INDIAN JOURNAL OF MALARIOLOGY

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

Editor :— S. P. RAMAKRISHNAN, M.B.B.S., D.P.H., D.Sc. (P.H.),
Director, Malaria Institute of India, Delhi.

Vol. XV, 1961.

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

Price per volume : Twelve rupees (or seventeen shilling six pence) including postage.
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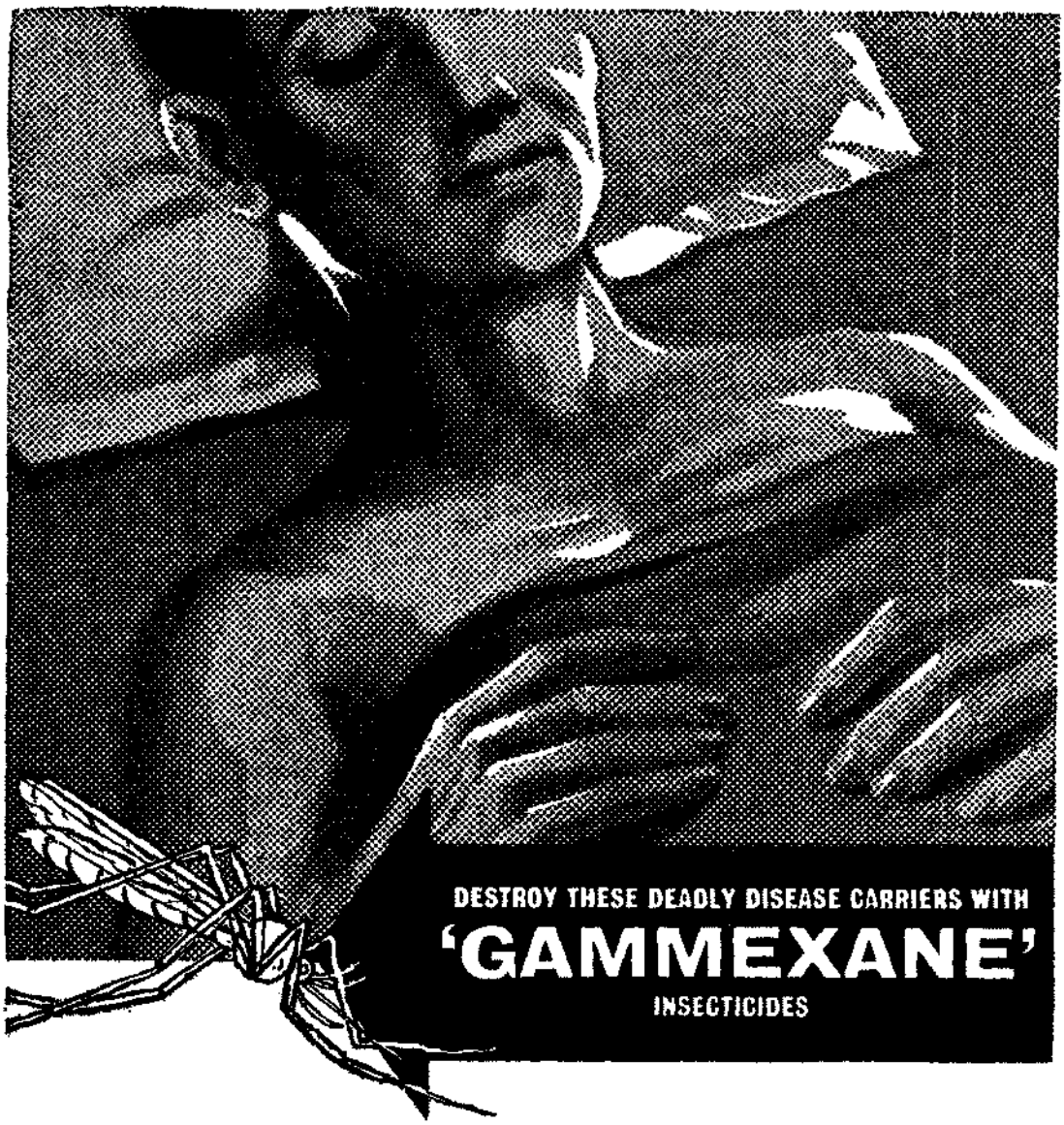

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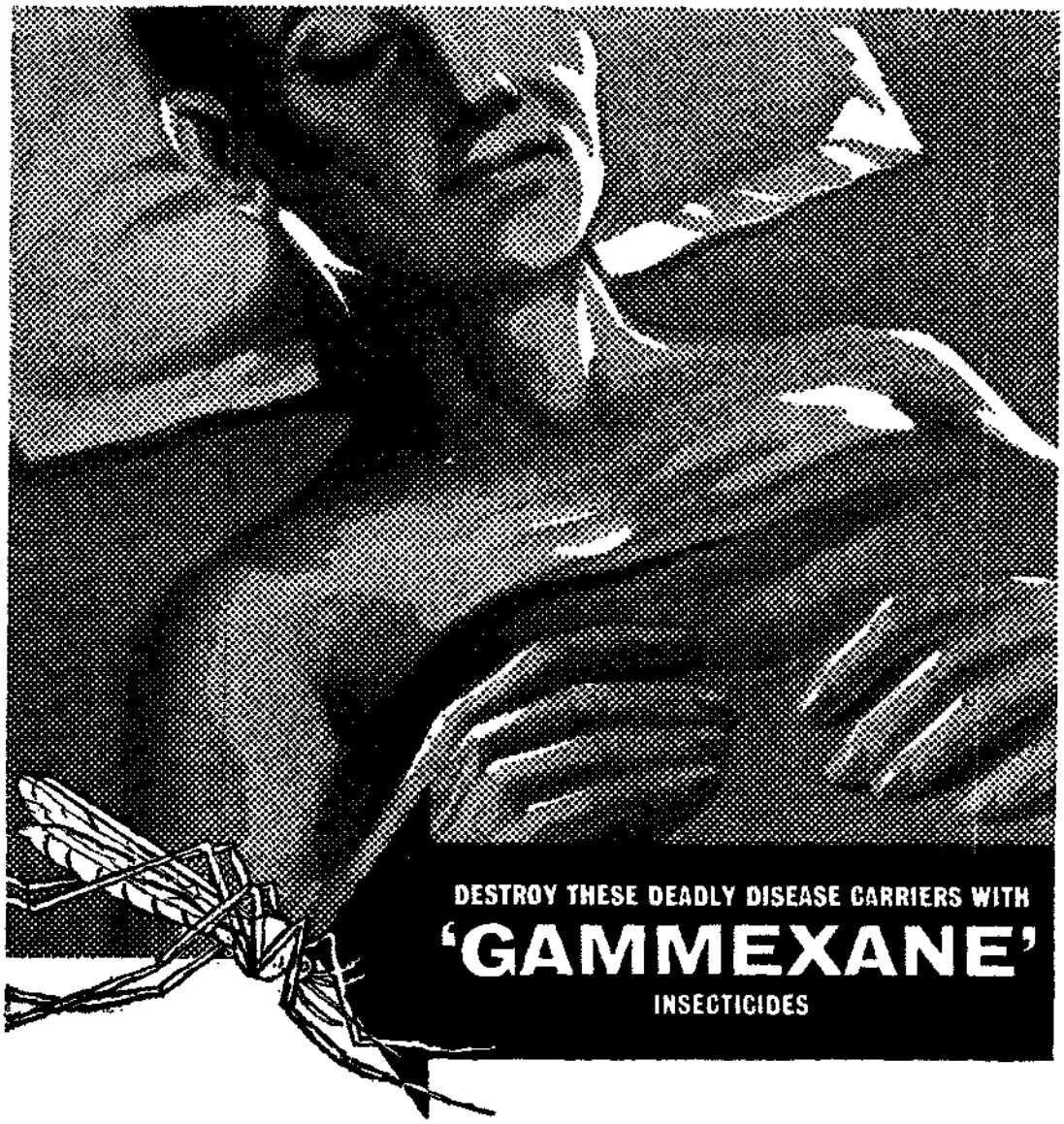

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Indian Journal of Malariology, **15**, *1, March 1961.*

A NOTE ON THE RESIDUAL FOCI OF MALARIA TRANSMISSION ENCOUNTERED IN THE COURSE OF EXPERIMENTAL SURVEILLANCE OPERATIONS IN COORG DISTRICT OF MYSORE STATE.

BY

M.T. AIAPPA.

(Assistant Public Health Officer, Coorg.)

[March 7, 1961.]

Coorg, formerly a centrally administered State, was merged with Mysore State, with the re-organisation of States in the country in 1956. It has since been called the Coorg District of Mysore State. The entire district is hilly, with an area of 1596 square miles and a population of 3·21 lakhs (1961 census). The climate of Coorg is temperate, the temperature ranging from 50°F. in December to about 90°F. in April. The district gets both the south-west and the north-east monsoons, with annual rainfall ranging from 120 to 250 inches in certain parts.

Coorg was one of the highly malarious areas of the western hill tracts with a spleen rate of 58·2 per cent in 1947. The main vector is *A. fluviatilis*. *A. culicifacies* plays a part in the transmission in the eastern part of the district which merges with the plains. Malaria control operations were started in Coorg in 1947 and became part of the National Malaria Control Programme in the country in 1953. The operations resulted in a dramatic absence of the disease as evidenced by the reduction in spleen rates which remained below one per cent during 1953, 1954 and 1955. The child parasite rate also recorded a steady fall as is shown in Table I.

TABLE I.

Child parasite rate.

Year.	Number of smears examined.	Number positive for malaria parasite.	Parasite rate, per cent.
1951	35	2	5·7
1952	249	7	2·8
1953	448	10	2·2
1954	493	4	0·8
1955	301	3	1·0
1956	131	0	Nil

Residual spraying with D.D.T. was suspended in the area in October, 1957, following the recommendations of an Expert Team appointed by the Government of India, who visited Coorg and certain other areas in Mysore and adjoining States. The criteria adopted by the expert team have been discussed in detail by Shama Sastry and Narayana Iyengar (1959). The Committee recommended the setting up of surveillance teams to take up active malaria surveillance in the rural parts of

Coorg District with a population of 1·58 lakhs, after excluding the population in compact areas around hospitals and the estate areas. The house visits were to be made at monthly intervals.

Malaria surveillance work in Coorg District was commenced from July, 1957, simultaneously with suspension of spraying operations. During the first year, the surveillance work had to be carried out exclusively by the 16 Health Inspectors and Sub-Inspectors. The population allotted to each worker ranged from 5 to 10 thousands. From August, 1958, however; eight surveillance workers were added, as recommended two years earlier by the Expert Committee of Malariologists. The surveillance sub-divisions are shown in Map 1.

PARASITE POSITIVE CASES.

For the first time, 11 months after suspension of spraying, two parasite positive cases were detected in the month of May, 1958. Both were from Srimangala Sub-Division. From September, 1958 onwards, parasite positive cases were coming up regularly every month as detailed in Table II below.

TABLE II.

Parasite positive cases from September, 1958 to March, 1959 in Srimangala Sub-Division.

Month and year.	Number of parasite positive cases.
May, 1958	2
July, 1958	4
August, 1958	2
September, 1958	5
October, 1958	1
November 1958	8
December, 1958	6
January, 1959	6
February, 1959	3
March, 1959	4

Thirty-three more positive smears were encountered in another Sub-Division (Balale) during the period September, 1958 to March, 1959, as detailed in Table III.

TABLE III.

Parasite positive cases from September, 1958 to March, 1959 in Balale Sub-division.

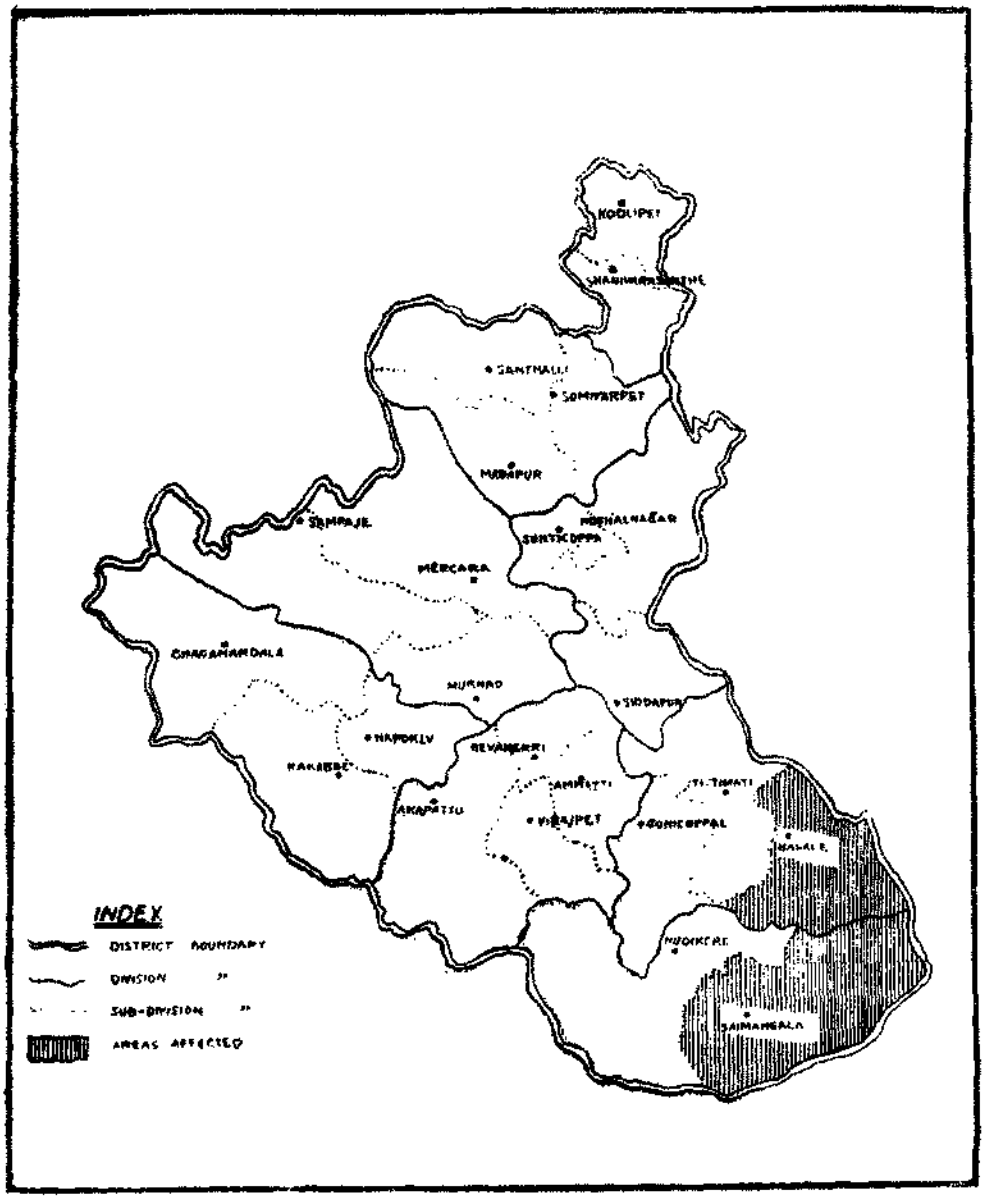
Month and year.	Number of parasite positive cases.
September, 1958	1
October, 1958	5
November, 1958	2
December, 1958	3
January, 1959	9
February, 1959	2
March, 1959	11

Isolated cases of parasite positive smears were recorded from the Gonikoppal and Virajapet sub-divisions.

MAP I.

COORG DISTRICT.

Surveillance Sub-Divisions (Units) and areas reporting malaria parasite positive cases.



The incidence of the species of plasmodia in the various sub-divisions is depicted in Table IV.

TABLE IV.

Incidence of the species of plasmodia in the various sub-divisions of Coorg district.

Sub-division.	POSITIVE SMEARS										Total number of cases.
	P. vivax.		*P. falciparum.*		*P. malariae.*		Mixed.		Species not determined.		
	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	
Srimangala	18	43·9	15	35·6	1	2·5	4	9·8	3	7·3	41
Balale	16	48·5	11	33·3	0	..	6	18·2	0	..	33
Gonikoppal	5	100	..	..	..	..	..	..	..	..	5
Virajapet	2	100	..	..	..	..	..	..	..	..	2

The details regarding the different villages where the positive cases were encountered are given in Table V. Most of the positive smears were collected from a few villages, namely, Badaga, Balale and Devanur in the Srimangala and Balale surveillance sub-divisions. Twenty-two of the 41 cases in the former sub-division were recorded from Badaga area. In the Balale Sub-division, 14 of the 33 positives were from Devanur while 9 were from Balale. All the positive cases occurred within an area confined to the south-eastern portion of Coorg District (Map 2) and have been among the Yerwars* and other tribal people.

EPIDEMIOLOGICAL INVESTIGATIONS.

The following investigations were carried out in March, 1959, in the areas where malaria parasite positive cases were recorded.

(*i*) Epidemiological investigations of the parasite positive cases to determine the source of infection.

(*ii*) General blood surveys, and

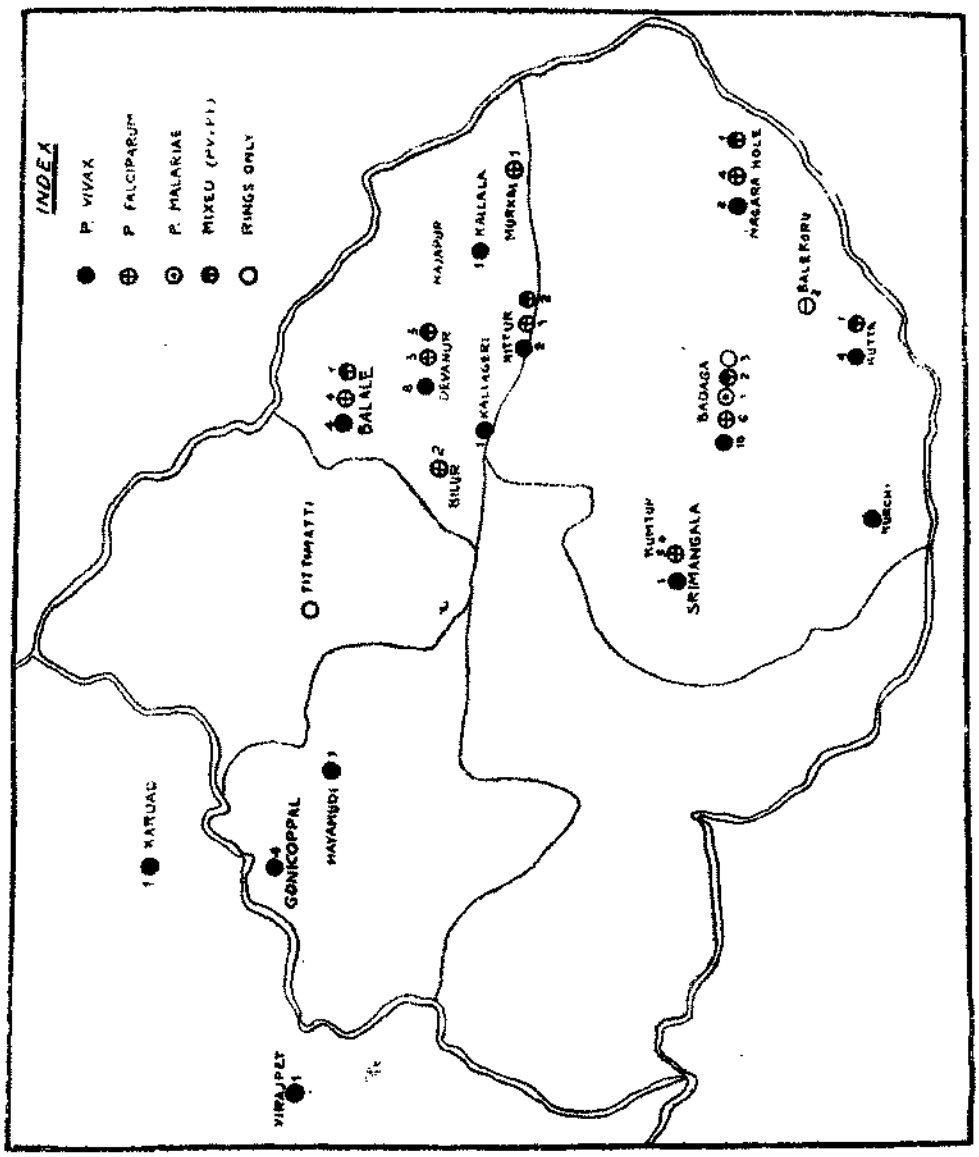
(*iii*) Entomological investigations.

Investigation to trace the source of the infections revealed that these cases were all of local origin and it appeared to indicate that autochthonous transmission was still going on in the areas, particularly those inhabited by the tribal people.

It was noted that five of the positive cases from Devanur area had been treated earlier with 8-aminoquinolines. The follow-up smears taken from them revealed that two of them still showed light infection (both *P. vivax*), one of them showing gametocytes.

Four-hundred-and-nine blood smears were collected during general surveys in the different villages. Eleven of these were found positive, two being *P. falciparum* infections, and the rest *P. vivax*. The details of the examinations from the different villages are depicted in Table VI.

*Yerwars are tribal population.



MAP 2.

Places where parasite positive cases were found in Coorg District.

TABLE V.

Village-wise distribution of malaria parasite positive cases reported from May, 1958 to March, 1959.

SRIMANGALA SUB-DIVISION (SURVEILLANCE UNIT).

Month and year.	KUTTA :			BADAGA :						NAGARAHOLE :				KUMTOOR :			BALEKOV :		KURCHI :		TOTAL :					
	P. vivax.	*P. vivax* plus *P. falciparum.*	Total.	*P. vivax.*	*P. falciparum.*	*P. malariae.*	*P. vivax* plus *P. falciparum.*	Rings only.	Total.	*P. vivax.*	*P. falciparum.*	*P. vivax* plus *P. falciparum.*	Total.	*P. vivax.*	*P. falciparum.*	Total.	*P. falciparum.*	Total.	*P. vivax.*	Total.	*P. vivax.*	*P. falciparum.*	*P. malariae.*	*P. vivax* plus *P. falciparum.*	Rings only.	Total.
1958																										
May	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1	..	..	1	1	..	..	..	2
June	..	..	..	2	..	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	2	..	..	..	..	2
July	..	..	..	..	..	..	..	..	..	..	1	1	2	..	..	..	..	..	..	..	..	1	..	..	..	3
August	..	..	..	1	..	..	..	1	2	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	3
September	..	..	..	1	1	..	..	2	4	1	..	..	1	..	..	..	..	..	..	..	2	1	..	..	..	6
October	..	..	..	..	..	..	1	2	3	..	..	..	..	..	..	..	..	..	..	..	2	..	..	1	..	3
November	..	..	..	1	2	..	..	..	3	1	3	..	4	..	..	..	1	1	..	..	2	6	..	1	..	9
December	1	..	1	5	..	..	..	..	5	..	..	..	..	..	..	..	..	..	..	..	6	..	..	..	..	6
1959																										
January	2	..	2	..	..	1	1	..	2	..	..	..	..	..	1	1	..	..	1	1	3	1	1	1	..	6
February	..	..	..	..	1	..	..	..	1	..	..	..	..	1	2	3	..	..	..	..	1	3	..	..	..	4
March	..	1	1	1	1	..	..	..	2	..	..	..	..	..	1	1	..	..	..	..	2	1	..	1	..	4
Total	**4**	**1**	**5**	**10**	**6**	**1**	**2**	**3**	**22**	**2**	**4**	**1**	**7**	**1**	**3**	**4**	**2**	**2**	**1**	**1**	**15**	**15**	**1**		**3**	**41**

TABLE V. (*Contd.*)

BALALE SUB-DIVISION (SURVEILLANCE UNIT).

Month and year.	BILUR :		NITTUR FOREST BLOCK :				DEVANUR :				BALALE :				KALLALA :		MURUKAL :		KOTTAGERI :		TOTAL :			
	P. falciparum.	Total.	*P. vivax.*	*P. falciparum.*	*P. vivax* plus *P. falciparum.*	Total.	*P. vivax.*	*P. falciparum.*	*P. vivax* plus *P. falciparum.*	Total.	*P. vivax.*	*P. falciparum.*	*P. vivax* plus *P. falciparum.*	Total.	*P. vivax.*	Total.	*P. falciparum.*	Total.	*P. vivax.*	Total.	*P. vivax.*	*P. falciparum.*	*P. vivax* plus *P. falciparum.*	Total.
1958																								
September	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1
October	..	..	2	..	..	2	1	..	..	1	..	..	..	..	1	1	1	1	..	..	4	1	..	5
November	..	..	..	..	..	..	..	..	..	..	1	1	..	2	..	..	..	..	..	..	1	1	..	2
December	..	..	..	..	2	2	..	..	..	..	..	..	..	..	..	..	..	..	1	1	1	..	2	3
1959																								
January	1	1	..	1	..	1	2	..	2	4	2	..	1	3	..	..	..	..	..	..	4	2	3	9
February	..	..	..	..	..	..	..	..	1	1	..	1	..	1	..	..	..	..	..	..	..	1	1	2
March	..	..	..	..	..	..	5	3	..	8	1	2	..	3	..	..	..	..	..	..	6	5	..	11
Total	2	2	2	1	2	5	8	3	3	14	4	4	1	9	1	1	1	1	1	1	16	11		33

M. T. Aiappa.

ISOLATED CASES REPORTED FROM OTHER AREAS.

Month and year.	GONIKOPPAL DIVISION :		VIRAJAPET DIVISION :		Remarks.
	Muyamudi.	Gonikoppal Hospital.	Karmad (Ammatti).	Virajapet Hospital.	
October, 1958	1 *P. vivax*	..	..	..	
February, 1959	..	..	1 *P. vivax*	..	
March, 1959	..	4 *P. vivax**	..	1 *P. vivax*	
Total	1 *P. vivax*	4 *P. vivax**	1 *P. vivax*	1 *P. vivax*	

*2 Cases were reported to be from Srimangala.
1 Case was reported to be from Palghat.
1 Case was reported to be from Gonikoppal.

TABLE VI.

Details of the examination of blood-smears.

Village.	Number of smears examined.	Number of smears positive.	SPECIES OF PLASMODIUM :	
			P. vivax.	*P. falciparum.*
Nanachi Badaga	63	4	2	2
Balale	178	0	..	..
Devanur	147	0	..	..
Navilgadde	21	7	7	..

Seven out of 26 children examined from Navilgadde showed enlarged spleens of varying sizes. The child spleen rate in this area worked out to 73·1 per cent.

Entomological investigations consisted of collection and dissection of mosquitoes. The collections were made during the day between 6·30 and 11·30 a.m. and at two-hourly intervals at night between 8 p.m. and 6 a.m. The different types of shelters were searched during the day collections ; night collections were restricted to human dwellings only.

Fourteen man-hours spent over the night collections at Navilgadde yielded a total of 123 anophelines, of which 89 were *A. fluviatilis*. The other species of anophelines collected were *A. jamesi*, *A. jeyporiensis*, *A. subpictus* and *A. vagus*.

The density of *A. fluviatilis* worked out to 6·35 per man-hour.

Mosquito collections were made from the villages of the Srimangala and Badaga sub-divisions also, both during day and night. *A. fluviatilis* was collected both during the day and night collections. The following anophelines were also collected during these visits : *A. aconitus*, *A. annularis*, *A. jamesi*, *A. jeyporiensis*, *A. pallidus*, *A. suplendidus*, *A. subpictus*, *A. tessellatus* and *A. vagus*.

Dissection of *A. fluviatilis* showed the presence of gland infection in Navilgadde village, where a sporozoite rate of 6·7 per cent was recorded. The details of the dissections are depicted in Table VII.

TABLE VII.

Details of dissections of A. fluviatilis.

Village.	Number of *A. fluviatilis* dissected.	Number with gland infection.	Sporozoite rate, per cent.
Kallahalla Kumri, Devanur and Badaga (night collections)	24	Nil	..
Navilgadde, Devanur and Badaga (day collections)	11	Nil	..
Navilgadde (night collections)	89	6	6·7

Devanur, Balale, Badaga and Balekov, where mosquitoes were collected on April 29, 1959, were all sprayed during February, 1959, because of appearance of positive cases. Morning collections made in these villages on April 29, 1959,

yielded anophelines in unsprayed structures. The per-man-hour densities of all anophelines and *A. fluviatilis* are depicted in Table VIII.

TABLE VIII.

Per-man-hour densities of all anophelines and A. fluviatilis. *(Morning collections).*

Village.	ALL ANOPHELINES :		*A. fluviatilis* :	
	Human dwellings.	Cattle-sheds.	Human dwellings.	Cattle-sheds.
Devanur	0·0	8·0	0·0	3·2
Balale	0·66	0·0	0·0	0·0
Badaga	0·65	0·0	1·0	0·0
Balekov	0·0	0·0	0·0	0·0

The night collections made in the first three villages and also at Kallahalla Kumri gave overall densities of all anophelines, and *A. fluviatilis*. These are depicted in Table IX :

TABLE IX.

Per-man-hour densties of all anophelines and A. fluviatilis *(Night collections).*

Viltage.	ALL ANOPHELINES :		*A. fluviatilis* :	
	Human dwellings.	Cattle-sheds.	Human dwellings.	Cattle-sheds.
Devanur	0·0	5·3	0·0	0·66
Balale	0·0	1·0	0·0	0·0
Badaga	6·8	13·3	0·0	2·33
Kallahalla Kumri	20·0	0·0	5·0	0·0

At Navilgadde (unsprayed village), the morning collections on April 30, 1959, revealed a per-man-hour density of 5·0 for *A. fluviatilis*. Night collections made on May 1-2, 1959, revealed a per-man-hour density of 6·35 for the same species.

RESULTS OF DISSECTION.

Thirty-five specimens of *A. fluviatilis* collected on the morning and night of April 29, 1959, and on the morning of April 30, 1959, did not show gland infection. Six out of 89 specimens of *A. fluviatilis* collected at Navilgadde (unsprayed village) on May 1-2, 1959, showed gland infection, yielding a sporozoite rate of 6·7 per cent.

DISCUSSION.

The investigations revealed that malaria transmission was going on in the areas inhabited by the tribal population. These areas are densely forested and thinly populated. The people live in scattered sylvan habitations which have never been sprayed in the past. About 35-40 per cent of the tribals live in the above-mentioned areas, while the rest reside in compact groups in thinner forest areas with habitations easily accessible and which appear to have received total coverage. It was learnt that in the former areas, the practice was that the requisite quantity of D.D.T. was handed over to the staff of the Forest Department who ensured that

the Government buildings were sprayed but not the other habitations. It is, therefore, obvious that the spraying was never according to the required standard, and transmission of malaria had continued in such areas.

The parasite positive cases were recorded from the Balale surveillance subdivision from September, 1958, when the malaria surveillance worker was posted to this locality for the first time. The parasite positive cases, as mentioned earlier, were mostly confined to the tribal areas in the south-eastern portions of Coorg District. General blood surveys in the area confirmed these observations; four positive smears out of 63 examined were from the *Yerwars* which is a tribal population in the area.

The two parasite-positive cases recorded in persons with a history of having received treatment with 8-aminoquinoline is a definite evidence that autochthonous transmission has been in progress. This was further substantiated during talks with a local estate owner who stated that he had been experiencing lot of difficulties in obtaining labour due to malaria. The situation, he stated, was so bad that he desired to sell away the estate and leave the area.

Maintenance of the infection in Badaga, Balale and Devanur villages (Table V) appears to be largely due to migration of *Yerwars* (tribal population) and labour population from these villages to the forest areas and *vice versa* in connection with forest labour.

It was, therefore, decided to start residual spraying in these areas, and to continue the same concurrently with the active surveillance programme for 3 years.

SUMMARY.

Spraying of D.D.T. was interrupted and active surveillance started in Coorg District in 1957. Parasite positive cases were recorded every month from some villages in the south-eastern portion of the district. Investigations gave evidence that autochthonous transmission was in progress. Spraying has been re-introduced and is to continue for 3 years concurrently with the active surveillance.

ACKNOWLEDGEMENT.

Grateful thanks are due to the Director of Public Health in Mysore, Bangalore, for permitting to publish this paper and to Dr. S.P. Ramakrishnan, (Director, Malaria Institute of India, Delhi), Dr. S.R. Bhombore (Regional Deputy Director, Regional Co-ordinating Organisation, National Malaria Eradication Programme, Coonoor), Dr. H. Shama Sastry (Deputy Director of Public Health, National Malaria Eradication Programme, Bangalore), and to the staff of the Bureau of Malariology, Bangalore, for their guidance and active participation in this investigation.

The faithful co-operation of the members of the Malaria Organisation of Coorg District is gratefully acknowledged.

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Indian Journal of Malariology, **15**, *1, March 1961.*

A NEW HOST RECORD FOR *PLASMODIUM RELICTUM* : THE SILVER GULL (*LARUS NOVAE-HOLLANDIAE* STEPHENS.)

BY

J.J. LAWRENCE*

AND

A.J. BEARUP.

(School of Public Health and Tropical Medicine, Sydney, Australia.)

[September 15, 1960.]

INTRODUCTION.

In January, 1958, parasites were noticed in blood films taken from a silver gull but although these parasites obviously belonged to the genus *Plasmodium*, they did not seem to be identical with any of the described species of avian plasmodia. This gull was one of a number of fledglings caught at Five Islands, off Wollongong, N.S.W., in November and December, 1957, which had been kept for the subsequent time in Sydney. The remaining five birds were then examined and two more infections found. An adult gull, caught at the same place in December, was negative. Seven more fledgling gulls were caught at Five Islands in November, 1959, but no parasites could be detected in their blood.

This paper describes the morphology of the parasite as seen in the gull together with the evidence that leads us to conclude that it is identical with *Plasmodium relictum* (Grassi and Feletti), and that the variation from the typical *relictum* morphology is due to the unusual host.

DESCRIPTION.

The following description is based mainly upon smears taken from one gull which showed the heaviest infection but the appearance of the parasite was similar in the other two naturally infected gulls.

Gametocytes.—These are usually elongate but may be oval to round. The elongate gametocytes, as a rule, showed no tendency to encircle the nucleus; only on very rare occasions did they curl slightly around one or both ends of the nucleus. The male gametocyte was much more often oval to round than the female, in which the modal form might be described as sausage-shaped. The male gametocyte often had an amoeboid appearance, giving it quite a ragged outline,

* It is regretted that Dr. J.J. Lawrence died on 26th May, 1960, after a long illness. Not long before he died, he completed the experimental work and prepared a draft of the present paper, almost in its final form.

—*A.J. Bearup.*

while the female rarely looked like this. The nucleus was usually displaced, even by the elongate gametocytes, and occasionally it was expelled. Not infrequently the gametocyte lay obliquely across the long axis of the red blood cell and, of course, displaced its nucleus. The pigment granules were normally round and coarse but varied somewhat in size and shape and usually numbered about nine. The pigment in the female gametocyte often tended to be concentrated at one end of the parasite.

Asexual forms.—The youngest trophozoites seen are small and solid-looking. Although vacuoles may develop later, typical ring forms are uncommon. The proportion of trophozoites in immature red blood cells did not seem high as judged in Giemsa-stained films (vital staining was not done). Pigment granules, which are coarse and round or irregular in shape, can be seen in the trophozoites when they are about one-quarter grown. The older trophozoites and the schizonts usually are amoeboid in appearance. They show no marked tendency to occupy a polar position or to be close to the nucleus. The number of merozoites seen in the segmenters was usually 8 but one with 14 was noted. The larger schizonts tended to displace the nucleus.

From the time that the infection was first detected, parasites continued to appear for one month in the blood of the only gull which was kept under continuous observation. No synchronicity was noted in the strain and no attempt was made to determine its periodicity.

It will be noticed that the main difference from *P. relictum* lies in the shape of the gametocyte which was usually elongate but it might be oval or round as is typical of *P. relictum*. *P. juxtanucleare* Versiani and Gomes is said to have round or elongate gametocytes and *P. durae* Herman, gametocytes which are elongate or irregular and which frequently take up an oblique position in regard to the long axis of the host-cell nucleus; but the gull parasite did not resemble either of these two species in other respects.

Transmission, via the mosquito, to a sparrow.—A number of mosquitoes, belonging to the species *Culex fatigans* Wiedmann, were fed on one of the infected gulls and kept at a temperature of 25°C. Sporozoites were present in the salivary glands after a fortnight. Two sets of positive salivary glands were triturated in fowl-serum Ringer's solution and the sporozoites were inoculated into a sparrow. This sparrow had been kept in captivity for a fortnight and blood smears made from it had always been negative. A sparrow was used in preference to a canary because the local canaries seem to be infected even more frequently than the sparrows. It was hoped that the sparrow used was free from infection when inoculated and the course of its malarial infection seems consistent with this assumption. Blood films made from the sparrow continued to be negative for 5 days but were positive on the eighth day.

The morphology of the gull plasmodium, as seen in the sparrow, was quite consistent with that of *P. relictum*. The gametocytes were round to oval and many

of the trophozoites were present in the immature red blood cells. The number of merozoites in segmenters seemed to be greater than when in the gull and a count confirmed this impression : in the sparrow the average number of merozoites was 14·1 (range 9 — 18), and in the gull 9·6 (range 8 — 12). This difference is significant (P 0·01). The number of merozoites in *P. relictum* varies greatly according to strain, from 8 up to 32.

Transmission of P. relictum *to gulls.*—Three fledgling gulls were kept for two months in a mosquito-proof room during which period their blood was consistently negative for parasites. The smallest gull (weight=215 grams) was then inoculated intramuscularly with about 0·6 ml. of blood from a sparrow which showed a typical infection with *P. relictum*. The calculated number of parasites injected was $9{\cdot}5 \times 10^7$. Parasites were first detected in the gull after 7 days and became very numerous after two weeks. This very heavy infection persisted for about a week and the number of parasites then diminished sharply. The bird was very anaemic at this stage and the great majority of red blood cells were immature. About a fortnight later, when the bird was recovering from its anaemia, the number of parasites increased again.

Before the infection had reached its height, it was passed on to a second gull by inoculation intramuscularly of about 0·6 ml. blood, containing about $2{\cdot}3 \times 10^7$ parasites. This gull appeared older than the other and weighed 320 grams. Parasites were detected in its blood after 6 days and continued to be present for over 3 weeks but the peak of parasitaemia was not as high as in the first gull.

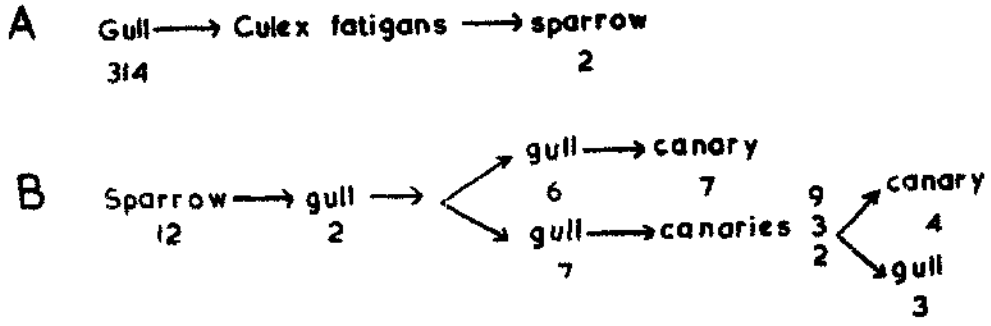
The appearance of the plasmodium in the sparrow had been quite typical of *P. relictum* but in the gulls it assumed a morphology quite like that of the plasmodium seen in the naturally infected gulls; most important, it showed the marked tendency towards elongation of the gametocytes, especially the female, which characterises the "gull plasmodium". The average number of merozoites per segmenter was 8·4 and the range, 5 — 16. In the canary, this strain showed an average number of merozoites per segmenter of 11·7 and a range of 9 — 15. The merozoites of *P. relictum*, when in the gull, did not show their usual predilection for young red blood cells. Using Hegner & Eskridge's (1938) method of vital staining with brilliant cresyl blue, followed by a Romanowsky stain, and also their system of classifying the cells it was found that only 6 per cent of the early trophozoites were present in the immature red blood cells (types I-III) and that these were 5·5 per cent of the total number of red blood cells. Only Giemsa stained films were available from the sparrow but cell types I-III may still be recognised, mainly by their nuclear structure. It was found that 93·5 per cent of the early trophozoites were found in the immature red blood cells which represented 6·8 per cent of the total red blood cells.

This strain of *P. relictum* was then passed from the second gull to a canary by the intramuscular injection of 0·5 ml. of blood, containing about 10^5 parasites. This canary was one of a batch of seven received from Hobart, Tasmania, which all

seemed free from infection. Blood films became positive after 4 days and the parasite resumed the morphology typical of *P. relictum*.

This strain showed little or no synchronicity whether in gull, sparrow or canary, and an attempt to see if there was any change in periodicity, when in the gull, was very difficult for this reason.

FIG. 1.
Plasmodium relictum.
Diagram of experimental work on two naturally-acquired infections.
(Gull 314 and Sparrow 12).



DISCUSSION.

P. relictum is one of those avian plasmodia that show little host specificity. It has been found present in quite a variety of species belonging to many different families of passerine birds and even to several different orders. For instance, Fantham and Porter (1944) recorded natural infections in four species of penguins and Rodhain (1939) found penguins in the Antwerp Zoo to be infected. In this latter case Rodhain produced good evidence that the penguins had been infected after their arrival at the Zoo. It is probable that this was the case with the gulls also, namely that they were infected in captivity, since they were only found to be infected after they had been kept for one to two months in an unscreened cage at a season of the year when both *C. fatigans* (a good vector for *P. relictum*) and infected sparrows are numerous.

Since it is not usual in survey work to subinoculate the plasmodium into canaries, unless the morphology was typical in the host bird, it would not be recorded as *P. relictum*, so it will be realised that this parasite retains its typical morphology in a great variety of hosts. However, *P. relictum* (and the closely related *P. cathemerium*) have been successfully adapted to chicks and ducks by means of repeated passage, and with some strains rather marked changes in morphology have been reported. Wolfson (1939) found that in ducks quite a number of gametocytes of both sexes were elongate, and even those that were not, exhibited a tendency to surround the nucleus rather than displace it. In most of the elongate gametocytes, the pigment granules were located either at the end or in the middle

of the parasite. Hegner and West (1941) found that *P. cathemerium* behaved rather similarly. Although very few of the gametocytes were long and slender, lying parallel to the nucleus as they do in *P. elongatum* and *P. rouxi*, most of them showed a concavity in the side nearest the nucleus as if their tendency was to grow around it rather than displace it. In the chicken, Manwell (1943) found that *P. relictum* failed to displace the nucleus at all except when it pushed it laterally. Fewer than 10 per cent of the gametocytes were round or nearly so : more usually they were elongate and could resemble those of *P. circumflexum* to a marked degree. However, in the duck such modifications of the normal morphology were much less noticeable than in the chicken.

These morphological changes are rather similar to those seen in the "gull plasmodium" and when *P. relictum* is transferred to the gull, but in this case there is no suggestion that the gametocyte becomes elongate because it is restricted by the nucleus; indeed, the nucleus is just as readily displaced by elongate gametocytes, which often lie across the long axis of the red blood cell, as by round ones. Schizonts and even segmenters might be elongate at times and fail to displace the nucleus but such forms are also seen in the canary. The tendency for the pigment in the elongate gametocytes to be concentrated, as noted by Wolfson in ducks, was also seen in female, and less commonly in male gametocytes in gulls. The pigment was always concentrated at one pole in elongate gametocytes or towards the edge in round female gametocytes.

The average number of merozoites per segmenter was 9·6 in the "gull plasmodium" but 14·1 when the infection was transferred to a sparrow. Similarly the average number of merozoites in our strain of *P. relictum*, when in the gull, was 8·4 but was 11·7 in the canary. Passage of a strain may affect the number of merozoites per segmenter but here the suggestion is that the gull is a less favourable host for the plasmodium.

Both the periodicity and the time of segmentation may be changed by passage to an abnormal host but the synchronicity of the strain of *P. relictum* used was so low that its periodicity could not be determined with any certainty. It is concluded that the plasmodium seen in the naturally infected gulls was *P. relictum*, modified by sojourn in abnormal host. The importance of this observation lies in the fact that the avian plasmodia are divided into two broad groups on the basis of whether their gametocytes are elongate or round. If the parasites seen in a blood film were few, it would be easy to mis-identify the species or at best, be unable to assign a specific name to it, unless resort was made to sub-inoculation.

Stained blood films of the gulls, canaries and sparrows used in this study, have been deposited in the Museum Collection of this School, numbered Mn. 1476; and in the Australian Museum, No. Z 2785.

EXPLANATION OF 17 SUB-FIGURES APPEARING ON THE OPPOSIT PAGE UNDER THE HEADING "FIG. 2".

Plasmodium relictum.

Comparison of parasites as they appear in the blood of the canary or sparrow; and in the gull. (Outline of parasites traced from projected colour film).

Sub-Figure

1. Trophozoites in the gull.
2. Trophozoites in the gull.
3. Trophozoites in the gull.
4. Young schizont in the gull.
5. Mature schizont, Canary 4.
6. Mature schizont, Canary 4.
7. Mature schizont, Sparrow 12. Uninfected cell below.
8. Mature schizont, Gull 2.
9. Mature schizont, Gull 314.
10. Mature schizont, Gull 314.
11. Male gametocyte, gull.
12. Female gametocyte, Gull 314.
13. Female gametocyte, Gull 314.
14. Female gametocyte, Gull 7.
15. Male gametocyte, Gull 7.
16. Male gametocyte, Canary 4.
17. Male gametocyte, Sparrow 12.

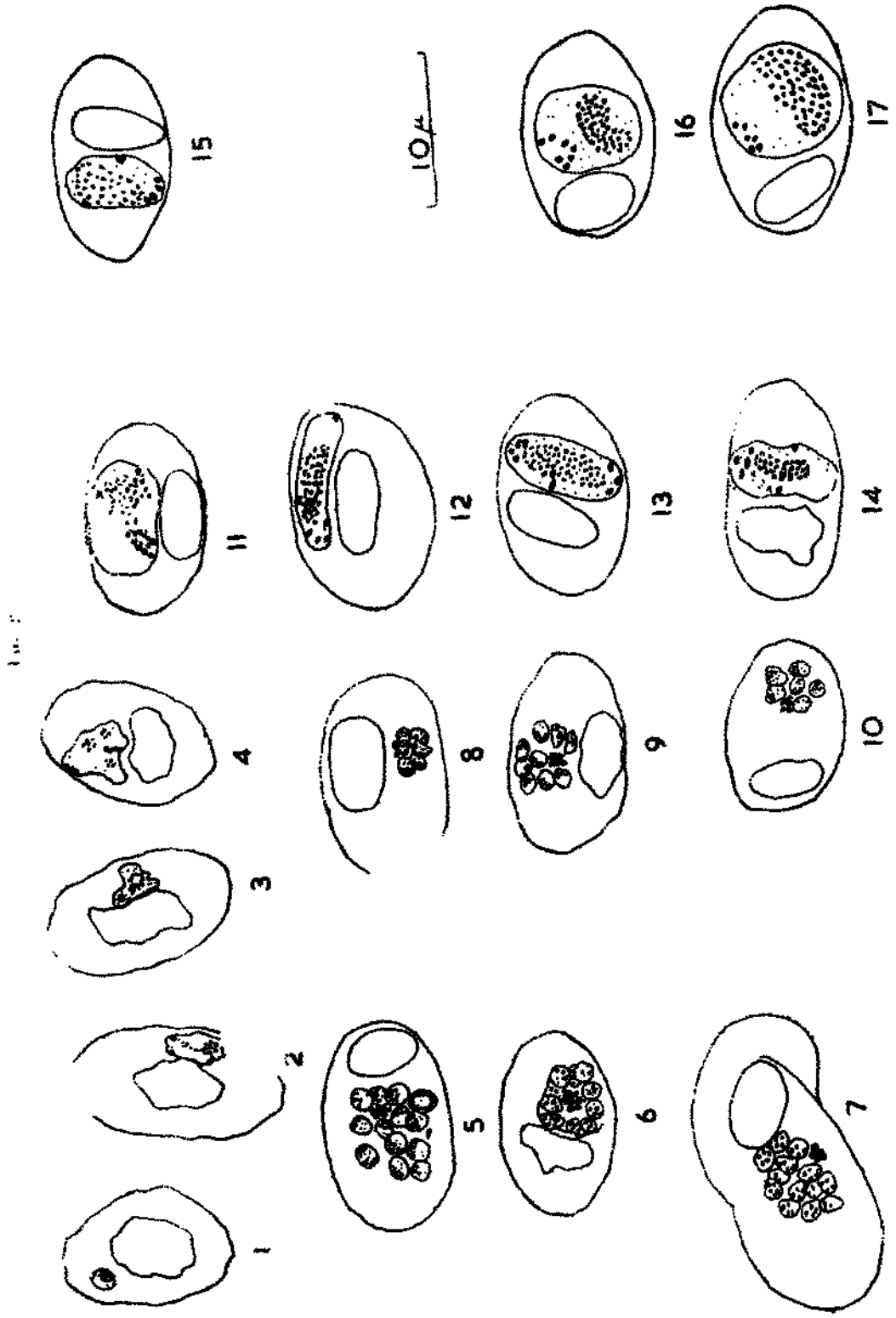

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PLATE I

Photomicrographs of *Plasmodium relictum* from the silver gull *Larus novae-hollandiae*.

Fig. A.—Gametocytes from Gull 2.

Fig. B.—Schizonts from Gull 3.

SUMMARY.

Three silver gulls (*Larus novae-hollandiae*) were found to be infected with a species of *Plasmodium* which differed morphologically from the hitherto described species. The parasite was transferred via the mosquito, *Culex fatigans*, to a sparrow, in which host it assumed the morphology normal to *P. relictum*.

A strain of *P. relictum* was passaged by blood inoculation in young gulls, and in these it assumed an abnormal morphology which made it closely resemble the plasmodium seen in the original gull infections. On passage back to a canary it resumed its original morphology. It is concluded that the parasite seen in the naturally infected gulls was *P. relictum* modified by its sojourn in a different host.

ACKNOWLEDGEMENTS.

The authors wish to thank the following persons for assistance in the project; Dr. B. Reid and Mr. Peter Blackwell of the Sydney University, and Mr. H.V. Golding of this School for microphotos of the parasites; Miss J. Oliver for assistance with the technical work and Mr. M. Olsen of the C.S.I.R.O. in Hobart for the supply of canaries.

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MANWELL, R.D. (1943) ... ... Malarial infections by four species of *Plasmodium* in the duck and chicken, and resulting parasite modifications. *Amer. Jour. Hyg.*, **38**, p. 211.

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Indian Journal of Malariology, **15**, *1*, *March 1961*.

FILARIASIS IN THE DISTRICT OF GHAZIPUR (UTTAR PRADESH).

BY

DIWAN CHAND*,

M.V. SINGH†

AND

V.K. PATHAK‡.

(April 16, 1960.)

INTRODUCTION.

A FILARIASIS survey was carried out in the district of Ghazipur by the Filaria Survey Unit, between April, 1956 and April, 1958. Results of the survey are recorded in this paper.

AREA, POPULATION AND CLIMATE.

The district of Ghazipur forms a part of Varanasi Division and lies north-east of Varanasi District. Its maximum length from east to west is about 56 miles and width from north to south is about 37 miles. The area of the district is about 1,392 square miles. It is divided into four tehsils having 2,129 villages (Map 1). The total population of the district is 11,41,278 (1951 Census) out of which 5,70,725 are males and 5,70,553 females.

The general slope of the district is from north-west to south-east. On the whole, the district is a fertile plain, the only marked surface variations are caused by the broad valley of the River Ganga and by channels of minor streams. There are no major forests but a few jungles are present.

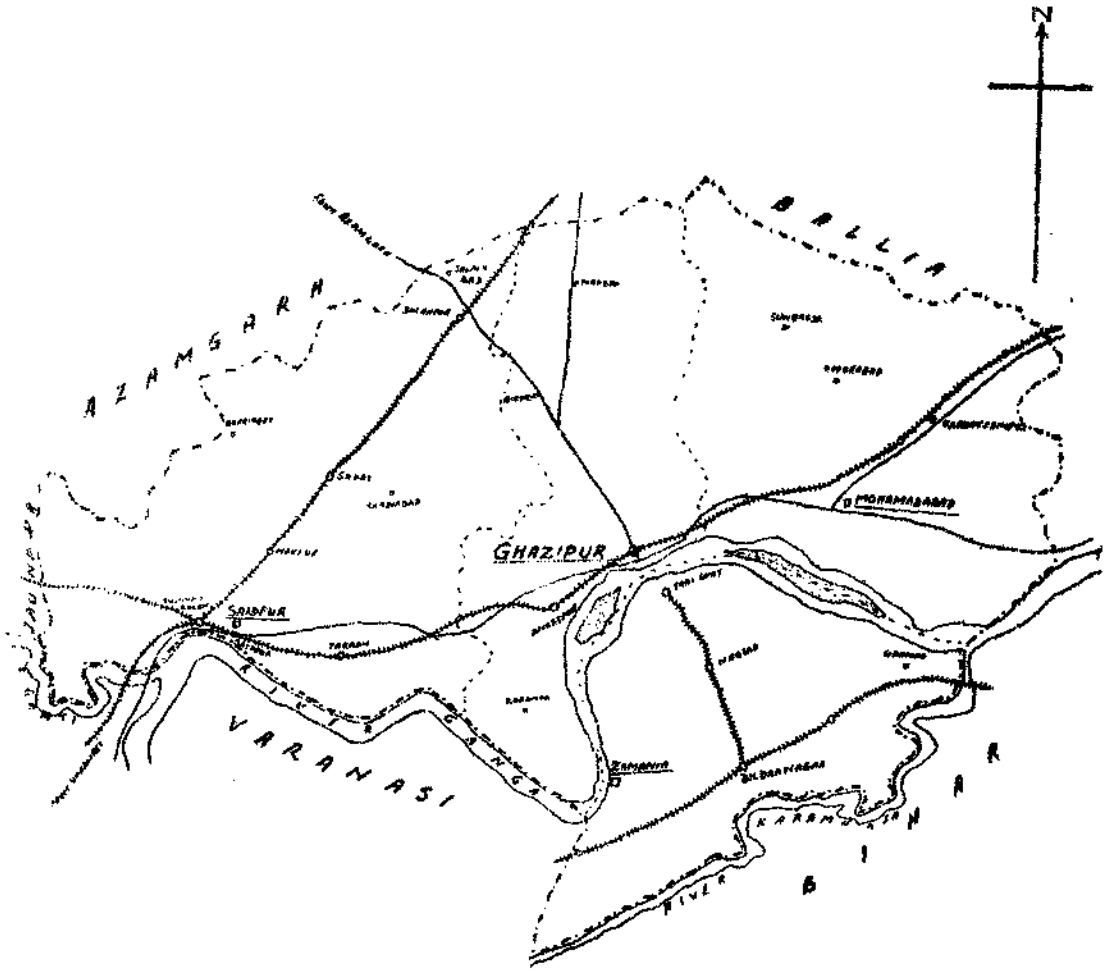
Ghazipur Town, the headquarter of the district, is a municipal town with a population of 28,322. It has a limited safe water supply. Large number of persons are still using hand-pumps and wells for their house-hold use. There is no planned drainage system and this results in the stagnation of waterin pits and small pools, forming ideal places for breeding of mosquitoes.

Climatically, the district has comparatively short cold weather and long, though not very hot, summer. The maximum and the minimum temperature, is 41·8°C. and 8·2°C. respectively. The average annual rainfall is about 43·1 inches

* Deputy Director, Malariology, Uttar Pradesh, Lucknow.
† Assistant Director, Malariology, Uttar Pradesh, Lucknow.
‡ Filaria Survey Officer, Ghazipur.

Map I.

MAP OF GHAZIPUR DISTRICT.



and relative humidity ranges from 71 to 89 per cent. The meteorological data of the district for the years 1957 and 1958 are depicted in Table I.

TABLE I.

Monthly maximum and minimum temperature, relative humidity and rain-fall of Ghazipur District for the years 1957 and 1958.

Month and Year.	Maximum temperature °C.	Minimum temperature °C.	Relative humidity, per cent.		Rain-fall (inches).
			08.30 hours.	17.30 hours.	
1957					
January	22·5	8·9	87	63	1·78
February	26·1	8·2	67	32	0·03
March	31·3	31·3	58	29	0·50
April	38·5	18·8	36	18	0·04
May	41·8	24·1	35	17	0·00
June	39·1	25·6	67	39	0·82
July	33·2	23·8	83	75	14·36
August	33·7	23·6	80	76	11·64
September	34·2	22·1	78	60	6·38
October	33·9	17·2	62	41	..
November	29·9	9·0	60	35	..
December	25·2	9·8	75	35	0·01
1958					
January	25·3	10·3	78	51	0·61
February	27·3	11·7	61	36	0·05
March	34·1	17·2	46	23	0·31
April	39·1	23·3	44	21	0·00
May	42·0	25·8	42	16	0·00
June	41·8	27·8	58	35	1·84
July	33·9	26·8	80	67	12·91
August	31·6	25·8	88	53	10·51
September	32·8	25·7	84	72	7·39
October	31·6	22·6	79	67	3·22
November	29·5	14·9	77	47	..
December	25·3	12·5	85	49	..

The district is connected with neighbouring areas by rail and road. Common festivals like Dasherra, Ramnaumi, Sheoratri are celebrated. Full moon of *Kartik*, a great bathing day, attracts thousands of people from neighbouring districts.

MEDICAL AND PUBLIC HEALTH ORGANISATION.

There is a district hospital and a maternity and child-welfare centre at the district headquarters. Besides, there are fifteen allopathic dispensaries, twelve dispensaries of indigenous medicine, nineteen maternity and child-welfare centres and two primary health units in the district. A Malaria Eradication Programme Unit has been established in the district since 1959-60. Cholera and small-pox are endemic throughout the district.

FILARIA SURVEY.

Elephantiasis has been known in the district for quite a long time but during the recent years the general public is more conscious of the disease, probably due to better general as well as health educational facilities. The survey of Ghazipur District (rural area) was conducted from April 14, 1956 to April 11, 1958 and of Ghazipur Town between July and October, 1956. Persons of all age-groups, and both sexes, were examined by house-to-house visits in the night between 8 p.m. and mid-night. Care was taken to examine all the persons in each house. Approximately 20 c.mm. of blood was obtained from each person for preparing thick smears which were examined after staining with J.S.B. Stain I. The disease manifestations were also recorded side by side.

INFECTION RATE.

In the rural areas of the district, the number of persons examined during the survey was 11,212 (2·4 per cent of the population) while in the urban areas of Ghazipur Town 1,838 (6·4 per cent of the population) were examined. Table II shows the infection rate in the rural areas according to different age-groups.

TABLE II.

Infection rate according to different age-groups (rural areas).

Age-groups (years).	Number of persons examined.	Number of persons showing infection.	Infection rate, per cent.	Average infestation per 20 c.mm. of blood.
0 to 1	34	Nil	Nil	Nil
2 to 5	619	5	0·80	9·6
6 to 10	1,909	38	0·99	9·4
11 to 20	2,973	192	6·45	8·2
21 to 30	2,188	225	10·23	8·2
31 to 40	1,670	198	11·85	8·8
41 to 50	1,107	117	10·60	9·3
Above 50	712	78	10·95	6·1
Total of all age-groups :—	11,212	853	7·6	8·7

The infection rate worked out to be 7·6 per cent. The lowest age at which the infection was found, was in a male child of 2 years. The infection rate increased with the age, being maximum in the age-group of 31 to 40 years, after which it showed a decline. The type of infection detected was *W. bancrofti*.

The average infestation was found to be 8·7 per 20 c.mm. of blood and the maximum number of microfilaria found in one blood smear was 116.

The results of blood examination in urban areas, according to various age-groups, are presented in Table III.

From the Table III, it is seen that the infection rate in urban area is higher than the rural rate. The highest infection was found in the age-group of 41-50 years. The average infestation was also higher in the urban area.

TABLE III.

Infection rate according to different age-groups (urban area).

Age-groups (years)	Number of persons examined.	Number of persons showing infection.	Infection rate, per cent.	Average infestation per 20 c.mm. of blood.
0 to 1	3	Nil	Nil	Nil
2 to 5	61	3	4·91	6·3
6 to 10	225	11	4·8	9·3
11 to 20	500	41	8·2	14·9
21 to 30	549	58	10·5	13·5
31 to 40	295	40	13·5	13·1
41 to 50	144	23	15·9	9·7
Above 50	61	5	8·2	8·4
Total of all age-groups :—	1,838	181	9·84	12·7

DISEASE MANIFESTATIONS.

During the survey, the persons from whom blood was taken were also examined for disease manifestations. Out of 11,212 persons in rural areas 1,007 were found to have various disease processes in the form of swelling of the scrotum, hydrocoele, elephantiasis of limbs and chyluria. The disease rate was 8·98 per cent.] Table IV shows the incidence of disease according to age-groups.

TABLE IV.

Disease rate at different age-groups (rural areas).

Age-groups (years).	Number of persons examined.	Number of persons showing disease manifestations.	Disease rate, per cent.
0 to 1	34	Nil	Nil
2 to 5	619	5	0·8
6 to 10	1,909	21	1·1
11 to 20	2,973	125	4·2
31 to 30	2,188	266	12·15
31 to 40	1,670	272	13·29
41 to 50	1,107	189	17·07
Above 50	712	129	16·71
Total of all age-groups :—	11,212	1,007	8·98

The earliest age at which the disease was found was in a child of 2 years having hydrocoele. The disease rate increased up to a limit with the advancing of age.

The results of disease manifestations in urban areas are shown in Table V.

The study of disease processes made during the present survey revealed that hydrocoele and other genital affections represented the maximum and then came the elephantiasis of lower limbs and the upper limbs. Chyluria was also noted in very few cases.

TABLE V.

Disease rate at different age-groups (urban areas.)

Age-groups (years).	Number of persons examined.	Number of persons showing disease manifestations.	Disease rate, per cent.
0 to 1	3	Nil	Nil
2 to 5	61	1	1·6
6 to 10	225	3	1·3
11 to 20	500	23	4·6
21 to 30	549	39	7·1
31 to 40	295	32	10·8
41 to 50	144	19	13·2
Above 50	61	11	18·03
Total of all age-groups :—	1,838	128	6·96

The number of cases, along with the percentage for each disease manifestation in rural and urban areas, is shown in Table VI.

TABLE VI.

Percentage with disease manifestation in rural and urban areas.

Areas.	Total with disease manifestation.	Hydrocoele and other genital diseases.		Elephantiasis of :				Chyluria.	
				Lower limbs.		Upper limbs.			
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Rural	1,007	852	78·7	112	11·1	38	3·7	5	0·42
Urban	128	89	69·5	26	20·3	10	7·8	3	2·3

ENDEMICITY RATE.

Out of 11,212 persons examined, 853 showed microfilaria in the blood and 1,007 were found showing disease manifestations. There were 65 persons showing both the disease manifestations and infection in their blood. They were from the age-groups 6 - 10 years (2 persons), 11 - 20 years (5 persons), 21 - 30 years (14 persons), 31 - 40 years (29 persons), 41 - 50 years (5 persons) and above 50 years (10 persons). This shows that the incidence of simultaneous presence of the infection in the blood and disease manifestations is maximum in the age-group between 31 - 40 years. The endemicity rate of Ghazipur District works out to 16 per cent which is moderate. The endemicity rate in rural and urban areas according to age-groups is depicted in Tables VII and VIII.

Further analysis of the data of infection, disease and endemicity rates has been made for the entire area surveyed, according to sex and the two main religious

denominations, *viz.*, Hindus and Muslims. The results are shown in Tables IX and X.

TABLE VII.

Endemicity according to different age-groups (rural area).

Age-groups (years).	Infection rate, per cent.	Disease rate, per cent.	Endemicity rate, per cent.
0 to 1	Nil	Nil	Nil
2 to 5	0·8	0·8	1·6
6 to 10	0·99	1·1	2·0
11 to 20	6·45	4·2	10·4
21 to 30	10·23	12·15	21·8
31 to 40	11·85	13·29	26·3
41 to 50	10·69	17·07	27·1
Above 50	10·95	16·71	27·6
All ages :	7·60	8·98	16·01

TABLE VIII.

Endemicity according to age-groups (urban area).

Age-groups (years).	Infection rate, per cent.	Disease rate, per cent.	Endemicity rate, per cent.
0 to 1	Nil	Nil	Nil
2 to 5	4·91	1·6	6·5
6 to 10	4·8	1·3	6·2
11 to 20	8·2	4·6	12·6
21 to 30	10·5	7·1	17·3
31 to 40	13·5	10·8	22·71
41 to 50	15·0	13·2	28·4
Above 50	8·2	18·03	26·2
All ages :	9·84	6·96	16·32

TABLE IX.

Incidence of disease, infection and endemicity according to sex in rural and urban areas.

Area.	NUMBER OF PERSONS EXAMINED.		NUMBER WITH DISEASE.		NUMBER WITH INFECTION.		NUMBER WITH BOTH DISEASE AND INFECTION.		ENDEMICITY RATE, PER CENT.	
	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.
Rural	3,588	2,624	950	57	708	145	62	3	17·3	7·5
Urban	1,508	330	110	10	162	19	8	1	18·07	8·48

On perusal of the Table IX, it is evident that the endemicity rate amongst males is higher than females. However, since the sample collected from the latter is small, no definite conclusion can be drawn.

TABLE X.

Incidence of disease, infection and endemicity amongst Hindus and Muslims in rural and urban areas.

Areas.	Number of persons examined.		Number with disease.		Number with infection.		Number with both disease and infection.		Endemicity rate, per cent.	
	Hindu.	Muslim.	Hindu.	Muslim.	Hindu.	Muslim.	Hindu.	Muslim.	Hindu.	Muslim.
Rural	9,905	1,807	888	119	758	95	58	7	15·02	15·7
Urban	1,403	435	104	24	136	45	7	2	16·6	13·1

A study of Table X indicates that the endemicity amongst Hindus and Muslims is almost the same.

ENTOMOLOGICAL OBSERVATIONS.

The mosquitoes were collected from human dwellings, cattlesheds and mixed dwellings. They were identified and dissected in the laboratory to study the infection and infectivity rates. The results are shown in Table XI.

TABLE XI.

Mosquitoes collected and the results of dissection.

Type of mosquito.	Number collected.	Number dissected.	Number found positive.	Number of mosquitoes showing larvae Stage-wise.			
				I	II	III	IV
Culex fatigans	7,322	2,060	174	10	88	68	8
Anopheles	5,244	105	Nil	..	..	..	..

It is evident from Table XI that the vector mosquito of filaria in the district of Ghazipur is *Culex fatigans*. The infection rate in the mosquitoes was 6·5 per cent and infectivity rate 0·3 per cent. The breeding places for the vector mosquitoes were also studied and it was found that it breeds profusely in stagnant waters with plenty of organic population, *viz.*, stagnant pools, cess-pits, ponds and stagnant drains, small nullahas and tanks.

SUMMARY.

The district and the township of Ghazipur were surveyed under the National Filaria Control Programme for delimitation of the filarial endemicity. Both the rural and urban areas show that there is moderate endemicity, the rate being 16·01 and 16·32 per cent respectively.

The type of infection detected is *W. bancrofti* all over the district. The youngest age at which the microfilaria and disease manifestations have been detected, is in a child of two years.

Both men and women, Hindus and Muslims, were almost equally affected by the disease.

The vector of filariasis was found to be *C. fatigans* in the district.

ACKNOWLEDGEMENT.

The authors are grateful to the staff of the Filaria Survey Unit, Ghazipur, for their untiring efforts in the collection of data from various areas of the district.

Indian Journal of Malariology, 15, 1, March 1961.

PROBLEM OF FILARIASIS IN THE DISTRICT OF DEORIA (UTTAR PRADESH).

BY

DIWAN CHAND,*

M.V. SINGH†

AND

V.K. PATHAK‡.

[June 4, 1960.]

INTRODUCTION.

DELIMITATION of the filarial problem in the district of Deoria has been carried out under the National Filaria Control Programme, launched in Uttar Pradesh during the year 1955-56. This paper deals with the results of survey carried out in the district.

AREA, PEOPLE AND CLIMATE.

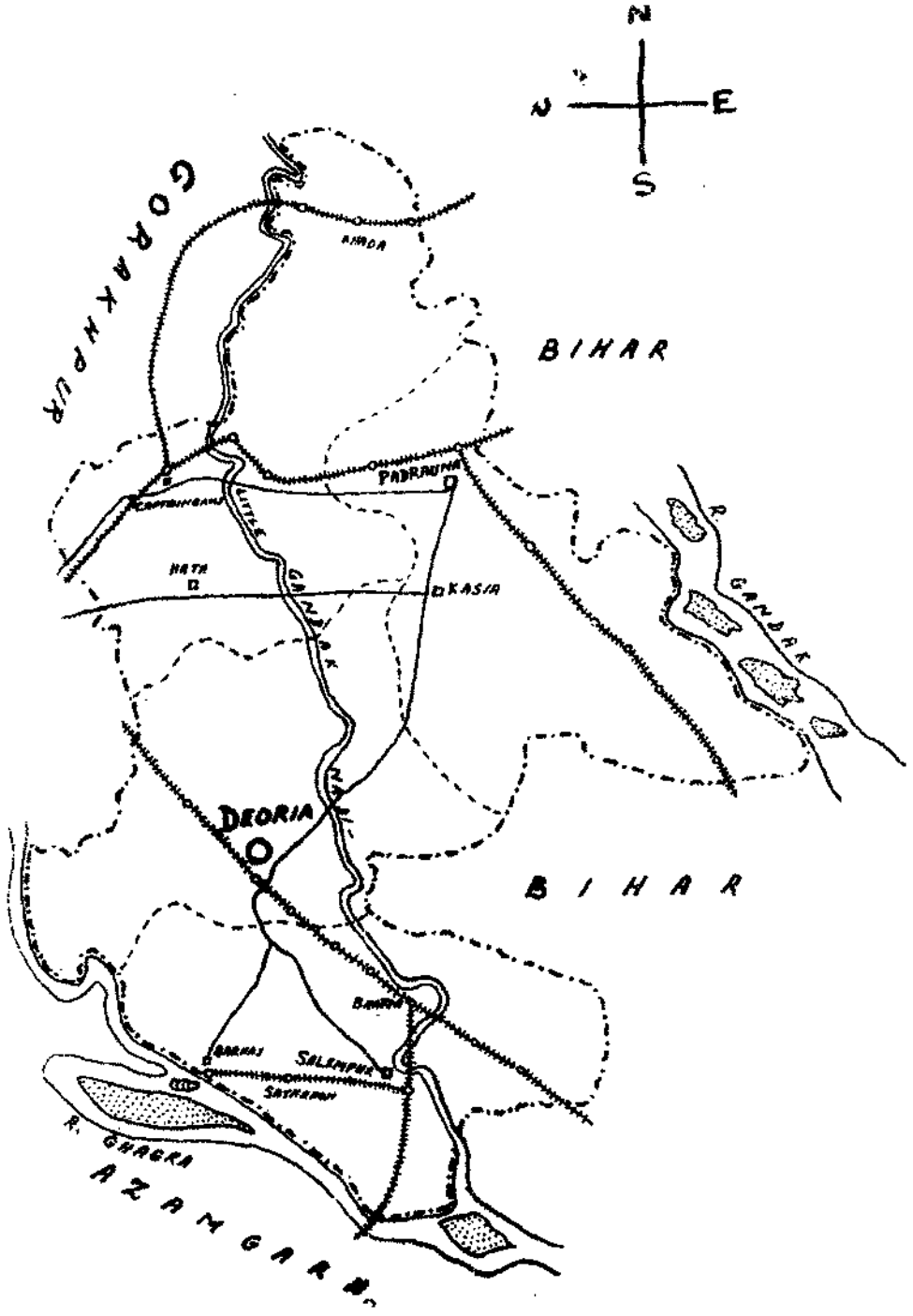
Before the creation of the district of Deoria in the year 1946, it was part of the district of Gorakhpur. The new district, having four tehsils, is the easternmost district of Uttar Pradesh and is located in Gorakhpur Division. It is bounded on the north by the district of Champaran (Bihar), south by the district of Azamgarh, east by the district of Chapra (Bihar) and the west by the district of Gorakhpur (Map 1). The district is thickly populated, the density of population per square mile being 1004. According to the 1951 census, the population of the district is 21,02,627 of which 10,49,928 are males and 10,52,699 are females, living in 3,554 villages. The headquarters of the district (Deoria Town) has a population of 30,156.

The district is well connected by rail and roads with the neighbouring districts. It comprises fairly compact plain and the height above sea level averages about 316 feet. There are two natural sand hills in the district. The valleys of large rivers like great Gandak and Rapti are wide and deep. There is inundation of the low land during rainy season, providing breeding places for mosquitoes. There are timber-forests and jungles, groves, pastures; specially in Padrauna Tehsil and along the banks of rivers.

* Deputy Director, Malariology, Uttar Pradesh, Lucknow.
† Assistant Director, Malariology, Uttar Pradesh, Lucknow.
‡ Filaria Survey Officer, Ghazipur.

MAP I.

MAP OF DEORIA DISTRICT (UTTAR PRADESH).



Comparatively heavy rainfall and easterly winds combine to avert the great heat during the summer, the temperature rarely rising above 100°F. during May and June. The average minimum temperature for December and January falls little short of 50°F. The average rainfall is about 50·68 inches. The meteorological data of the district from 1954 to 1958 are depicted in Table I.

TABLE I.

Meteorological data of Deoria District for the years 1954 to 1958.

Month and years	Maximum temperature. °F	Minimum temperature. °F	Relative humidity, per cent.		Rainfall. (inches)
			08·30 hours.	17·30 hours.	
1954					
January	69·5	49·7	85	62	0·68
February	79·2	58·6	83	57	0·36
March	90·0	61·5	56	37	0·00
April	102·4	74·3	40	30	0·00
May	104·3	80·8	61	39	2·63
June	96·8	81·1	75	62	1·71
July	91·8	79·7	88	73	12·52
August	88·9	80·4	85	79	8·85
September	90·9	79·9	82	78	4·07
October	87·3	69·1	75	53	0·93
November	80·7	50·7	70	51	0·00
December	73·9	50·7	83	60	0·00
1955					
January	71·1	50·1	86	59	0·85
February	76·4	53·5	71	42	0·28
March	92·4	66·2	53	27	0·11
April	97·4	70·2	33	19	0·49
May	102·9	73·8	52	25	0·02
June	94·0	80·2	77	62	14·77
July	87·1	78·5	89	78	11·18
August	87·1	79·0	92	82	16·94
September	88·0	78·6	78	78	21·66
October	86·0	72·1	79	64	3·34
November	81·3	60·8	66	55	0·00
December	71·9	51·7	80	60	0·69
1956					
January	69·8	51·5	84	56	0·63
February	76·5	53·8	63	40	0·31
March	90·0	64·9	57	27	0·36
April	101·1	74·4	44	21	0·00
May	99·7	80·7	69	46	5·35
June	91·2	79·0	85	71	11·84
July	89·9	79·8	83	71	8·81
August	90·0	78·3	86	81	16·00
September	86·2	77·9	85	78	22·37
October	84·7	72·3	88	74	6·66
November	76·6	59·5	74	64	3·38
December	69·5	52·4	79	65	1·00

(*Contd.*)

TABLE I. (*Concld.*)

Month and years.	Maximum temperature. °C.	Minimum temperature. °C.	RELATIVE HUMIDITY, PER CENT.		Rainfall. (mm.)
			08·30 hours.	17·30 hours.	
1957					
January	20·3	11·6	89	68	30·1
February	23·0	11·0	68	39	0·0
March	29·3	16·3	55	34	0·0
April	37·1	22·1	38	21	0·0
May	40·8	26·6	40	26	0·0
June	37·4	26·7	65	45	53·2
July	..	26·6	86	79	475·6
August	33·0	26·1	84	81	361·6
September	33·4	25·0	79	67	150·7
October	32·3	21·1	58	56	14·2
November	29·2	14·6	70	55	0·0
December	24·3	11·9	84	56	10·0
1958					
January	24·6	11·3	81	56	31·0
February	26·5	13·0	65	42	0·0
March	24·4	17·9	46	23	2·5
April	39·2	23·8	44	26	34·3
May	41·3	25·4	50	23	0·0
June	42·1	27·5	62	43	39·9
July	33·8	25·7	81	75	197·9
August	31·7	24·7	88	86	336·7
September	33·2	25·1	81	77	221·8
October	31·8	22·3	75	70	70·6
November	29·7	15·1	60	55	0·0
December	24·3	12·4	80	71	0·3

The usual Hindu and Muslim religious fairs are celebrated in the district. The district trades in sugar, indigo and cloth.

MEDICAL AND HEALTH ORGANISATION.

The noticeable feature among the health problems of the district is the prevalence of hydrocoele, elephantiasis and goitre. Cholera, small-pox and malaria are also prevalent. There are 19 allopathic dispensaries, 5 primary health units each having a dispensary, and 19 maternity and child-welfare centres. There are two National Malaria Eradication Programme Units operating in the district.

FILARIA SURVEY.

The history of the presence of filariasis is quite old in the district as mentioned in the Gorakhpur District Gazette (1909), but during the recent years more public attention has been drawn towards the disease. The general belief is that the disease has been imported from the adjoining areas of Bihar State. Before commencing the survey in the district, the crude disease rate was studied and the areas were marked out on the map where the work was to be undertaken.

The survey was carried out in the selected villages by house-to-house visits between 8 p.m. and mid-night. Persons of all age-groups and both sexes in the families were examined for disease manifestations, and parasite in the peripheral blood, by taking about 20 c.mm. of blood from each individual. The thick-films prepared were examined after staining with J.S.B. stain. Every effort was made to examine the children below 2 years of age also.

INFECTION RATE.

9,012 persons of all age-groups were examined during the present survey. The result of the blood examination is depicted in Table II according to various age-groups.

TABLE II.

Infection rate according to various age-groups.

Age-groups, (years).	Number of persons examined.	Number of persons showing infection in their blood.	Infection rate, per cent.	Average infestation.
0 to 1	8	Nil	Nil	Nil
2 to 5	443	6	1·35	11·5
6 to 10	1,179	33	2·79	5·9
11 to 20	2,447	117	4·78	7·5
21 to 30	1,781	171	9·6	6·3
31 to 40	1,589	161	10·13	6·2
41 to 50	999	125	12·51	6·0
Above 50	566	59	10·42	9·0
Total	9,012	672	7·45	11·0

The infection rate for all ages worked out to 7·45 per cent. The lowest age at which the infection was found, was in a male child of 3 years. The infection rate increased with the age, being maximum in the age-group of 41-50 years, after which it declined. The type of infection detected was only *W. bancrofti*.

The average infestation was found to be 11 microfilaria per 20 c.mm. of blood and the maximum number of microfilaria found in one slide was 87.

DISEASE RATE.

During the surveys, the same persons from whom blood was taken were examined for disease manifestations. Out of 9,012 persons examined, 1,227 persons were found to have various disease processes, namely, hydrocoele, swelling of genitals, elephantiasis of both the limbs and chyluria. The disease rate worked out to be 13·61 per cent. Table III shows the incidence of disease according to age-groups.

The earliest age at which the disease was found, was in a male child aged 3 years having hydrocoele. On perusal of Table III, it is clear that the disease rate increased with the advancement of age, the highest being in the age-group of above 50 years.

TABLE III.

Disease rate at different age-groups.

Age-groups, (years).	Number of persons examined.	Number of persons showing disease manifestation.	Disease rate, per cent.
0 to 1	8	Nil	Nil
2 to 5	443	11	2·46
6 to 10	1,179	24	2·03
11 to 20	2,447	134	5·47
21 to 30	1,781	263	14·76
31 to 40	1,589	368	23·15
41 to 50	999	263	26·32
Above 50	566	164	28·97
Total	9,012	1,227	13·61

The distribution of the disease processes was also studied and Table IV shows the percentage of different disease manifestations noticed during the survey.

TABLE IV.

Percentage with disease manifestations.

Disease manifestations.	Number showing disease manifestations.	Per cent.
Hydrocoele and other genital affections.	951	77·12
Right lower limb.	133	10·78
Left lower limb.	106	8·59
Right upper limb.	16	1·29
Left upper limb.	20	1·62
Chyluria.	7	0·05

A study of the Table IV reveals that the commonest manifestation of filariasis was hydrocoele and other genital affections, followed by the elephantiasis of lower limbs and upper limbs. Chyluria was observed in 7 cases only.

ENDEMICITY RATE.

Out of 9,012 persons examined during the present survey, 672 showed microfilaria in their blood and 1,227 were found showing disease manifestations. There were 134 persons showing both, the disease manifestations and infection, in their blood. They were from the age-groups 6 - 10 years (2 persons), 11 - 20 years (12 persons), 21 - 30 years (23 persons), 31 - 40 years (35 persons), 41 - 50 years (47 persons) and above 50 years (13 persons). This shows that the incidence of simultaneous presence of the infection in the blood and disease manifestations is maximum in the age-group between 41-50 years. The endemicity rate in the district of Deoria works out to 19·58 per cent which is moderate. The endemicity rate according to age-groups is shown in Table V.

TABLE V.

Endemicity according to different age-groups.

Age-groups (Years).	Infection rate, per cent.	Disease rate, per cent.	Endemicity rate, per cent.
0 to 1	Nil	Nil	Nil
2 to 5	1·35	2·48	3·83
6 to 10	2·79	2·03	4·66
11 to 20	4·78	5·47	9·64
21 to 30	9·6	14·70	23·07
31 to 40	10·13	23·15	31·08
41 to 50	12·51	26·32	34·13
Above 50	10·42	28·97	37·1
Total of all age-groups	7·45	13·61	19·58

The data collected during the survey have been further analysed to study their relation with sex and religion. Table VI shows the infection, disease and endemicity rates amongst male and female population.

TABLE VI.

Infection, disease and endemicity rates according to sex.

Sex.	Number examined.	DISEASE MANIFESTATIONS.		INFECTION IN BLOOD.		BOTH DISEASE AND INFECTION.		Endemicity rate, per cent.
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	
Male	7,129	1,140	15·99	558	7·96	120	1·68	22·13
Female	1,883	87	4·62	114	6·05	14	0·74	9·93

From Table VI, it is noticed that the disease, infection and endemicity rates are higher amongst the male population, but as the number of females examined is comparatively too small no definite conclusion can be drawn.

Table VII shows the incidence of disease, infection and endemicity rates amongst Hindus and Muslims, the two main religions in the district.

TABLE VII.

Incidence of disease, infection and endemicity rates in Hindus and Muslims.

NUMBER OF PERSONS EXAMINED.		NUMBER OF PERSONS WITH DISEASE.		NUMBER OF PERSONS WITH INFECTION.		NUMBER OF PERSONS WITH BOTH (DISEASE AND INFECTION).		ENDEMICITY RATE, PER CENT.	
Hindu.	Muslim.	Hindu.	Muslim.	Hindu.	Muslim.	Hindu.	Muslim.	Hindu.	Muslim.
8,018	994	1,128	99	603	69	103	31	20·3	13·78

On perusal of Table VII, it is clear that the disease, infection and endemicity rates amongst Hindus are higher than the Muslims, but since the sample collected from Muslims is comparatively small, no definite conclusion is possible.

ENTOMOLOGICAL OBSERVATIONS.

The mosquitoes were captured from different areas of the district from human dwellings, cattle-sheds and mixed dwellings. A total of 2,517 mosquitoes were caught, out of which 1,482 were identified as *Culex fatigans* and 1,035 as anophelines. Female mosquitoes of both varieties were dissected to determine the vector species. Out of 485 *Culex fatigans* dissected, 76 were found positive with developing forms of larvae, giving the infection rate as 15·7 per cent. The infectivity rate works out to 2·8 per cent. None of the anopheline mosquitoes was found positive. The result of dissection is shown in Table VIII.

TABLE VIII.

Result of mosquitoes dissection.

Type of mosquito.	Number collected.	Number dissected.	Number found positive.	Number of mosquitoes with larvae, stage-wise :			
				I	II	III	IV
Culex fatigans ...	1,482	485	76	9	32	21	14
Anopheles sp. ...	1,035	335	Nil	Nil	Nil	Nil	Nil
Total ...	2,517	820	76	9	32	21	14

It is evident from Table VIII that *Culex fatigans* is the vector of *W. bancrofti* infection detected in the district of Deoria. A thorough search was made to find out the breeding places of the vector mosquitoes which consist of stagnant pools, domestic cess pools, ponds and stagnant drains, small nālās and inundated low lands where water stagnates.

SUMMARY.

The filaria survey of the District of Deoria was carried out under the Filaria Survey Unit, Ghazipur, where 9,012 persons of all age-groups and both sexes were examined.

The infection and disease rates were found to be 7·45 per cent and 13·61 per cent respectively. The average infestation is 11 microfilaria per 20 c.mm. of blood. The endemicity rate is 19·58 per cent which is moderate.

The relationship of disease to the sex and religion have been studied, but no definite conclusion has been drawn as the samples of females and Muslims were too small. The type of infection is only *W. bancrofti* and the vector is *C. fatigans*.

The vector mosquito breeds in dirty water collections where organic pollution is plenty.

ACKNOWLEDGEMENT.

The authors are thankful to the staff of the Filaria Survey Unit, Ghazipur, for all the assistance given for collection of data in the field.

REFERENCE.

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Indian Journal of Malariology, **15**, *1*, *March 1961*.

A NOTE ON FILARIASIS IN GONDA TOWN (UTTAR PRADESH).

BY

DIWAN CHAND,

M.V. SINGH,

B.B. GUPTA

AND

R.N. SRIVASTAVA.

(*Malaria Organization, Uttar Pradesh*).

[June 23, 1960.]

INTRODUCTION.

GONDA Town, the headquarter of Gonda District, is a municipal town with a population of 32,749, out of which there are 18,323 males and 14,426 females. The whole town is divided into seven wards (Map 1).

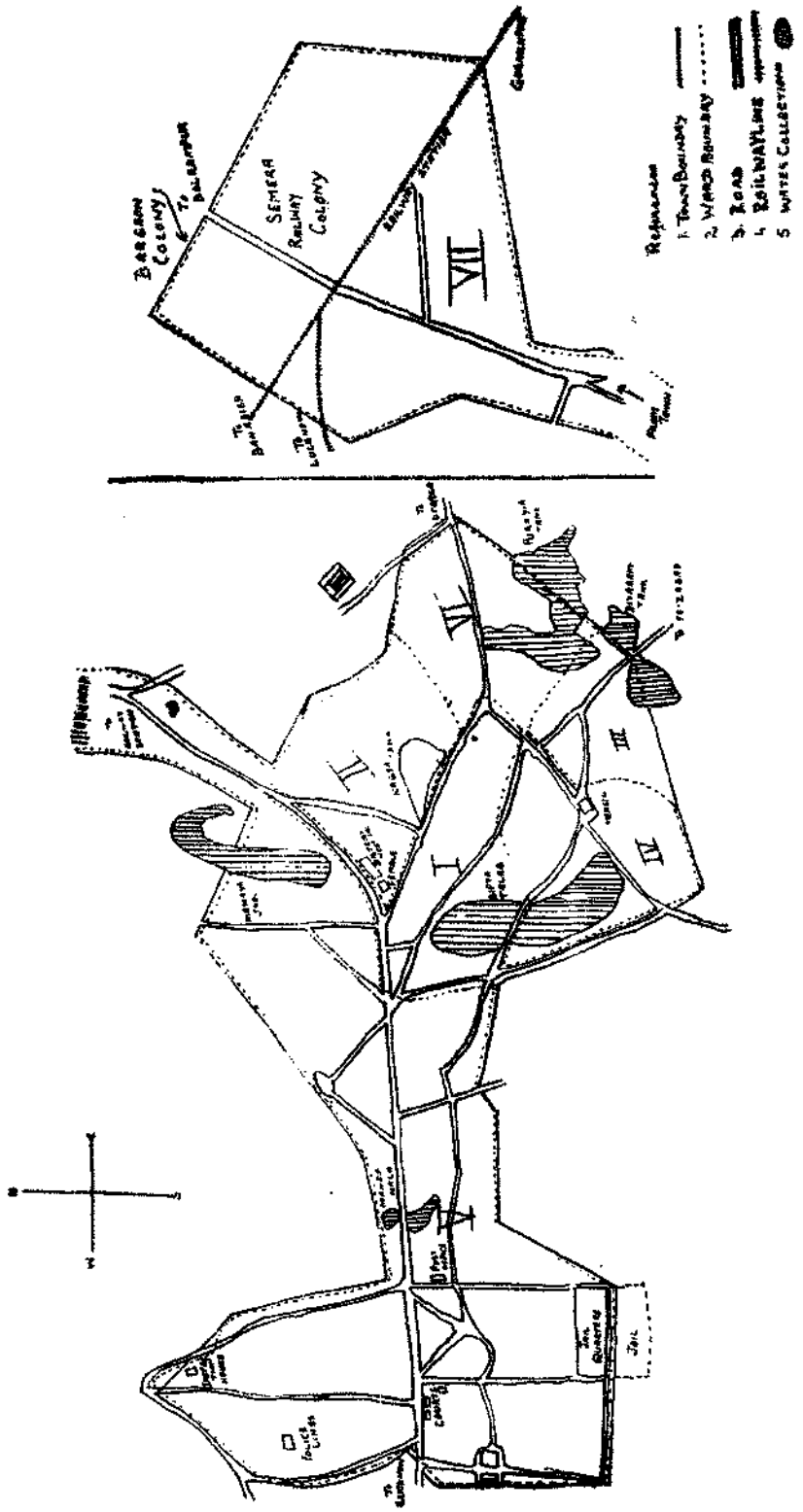
The climate of the town is moderate, the maximum and minimum temperatures being 42·4°C. and 7·4°C. respectively. The rainy season extends from the middle of June to the end of September, the average annual rainfall being 1385·3 mm., and relative humidity 70 to 90 per cent. The meteorological data of Gonda Town for the years, 1955 to 1959 are depicted in Table I.

The town has not got a safe water supply. Wells and hand-pumps are the present sources of water supply. There is, however, a proposal to establish a water works for the town. There is no organised system of drainage for the sullage and ablution water which stagnate at places in pits and small pools, forming ideal places for the breeding of the mosquitoes. The sub-soil level of water is 15 to 20 feet.

The town is connected with the neighbouring areas by rails and roads. There is no big fair held in the town, which is of significance as regards migration, except the annual development exhibition in which people mainly from the district are attracted.

The town is provided with a modern hospital and a maternity and child health centre. The municipality has got its own Medical Officer of Health who carries out some anti-mosquito measures in the town.

It is stated that the disease was not prevalent in the area about 50 to 60 years back. It is for the last 10 to 15 years only that an upward trend in the incidence of the disease has been noted.



MAP 1.

GONDA TOWN.

TABLE 1.

Meteorological data of Gonda Town.

Month and year.	Maximum temperature °C.	Minimum temperature °C.	Relative humidity.		Rainfall (in millimeters).
			08·30 hours.	17·30 hours.	
1955					
January	22·3	7·4	88	62	26·2
February	26·6	8·7	71	46	6·6
March	34·1	15·6	55	63	...
April	37·2	18·4	50	37	...
May	40·2	24·1	40	18	0·8
June	35·9	26·1	73	59	208·2
July	30·8	25·2	92	84	775·8
August	31·3	25·2	85	81	704·6
September	32·1	25·1	82	80	257·8
October	30·2	21·0	83	70	152·7
November	28·4	14·0	82	63	5·1
December	23·8	9·6	89	64	8·1
					Total 2235·4 mm.
1956					
January	22·6	9·5	87	64	16·3
February	26·0	10·3	69	53	11·2
March	32·0	16·8	55	35	18·5
April	38·7	22·1	30	20	...
May	37·7	26·4	65	46	232·4
June	33·1	25·9	89	77	142·0
July	32·4	25·7	82	73	234·9
August	32·3	25·8	84	81	308·1
September	31·6	24·6	83	78	274·3
October	30·6	20·9	84	78	288·0
November	26·7	12·3	77	66	6·3
December	23·5	9·2	82	72	14·5
					Total 1547·5 mm.
1957					
January	21·1	9·9	90	75	84·9
February	24·1	8·6	68	46	...
March	29·6	14·8	69	68	24·2
April	37·2	19·6	34	17	...
May	41·1	24·7	34	20	...
June	38·1	25·8	65	42	133·8
July	33·2	25·7	84	73	414·1
August	32·2	25·2	84	80	222·0
September	32·3	23·6	83	73	187·7
October	32·4	18·6	73	56	...
November	28·8	12·0	74	55	...
December	24·1	9·0	80	65	0·1
					Total 1072·8 mm.

(Contd.)

TABLE I. (*Concld.*)

Meteorological data of Gonda Town.

Month and year.	Maximum temperature °C.	Minimum temperature °C.	RELATIVE HUMIDITY.		Rainfall (in millimeters).
			08·30 hours.	17·30 hours.	
1958	24·0	8·6	80	61	22·8
January	26·1	9·5	62	40	...
February	32·8	14·5	45	23	...
March	38·3	21·4	36	19	...
April	42·4	23·9	41	19	...
May	41·8	28·0	53	35	21·6
June	33·2	24·7	82	72	259·6
July	31·5	23·8	90	83	441·4
August	32·1	23·7	84	80	214·0
September	31·4	19·4	83	70	76·8
October	28·3	12·3	78	59	...
November	23·7	11·0	90	70	7·0
December					
					Total 1043·2 mm.
1959					
January	21·6	9·6	88	70	54·0
February	23·9	10·7	75	52	...
March	32·6	15·5	55	36	6·0
April	38·3	21·3	45	27	1·6
May	40·1	25·4	53	36	26·8
June	37·5	27·3	68	51	60·3
July	33·0	26·6	85	74	338·4
August	32·4	26·6	89	78	253·0
September	33·0	25·7	80	72	143·6
October	30·6	22·3	84	75	125·2
November	28·2	13·6	80	62	...
December	24·6	8·9	82	71	...
					Total 1008·9 mm.

FILARIA SURVEY.

The survey of Gonda Town was conducted during the months of May—June, 1958 and October, 1959 to May, 1960. It included all the wards of the town. Persons of all the age-groups and both the sexes were examined by house-to-house visit between 20·00 hours and 24·00 hours. Approximately 20 c.mm. of blood was obtained from each individual for preparing thick smears which were examined after staining with JSB—I stain. The incidence of disease manifestations was also investigated during the survey.

INFECTION RATE.

Out of the total population of 32,749 the number of persons examined was 3,098 which represents 9·9 per cent of the population. Table II shows infection rate according to different age-groups.

TABLE II.

Infection rate according to different age-groups.

Age-groups (years).	Number of persons examined.	Number of persons with infection.	Infection rate, per cent.	Average infestation rate per 20 c.mm. of blood.
0 to 1	13	Nil	Nil	Nil
2 to 5	205	5	2·43	7·2
6 to 10	443	23	5·19	5·65
11 to 20	807	65	8·05	10·67
21 to 30	773	88	11·38	7·73
31 to 40	500	57	11·19	8·34
41 to 50	206	19	9·22	9·36
Above 50	142	14	9·85	10·70
Total	3,098	271	8·75	9·15

The infection rate for the town worked out to be 8·75 per cent. The lowest age at which infection was noted was in a male child of two years of age. The infection rate increased as the age advanced, reaching the maximum in the age-group of 21-30 years after which it started declining. The type of infection noted was *W. bancrofti*.

The average infestation was found to be 9·15 per 20 c.mm. of blood, and the maximum number of microfilariae found in one blood smear was 80.

DISEASE RATE.

During the blood survey, the same persons were examined for disease manifestations. 157 persons, out of 3,098 persons examined, were found with disease processes in the form of hydrocoele, elephantiasis of the scrotum, limb affection and chyluria. The disease rate worked out to be 5·06 per cent. The youngest age at which the disease was noted was a boy of 8 years of age, having hydrocoele.

Table III depicts the incidence of disease according to age-groups.

TABLE III.

Disease rate according to different age-groups.

Age-groups (years).	Number of persons examined.	Number of persons showing disease manifestations.	Disease rate, per cent.
0 to 1	13	Nil	00·00
2 to 5	205	Nil	00·00
6 to 10	443	2	00·45
11 to 20	807	15	1·85
21 to 30	773	52	6·72
31 to 40	500	51	10·02
41 to 50	206	15	7·4
Above 50	142	22	15·49
Total :	3,098	157	5·06

Table III shows that the disease started to appear in the age-group 6—10 years and reached the maximum in the age-group 21—30 years. It remained stable in the age-group 31-40 but declined in the later age-groups. The sample size of persons of "41 years and above" was, however, small.

Table IV shows the incidence of the various disease manifestations noted in the persons examined.

TABLE IV.

Percentage with disease manifestations.

Disease manifestations.	Number showing the disease manifestations.	Percentage of all disease manifestations.
Hydrocoele and other genital affections.	106	64·24
Right lower limb.	21	12·72
Left lower limb.	27	16·36
Right upper limb.	3	1·82
Left upper limb.	4	2·43
Chyluria.	4	2·43

It is seen from Table IV that the hydrocoele and other genital lesions were the commonest in the area, and next to it was the affection of the lower limbs.

ENDEMICITY RATE.

3,093 persons were examined for microfilaria in the blood and disease manifestations. Out of them, 271 showed microfilaria in the blood and 157 were found with disease manifestations. There were 9 persons showing both, the disease and infection in their blood, out of which 7 were Hindus and 2 Muslims. They were from the age-groups 21-30 (4 persons), 31-40 years (3 persons) and above 50 years (2 persons). This shows that the incidence of simultaneous presence of infection in the blood and the disease manifestations is maximum in the age-group 21—30 years. The endemicity rate of Gonda Town worked out to 13·52 per cent which is moderate endemicity. The endemicity rate according to the different age-groups is set out in Table V.

TABLE V.

Endemicity rate according to different age-groups.

Age-groups (years).	Infection rate, per cent.	Disease rate, per cent.	Mixed rate, per cent.	Endemicity rate, per cent.
0 to 1	00·00	00·00	00·00	00·00
2 to 5	2·43	00·00	00·00	2·43
6 to 10	5·19	00·45	00·00	5·64
11 to 20	8·05	1·85	00·00	9·50
21 to 30	11·38	6·72	00·52	17·58
31 to 40	11·19	10·02	00·59	20·63
41 to 50	9·22	7·40	00·00	16·62
Above 50	8·75	15·49	1·40	23·94
Total	8·75	5·06	00·29	13·52

The data collected during the night surveys was further analysed to determine the difference, if any, between the sexes and the religious groups of the town, namely, Hindus and Muslims.

Table VI below shows the infection, disease and endemicity rates amongst males and females.

TABLE VI.

Infection, disease and endemicity rates according to sex.

Sex.	Number examined.	DISEASE MANIFESTATIONS.		INFECTION IN BLOOD.		BOTH DISEASE AND INFECTION.		Endemicity rate.
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Per cent.
Male	2,217	135	6·08	218	9·83	9	0·41	15·51
Female	581	22	3·76	53	9·12	Nil	Nil	12·88

From Table VI it is evident that the disease, infection and the endemicity rates amongst the males were higher than those for the females. However, the sample sizes of the two sexes differed significantly.

Table VII shows the incidence of disease, infection and endemicity amongst the two main religious groups, *viz.*, Hindus and Muslims.

TABLE VII.

Incidence of disease, infection and endemicity in Hindus and Muslims.

NUMBER OF PERSONS EXAMINED.		NUMBER OF PERSONS WITH DISEASE.		NUMBER OF PERSONS WITH INFECTION.		NUMBER OF PERSONS WITH BOTH (DISEASE AND INFECTIONS).		ENDEMICITY RATE, PER CENT.	
Hindus.	Muslims.	Hindus.	Muslims.	Hindus.	Muslims.	Hindus.	Muslims.	Hindus.	Muslims.
1,793	1,305	98	59	183	98	7	2	15·27	11·11

Table VII indicates that there was no appreciable difference between the endemicity rates in the two main communities, *viz.*, Hindus and Muslims, residing in the town.

The results of the surveys carried out in each of the seven wards, in which the town has been divided for administrative purposes, are set in the Table VIII.

ENTOMOLOGICAL OBSERVATIONS.

72 catching stations, comprising human dwellings, cattle-sheds and mixed dwellings, were established during the survey for adult mosquito collections in the various wards of the town. The mosquitoes caught were identified and later dissected for determining the vector species. A total number of 2,548 mosquitoes were collected. The per man-hour density of *C. fatigans* was 39, while of the Anophelene it was 11. The results are depicted in Table IX.

TABLE VIII.

Infection, disease and endemicity rates, and the average infestation in different wards of the town.

Ward Number.	Total population.	Population examined.	Infection rate, per cent.	Disease rate, per cent.	Endemicity rate, per cent.	Average infestation per 20 c.mm.
I	5,316	636	8·48	5·5	13·67	7·00
II	4,490	210	12·85	3·66	15·23	11·56
III	5,264	522	8·42	2·49	10·91	7·29
IV	4,238	514	6·61	5·44	11·67	8·65
V	4,535	576	11·43	6·38	17·55	7·67
VI	4,740	315	6·98	3·17	10·15	8·64
VII	4,166	421	11·16	9·26	20·19	9·9
Total	32,749	3,093	8·75	5·03	13·53	8·09

TABLE IX.

Results of mosquito dissection.

Type of mosquito.	Number collected.	Number dissected.	Number positive.	Stage of larvae.			
				I	II	III	IV
Culex fatigans	2,253	536	6		1	4	2
Anophelene (all species)	295	12	Nil		Nil	Nil	Nil

It is evident from Table IX that the vector species of filaria in Gonda town was *C. fatigans*. The infection rate in the mosquito was found to be 1·3 per cent. The infectivity rate was 0·37 per cent.

A thorough search was made throughout the town to study the breeding places of the mosquitoes, specially the *Culex sp.* The breeding places consisted mainly of domestic cess pools, stagnant pools from drains, nullahs and tanks.

SUMMARY.

A filaria survey was carried out in Gonda Municipal Town. 9·9 per cent of the population was examined. The town was found to be endemic, the endemicity rate being 13·52 per cent. The infection and disease rates were found to be 8·75 and 5·06 per cent, respectively.

The youngest age at which the infection was noted was a child of 2 years, and the disease in a male child of 8 years of age.

Persons from both the communities (Hindus and Muslims) were examined during this survey, and the endemicity rate amongst Hindus was found to be 15·27 and amongst Muslims 11·71 per cent. Sex-wise, endemicity rate in males was found to be 15·51 while in females it was 12·88.

The chief disease manifestation was hydrocoele which constituted 04·24 per cent and next common to it was elephantiasis of the lower limbs (29·08 per cent).

The type of filarial infection was found to be *W. bancrofti* and the vector *Culex fatigans*.

ACKNOWLEDGEMENT.

The authors are thankful to Shri S. Wasi Uddin, Laboratory Assistant, and other staff of the Filaria Survey Unit, Bahraich, Uttar Pradesh for their active cooperation in the field and laboratory.

Indian Journal of Malariology, **15**, 1, March 1961.

SUSCEPTIBILITY OF *AEDES ALBOPICTUS* TO *DIROFILARIA REPENS* INFECTION IN CATS.

BY

C.P. NAIR,

RADHAGOVINDA ROY

AND

N.G.S. RAGHAVAN.

(*Malaria Institute of India, Delhi.*)

(July 29, 1959.)

DIROFILARIAL infections in cats in Central Kerala (Ernakulam and surrounding areas) were noted during a routine survey of animals for filarial infections (Annual Report of the Malaria Institute of India, 1957). These studies were extended to the year 1958 during which 2 out of 15 cats were found positive for Mf. *D. repens*.

Bhalerao (1935) had mentioned that *Aedes aegypti* is a good vector for *D. repens* in the dog. *Aedes albopictus* were noted in fairly large numbers in Ernakulam area where this filarial infection exists in cats. It was decided to study the results of infecting this mosquito experimentally with cat filaria. The results obtained are set out in this paper.

METHODS AND MATERIALS.

Two cats (Numbers 1 and 2) with 25 and 70 microfilariae, respectively, per 20 c.mm. of blood, and *Aedes albopictus* of Coonoor origin maintained in the laboratory, were used in this investigation. The cats were immobilised and kept in large mosquito-breeding cages into which a number of female mosquitoes, starved previously for 24 hours, were released. After 30 minutes the animals were removed, the fully-fed mosquitoes collected separately and kept in glass chimneys and subsequently fed on glucose. The temperature and the relative humidity of the insectarium, during the experiment, was 72°F. to 84°F. and 85 to 95 per cent respectively. The chimneys were examined daily in the morning and evening. Recently-dead or moribund mosquitoes were removed and dissected, and a thorough search made for the developmental stages of the parasite in the malpighian tubules, abdomen, thorax, head and proboscis. The stages of development, their respective numbers, and the sites where they developed, were carefully recorded.

RESULTS.

The details of the findings are depicted in Tables I and II. Of 274 mosquitoes fed on the two cats, 224 were dissected between the 3rd and 24th day (both

TABLE I.

Results of feeding aedes Albopictus *on cat Number 1 with* Dirofilaria repens *infection.*

Days after feeding.	Number dissected.	Percentage positive.	Average number of the developmental forms found.												Range of the developmental forms noted.
			Stage I.			Stage II.			Stage III.			Stage IV.			
			M. tubules.	Thorax.	Head/ proboscis.	M. tubules.	Thorax.	Head/ proboscis.	M. tubules.	Thorax.	Head/ proboscis.	M. tubules.	Thorax.	Head/ proboscis.	
3	3	66·6	4	..	..	..	..	..	..	..	..	..	..	..	2 – 6
5	4	75	..	..	..	1·5	..	..	..	..	..	..	..	..	1 – 2
6	1	0	..	..	..	..	..	..	..	..	..	..	..	..	..
8	4	0	..	..	..	..	..	..	..	..	..	..	..	..	..
9	9	44·4	..	..	..	2	..	..	2	..	..	..	..	..	1 – 3
10	14	26·5	1	..	..	2	..	..	3·5	..	..	3	..	..	1 – 6
12	8	25	1	..	..	1	..	..	..	..	..	..	..	..	1
13	5	20	..	..	..	7	..	..	..	..	..	..	..	..	7
14	4	0	..	..	..	..	..	..	..	..	..	..	..	..	..
15	7	14·2	..	..	..	..	..	..	..	..	..	1	1	2	1 – 2
17	14	7·1	..	..	..	..	..	..	..	..	..	..	..	2	2
18	2	0	..	..	..	..	..	..	..	..	..	..	..	..	..
19	25	16	..	..	..	..	..	..	..	..	..	2·3	2	1	1 – 4
21	7	14·2	..	..	..	..	..	..	..	..	..	1	..	..	1
25	0	0	..	..	..	..	..	..	..	..	..	..	..	..	..
27	11	0	..	..	..	..	..	..	..	..	..	..	..	..	..
28	15	0	..	..	..	..	..	..	..	..	..		..	..	..
29	8	0	..	..	..	..	..	..	..	..	..	..	..	..	..
30	7	14·2	..	..	..	..	..	..	..	..	..	..	..	2	2

TABLE II.

Results of feeding Aedes albopictus *on cat Number 2 with* Dirofilaria repens *infection.*

Days after feeding.	Number dissected.	Percentage positive.	Average number of developmental forms found:												Range of the developmental forms noted.
			Stage I.			Stage II.			Stage III.			Stage IV.			
			M. tubules.	Thorax.	Head/ proboscis.	M. tubules.	Thorax.	Head/ proboscis.	M. tubules.	Thorax.	Head/ proboscis.	M. tubules.	Thorax.	Head/ proboscis.	
3	3	100	2·6	..	..	..	..	..	..	..	..	..	..	..	1 – 3
5	4	100	16·75	..	..	21	..	..	..	..	..	..	..	..	2 – 50
6	4	50	1	..	..	3	..	..	..	..	..	..	..	..	1 – 5
8	3	66·6	..	..	..	3	..	..	4	..	..	..	..	..	2 – 4
9	9	55·5	..	..	..	7·6	..	..	8·3	..	..	..	..	..	1 – 22
10	14	14·2	..	..	..	5·5	..	..	5·5	..	..	..	..	..	3 – 8
11	10	30·0	1	..	..	2	..	..	1	..	..	..	..	..	1 – 2
12	22	31·8	..	..	..	2·3	..	..	6·3	..	..	4·25	..	3	1·7
13	4	0	..	..	..	..	..	..	..	..	..	..	..	..	..
15	6	33·3	..	..	..	1	..	..	..	3	..	2	3	6	1 – 6
17	8	25	..	..	..	..	..	..	2	..	..	3	1	1	1 – 3
..	..	0	..	..	..	..	..	..	..	..	..	1	1	..	1
			1·5	..	..	1	..	..	..	..	..	1	1	2	1 – 2
			..	..	..	..	..	..	..	..	..	1·5	2	1·25	1 – 2

days inclusive) after feeding. From the Tables, the following observations are observed :—

1. All the developmental stages were noted in the malpighian tubules, and only the infective larvae were found in the abdomen, thorax, head and proboscis. Up to the 11th day, the developmental stages were noted in the malpighian tubules only, whereafter infective larvae appeared at this as well as other sites.

2. The complete development of microfilariae to the infective stage was possible within 10 to 12 days.

3. *Aedes albopictus* is a good vector of *Dirofilaria repens* infection in the cat.

Indian Journal of Malariology, **15**, *1*, *March 1961*.

ADRENAL INSUFFICIENCY OF THE HOST IN *P. KNOWLESI* MALARIA.

BY

NAU-NIHAL SINGH

AND

P.C. BASU.

(*Malaria Institute of India, Delhi.*)

[June 3, 1960.]

In a previous communication, Jaswant Singh *et al.* (1956) had shown that in *P. knowlesi* (Nuri strain) infection, which runs a progressively rapid and fatal course the blood sugar level in the host (*M. mulatta mulatta*) falls precipitously at the final stage of infection (fifth or sixth day) and the level attained varies between 12·7 and 40 mg. per cent (the normal values in monkeys varying between 62 and 111 mg. per cent). They had also noted that the abrupt fall in blood sugar level seemed to coincide with the gross liver damage in shape of centrilobular necrosis, practically involving all lobules, and spread well beyond the mid-zonal region. On observing this coincidence of the liver pathology and the precipitous fall in blood sugar level, they suggested that the fall in blood sugar level was largely due to liver damage. But in view of the fact that some healthy glycogen containing liver tissue always remains available, the probable role of adrenal insufficiency in the causation of precipitous hypoglycaemia has to be considered.

This paper relates to the investigation in which indirect evidence has been obtained on the functional insufficiency of the adrenals during the terminal stage of *P. knowlesi* (Nuri strain) infection. This could be a further contributory and concomitant factor in precipitating the terminal hypoglycaemia.

MATERIALS AND METHODS.

Host.—Ten tuberculin negative *M. mulatta mulatta* monkeys, weighing between 2·5 and 5·68 kg., were used for this investigation. The diet provided to the animals consisted of gram, wheat flour and vegetables or fruits. The total caloric value ranged between 760 and 800 per day.

Parasite.—The monkeys were inoculated intravenously through the dorsal leg-vein with a standard dose of 5×10^6 parasitized cells (Nuri strain of *P. knowlesi*) per kg. body weight. The dose invariably proves fatal in five to six days from the date of inoculation. The cell infection reaches between 60 and 96 per cent. It is also at this stage that the precipitous fall in blood sugar level and gross liver damage, similar to that noted by Ray (1954), are recorded.

Blood smears were collected in the morning on the 4th day of infection in two monkeys, 5th day in six monkeys and on the 6th day of infection in two. The degree of parasitaemia was determined by counting the number of parasitized cells per 10,000 erythrocytes, Ehrlich's eye-piece being used for the purpose. The degree of parasitaemia is expressed as percentage of cell infection.

Estimation of blood sugar.—Fasting samples of blood were drawn on the 4th, 5th and 6th day of infection from the respective monkeys mentioned in the protocol. Further samples of blood were drawn at intervals of 10, 30 and 50 minutes, following a single injection of adrenaline given immediately after the withdrawal of the initial samples. The animals were kept fasting till the last sample was drawn. Blood sugar was estimated according to the technique of Folin and Wu (1919: 1920), using a Klett-Sommerson photo-electric calorimeter.

Adrenaline administration.—Adrenaline B.P. 1 : 1000 w/v [May and Baker Ltd. (Batch No. 131, 1957)] was used. A single intravenous injection of adrenaline, at the rate of 50 microgram per kg. body weight, was given immediately after drawing the initial blood samples on the 4th, 5th or 6th day of infection, as noted against the individual monkeys (Table I).

TABLE I.

Response of blood sugar level to extraneous adrenaline in monkeys with advanced P. Knowlesi *infection (dose of adrenaline 50 microgram per kg. body weight).*

Serial Number.	Monkey number.	Day of infection.	Degree of parasitaemia (Percentage of cell infection).	Initial fasting blood sugar value mg. per cent. just before adrenaline injection.	BLOOD SUGAR IN MG. PER CENT VALUES AFTER 10, 30 AND 50 MINUTES OF ADRENALINE INJECTION.		
					10 minutes.	30 minutes.	50 minutes.
1	8,113	5th	63	45·4	96·3	65·3	68·1
2	8,124	6th	71	41·2	66·05	54·1	64·2
3	8,141	5th	64	47·4	72·7	88·8	116·1
4	8,147	6th	64	75·0	90·0	95·0	95·0
5	113	5th	60	47·0	55·0	60·0	70·0
6	133	5th	80	45·0	52·0	65·0	50·0
7	29	4th	50	30·0	40·0	65·0	58·0
8	264	5th	65	46·0	52·0	53·0	55·0
9	212	5th	58	42·0	43·0	53·0	51·9
10	326	4th	78	37·0	43·75	54·25	55·1

EXPERIMENTS AND RESULTS.

The day of infection, the degree of parasitaemia, the initial fasting blood sugar value and response to extraneous adrenalines given, are shown in Table I.

It would be observed that the degree of parasitaemia in all the monkeys, immediately before adrenaline was administered, exceeded 50 per cent cell infection. During the same period, considerable hypoglycaemia, which ranged from 75·0 to 30·0 mg. per cent, was observed. Estimation of blood sugar levels in

different monkeys, 10 minutes after adrenaline injection, showed a rise in blood sugar values in all the monkeys and these values ranged between 40·0 and 96·3 mg. per cent. The data were, however, put to statistical test and the analysis of variance technique showed that on the whole there was significant increase in blood sugar level following a single injection of adrenaline and that this higher blood sugar level was maintained up to 50 minutes as was evident from the subsequent determinations.

DISCUSSION.

Clinical signs such as asthenia, hypotension and bradycardia have been observed in algid and comatose malaria, and the suggestion that these could be due to adrenal insufficiency, has been made by many workers (Paisseau and Lemaire, 1916 ; Fraga and Motta, 1917 ; MacDowell, 1917). Various histo-pathological evidences of damage to the adrenal glands, both in human beings and in monkeys, in the shape of degeneration, necrosis, haemorrhage, vascular congestion and thrombosis, cellular infiltration and oedema, involving both medulla and to some extent the cortex, have been put forward in support (Paisseau and Lemaire, 1916 *loc. cit.* ; Natali, 1934). Careful autopsies on 26 monkeys, infected with *P. knowlesi*, have shown relatively inconspicuous pathological changes like small scattered haemorrhages, mostly in the region of the corticomedullary junction, in a few monkeys (Rigdon and Stratmen-Thomas, 1942). In monkey malaria, therefore, histopathological evidence of constant damage to adrenals has not been provided.

Changes in the chloride and sugar content of blood in acute malignant tertian malaria, during the paroxysm, have been claimed to be due to adrenal insufficiency by Miyahara (1936) and others. In about 1/3rd of his cases of acute and chronic benign and malignant tertian malaria, showing signs of adrenal insufficiency, Chessa (1938) found a definite increase in sensitivity to insulin and deduced that there was in these cases, some degree of adrenal dysfunction.

The degree of hypoglycaemia in the terminal stage of *P. knowlesi* (Nuri strain) infection in this investigation was as much as 30·0 mg. per cent. In a larger number of observations made previously, the hypoglycaemia had been noted to be as much as 12 to 15 mg. per cent., with no appreciable tendency to lessen during the residual survival period of the host (Jaswant Singh *et al.*, 1956 *loc. cit.*). The hypoglycaemia by itself should provide necessary physiological stimulus for increased adrenaline secretion (Cannon *et al.*, 1924) and mobilisation of reserve glucose (glycogen). In spite of the extensive liver necrosis found during the terminal stage of infection (60 per cent or more cell infection), raising of the blood sugar level, following adrenaline administration, shows the availability of residual glycogen, probably along the periphery of the liver lobules, as the glycogen stores in the muscles are unable to contribute to the blood sugar. Probably insufficient adrenal medullary function prevented the glycogenolysis, and the hypoglycaemia was precipitous. *Vis-a-vis* adrenal cortex, experimentally better concentration of glycogen in the liver and in the skeletal muscles of heavily infected monkeys,

following intravenous injection of glucose, was noticed in animals which received adrenal cortical hormones (Devakul and Maegraith, 1958).

Conversely, in the terminal stages of *P. knowlesi* infection, dramatic resuscitation of the shocked animals after administration of extraneous nor-adrenaline and partial resuscitation after the administration of extraneous adrenaline, have been demonstrated (Maegraith, Devakul and Leithead, 1959*a*). High dosage of adrenaline (640 microgram) was required to cause a slow rise of blood pressure in the monkeys in the terminal stages (Maegraith, Devakul and Leithead, 1959*b*). The vasomotor response to extraneous nor-adrenaline and adrenaline may indicate insufficiency of the medullary secretion of the adrenal glands in the terminal stages.

SUMMARY.

On the fourth, fifth and sixth day of *P. knowlesi* (Nuri strain) infection, the parasitaemia ranged from 50 to 80 per cent cell involvement in *M. mulatta mulatta* monkeys. Concomitantly the hypoglycaemia ranged from 30·0 to 75·0 mg. per cent (fasting values).

Administration of a single dose of extraneous adrenaline raised the blood sugar values and the effect lasted for at least 30 to 50 minutes.

Hypoglycaemia *per se* should provide the required physiological stimulus for the mobilisation of reserve glucose via adrenaline.

Response to extraneous adrenaline showed the availability of glycogen in the liver in spite of its gross damage.

It is inferred that adrenaline insufficiency could be a contributory factor in causing the precipitous fall in blcod sugar values noted in the terminal phase of *P. knowlesi* (Nuri strain) infection in *M. mulatta mulatta* monkeys.

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Indian Journal of Malariology, **15**, *1*, *March 1961*.

RESULTS OF A RAPID SUSCEPTIBILITY SURVEY OF *ANOPHELES CULICIFACIES* IN BOMBAY STATE, INDIA, DURING 1959, REVEALING CONTINUED SUSCEPTIBILITY TO DDT, EXCEPT IN A FEW SCATTERED POCKETS*.

BY

T.B. PATEL,

T. RAMACHANDRA RAO

AND

S. C. BHATIA.

(*Malaria Organisation, Bombay State, Poona, India.*)

[June 23, 1960.]

WORK on measuring of susceptibilities to DDT of *Anopheles culicifacies*, a major malaria vector, was started in Bombay State, India, during 1955 and has since then been regularly carried out and the results published, *vide* Ramachandra Rao and Bhatia (1957) and Bhatia *et al.* (1958), and enough of base-line data has been collected so far. But hitherto the procedure had involved trials of series of dosages of insecticides with a good number of replications at each place of test. The staff allocated for this purpose being limited, the speed of detecting the development of resistance, if any, was not considered adequate for a State of such a vast size, measuring approximately 200,000 square miles. Besides, during the months of August and September, 1959, lowered susceptibilities of *A. culicifacies* to DDT were observed in three or four widely separated localities of the State. This caused some concern and created apprehensions about the possibility of vast areas being involved in this new situation of lowered susceptibility. Therefore, it became essential to carry out a rapid susceptibility survey of *A. culicifacies* in as large a number of places as possible, using a less elaborate procedure.

In consultation with Dr. B.A. Rao, the then Director, National Malaria Eradication Programme, Delhi, it was decided that a number of teams should be organised immediately to survey all the districts of the State in as short a period as possible. Some teams were to be formed from the staff deputed by the Malaria

* After the submission of this report to the press, further extensive work has been carried out in the States of Gujerat and Maharashtra to determine the susceptibility status of *A. culicifacies* to DDT and the results have been classified, according to the recent World Health Organization criteria, as resistant, intermediate or susceptible. In the year 1960-61, a high degree of resistance has been found in an area of approximately 10,000 to 15,000 sq. miles in a contiguous area in the districts of Jalgaon, Dhulia, Broach, Baroda and Panch Mahals. The results of these investigations will be published in due course. —*Authors*

Institute of India and some by the Bombay State Malaria Organisation, and each team was assigned a certain region for rapid testing of the susceptibility of *A. culicifacies* to DDT.

It was decided to employ a single dosage for these purposes. The dosage selected was 2 per cent DDT, which had earlier given approximately a mortality of 100 per cent in *A. culicifacies* with one hour's exposure. The employment of a single dosage under such a situation, as described above, was considered justifiable as we had enough of base-line data and as we had to cover a vast area in short time. Any lowered susceptibility would be reflected in the percentage of survivors at this concentration. The method employed proved itself to be quite useful in covering a vast number of places within a short period with a limited staff.

This paper reports the results of this rapid survey carried out, using a single dosage of DDT, together with the results of tests with full series of dosages in places where lowered susceptibility was noticed. The data presented relate only to the tests carried out by the staff of the Bombay State Malaria Organisation. Some data which had been previously collected, have also been included in this report in order to provide a comparative picture of the situation.

MATERIAL AND METHOD.

World Health Organization kits and impregnated papers for testing susceptibility of adult mosquitoes were employed. At each place of test, two or more replications were tried with a single dosage of 2 per cent DDT, along with controls. The exposure period was one hour and as usual the observations on mortality were made 24 hours after the exposure. Only uninjured wild-caught females of *A. culicifacies* which had fully engorged on the previous night, with all legs, wings, etc., in tact, were selected for the experiments. The number of mosquitoes tested at each place varied according to their availability in that area, the number being very small in a few places.

RESULTS.

The tests on *A. culicifacies* with a single dosage of DDT exposure were carried out in 49 places, scattered throughout the State, during the year 1959 and the data collected are summarized in Table I. As already pointed out, papers impregnated with 2 per cent DDT were used to give an approximately 100 per cent mortality of *A. culicifacies* in earlier years. In the present series, 100 per cent mortality was obtained in only three out of 49 places. As a sample of the dosage-mortality relationship in a highly susceptible population, the data in respect of *A. culicifacies* collected in 1957 are presented in Table II and plotted in Graph 1. The lower mortalities obtained now (in 1959) with the same concentration, therefore, are suggestive of the development of a certain degree of tolerance. It was extremely difficult to set any arbitrary limit of percentage mortality below which a species could be declared resistant and above which it could be declared susceptible. Unless

one has a detailed knowledge of the genetic composition of the population, it would be hazardous to make precise classifications. But for practical purposes, the degree of tolerance would have to be classified. Therefore, we have utilised the following classification.

Grade of tolerance.	Range of mortality with 2 per cent DDT dosages.
1 Susceptible.	Above 75 per cent.
2 Moderately tolerant.	20-75 per cent.
3 Resistant.	Below 20 per cent.

Data of Table I, based on this classification, are presented in Map 1, showing localities where tests were performed.

Based on this classification, *A. culicifacies* was not regarded as highly resistant in any place in Bombay State. In 42 out of 49 localities, the mortalities were above 75 per cent and, therefore, the species may be regarded as still susceptible in such places. In 7 out of 49 localities, the susceptibilities have ranged between 37 and 75 per cent. These areas present pockets in which *A. culicifacies* populations have become moderately tolerant. The list of such places is :—

District.	Villages.	Percentage mortality.	Month of experiment.
Aurangabad.	Fardapur.	68	September, 1959.
Panchmahals.	Khumpir and Sindbai.	37	September, 1959.
Nasik.	Bortembhe and Titoli.	55	September, 1959.
Panchmahals.	Navakua.	49	September, 1959.
Parbhani.	Deosadi.	65	December, 1959.
Nanded.	Degloor, Mujalda.	61	December, 1959.
Kaira.	Pali.	60	December, 1959.

It is of interest that when the tests were repeated in December, the mortality was 85 per cent in Bortembhe and it was 79 per cent in Fardapur. In these two localities at least, there was some degree of reversion towards susceptibility.

The full series of dosages of DDT were tried in a few places where high tolerance to DDT was detected and the data obtained are depicted in Table III, along with the probit-analysis. Log dosage probit mortality regression lines for these data are presented in Graph 1. At Khumpir of Halol Taluka of Panchmahals District, where *culicifacies* was highly tolerant to DDT, additional tests were carried out with longer times of exposure at 2 per cent concentration. The time mortality data with 2 per cent DDT are summarised in Table IV. At the same place, a full series of Dieldrin dosages with the W.H.O.-impregnated papers was also tried and the data, along with probit-analysis, are depicted in Table V. A few tests with Lindane (with papers locally impregnated) were also carried out on the same mosquito at the same place, and the results are shown also in Table V. *A. culicifacies* of Khumpir, although highly tolerant to DDT, was still highly susceptible to both Dieldrin and Lindane.

TABLE I.

Results of susceptibility tests carried out on A. culicifacies *with DDT, using the World Health Organization kit and control and 2 per cent DDT impregnated papers only with an exposure of one hour.*

Year 1959.

D=Number dead. T=Total exposed.
D/T=Number dead out of the total number exposed.

S=Susceptible (76 to 100 per cent mortality with 2 per cent DDT.)
T=Tolerant (20 to 75 per cent mortality with 2 per cent DDT.)
R=Resistant (Less than 20 per cent mortality with 2 per cent DDT.)

Serial number.	Dates of experiment.	Villages of experiments.	Talukas.	District.	Sprayed with DDT since.	Mosquitoes collected from sprayed (SP) or Unsprayed (U/S) or both structures.	Mortality with 2 per cent DDT.		Control mortality.		Remarks.
							D/T	Kill percentage.	D/T	Kill percentage.	
	1959										
1	Feb. 26-28	Manjri	Haveli	Poona	1953	SP	49/50	98	2/48	4	S
2	May 1-3	Vithalwadi	Haveli	Poona	1953	U/S	75/75	100	0/65	0	S
3	May 8-10	Potegaon	Murbad	Thana	1949	SP	56/59	95	0/45	0	S
4	July 23 to Aug. 5	Nara	Nagpur Corporation	Nagpur	1953	U/S	76/83	90	12/88	13	S
5	Aug. 3-7	Ghasia Malgingva	Veraval	Sorath	1953	U/S	38/46	83	1/78	1	S
6	Aug. 23-26	Nirgude	Junnar	Poona	1953	U/S	52/67	78	0/61	0	S
7	Sep. 15-18	Pardapur	Sillod	Aurangabad	1954	SP	56/82	68	0/75	0	T
8	Sep. 24-28	Khumpir and Sindbai	Halol	Panchmahals	1950	SP	33/88	37	2/93	2	T
9	Sep. 26-30	Bortembhe and Titoli	Igatpuri	Nasik	1953	U/S	44/73	55	0/70	0	T

10	Oct. 1-6	Navakua	Halol	Panchmahals	1950	SP	25/51	49	0/49	0	T
11	Oct. 17-23	Talewadi	Mubad	Thana	1949	SP	37/45	82	1/30	3	S
12	Oct. 28	Khed Shivpur and Shivra	Haveli Purandhar	Poona	1953	U/S	35/37	94	2/39	5	S
13	Oct. 29	Ambegaon	Ambegaon	Poona	1953	U/S	78/80	97	0/19	0	S
14	Nov. 5-6	Pimpalwandi	Junnar	Poona	1953	U/S	57/72	79	0/41	0	S
15	Nov. 5-6	Mahatma-Phulenagar	Wai	North Satara	1959 (1955-56)	U/S	81/82	99	0/41	0	S
16	Nov. 6-8	Ghargaon	Sangamner	Ahmednagar	1953	U/S	59/70	84	0/37	0	S
17	Nov. 11-12	Jawle	Sangamner	Ahmednagar	1953	U/S	58/70	83	0/25	0	S
18	Nov. 11-14	Manpur Kharad	Yeotmal	Yetomal	1956	SP	27/30	89	1/29	3	S
19	Nov. 12-13	Kashil	Satara	North Satara	1959 (1955-56)	U/S	54/60	90	0/35	0	S
20	Nov. 13-14	Vithe	Akola	Ahmednagar	1953	U/S	54/69	78	0/32	0	S
21	Nov. 14-16	Chanasar Wami Dhudhwa Bhakhri	Tharad	Banaskantha	1959 (1955-56)	SP	59/74	79	0/20	0	S
22	Nov. 14-16	Karandwadi and Bavchi	Islampur	South Satara	1959 (1955-56)	U/S	31/34	91	0/25	0	S
23	Nov. 16-17	Dahigaon and Nandurk	Kopergaon	Ahmednagar	1953	U/S	62/76	81	0/25	0	S
24	Nov. 18-19	Kothali Parithe Kerva Devla	Karvir	Kolhapur	1959 (1956-57)	U/S	26/26	100	1/20	5	S
25	Nov. 19-23	Mathapur	Bhuj	Kutch	1953	U/S	60/70	86	1/75	1	S
26	Nov. 21-23	Charata-Karade	Sawantwadi	Ratnagiri	1953	SP	61/65	93	1/42	2	S

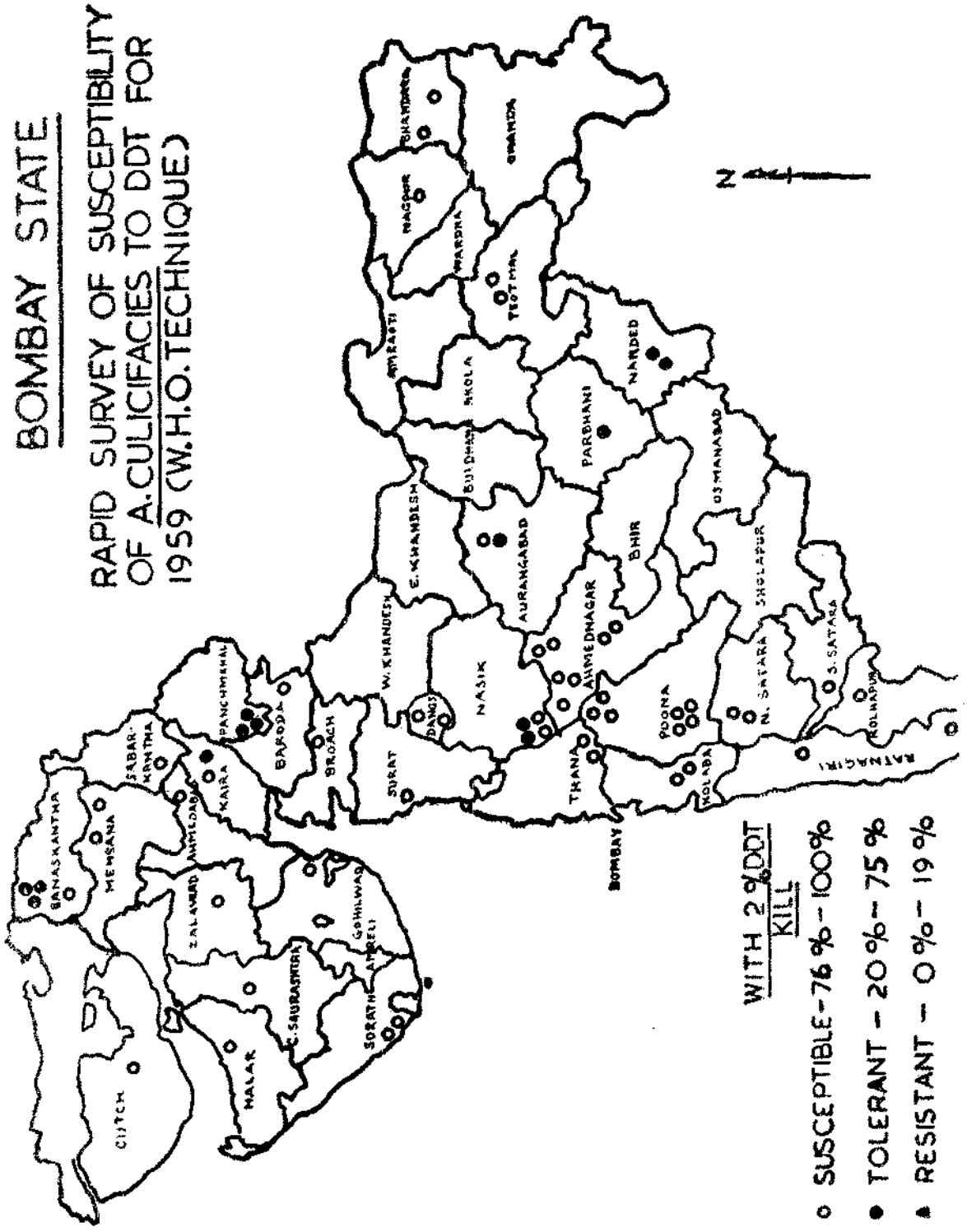
(Contd.)

TABLE 1 (*Contd.*).

Serial Number.	Dates of experiment.	Villages of experiments.	Talukas.	District.	Sprayed with DDT since	Mosquitoes collected from sprayed (SP) or Unsprayed (U/S) or both structures.	Mortality with 2 per cent DDT.		Control mortality.		Remarks.
							D/T	Kill Percentage.	D/T	Kill Percentage.	
27	Nov. 22	Pimpalgaon-Ujni	Ahmednagar	Ahmednagar	1953	U/S	78/83	94	0/21	0	S
28	Nov. 27	Vajarkhol	Sangamneshwar	Ratnagiri	1959 (1956-57)	SP	55/67	90	1/20	5	S
29	Nov. 29	Dasgaon	Mahad	Kolaba	1959 (1956-57)	SP	56/60	92	1/20	5	S
*30	Dec. 1	Bortembhe	Igatpuri	Nasik	1953	U/S	12/14	85	0/10	0	S
*31	Dec. 5-6	Fardapur	Sillod	Aurangabad	1954	U/S	104/131	79	0/42	0	S
32	Dec. 6	Bedi	Rajkot	Rajkot (Madhya Saurashtra)	1953	SP	38/40	94	1/20	5	S
33	Dec. 5-6	Mahal	Ahwa	Dangs	1953	SP	52/52	100	0/28	0	S
34	Dec. 7	Kashindra	Daskori	Ahmedabad	1950	SP	17/20	85	0/15	0	S
35	Dec. 5	Paladi Jambhadi	Bhandra Sakoli	Bhandara	1956	SP	47/47	100	1/19	5	S
36	Dec. 7	Duva	Jamnagar	Halar (Jamnagar)	1953	U/S	34/36	94	0/18	0	S
37	Dec. 9-10	Navalpur	Himatnagar	Sabarkantha	1953	U/S	38/40	95	0/40	0	S
38	Dec. 10	Vertej	Bhavnagar	Gohilwad	1953	U/S	36/40	90	0/20	0	S
39	Dec. 12	Nama Karela	Surendra Nagar	Zalawad	1953	U/S	35/40	88	0/20	0	S

40	Dec. 12	Valasna	Kheralu	Mehsana	1959 (1955-56)	U/S	18/20	90	0/20	0	S
41	Dec. 13	Sidhpur	Sidhpur	Mehsana	1959 (1955-56)	U/S	24/25	96	0/20	0	S
42	Dec. 9-10	Umra	Chorashi	Surat	1959 (1955-56)	SP	75/80	94	0/38	0	S
43	Dec. 14	Indapur	Mangaon	Kolaba	1953	SP	79/80	99	0/20	0	S
44	Dec. 10	Kardi	Chhota Udepur	Baroda	1953	SP	66/80	85	0/20	0	S
45	Dec. 4-6	Piraman and Kapodra	Ankleshwar	Broach	1953	SP	48/51	96	23/29	79†	S
46	Dec. 14	Deosadi Rameshwar	Jintur	Parbhani	1953	SP U/S	44/67	66	0/32	0	T
47	Dec. 10-11	Patoda	Patoda	Bihar	1958	U/S	60/62	97	0/21	0	S
48	Dec. 24-26	Gharni	Ahmedpur	Osmanabad	1958	U/S	72/73	99	0/41	0	S
49	Dec. 18-19	Degloor and Mujalda	Degloor	Nanded	1958	U/S	43/70	61	0/47	0	T
50	Dec. 15-16	Pali	Thasra	Kaira	1953	U/S	48/80	60	1/20	5	S
‡51	Dec. 17-18	Talawadi	Murbad	Thana	1949	SP	16/16	100	1/10	0	S
52	Dec. 21-25	Nanded	Haveli	Poona	1946		33/34	97	0/21	0	S

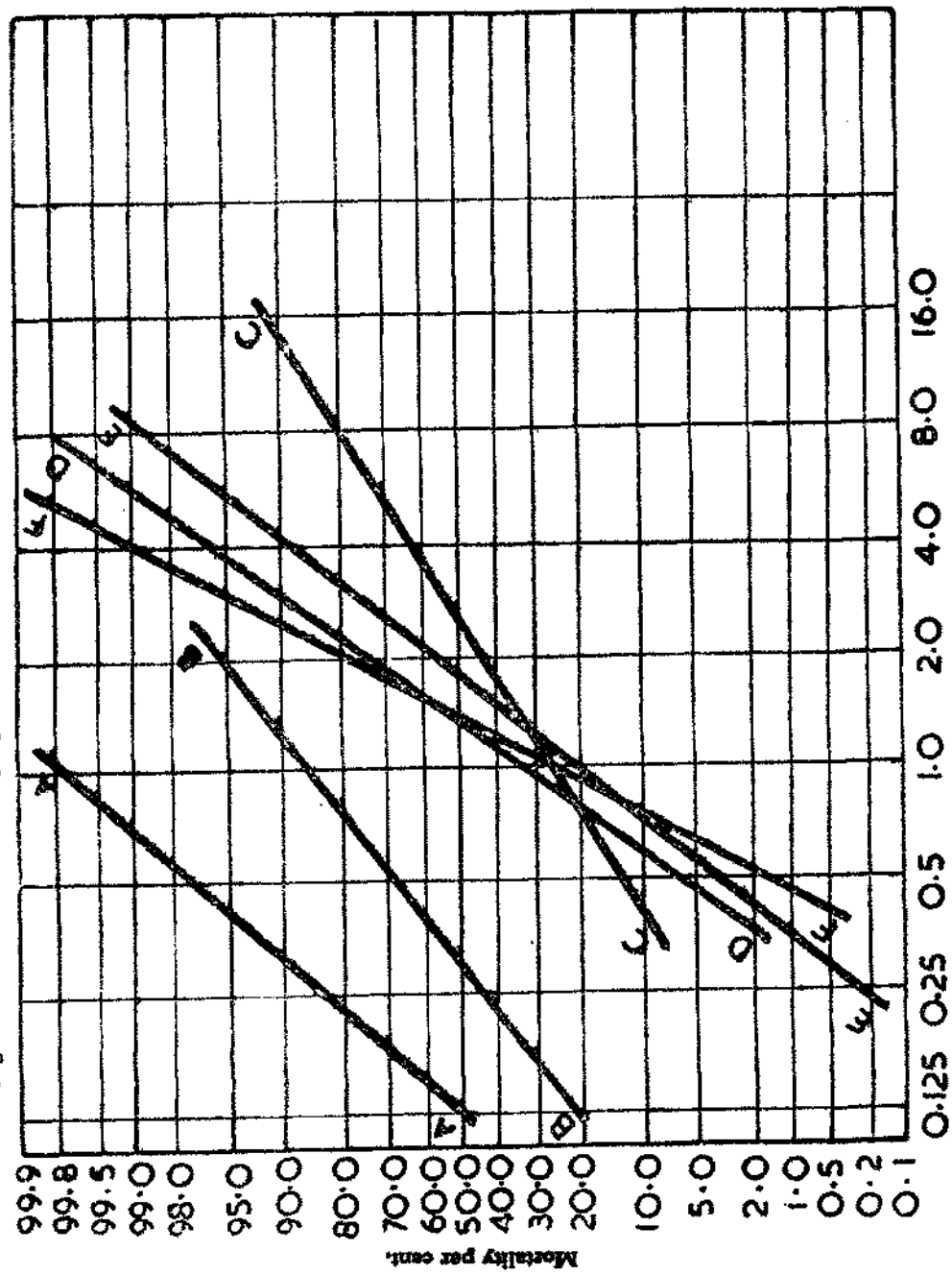
* Repetition of experiment.
† High mortality in control due to bad handling during collection. Result may be ignored.
‡ Experiments repeated.



MAP 1.
Bombay State showing localities where susceptibility tests were carried out.

GRAPH

Log Concentration Probit-mortality regression lines relating to Anopheles culicifacies and DDT.



Regression lines A—A and B—B relate to the findings observed in the year 1957, while the remaining 4 regression lines pertain to the year 1959.

Lines. A—A : Chorbaoli (Nagpur). B—B : Kada (Bhir). C—C : Khumpir (Panchmahals).
D—D : Nirgunda (Poona). E—E : Bortembha (Nasik). F—F : Pandapur (Aurangabad).

For regression equations please see Tables II and III.

TABLE IV.

Time-mortality data of A. culicifacies *(wild-caught females) with 2 per cent DDT at Khumpir, Halol Taluka, Panchmahals District.*

Exposure (in hours).	Mortality in controls.		Mortality with 2 per cent DDT.			Remarks.	Approximate value of	
	D/T	Per cent kill.	D/T	Per cent kill.	Adjusted percentage of kill.		LT_{50}	LT_{90}
One hour.	2/93	2·2	33·88	37	36	Data taken from Table III.	One hour 36 minutes.	Four hours.
Three hours.	0/36	0	34/43	79	79		"	"
Six hours.	0/38	0	61/61	100	100		"	"

TABLE V.

Dosage-mortality data of DDT tolerant A. culicifacies *(wild-caught females) of Khumpir, Halol Taluka, Panchmahals District. Bombay State, India with dieldrin and lindane. Date of experiment :- September 29 to Oct. 2, 1959.*

Insecticide.	Exposure (in hours).	Dosages tried (in per cent.)	Number dead/Total exposed.	Percentage of kill.	Adjusted mortality (Per cent).	Log (dosage × 100) probit-kill regression equation.	X^2 (degree of freedom)	Value of	
								LC_{50}	LC_{90}
Dieldrin.	One hour.	Control.	0/55	0	..	Y = 4·1307X + 1·993	$X^2(2)$ = 5·6866 Not significantly heterogeneous.	0·053 per cent.	0·11 per cent.
		0·05	26/60	43	43				
		0·10	59/63	94	94				
		0·20	56/58	97	97				
		0·40	62/62	100	100				
		0·80	25/25	100	100				
		1·60	20/20	100	100				
Lindane (γ-BHC)	One hour.	0·05 (γ-isomer)	64/64	100	100	Cannot be worked out.	..	..	..
		1·6 (γ-isomer)	19/19	100	100				
	½ hour.	0·05 (γ-isomer)	20/20	100	100		..	..	..

As the mosquitoes, in the W.H.O. exposure tubes, were found somewhat restless during the exposure at Khumpir (Halol Taluka), tests with the same W.H.O. impregnated papers were performed with Busvine-Nash exposure tubes (3 inch × 1 inch) and the comparative results with the two types of exposure tubes are presented in Table VI. There was no marked difference seen between the two types of exposure tubes (Table VI). We also used our own papers impregnated in our laboratory with technical grade of DDT supplied by the Malaria Institute of India, in Busvine-Nash exposure tubes (3 inch × 1 inch) and the results obtained are presented in Table VI. We got somewhat higher mortalities with papers impregnated in our laboratory.

TABLE VI.

Comparative tests on A. culicifacies *of Khumpir, Halol Taluka, Panchmahals District, Bombay State, India, with the World Health Organization kit and Busvine-Nash exposure tubes, using WHO-impregnated papers and papers prepared in the laboratory. Exposure one hour. Insecticide : DDT.*

Dosage of DDT (Per cent).	W.H.O. KIT AND IMPREGNATED PAPERS.		BUSVINE-NASH TUBES (3 INCH × 1 INCH). W.H.O.-IMPREGNATED PAPERS.		BUSVINE-NASH TUBES (3 INCH × 1 INCH) AND LABORATORY-IMPREGNATED PAPERS.	
	D/T	Percentage of kill adjusted.	D/T	Percentage of kill.	D/T	Percentage of kill.
Control. No DDT	2/114	2	0/35	0	0/35	0
0·5	18/100	16	4/36	11	2/36	6
1·0	27/102	24	12/36	33	13/31	42
2·0	33/88	36	17/35	49	24/34	71
4·0	70/106	66	25/36	69	26/30	86

D = Number of mosquitoes dead.

T = Total number of mosquitoes exposed.

DT = Number of mosquitoes dead out of total number of mosquitoes exposed.

DISCUSSION.

The rapid survey of the status of susceptibility, carried out in Bombay State, has shown that while in the vast majority of the localities in which tests were carried out *A. culicifacies* was found to be still highly susceptible to DDT, widely scattered and extremely circumscribed pockets were found in which there was strong evidence of a lower susceptibility. Papers impregnated with 2 per cent DDT had in earlier tests given mortality rates of 100 per cent or nearly 100 per cent in areas which had never been treated by any insecticide, *vide* Table I of Ramachandra Rao and Bhatia (1957) and the unpublished data presented by Bhatia *et al.* (1958). Similarly the two per cent concentration had also given nearly 100 per cent mortalities of *A. culicifacies* in many areas which had received intradomiciliary DDT spraying from 1 to 10 years, except in a few localities where mortalities ranged between 88

and 94 per cent. Making fair allowances for experimental errors, it is thought advisable for *practical* purposes to consider a population of *A. culicifacies* still susceptible to DDT if there was a 76 to 100 per cent mortality with papers impregnated with 2 per cent DDT with an exposure of one hour.

The most important question now is whether to consider the mortalities of 37 per cent and 55 per cent obtained in Khumpir (Halol) and Bortembhe (Igatpuri), respectively, with 2 per cent DDT-impregnated paper as an indication of definite development of true resistance to DDT or as merely examples of vigour tolerance ? Unfortunately there is no definite standard by means of which one could declare a population to have become resistant as distinguished from having developed vigour tolerance. Unless genetic experiments are carried out, it may not be possible to give any answer at all. Swaroop (1959) has indirectly hinted that if mortalities are found to be lower by about 50 per cent below that obtained with an extremely effective dosage (2 per cent DDT in the present case) it may indicate a high degree of physiological resistance. According to this criterion, populations of *A. culicifacies* giving kills of less than 50 to 60 per cent with 2 per cent DDT-impregnated paper could be regarded as resistant ones. If so, the *A. culicifacies* of Khumpir and Bortembhe would have to be declared as resistant. Trials with the full series of dosages of DDT in these places gave LC_{50}'s of 2·75 and 1·8 per cent., respectively. These values are about 3 to 5 times the values of LC_{50} in other sprayed areas (0·5 to 0·6 per cent) and about 8 to 10 times in totally unsprayed areas (0·25 per cent) *vide* Ramachandra Rao and Bhatia (1957). Whether these figures would justify calling the populations at Khumpir and Bortembhe as resistant to DDT, is doubtful. Further, the slope of log dosage-probit mortality regression lines of *A. culicifacies* of the above mentioned two places (Graph 1) are not so characteristic of a truly resistant population. The steepness of the line has somewhat declined ; perhaps indicating a mixture of populations.

After the low mortalities in the susceptibility test were noticed, Khumpir and Bertembhe areas were immediately re-sprayed with DDT at 112 mg. per sq. ft., which brought down the densities of *culicifacies* in both the localities. But the densities did not come up again in the succeeding months to yield enough mosquitoes for purpose of retesting, thereby pointing out that possibly *culicifacies* in these areas had not developed any specific resistance to DDT and what we had experienced was rather an example of tolerance. Making an allowance for the fact that the rapid drying up of all the breeding places was to a great extent responsible for the failure of the re-build up of adult *A. culicifacies* populations, the fact that DDT spraying itself produced an immediate decline in density is suggestive that DDT was still quite effective. This consideration leads one seriously to doubt whether what we have noticed in Khumpir and Bortembhe during the year 1959, was a true case of resistance. Further studies, to be carried out in these two localities during the succeeding years, perhaps will help to give precise answer to this question.

Tests have also been carried out at Khumpir with Dieldrin- and Lindane-impregnated papers. The species was found to be highly susceptible to both these insecticides.

It is appropriate to mention here that rapid susceptibility tests were carried out by the teams of the Malaria Institute of India in other parts of Bombay State, which have been separately reported* by them.

Now the intriguing question is how and why lowered susceptibility has occurred in a few widely scattered localities. The insecticidal treatment has been more or less uniform in the whole State. Khumpir and surrounding areas in Panchmahals District have been under DDT spray for over ten years but so also have been Poona, Thana and West Khandesh districts. Bertembhe in Nasik District has been receiving DDT spraying for seven years. The appearance of lowered susceptibility has, therefore, no relation to the number of years of spraying. The two localities, where the phenomenon has occurred, are separated from each other by a distance of over 200 miles and the two cases are not related to each other. It would be rather far fetched to believe that the genes for resistance were present only in these two localities.

The matter certainly needs further detailed investigations. The present indications are that these two instances are perhaps examples of an extreme case of vigour tolerance in response to purely local conditions. But the findings are extremely important, highlighting the need for constant vigilence in view of the programme of malaria eradication which has been launched and is in progress.

CONCLUSION AND SUMMARY.

A rapid susceptibility survey of *A. culicifacies* to DDT was carried out in 1959 with the World Health Organization kit, using a single dosage of 2 per cent DDT, in about 49 places scattered over whole of Bombay State comprising an area of about 200,000 sq. miles. Baseline data, using the full series of dosages, had been obtained previously. The method proved itself to be very useful in covering large areas of the State in a short time. The studies showed that *A. culicifacies* was still highly susceptible to DDT throughout the State, except in a couple of small localities in Panchmahals and Nasik districts which were widely separated by a distance of about 200 miles.

In the places where lowered susceptibility to DDT was encountered, a full series of DDT dosages were also tried and the results indicated that the LC_{50} in these places showed a 5 to 8-fold increase. It is still doubtful whether it is a true case of resistance. Indications are that it may be a case of vigour tolerance to DDT rather than specific resistance. The DDT tolerant *A. culicifacies* did not exhibit cross-tolerance to Dieldrin and Lindane (γ-BHC).

*Das, M. and Mammen, M.L. (1959) *Bull. Nat. Soc. Ind. Mal. Mosq. Dis.*, 7, 6, pp. 157-168.

ACKNOWLEDGEMENT.

Grateful acknowledgement is made of the work of several members of the staff of this organisation who were entrusted with the task of carrying out the tests at several places.

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Indian Journal of Malariology, 15, 1, March 1961.

AN OUTBREAK OF MALARIA IN PARTS OF THANA DISTRICT, BOMBAY STATE, INDIA, AFTER SEVERAL YEARS OF SUCCESSFUL CONTROL.

BY

T.B. PATEL,

T. RAMACHANDRA RAO

AND

G.J. AMBWANI.

(Malaria Organisation, Public Health Department, Bombay State, Poona.)

[May 5, 1960.]

INTRODUCTION.

An outbreak of malaria occurred in a small part of Thana District, Bombay State, India, between the months of July and October, 1958. This outbreak, though it had all the characteristics of an epidemic, should perhaps be more aptly described as a recrudescence because the area affected was originally hyperendemic for malaria till 1949 when a malaria control scheme by the use of DDT residual insecticide was started. The reduction in the incidence of malaria since 1949, till the outbreak referred to now, had been spectacular.

The outbreak has more than ordinary interest to malaria workers for two reasons; firstly, it represents the type of 'epidemic' from very small origins visualised by Macdonald (1956) in the later phases of a malaria eradication programme ; secondly, it represents, perhaps the first instance in India of a large scale failure of an insecticide (dieldrin) due to precipitate selection of resistant strain of a vector species. The outbreak also provides a much needed warning against complacence both in technical and administrative matters connected with the malaria eradication programme. This paper has been prepared with a view to presenting the salient features of the outbreak and it is hoped that the data will be of use to the other malaria workers as it has been to us.

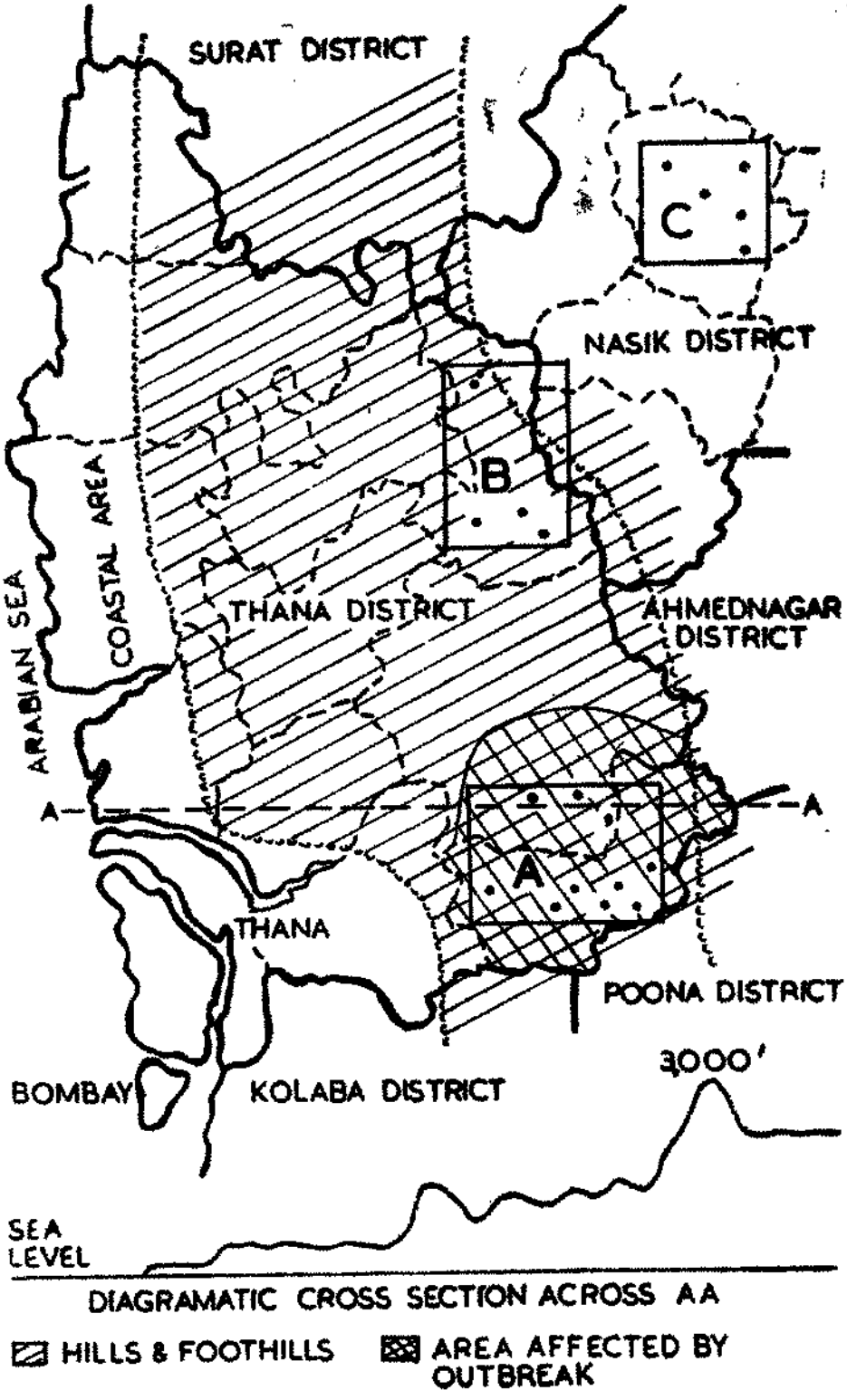
AREA, CLIMATE AND EPIDEMIOLOGY.

The area of the outbreak was restricted to two talukas (divisions) of Thana District, Bombay State, India, and had a total population of approximately 86,000 in an area of about 500 square miles (Map I). The district, as a whole, had a population of 1·6 million (1951 census) with a total area of 3,756 square miles.

Thana District forms a hinterland to Bombay City. Approximately one-third of the area of the district consists of coastal plains which are fairly well populated. The remaining two-third consists of hills and foot-hills much of which

MAP I.

Thana District, Bombay State.



is fairly well wooded by deciduous forest. The villages are scattered throughout the area and rice is the principal crop grown. The annual rainfall ranges from about 80 inches at the coast to about 200 inches at the foot of the hills. The rainfall occurs almost wholly between middle of June and the end of September ; July and August being the months of the heaviest rain-fall.

The climatic conditions are such that transmission can take place throughout the year, summer being not too warm nor winter too cold. The range of temperature is : minimum 20°C. in December and January and maximum 40°C. in May and June. The transmission season, therefore, is limited more by the availability of breeding places rather than the temperature and humidity conditions. In the major part of the district the transmission season is from July to December, coincident with the formation of ideal breeding conditions during and immediately after the monsoon. From November onwards the district dries up very rapidly and except for a few perennial streams no suitable breeding places remain. As a matter of fact there is acute scarcity even of drinking water in a large majority of the villages in the hilly and foot-hill sections of the district during the summer months of March, April and May.

A. culicifacies and *A. fluviatilis* are the vectors in this area, the former being more predominant and occurring in enormous numbers during monsoon and immediate post-monsoon months. The malaria surveys carried out in this district prior to 1949 had shown that practically the entire hill and foot-hill region of the district was endemic for malaria, the degree ranging from meso- to hyperendemicity.

MALARIOMETRIC DATA.

Since the commencement of the DDT spraying operations in 1949, there has been a rapid reduction in the incidence of the disease. Periodical annual malariometeric surveys indicated downward trends in all the indices, and towards the end of 1956 the indices were zero or very near zero (Table I). Therefore a question under consideration was whether the district as a whole was not fit for experimental interruption of spraying as was done in the case of Kanara District, Bombay State, India (Patel, Ramachandra Rao and Paranjpey, 1957). Such an interruption was however, not brought about as we were not quite certain of the epidemiological status of the district, particularly in respect of the parasitic reservoir and the manner in which the vector species may react to the release of the DDT pressure.

The dispensary statistics also have shown a remarkable reduction in the incidence of malaria. The available year-to-year data for 25 public dispensaries are depicted in Table I-A. It may be mentioned here that the figures do not refer to malaria cases as determined by blood examination, but those which were clinically diagnosed as malaria by dispensary doctors. The figures reveal that the number of malaria cases treated in 1957 was, till then, the lowest on record in the district. The sudden rise in the number of cases in 1958 was a result of an outbreak which forms the subject of this paper.

TABLE I.

Malariometric indices in Thana District, cumulative rates.

Taluka.		Prior to control measures (1947)	1955	1956	1957	1958	1959
1. Kalyan	S.R.	22·1	1·08	0·0	0·0	0·3	0·5
	C.P.R.	...	0·0	0·0	...	0·0	0·0
	I.P.R.	...	0·0	0·0	...	0·0	0·0
2. Thana	S.R.	40·1	4·07	0·27	0·14	0·0	0·7
	C.P.R.	...	0·0	0·0	...	0·5	0·0
	I.P.R.	...	0·0	0·0	...	0·0	0·0
3. Bhiwandi	S.R.	37·1	4·2	0·0	0·0	0·0	0·09
	C.P.R.	...	0·0	0·0	...	0·0	0·09
	I.P.R.	...	0·0	0·0	...	1·5	0·0
4. Bassein	S.R.	48·0	2·6	Not available	0·0	0·1	0·0
	C.P.R.	...	0·0		...	0·0	0·0
	I.P.R.	...	0·0		...	0·0	0·0
5. Shahapur	S.R.	35·1	1·9	0·13	0·0	5·7	0·9
	C.P.R.	...	10·0	0·0	0·0	0·0	0·2
	I.P.R.	...	0·0	0·0	0·0	0·0	0·0
6. Murbad	S.R.	41·3	4·2	0·0	0·0	17·5	2·7
	C.P.R.	...	0·0	0·0	0·0	0·62	0·4
	I.P.R.	...	0·0	0·0	0·0	1·2	0·0
7. Wada	S.R.	53.1	2·7	0·0	0·0	0·0	0·3
	C.P.R.	...	0·0	0·0	...	0·0	0·0
	I.P.R.	...	0·0	0·0	...	1·2	0·0
8. Palghar	S.R.	27·7	1·2	0·0	0·05	0·0	0·3
	C.P.R.	...	0·0	0·0	...	0·0	0·0
	I.P.R.	...	0·0	0·0	...	0·76	0·0
9. Jawahar	S.R.	18·6	1·9	0·0	0·0	N.A.	0·6
	C.P.R.	...	0·0	0·0	...	0·0	0·2
	I.P.R.	...	0·0	0·0	...	0·0	1·1*
10. Dahanu	S.R.	85·1	4·96	0·0	0·0	0·2	0·07
	C.P.R.	...	0·0	0·0	...	0·0	0·07
	I.P.R.	...	0·0	0·0	...	0·0	0·6
11. Umbergaon	S.R.	40·8	5·06	0·25	0·0	0·4	0·13
	C.P.R.	...	0·0	0·0	...	N.A.	0·0
	I.P.R.	...	0·0	0·0	...		0·0
12. Mokhada	S.R.	41·0	3·7	0·09	0·29	0·0	1·03
	C.P.R.	...	0·0	0·0	...	0·0	1·2
	I.P.R.	...	0·0	0·0	...	0·0	0·0

S.R.=Spleen rate.

C.P.R.=Children parasite rate.

I.P.R—Infant parasite rate. (All rates are in percentages.)

*One positive out of 90 infants.

TABLE I-A.

Year-to-year data for 25 public dispensaries, Thana District, Bombay State.

Year.	Number of cases of all diseases.	Number of malaria cases only.	Percentage of malaria cases.
1946	...	56,169	...
1947	...	59,243	...
1948	...	59,068	...
1949	193,825	35,324	18·2
1950	211,833	35,695	16·9
1951	191,728	32,683	17·1
1952	222,286	29,225	13·2
1953	254,481	24,175	9·5
1954	220,633	18,356	8·3
1955	229,758	16,604	7·2
1956	255,883	11,707	4·6
1957	296,720	7,433	2·5
1958	288,951	13,442	4·7
1959	303,932	6,086	2·0

The malariometric indices for 1957, i.e., the year prior to the outbreak, are unfortunately incomplete except for the spleen rates which are fully available for all sections of the district. But the parasite rates, both for children and infants, are available only for the two talukas (divisions) in which the outbreak occurred. There was, however, no indication that in 1957 there was any widespread prevalence of malaria in any part of the district. Even if there was any increase in malaria cases in the Murbad and Shahapur talukas (divisions), it must have been so slight as not to be reflected in the dispensary figures or in the spleen parasite and infant parasite rates.

HISTORY OF SPRAYING.

The history of spraying in the district may be summarised as follows :

1948-1952 : DDT water-aromex-soap emulsion, three rounds per year during the transmission season, at a dosage of 56 mg. per square foot.

1953-1956 : DDT 75 per cent water wettable powder, two rounds each year, at a dosage of 100/112 mg. per square foot.

1957 Dieldrin 50 per cent water wettable powder, two rounds at 28 mg. per sq. foot. Some sections of the district (not the area in which the outbreak occurred) had received the second round of spraying, during the period September-November with DDT wettable powder instead of dieldrin because of cases of dieldrin toxicity noticed among the workers.

1958 One round of dieldrin, 50 per cent water wettable powder in May-June, at 28 mg. per square foot. The second round of spraying was entirely with DDT in all sections and a special third round also with DDT in selected areas.

1959 DDT 50 per cent water wettable powder, two rounds.

RECRUDESCENCE OF MALARIA.

The above mentioned brief account would be adequate to show that malaria had been very successfully controlled in the district and at the end of 1957, i.e., at the end of ten years of control, the district was practically fit for establishment of a surveillance organisation to detect and wipe out the remaining foci, if any.

It was, therefore, disturbing to receive reports in September, 1958, that there was a number of cases of fevers occurring in the two talukas of the district, *viz.*, Murbad and Shahapur, presenting typical clinical symptoms of malaria. Investigations soon revealed that the fever cases were due to malaria. Immediate steps were taken to interrupt further transmission by (*1*) abandoning the use of dieldrin and reversion to the use of DDT wettable powder, and (*2*) widespread distribution of anti-malarials, chiefly chloroquine, in the entire affected area.

Out of 1,665 villages scattered throughout 3,767 sq. miles of the district, only 209 villages with a total area of 500 sq. miles were affected by this recrudescence (Map 1). The area affected was a part of the foot-hill zone of the district. The recrudescence was not restricted to one or more of a few groups of villages but was uniformly spread throughout the entire affected area. In Table I are presented the spleen, child parasite, and infant parasite rates in this district from time to time and including those found in the period prior to the institution of any control measures. In Table II is presented a summary of the malariometric data collected towards the end of 1958 in the several talukas (divisions) of Thana District. These data clearly show the manner in which malaria had receded in the district as a whole till 1957 as a result of the control measures. It can also be seen that it was only in the two talukas of Murbad and Shahapur that increased spleen and parasite rates were noticed in 1958.

THE ORIGIN, EXTENT AND COURSE OF THE OUTBREAK.

Apart from the annual malariometric survey carried out at the end of the transmission season of 1958, a special house-to-house enquiry was made during the epidemic period in a number of villages to determine the extent of morbidity and the general course of the outbreak. From the replies given by the house-holders it was noticed that between 50 and 60 per cent of the entire population of all villages had been affected by the outbreak by the end of September and had suffered at least one attack of fever between the months of July and September, 1958. The spleen rates were also substantially high during the epidemic, indicating that spleen had enlarged rapidly due to the new infections.

The monsoon normally bursts over Thana District at the end of the first week of June. But in 1958, the first burst occurred on June 21. The first new cases of fever seem to have occurred in the fourth week of July, i.e., some four to five weeks after the onset of the rains. By the third week of August the prevalence of fevers was quite widespread and the 'epidemic' had reached its peak by the end of September, i.e., about 100 days after the onset of the monsoon and about 70 days after the appearance of the first new case.

TABLE II.

Cumulative malariometric rates—Thana District, 1958-59.

Taluka.	SPLEEN RATE.				NUMBER OF VILLAGES WITH SPLEEN RATES OF						CHILD PARASITE RATE.				INFANT PARASITE RATE.			
	Number of villages visited.	Number of cases examined.	Number positive.	Percentage.	0·0 per cent.	Less than 5 per cent.	5—9 per cent.	10—24 per cent.	25—49 per cent.	50 per cent and over.	Number of villages visited.	Number of cases examined.	Number positive.	Percentage.	Number of villages visited.	Number of cases examined.	Number positive.	Percentage.
1. Kalyan	16	895	3	0·3	13	2	1	..	..	..	7	462	0	0	3	51	0	0
2. Murbad	16	810	142	17·5	3	2	..	6	3	2	7	484	30	0·6	7	84	1	1·2
3. Thana	10	613	0	0	10	..	..	..	..	..	7	407	2	0·5	3	59	0	0
4. Shahapur	6	394	23	5·7	1	2	1	2	..	..	7	544	0	0	9	91	0	0
5. Mokhada	4	457	0	0	4	..	..	..	..	..	3	67	0	0	3	45	0	0
6. Bhiwandi	9	593	0	0	9	..	..	..	..	..	8	572	0	0	8	65	1	1·5
7. Bassein	5	600	1	0·1	4	1	..	..	..	..	3	240	0	0	3	36	0	0·0
8. Palghar	4	690	0	0	4	..	..	..	..	..	9	606	0	0	8	131	1	0·8
9. Dahanu	5	413	1	0·2	4	1	..	..	..	..	3	227	0	0	4	31	0	0·0
10. Wada	1	55	0	0·0	0	..	..	..	..	..	6	405	0	0	7	85	1	1·2
11. Umbergaon	11	1,397	6	0·4	7	N.A.	N.A.	..	..	..	2	N.A.	0	0	..	N.A.	0	0·0
12. Jawahar	0	0	0	0	..	..	..	..	..	..	5	488	0	0	5	98	0	0·0

N.A.=Not available.

The villagers, who had ceased to experience malaria over a period of years, did not at first suspect that the fevers were due to malaria. They, and even most of the local officials, attributed the rise in the incidence of fevers in July, 1958, to influenza, an epidemic of which had passed all over the country in the preceding year. It was only in the later part of August that malaria was suspected as a cause of the fevers. As the Malaria Department did not have a surveillance or vigilance organisation located in the area to keep a watch on the incidence of fevers and to investigate the causes thereof, the outbreak did not come to our notice till the middle of September when investigations were started. As already stated, the epidemic was quickly brought under control by wide-spread use of anti-malarials and by rapid spraying of the area with DDT.

The origin of this outbreak can be attributed to the failure of the dieldrin spraying which was carried out between May 16 and June 3 of that year, to control the building-up of mosquito densities after the onset of the forthcoming monsoon on June 21. Later investigations showed that *A. culicifacies* of this area had definitely developed resistance to dieldrin. The LC_{50} was very well above 1·6 per cent., while normally it was about 0·05 per cent for susceptible populations (Patel *et al.*, 1958 : 1961). A contributory cause, not for the origin of the outbreak but for its persistence and further build-up, was perhaps the rather late commencement of the second round of spraying. Such delayed spraying had been a practice in parts of Thana District because of the general non-approachability of the villages during the height of the monsoon and the practice had not, in previous years, given rise to any outbreak of the kind now reported. However, the late spraying could not have had any effect on the origin of the outbreak because the first cases of malaria undoubtedly occurred long before the normal interval of 10 to 12 weeks required for the second round of spraying.

A question of some interest arises whether the outbreak could have been partly or wholly precipitated by the inadequacy of coverage by the insecticide in space and time year after year. A careful study of the available data from 1953-54 to 1958-59 has shown that there was no unusual increase in the percentage of missed houses due to refusal or due to being locked. In Murbad Taluka the percentage of missed houses was 8·4 per cent in the first round for 1958, as against 9·7 per cent in 1957. The figure for Shahapur Taluka for 1958 was 11·8 per cent as against 9·4 in 1957. The percentages of missed houses year after year from 1953-54 were as under :

	1953	1954	1955	1956	1957	1958
Shahapur	1·7	9·3	7·9	11·3	9·4	11·8
Murbad	1·3	10·7	8·9	11·1	9·7	8·4

The figures do not indicate any significant change in the work in 1958. The figures for the rest of the district are also more or less similar, the percentage of missed houses ranging from 17·9 in Bhiwandi Taluka to 4·5 in Wada Taluka during the first round in 1958.

Similarly a study of the actual data of spraying in respect of the first and second rounds in the district as a whole, and particularly in these two talukas, do not show any significant change in 1958. The period of spraying for the two talukas from 1953-54 onwards are shown below :—

Murbad Taluka.

Year.	Date of commencement of		Interval.
	First round.	Second round.	
1953–54	May 15	September 3	15 weeks
1954–55	May 19	August 16	13 weeks
1955–56	May 19	August 16	13 weeks
1956–57	May 14	September 16	16 weeks
1957–58	June 5	September 23	15 weeks
1958–59	May 16	September 24	18 weeks
1959–60	May 29	July 15	6 weeks

Shahapur Taluka.

Year.	Date of commencement of		Interval.
	First round.	Second round.	
1953–54	June 3	September 29	16 weeks
1954–55	May 30	September 22	16 weeks
1955–56	June 3	September 25	16 weeks
1956–57	May 27	October 10	18 weeks
1957–58	June 16	October 13	16 weeks
1958–59	May 31	September 13	15 weeks
1959–60	May 16	July 15	8 weeks

While there is nothing to indicate that the intervals between the sprayings had any significant role in the precipitation of the 'epidemic' in 1958, there is no reason to disregard the possibility that the intervals were long enough to maintain a low degree of transmission year after year and that the outbreak was precipitated when the mosquito factor became very favourable in 1958.

SPECIAL FEATURES OF THE OUTBREAK.

The outbreak had several interesting features :

Firstly, it had arisen uniformly in a large number of villages almost simultaneously. Such a simultaneous increase in malaria prevalence in all the villages of the area could not have taken place without the presence of gametocyte carriers, spread throughout the area. If the outbreak was due, on the other hand, only to a very few foci of gametocyte carriers limited to few villages it would not have assumed the proportions which it did in such a short period and it would have been perhaps possible to follow the course of the epidemic from village to village. On the basis of this reasoning, the possibility of the outbreak having arisen from only a few very scattered gametocyte carriers, can be ruled out.

Secondly, if it is inferred that the outbreak occurred as a result of wide distribution of gametocyte carriers throughout the area, the next question naturally arises as to what was the quantum of reservoir at the beginning of the 1958 season. As has already been shown, all malariometric indices were practically zero at the end of 1956. At the end of 1957, the spleen rate was zero in the entire district. Nor was there any indication of any increase in the incidence of fevers in any of the dispensaries in the district in 1957. Fortunately the child parasite rate and the infant parasite rate for 1957 are available for the affected talukas of Murbad and Shahapur (Table 1). Out of 360 children and 192 infants examined, none was positive for malaria parasites within this area. Quite obviously, therefore, the gametocyte carriers must have been extremely few in the entire community towards the beginning of the year 1958 and were low enough not to be detected in the usual malariometric surveys.

The *third* feature of considerable interest is the extreme rapidity with which the outbreak assumed epidemic proportions. The first *new* cases of the season occurred hardly within four to five weeks after the onset of the monsoon. Within 70 to 80 days of the first appearance of the new cases or 90 to 100 days after the onset of the monsoon, the outbreak had reached its peak. There is no doubt that a number of fresh infections took place even after the peak period was reached but in diminishing numbers. Our studies, reported later in this paper, have shown that the epidemic was due both to *vivax* and *falciparum* infections, the latter being more predominant.

Such a rapid build-up of the 'epidemic' could have occurred only if the reproduction rate of the disease was very high. A high rate was quite possible taking into consideration the fact that the densities of *A. culicifacies* were enormous.

The absolute urgency of taking immediate steps to interrupt the 'epidemic', and our ignorance about the outbreak at the time of its origin, provided us no opportunity to study the interplay of the several epidemiological factors as the 'epidemic' progressed. Even if one could have foreseen the possibilities of such an 'epidemic', it would have been impossible to collect the requisite data in view of the overwhelming urgency for measures of prompt control of the epidemic and relief to the people.

The *fourth* interesting feature is that the outbreak occurred only in a small portion of the district though dieldrin had been sprayed in other sections also. It would be rather difficult to believe that both resistance to dieldrin and the reservoir of infection were so conveniently prevalent together only in this area. As the entire district practically had the same history of antimalaria measures, till 1957 it would be difficult to expect that gametocyte reservoir was restricted to this area only.

COMPARATIVE STUDIES IN THE AFFECTED AND NON-AFFECTED AREAS.

The above mentioned features cannot be fully explained without detailed information of such matters as actual weekly mosquito densities, the degree of contact of the vector with man, i.e., biting rate, the actual number of persons affected week after week, and a numerical estimate of the actual gametocyte reservoir just prior to the onset of the monsoon in 1958. Unfortunately such information is not collected routinely and is not available for the area. However, in order to gain some insight into some of the related problems, particularly regarding the reservoir of infection to be expected in areas where the control measures, extending over five to six years, had reportedly been very successful, a special comparative study was undertaken in three groups of villages.

Group 'A' consisted of 10 villages within the area affected by the above mentioned outbreak (Map 1).

Group 'B' consisted of five villages in an area of the same district, but outside the area affected by the outbreak, but having very similar topographical and epidemiological conditions and history of spraying operations including the use of dieldrin.

Group 'C' consisted of five villages situated in another originally hyperendemic area in the neighbouring district of Nasik, situated some 80 miles away from the area affected by the outbreak and in which continuous use of DDT, only for six years, had led to an extremely good control of malaria.

In these special investigations, an attempt was made :

(1) to gather information regarding the prevalence of fevers in 1957 and 1958,

(2) to collect blood smears from as many persons as possible in the entire population of the selected villages, irrespective of sex and age or history of fevers, and

(3) spleen examination of all the children between the ages of 2 and 9.

The data collected from each of these areas are described below :

GROUP 'A' (Tables III and IV).

This group consists of 10 villages of Shahapur and Murbad talukas of Thana District with a total population of 5,270. All of them are located within the area in which the malaria outbreak occurred. The special investigations were carried out in January and February, 1959, i.e., three months after the 'epidemic' had been brought under control and when there was no evidence of fresh transmission. This survey also afforded a further opportunity to distribute antimalarials in the villages. The spleen rates of the villages varied from 7·5 per cent to 92 per cent. The blood smears of 2,294 persons of all age-groups were actually examined and 213 of them were found to be positive for malaria parasites (*P. vivax* 74, and *P. falciparum* 129), giving a total parasite rate for the entire population as 8·8 per cent.

TABLE III

Mass blood survey in Group 'A' area—Thana District—Area affected by the outbreak of malaria.

Serial number.	Name of the village in Shahapur and Murbad talukas.	Census population.	Spleen rate per cent.	Number of persons whose blood smears were examined.				Results of examination. Persons found with malaria parasites.				Parasite rate, per cent.			Species of parasites.	
				Below 1 year.	1 to 9 years.	Above 9 years.	Total.	Below 1 year.	1 to 9 years.	Above 9 years.	Total.	Infant.	Child.	Entire population.	*P. vivax.*	*P. falciparum.*
1	Sapgaon	680	7·5	2	10	31	43	0	0	0	0	0·0	0·0	0·0	0	0
2	Satgaon	439	N.A.	6	74	122	202	0	9	11	20	0·0	12·2	9·9	8	12
3	Khutgar	338	10·3	4	31	85	120	0	3	3	6	0·0	9·7	4·6	2	4
4	Pimpalghar	48	N.A.	3	8	21	32	2	3	3	8	66·6	37·5	24·2	3	5
5	Nandgaon	270	92·0	4	31	81	116	3	23	32	58	75·0	74·2	50·0	22	36
6	Vanzala	410	20·4	7	45	141	193	0	8	8	16	0·0	17·8	8·3	5	11
7	Kisor	314	51·1	5	58	127	190	2	15	11	28	40·0	25·9	14·7	15	13
8	Umbroli Bk.	329	13·6	5	63	166	234	0	6	17	23	0·0	9·5	9·8	8	15
9	Vaishakhara	697	35·1	18	90	306	414	0	8	15	23	0·0	8·9	16·4	3	20
10	Dhasai	1,745	28·8	20	238	492	750	0	11	20	31	0·0	8·4	5·0	8	23
	Total	5,270	..	74	648	1,572	2,294	7	86	120	213	9·0	13·3	8·8	74	129

N.A.=Not available.

TABLE IV.

Fever incidence in Group 'A' area, affected by the outbreak of malaria.

Serial Number.	Names of the villages in Shahapur and Murbad talukas.	Census population.	Number of persons surveyed.	Number with history of fever in 1957.	Number with history of fever in 1958.	INCIDENCE OF FEVER PER 1,000 OF POPULATION.	
						1957	1958
1	Sapgaon	680	43	4	25	93·0	581·4
2	Satgaon	439	202	4	157	19·8	777·2
3	Khutgaon	338	120	4	66	33·3	550·0
4	Pimpalghar	48	33	0	31	0·0	939·4
5	Nandgaon	270	116	1	108	8·6	931·0
6	Vanzala	410	193	0	158	0·0	818·7
7	Kesor.	314	190	0	153	0·0	805·3
8	Umbroli Bk.	329	234	0	164	0·0	700·9
9	Vaishakhara	697	414	3	343	7·2	828·5
10	Dhasai	1,745	750	0	428	0·0	570·7
	Total	5,270	2,295	16	1,633	7·0	710·2

Seven out of 74 infants examined were found to be infected, giving an infant parasite rate of 9 per cent. Out of 2,295 persons from whom enquiries were made regarding the history of fevers, 16 reported that they had fevers in the year 1957 and 1,633 in the year 1958, giving an incidence of fevers of 7·0 and 710·2 respectively for a population of 1,000. The difference between the figures for the years 1957 and 1958 is remarkable, even after making allowances for the natural likelihood of some persons having forgotten any slight fever which they might have experienced over a year earlier. But malaria, with its characteristic features and relapses, is not so easily forgotten. These figures amply bear out the previous statement that there was no marked malaria prevalence in 1957 in the area. Roughly, therefore, 71 per cent of the population had experienced fevers in 1958. After making allowances for other fevers, one may safely estimate that at least 60 per cent of the population of the area was actually directly affected by the outbreak.

GROUP 'B' (Tables V and VI).

This group consists of five villages of Mokhada Taluka of Thana District with a total population of 5,089. These villages are approximately 30 to 40 miles away from the area of the outbreak but have the same topography, climate and history of insecticidal treatment, including the use of dieldrin. The study was made in January-Feburary, 1959. The spleen rate was zero in all these villages. Out of 2,016 persons of all age-groups whose blood smears were examined, 21 were found to have malaria parasites, giving the total parasite rate, for the entire community, of 1·0 per cent. None of the 46 infants, and only eight out of 570 children, showed malaria parasites. In this case also the bulk of the few positive were actually found in only one village, Poshera. The history of fevers showed that none of the 2,019 persons investigated reported having had fevers in the year 1957, while 23 (or 11·4 per thousand) reported having suffered from fever in 1958. Here again, allowance has to be made for forgetfulness on the part of the people regarding the fevers

TABLE V.

Mass blood survey in villages of Mokhada Taluka (non-affected area, Group 'B'), Thana District.

Serial number.	Name of the village.	Census population.	Spleen rate.	Number of persons whose blood smears were examined.				Results of examination. Persons found with malaria parasites.				Parasite rate, per cent.			Parasite species.	
				Below 1 year.	1 to 9 years.	Above 9 years.	Total.	Below 1 year.	1 to 9 years.	Above 9 years.	Total.	Infant.	Child.	Entire population.	*P. vivax.*	*P. falciparum.*
1	Poshera	1,774	0·0	35	305	635	975	0	7	7	14	0·0	2·3	1·4	1	14
2	Khodala	1,154	0·0	2	113	377	492	0	0	4	4	0·0	0·0	0·8	2	2
3	Gonda Bk.	635	0·0	8	75	179	262	0	1	1	2	0·0	1·3	0·8	0	2
4	Gomghar	882	0·0	1	77	152	230	0	0	1	1	0·0	0·0	0,4	0	1
5	Koregaon	644	0·0	0	0	57	57	0	0	0	0	0·0	0·0	0·0	0	0
	Total	5,089	..	46	570	1,400	2,016	0	8	13	21	0·0	1·4	1·0	3	19

TABLE VI.

Incidence of fever in Mokhada Taluka (non-affected area, Group 'B'), Thana District.

Serial Number.	Name of village.	Census population.	Number of persons surveyed.	Number with history of fever in 1957.	Number with history of fever in 1958.	Incidence of fever per 1,000 of population.	
						1957	1958
1	Poshera	1,774	975	0	6	0·0	6·2
2	Khodala	1,154	492	0	4	0·0	8·1
3	Gonda Bk.	635	262	0	2	0·0	7·6
4	Gomghar	882	230	0	11	0·0	47·8
5	Koregaon	644	60	0		0·0	0·0
	Total	5,089	2,019	0	23	0·0	11·4

experienced in the previous year. But the figures are fully supported by the malariometric data. This area, it should be remembered, was also sprayed twice with dieldrin in the year 1957 and once in 1958. But there was no report of outbreak or any unusual prevalence of any fevers in this area.

In this connection, it is interesting to compare the spleen and parasite rates of these five villages, which had been recorded in the year 1948, i.e., prior to the commencement of the malaria control scheme with the use of DDT, with those obtained during this study. The rates are depicted below :

Name of the village.	1948		1958	
	Spleen rate.	Parasite rate.	Spleen rate.	Parasite rate.
Poshera	16·0	4·7	0·0	2·3
Khodala	38·0	4·0	0·0	0·0
Gonda Bk.	70·0	8·9	0·0	1·3
Gomghar	34·5	6·0	0·0	0·0
Koregaon	15·0	8·3	0·0	0·0

These figures show that over these years, there has been (*1*) spectacular reduction in the spleen rates leading to the practical disappearance of enlarged spleen (*2*) a great reduction but still a slight persistence of parasites in the community. Whether the persistence of the parasites in this community was a feature of the year 1958 alone, cannot be positively stated.

GROUP 'C' (Tables VII and VIII).

This group consisted of five villages of Dindori Taluka, Nasik District, with a total population of 4,842. This area was hyperendemic for malaria prior to 1953 and since then has been regularly under DDT indoor residual spraying, twice a year between June and November, at dosages of 100-112 mg. per sq. foot. The special study which was carried out in December, 1958, and January, 1959, has now indicated that, in 1958, the spleen rate was zero in all the villages. Out of 2,182 persons of all age-groups whose blood smears were examined, only four were positive (one *P. vivax* and three *P. falciparum*), giving the parasite rate for the entire population as 0·18 per cent. None of the 91 infants was positive. Only one, out of 602 children between the ages of one and nine years, was positive ; giving a childhood parasite rate of 0·2 per cent. The history of fevers is extremely interesting in that out of 2,767 persons investigated 21 reported having had fever in the year 1957 and 82 in 1958, giving an average incidence of fever 7·6 and 30·1 per thousand respectively.

This area was also, as already stated, highly malarious, as can be seen from the spleen and parasite rates pertaining to the same villages for the years 1947-48 presented in Table IX, along with those of 1958, for purposes of comparison.

TABLE VII.

Mass blood survey in villages of Dindori Taluka, Nasik District, Group 'C'.

Serial number.	Name of the village.	Census population.	Spleen rate.	Number of persons whose blood smears were examined.				Results of examination. persons found with malaria parasites.				Parasite rate, per cent.			Species of parasites.	
				Below 1 year.	1 to 9 years.	Above 9 years.	Total	Below 1 year.	1 to 9 years.	Above 9 years.	Total.	Infant.	Child	Entire population.	*P. vivax.*	*P. falciparum.*
1	Umrala	1,644	0·0	55	281	647	983	0	..	2	2	0·0	0·36	0·2	0	2
2	Mahaja	601	0·0	18	119	338	475	0	0	0	0	0·0	0·0	0·0		
3	Krishnagaon	527	0·0	12	84	263	359	0	0	1	1	0·0	0·0	0·28	..	1
4	Palkhed	1,150	0·0	6	92	170	268	0	1	..	1	0·0	0·0	0·37	1	0
5	Amba Dindori	920	0·0	0	26	71	97	0	0	0	0	0·0	0·0	0·0		
	Total	4,842		91	602	1,489	2,182	0	1	3	4	0·0	0·7	0·18	1	3

TABLE VIII.

Incidence of fevers outside the epidemic area—Dindori Taluka, District Nasik, Group 'C'.

Serial number.	Name of the village.	Census population.	Number of persons surveyed.	Number with history of fever in 1957.	Number with history of fever in 1958.	Incidence of fever per 1,000 of population.	
						1957.	1958.
1	Umrala	1,644	1,388	19	39	13·7	26·7
2	Mahaja	601	559	0	0	0·0	0·0
3	Krishanagaon	527	435	2	6	4·6	13·8
4	Palkhed	1,150	277	0	19	0·0	68·6
5	Amba Dindori	920	98	0	18	0·0	183·7
	Total	4,842	2,757	21	82	7·6	30·1

TABLE IX.

Spleen and parasite rates of villages in Dindori Taluka, Nasik District.

Name of the village.	1947-48		1958	
	Spleen rate.	Parasite rate.	Spleen rate.	Parasite rate.
Umrala	20·3	6·3	0·0	0·4
Mahaja	54·8	16·3	0·0	0·0
Krishanagaon	36·1	8·3	0·0	0·0
Palkhed	70·2	19·6	0·0	0·0
Amba Dindori	26·6	20·0	0·0	0·0

The figures show that there has been a clear reduction in malaria prevalence. There was no report of any unusual prevalence of fevers and the few cases of malaria which were detected, would have perhaps gone unnoticed in the usual malariometric surveys. The four cases of malaria-parasites positives included one child of seven years of age and the remaining three were adults. Whether these cases were due to fresh infections in the year 1958, or were relapses, cannot be stated with certainty. The proportion of the species of parasites prevalent in 1947-48 was approximately, *P. vivax* 12 : *P. falciparum* 22 : *P. malariae* 4. In the year 1958, the proportion was, *P. vivax* one : *P. falciparum* 3. There were no cases of *P. malariae.*

This study has shown that even in parts of Nasik District which, judged by all standards, were definitely highly malarious and in which malaria has been thoroughly brought under control, there are still a few persons positive for malaria-parasites and who would have gone unnoticed in the routine assessment surveys. As similar conditions were prevalent in Thana District in the years 1955, 1956, and 1957, it would not be unreasonable to assume that even in that district there might have been a few cases of malaria persisting in the community but which had gone unnoticed in the routine surveys. The conditions in Mokhada Petha of Thana District (Group *B*), where no malaria outbreak had occurred, were quite similar to those in the Murbad and Shahapur talukas prior to 1957, but were found very different in the year 1958 both as regards parasite prevalence and the prevalence of fevers. But in both these respects, the figures for Mokhada (Group *B*) are slightly higher than that of Dindori (Group *C*).

Accurate quantitative determinations of the parasite persistence certainly require more elaborate studies than we have been able to undertake. But the observation, made in the Group '*C*' villages of Nasik District, have brought an important fact, *viz.*, that even a community which by normal standards is considered to be free from malaria there may be a few lurking carriers. In the event of the densities of vectors being suddenly built-up, either because of suspension of spraying or because of the development of resistance, it may lead to a very rapid recrudescence of malaria.

DISCUSSION.

Macdonald (1957) while dealing with epidemics during and after eradication of malaria, has stated "There is little experience of this form of epidemic but it may well become the most common or almost the only type. Any recrudescence during the eradication programme would be an epidemic, and a main object of workers in such a programme would be to discover the epidemic whilst it was still in, what in other circumstances be called, negligible size". The outbreak which occurred in Thana District is an example of the type of epidemic visualized by Macdonald. The experience of this 'epidemic' has many lessons, not the least important of which is the need for very strict vigilance for the early detection of the new cases, which may arise in such areas, for taking prompt measures to prevent the further development of the epidemics.

One of the chief points of scientific interest of this outbreak is the extreme rapidity with which it reached its peak. Assuming the zero point as the date of the first appearance of the monsoon, i.e., about June 20, the peak affecting about half the entire population was reached in about 100 days, i.e., at the end of September. The period is roughly equal to 5·0 incubation intervals for *P. vivax* and 3·0 incubation intervals for *P. falciparum*. If the zero point is taken as the time when the first fresh cases occurred, i.e., July 20, the peak was reached in 3·5 incubation intervals for *P. vivax* and 2·0 intervals for *P. falciparum*. This could not have occurred without a very high reproduction rate. As both the species participated in the outbreak, it is difficult to assess the exact role of each species. If the outbreak was due to one species only, the reproduction rate of the disease would have been not less than 30 in the case of *P. vivax* and not less than 100 in the case of *P. falciparum*, if the original reservoir of infection was approximately of the order of 0·1 per cent. But neither species, by itself, infected 50 per cent of the entire community and, therefore, the reproduction *rate for each* species was certainly less than these figures. But the cumulative effect of the reproduction rates of the two species was that 50 per cent of the community was infected in about 100 days.

A mathematically minded epidemiologist may attempt to evaluate the course of the outbreak better, bearing in mind that the final proportion of the two species, i.e., *P. vivax* to *P. falciparum* was 74 to 129. But even here, the figures are somewhat vitiated by the fact that a considerable amount of chloroquin had already been distributed in the area by the time the investigations were commenced.

Moreover, there is no precise information regarding the exact status of the reservoir of the infection in the area at the commencement of the season. One can surmise that it would have been somewhere between what was found in the Group '*C*' villages, i.e., 0·18 per cent, and the figure for the group '*B*' villages, i.e., 1·0 per cent. But as Macdonald (1950 *loc. cit.*) has shown, it is not the actual gametocyte reservoir in such cases which is so important as the reproduction rate. With a high reproduction rate, the peak will be reached rapidly whether the reservoir was

low or moderate. Using the formula given by him, it is noticed that if the reproduction rate is 50 and the original reservoir of infection is only 110·001, 55 per cent of the population would be infected in three incubation intervals in the case of *P. vivax*. If the reservoir of infection is 0·01, i.e., 1·0 per cent, the same reproduction rate will infect about 80 per cent of the population with two incubation intervals. The difference between the two initial reservoirs, *for practical purposes*, is very small. Whether the infection rate, to start with is 0·001 or 0·01, the outbreak will be very rapidly built-up and unless there is a very special vigilance in the early stages, the outbreak will assume epidemic proportions before any action can be taken. The fresh infections should be detected and tackled in the first incubation interval itself.

Our studies have now shown that even in areas, such as Group '*C*', where the results of the malaria control work so far done have reportedly been very satisfactory and where there was no indication of any malaria prevalence as judged by the ordinary survey procedures, there is still a measurable reservoir of infection. If the vector control becomes ineffective either because of insecticidal resistance or administrative failures, a serious 'epidemic' can occur within the course of a couple of months. It is, therefore, extremely necessary to take note of this point in the Malaria Eradication Programme and to ensure that the surveillance organisations, which are to be established, work with the utmost efficiency.

SUMMARY.

An outbreak of malaria, involving over 60 per cent of the entire population, occurred in 209 villages in an area of 500 sq. miles (Total population 86,000) in Thana District, Bombay State, India, between July and September, 1958. The area was originally highly endemic, but from 1948 it was under very successful control by the use of DDT residual spray, till 1956, when all malariometric indices were zero or near zero. In 1957, DDT was replaced by dieldrin but there was no indication of any prevalence of malaria during that year also. In 1958, dieldrin was sprayed in May-June, just prior to the onset of the monsoon. But fresh malaria cases started appearing in July and an outbreak soon developed, reaching its peak at the end of September. The outbreak was soon brought under complete control by the use of DDT and distribution of anti-malarials.

The outbreak is mainly attributed to the failure of dieldrin to control building-up of mosquito densities as a result of development of resistance in the vector species, *A. culicifacies*.

A comparative study of three groups of villages, i.e., Group (*A*) within the affected area, Group (*B*) in an unaffected neighbouring area of the same district with the same history of insecticidal treatment, and Group (*C*) in an unaffected area of a neighbouring district in which only DDT had been used, was made to study the incidence of fevers and the status of parasite prevalence by mass examination of blood smears from the population. It was found that in the affected area, the

incidence of fevers was 7·0 and 710·2 per thousand in 1957 and 1958, respectively, indicating the widespread nature of the 'epidemic' in 1958. In the other groups, the incidence of fevers for 1957 and 1958 was :—

Group 'B' 0 and 11·4 and *Group 'C'* 7·6 and 36·1, respectively, indicating that these two areas had no unusual history of fevers.

The mass blood surveys revealed that in the affected areas, 213 (*P. vivax* 74, *P. falciparum* 129), out of 2,294 persons of all age-groups, had malaria parasites. The spleen rates ranged from 7·5 to 92 per cent. In the other two groups, there were no enlarged spleens. The mass blood smears showed that, in Group *'B'*, 21 (*P. vivax* 18, *P. falciparum* 2, mixed 1) were positive out of 2,016 persons and in Group *'C'* 4 (*P. vivax* 1, *P. falciparum* 3) out of 2,767 were positive.

Some theoretical and practical aspects of the outbreak have been discussed and it is shown that the outbreak was an "epidemic", of the type visualised by Macdonald (1956 *loc. cit.*) as likely to occur during or after the eradication of malaria. One of the main features of the epidemic is the very rapid build-up and it is emphasised that even a very low reservoir of infection may lead to a serious epidemic within one season, if vector control is not perfect, either because of insecticide resistance or administrative failures.

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OBITUARY.

PROF. G.G. MER, O.B.E., M.D., D.T.M. & H.

GIDEON GERNIMO MER, who died suddenly on March 22, 1961, was one of Israel's outstanding malariologists and insect toxicologists. He was not only an excellent investigator and inspiring teacher, but also a tireless pioneer and a valiant soldier.

Born in Poswol (Lithuania) in 1894 of a family of physicians, he interrupted his medical studies at Heidelberg, Germany, in 1914 and went, via Palestine, to Alexandria, where he joined the Jewish Legion of the British Army. He served subsequently in the Zion Mule Corps and was wounded in the Gallipoli campaign. After the war, he became, for several years, a member of Kibbutz (collective settlement) Amir in Upper Gallilee, Palestine, and participated in the country's anti-malaria campaigns. In 1924 he continued his studies in medicine at Naples, graduating as M. D. in 1927. Subsequently he specialized in Tropical Medicine in Rome (under Grassi), at the Malaria Research Station of Medemblik, Holland, and also at Paris, receiving there the D. T. M. & H. in 1928. Returning to Palestine, he took up research under the late Prof. I. J. Kligler at the Malaria Research Station of the Hebrew University at Rosk-Pina. This station is located in the mountains of Upper Gallilee and overlooks the Hule, Jordan and Beit Shean Valleys, which were then hyperendemic malaria areas. He was appointed junior assistant in 1929, instructor in 1930, became lecturer in epidemiology in 1934 and received his full professorship in 1938.

His first research period at Rosh-Pina, which extended until he joined the British Army during the Second World War, was devoted mainly to the study of malaria. G. G. MER is best known for his outstanding work on the experimental transmission of quartan malaria to man by *Anopheles elutus*. It was characteristic of MER that he used himself and his wife, the faithful companion of his privations, as the first volunteers for these transmission studies. Not less important were his studies on the female sex organs of anophelines, culminating in the development of his method for the age determination of mosquitoes by measurement of the size of the common oviduct. These investigations became the corner-stone of the subsequent work of Dr. T. S. Detinova and her associates in the U.S.S.R. on age determination of anophelines and various other medically important insects.

Other subjects of MER's scientific activity at that time, which grew out of the necessity to colonize the malaria-stricken areas of the Hule swamps, were anopheline ecology and malaria chemotherapy and prophplaxis. It was MER who advocated and made possible the establishment of the first settlements in this dangerous region many years before the advent of DDT. He did not deem it beyond his dignity to

spend much time, thought and effort on devising various protective measures (mechanical and others) to prevent entry of anophelines into houses and he submitted the settlers in the Hule area to a strict military-like regime, keeping all of them indoors during the night. This pioneering feat was crowned by success.

At the same time, true to his calling, he was always ready to come to the aid of the sick, especially the malaria-stricken, be it in Jewish settlements, Arab villages or Bedouin encampments in Gallilee.

In the Second World War, he joined the Medical Corps of the British Army and advanced to the rank of Lt. Colonel. He combated malaria in the Middle and Far East and rendered particularly outstanding services to the British troops fighting in Burma. For his decisive intervention in this theatre of operations, he was awarded the O. B. E. (military). After the war he returned to his laboratory at Rosh-Pina, remaining there until his death, except for two interludes : During the Israeli War of Liberation (1947-49) he served his country as Head of the Division of Preventive Medicine, Medical Corps, Israel Defence Forces, holding the rank he had in the British Army ; always ready to lend his services and experience to the Israeli Ministry of Health, he consented to act as its Director-General during 1956/57.

Apart from continuing research on the biology of mosquitoes, MER commenced to work, following the end of World War II hostilities, on environmental sanitation and, in particular, the use of insecticides. To many insect toxicologists, MER's name is associated with studies on insect control, insecticide resistance, synergists and attractants. Most of these investigations were again motivated by practical considerations, but others belong to the category of basic research. Being intrigued by the physiological and behaviouristic aspects of resistance, MER also worked on the influences of nutrients, particularly lipids, on insecticide tolerance; it was this study among others, which led ultimately to Wiesmann's "fat barrier" theory of insecticide resistance.

G. G. MER was an impressive personality and outspoken and direct in speech. He was a born leader of men and a great number of Israeli, Indian and British malariologists, public health specialists and insect toxicologists, who worked under him at some time or other, are proud to call themselves his disciples. He was of towering physical structure and one would not have suspected him of possessing such excellent manual dexterity even for the most delicate manipulations. He never failed to impress others with his outstanding intelligence, wisdom, richness of ideas and scientific integrity: his singlemindedness of purpose, where scientific and public health matters were concerned, was conspicuous. MER's scientific and moral standing is reflected by the fact that he was repeatedly appointed member of the Expert Panels of Malaria and Insecticides of the World Health Organisation. Prof. MER was an undefatiguable worker, spending day and night in the laboratory and at his other duties. He died in harness. He is survived by his wife, two married daughters and a son.

K. R. S. ASCHER
Z. H. LEVINSON

INDIAN COUNCIL OF MEDICAL RESEARCH

Colonel Amir Chand Trust Fund Prizes for Medical Research

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

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

It has been decided to award *four prizes in 1961* each of Rs. 300.00 *to graduates of not more than 40 years of age on 1st January, 1961, for the best* research papers in medical sciences published by them during 1960. These prizes will be known as 'Shakuntala Amir Chand Prizes'.

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

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

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

The AWARD of the prizes will be announced at the meetings of the Council's Advisory Committees in November/December, 1961.

CANDIDATES for award of prizes are required to submit, 10 REPRINTS of their papers published during 1960. These should be sent to the DIRECTOR, INDIAN COUNCIL OF MEDICAL RESEARCH, P.O. BOX 494, NEW DELHI, so as to reach him NOT LATER THAN 1st SEPTEMBER, 1961. The PAPERS should be accompanied by a short biographical sketch and two copies of passport size photographs of the candidate/candidates concerned.

INDIAN COUNCIL OF MEDICAL RESEARCH.

Applications are invited for the post of *Assistant Editor* of the *Indian Journal of Medical Research* in the scale of Rs. 900-50-1200 plus admissible dearness and other allowances.

Qualifications :—A degree in medicine recognised under the Indian Medical Council Act (1956) or a degree in allied sciences, such as Biochemistry, Physiology, Microbiology, Pharmacology, etc., with experience in research and medical journalism. Other things being equal, preference will be given to medical graduates. In the beginning, the candidate may be required to work at Kasauli for a few months.

Age below 50 years, relaxable in the case of otherwise suitable candidates.

The selected candidate will be appointed on five years' contract, renewable thereafter. Probationary period is one year. Benefits of Provident Fund admissible. Private practice or compensation in lieu thereof will not be allowed. Candidates called for interview will be granted one return Second Class rail fare. Only concessional rail fare, if available, will be allowed. No travelling allowance admissible for joining appointment or on termination of appointment.

Application on the prescribed form, obtainable from the Director, Indian Council of Medical Research, Medical Enclave, Post Box No. 494, New Delhi, should be sent to him accompanied by a crossed postal order for Rs. 7.50nP. (Rs. 1.87nP. only for Scheduled Castes/Tribes and other backward classes) made out in the name of the Indian Council of Medical Research, New Delhi. Applications without postal orders will not be considered. The last date for receipt of applications is *31st July, 1961.*

Statement about ownership and other particulars about newspaper (*Indian Journal of Malariology*) to be published in the first issue every year after the last day of February.

FORM IV.

(*See Rule 8*)

1.	Place of publication	Delhi.
2.	Periodicity of its publication	Quarterly.
3.	Printer's name	Shri B.R. Sardana.
	Nationality	Indian.
	Address	Cambridge Printing Works, Kashmere Gate, Delhi-6.
4.	Publisher's name	Shri T.D. Joshi.
	Nationality	Indian.
	Address	Assistant Director, Indian Council of Medical Research, P.O. Box No. 494, New Delhi.
5.	Editor's name	S. P. Ramakrishnan, M.B.B.S., D.P.H., D.Sc. (P.H.).
	Nationality	Indian.
	Address	Director, Malaria Institute of India, Delhi.
6.	Names and addresses of individuals who own the newspaper and partners or shareholders holding more than one per cent of the total capital.	Indian Council of Medical Research, Post Box No. 494, New Delhi.

I, T.D. Joshi, hereby declare that the particulars given above are true to the best of my knowledge and belief.

Dated 27.2.1961.

(Sd.) T.D. Joshi
Assistant Director,
Indian Council of Medical Research.

Indian Journal of Malariology, **15**, *2, June 1961.*

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

Part XXIX.

THE SIZE OF PARASITE POPULATION AND ITS RELATION TO THE SELECTION OF A STRAIN RESISTANT TO SULPHADIAZINE.

BY

S.P. RAMAKRISHNAN,

SATYA PRAKASH,

D.S. CHOWDHURY

AND

P.C. BASU.

(Malaria Institute of India, Delhi.)

[July 14, 1960.]

Plasmodium berghei has lent itself to the selection of resistant strains to various drugs, like sulphadiazine (Krishnaswami, Satya Prakash *et al.*, 1954) and chloroquine (Ramakrishnan, Satya Prakash *et al.*, 1957), etc. As a corollary to sulphadiazine resistant, a "milk" resistant strain was selected (Ramakrishnan *et al.*, 1956). In all these investigations, the procedure was orthodox inasmuch as the exposure to selecting agent was gradual, commencing with sub-minimal doses. Such a procedure was based on the impression that resistance was a gradual adaptation of the parasites to the drug.

In most instances of experimental selection of resistance to anti-malarials, the resistant strain of parasites was found to be stable in many cases even in the absence of selection pressure. Further, the resistance was retained for long periods even after successive mosquito passages (Bishop and Birckett, 1947; Adams and Seaton, 1949; Hawking and Perry, 1948; Hawking and Thurston, 1951; Schmidt, Genther *et al.*, 1949; Seaton and Adams, 1949; Seaton and Lourie, 1949; and Williamson, Bertram and Lourie, 1947).

The fact that experimental drug resistance of parasites was stable even after mosquito passage, lent itself to the presumption that the resistant characteristic should be exhibited by the pre-erythrocytic forms arising from sporozoites derived from a resistant strain. Experimental evidence to confirm this presumption was obtained in studies on *P. gallinaceum* (Jaswant Singh, Ramakrishnan *et al.*, 1952).

Covell, Coatney *et al.* (1955) considered the mechanism of drug resistance in malaria. The observed phenomena of drug resistance suggested a genetic origin.

The character was carried throughout the life cycle from generation to generation in vertebrate hosts. They considered that spontaneous mutation, induced mutation, or clonal variation could equally explain the drug resistance satisfactorily.

The volume of experimental work on the mode of action of anti-malarials has shown that, in general, it is related to the metabolism of the parasite cell. The action in human malaria is by disturbance of the synthesis or substitution of some essential metabolite. Conceivably, therefore, the case and speed of selection of resistance to a given drug are related to (1) the diverse metabolite pathways of the species of the parasite; (2) their variations within members of the species being true genetic characters; and (3) their susceptibility of interference by the drug. Such a presumption can explain drug resistance of malaria parasites without the invocation of spontaneous or induced mutation. Such is the theory of Yudkin (1953) which assumes that cell division need not necessarily distribute the systems responsible for resistance equally in daughter cells. Repeated divisions can produce a clone of cells with the average resistance of the parent cell, but with a distribution of greater or less around that of the parent cell. It, therefore, seemed that members of a species, inherently resistant to a drug, are more likely to be present in a large parasite population than in smaller ones. The work reported here, was planned primarily to investigate this point. It also seemed that if members with inherent resistance were extant in a large population, the orthodox method of commencing drug treatment, in sub-minimal doses with gradual increase, should not be necessary for the selection. Therefore, different combinations of the size of the parasite population and dosage schedules were studied. The work was actually commenced and completed in 1957. The paper could not be written up earlier due to the preoccupations of the authors.

MATERIAL AND METHODS.

Host.—Adult mice were obtained from the colony maintained at the Malaria Institute of India, Delhi. No choice was exercised with regard to the sex of the animals.

Parasite.—The parent strain of the parasite has been maintained by rat to rat passage since 1952 and had undergone 325 passages when taken over in mice for the present studies.

Inoculation.—The route of inoculation was always intraperitoneal and the standard inoculum was 5×10^6 parasites per animal.

Drug.—Injectable sulphadiazine solution (May and Baker), containing one gramme in 4 c.c., was diluted with distilled water to get the required concentration and administered intraperitoneally.

Normal course of infection in mice.—Infections of *Plasmodium berghei* in albino mice are invariably fatal. The course of infection was studied in six mice inoculated with the parent normal strain. The infection was found to be fatal during a course of 14 to 21 days (Table I).

TABLE I.

Details showing course of untreated infection in animals inoculated with the parent strain of Plasmodium berghei *(inoculum—5 million parasites per animal).*

Mouse Number.	Number of parasites per 10,000 RBC on days following inoculation.																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
M78	2	28	354	256	204	336	906	1,732	1,146	1,512	2,112	1,976	4,578	*D	..	..	..	..	.	..	..
M79	†N	N	N	N	12	158	716	408	322	728	1,266	1,510	2,116	4,872	6,454	5,788	7,342	8,754	7,950	D	..
M80	N	N	6	16	104	714	1,320	916	1,834	2,006	2,054	2,502	5,948	7,486	9,368	7,426	11,006	9,874	D	..	..
M81	N	P	34	54	316	378	314	632	778	656	932	1,128	944	1,540	1,934	1,672	1,982	1,538	2,734	3,548	D
M82	N	‡P	26	76	648	402	726	1,306	1,968	1,846	2,136	2,418	3,176	2,692	4,546	5,232	7,196	4,872	D	..	..
M83	P	32	240	264	254	328	1,194	1,766	954	1,372	1,852	3,004	5,218	4,254	4,916	8,478	12,034	D	..	..	..

*D=Died.
†N=No parasite seen in 100 oil immersion fields.
‡P=1 parasite in 100 oil immersion fields.

TABLE II.

Details showing course of infection in mice inoculated with the parent strain of Plasmodium berghei *and treated with* 0·5 mg./20 g. *sulphadiazine intraperitoneally.*

Mouse Number.	Number of parasites per 10,000 RBC's from the first day of drugging.									
	1	2	3	4	5	6	7	8	9	10
M659	192	176	56	96	272	248	456	352	*S	.
M660	232	58	†N	N	N	4	208	S	..	..
M662	784	144	N	N	N	‡P	6	S	..	..
M701	296	160	16	N	N	N	368	680	880	1,080
M703	244	120	2	N	N	N	68	560	896	1,080
M705	320	38	N	N	N	N	N	N	32	280
M957	208	204	6	P	N	N	N	218	S	..
M958	260	212	N	N	N	56	648	S	..	..
M959	292	128	26	80	642	616	812	878	S	..
M960	208	120	6	N	N	P	96	S	..	..
M961	298	42	44	384	718	256	168	280	S	..
M962	288	132	8	N	N	N	P	P	64	S

*S=Sacrificed.
†N=No parasite seen in 100 oil immersion fields.
‡P=1 parasite in 100 oil immersion fields.

Sensibility to sulphadiazine.—The minimum effective single dose of sulphadiazine, administered intraperitoneally to produce a Class II* effect, was determined to be 0·5 mg./20 g. according to the technique described by Ramakrishnan, Krishnaswami *et al.* (1951). The course of parasitaemia in 12 mice inoculated with the normal strain of *Plasmodium berghei* and treated with 0·5 mg./20 g. of sulphadiazine, administered intraperitoneally, is given in Table II. It was observed that out of 12 animals, 8 showed a clear Class II effect.

Selection of resistant strains.—Three groups of mice were inoculated from the same donor with the standard dose of 5×10^6 parasites. The experimental procedure was different for each of the groups and constituted the basis for selection of 3 strains. The first strain, low density low dose (LL Strain), was selected by exposing the parasites in low density to low doses of drug administered once daily through the intraperitoneal route as described by Jaswant Singh, Ramakrishnan *et al.* (1952). At the commencement of the selection of the resistant strain, the parasites inoculated in the mice were exposed to a dose of 0·01 mg./20 g. intraperitoneally (1/50th of the single dose required to produce Class II effect). The drug was administered when the density of parasitaemia was in the region of 100 parasites per 10,000 erythrocytes. The dose of the drug was gradually increased and was so adjusted as not to clear the peripheral blood totally of the parasites and sub-inoculated the strain before such a clearance occurred. After completing the passage from one animal (donor) to the other, the donor was given an increasing dose of the drug to ensure that the parasite could still tolerate that dose. The drug was further increased in the next passage.

In the selection of the second strain, high density low dose (HL Strain), the drug was administered when the density of parasitaemia was in the region of one thousand parasites per 10,000 erythrocytes. The gradually increasing dosages of the drug were similar to those used in the selection of LL Strain.

The third strain, high density high dose (HH Strain), was isolated by exposing the parasites inoculated in the mice to 10 mg./20 g. of the drug intraperitoneally when the density of the parasites was one thousand per 10,000 erythrocytes. The above procedure was repeated in almost all the 27 passages, except in a few where the drug could not be administered, and in one animal administered twice in the same passage as there was an apprehension that the strain might be lost.

Tables III, IV and V show the serial passages and the dosage schedules, adopted during the isolation of low density-low dose (LL), high density-low dose (HL) and high density-high dose (HH) strains, respectively.

Evaluation of the degree of resistance.—It was decided to evaluate the degree of resistance at the stage when all the strains were being exposed to 10 mg./20 g. Groups of three mice were inoculated with 5×10^6 parasites, each from an experimental animal carrying one of the three strains. Sulphadiazine (15 mg./ per 20 g.)

* Class II effect is defined as clearance of the peripheral blood of parasite for a minimum of three consecutive days following the treatment.

TABLE III.

Protocols of the LL strain.

Serial number of passage.	Date of passage. (1957)		Details of drug administration :	
			Dose in mg. per 20 g. I.P.	Number of courses.
0	May	14	0·01	3
1		28	0·01	2
2	June	4	0·01	2
		,,	0·02	1
3		12	0·02	3
4		19	0·03	2
5		28	0·03	1
6		29	0·03	1
7	July	3	0·03	2
8		9	0·1	1
9		12	0·1	1
10		16	0·2	1
11		20	0·8	1
12		24	1·5	1
13		28	1·5	1
14		31	1·5	1
15	August	3	1·5	1
16		6	1·5	1
17		9	1·5	1
18		13	2·5	1
19		17	3	1
20		21	3	1
21		26	4·5	1
22		31	4·5	1
23	September	3	4·5	1
24		7	6	1
25		10	6	1
26		14	7·5	1
27		18	7·5	1
28		24	7·5	1
29		29	7·5	1
30	October	4	7·5	1
31		8	7·5	1
32		12	7·5	1
33		16	7·5	1
34		20	7·5	1
*35		25	7·5	1
36		29	10	1
37	November	1	10	1
38		5	10	1
*39		9	10	1
40		13	10	1
41		18	10	1
*42		23	10	1
43		28	10	1
44	December	2	10	1
45		7	10	1
46		11	10	1
47		15	10	1
*48		19	10	1

* Degree of resistance evaluated.

TABLE IV.

Protocols of the HL strain.

Serial number of passage.	Date of passage. (1957)		Details of drug administration :	
			Dose in mg. per 20 g. I.P.	Number of courses.
0	May	14	0·01	2
1		25	0·01	4
2	June	8	0·01	1
3		15	0·02	3
4		25	0·02	1
5		29	0·03	1
6	July	3	0·1	1
7		8	0·1	1
8		13	0·4	1
9		17	0·5	1
10		23	1·5	1
11		28	2·5	1
12	August	7	2·5	1
13		17	3	1
14		27	4·5	1
15	September	3	6	1
16		10	6	1
17		17	7·5	1
18		24	7·5	1
19		30	7·5	1
20	October	5	7·5	1
21		11	7·5	1
22		16	7·5	1
23		22	7·5	1
*24		29	10	1
*25	November	5	10	1
26		13	10	1
27		19	10	1
*28		25	10	1
29		30	10	1
30	December	5	10	1
31		10	10	1
*32		19	..	..

* Degree of resistance evaluated.

was administered intraperitoneally to each of the inoculated mice when the density of the parasites was in the region of 200 per 10,000 erythrocytes. Four replicates of the same experiment were carried out.

It is evident from Table VI, VII and VIII that the infections in animals, inoculated from the LL and HH strains, were resistant to 15 mg./20 g. of the drug. The infection in animals inoculated from HL strain was, however, susceptible to the above dose of the drug.

TABLE V.
Protocols of the HH strains.

Serial number of passage.	Date of passage. (1957)		Details of drug administration	
			Dose in mg. per 20 g. I.P.	Number of courses.
0	June	3	10 mg.	1
1		16	10 ,,	1
2		25	10 ,,	1
3		29	10 ,,	1
4	July	2	10 ,,	1
5		9	10 ,,	1
6		14	..	..
7		20	10 mg.	1
8		25	10 ,,	1
9		30	10 ,,	1
10	August	7	10 ,,	1
11		13	10 ,,	1
12		20	10 ,,	1
13		29	10 ,,	2
14	September	26	10 ,,	1
15		30	10 ,,	1
16	October	8	10 ,,	1
17		15	..	..
18		22	10 mg.	1
*19		29	..	..
20	November	9	10 mg.	1
21		14	10 ,,	1
22		20	10 ,,	1
*23		25	10 ,,	1
24		30	10 ,,	1
25	December	6	10 ,,	1
26		11	10 ,,	1
*27		17	10 ,,	1

*Degree of resistance evaluated.

RESULTS.

It would be observed that resistance in the HH strain manifested after 19 passages, during which period the parasites were exposed to 18 doses of the drug; whereas HL strain did not attain the same degree of resistance although passaged 32 times, during which period the parasites were exposed to 37 doses of the drug, of course, at a lower rising concentration.

The LL strain was observed to acquire the same degree of resistance as the HH strain in the course of 35 passages, during which period the parasites were exposed to 45 doses of the drug in the same rising concentration as for the HL strain. Whether selection had occurred earlier than the 19th passage in strain HH, we are not in a position to state as no evaluation was done at that stage.

Thus, it appeared that the number of passages and duration of drug exposure required for selection of resistant strain were small with a high parasite population and higher concentration of drugs. Under such circumstances the selection was rapid. Correspondingly, if the exposure to drug was small, even in the presence of a high parasite population, the selection of resistant strain was slow.

TABLE VI.

Details showing course of infection in animals inoculated with the experimental LL strain treated with 15 mg./20 g. sulphadiazine intraperitoneally (inoculum—5 million parasite per animal).

Mouse Number.	Passage Number.	Parasites per 10,000 RBCs from the first day of drugging.														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MIII4	36	368	384	10	*P	†N	12	88	328	1,336	>2,000	..	..	..	..	..
MIII5	do	216	118	P	P	N	4	10	216	1,148	820	548	708	>2,000	..	..
MIII6	do	228	248	184	42	12D‡	..	..	..	..	..	..	..	..	..	..
MII79	40	208	680	1,080	D	..	..	..	..	..	..	..	..	.	..	..
MII80	do	232	520	880	D	..	..	..	..	..	..	..	..	..	..	..
MII81	do	296	D	..	..	..	..	..	..	..	..	..	..	..	..	..
MII82	do	252	D	..	..	..	..	..	..	..	..	..	..	..	..	..
MI244	43	240	680	380	128	460	500	880	908	1,080	1,100	1,120	D	..	..	..
MI245	do	260	560	468	112	408	560	688	996	1,200	1,480	1,660	>2,000	..	..	..
MI246	do	366	580	308	196	328	408	560	880	600	808	1,860	>2,000	..	..	..
MI247	do	348	560	360	>2,000	128	96	108	60	8	6	4	88	260	628	1,020
MI248	do	268	528	396	280	380	568	800	680	608	D	..	..	..	..	..
MI352	49	280	D	..		..	..	.	..	.	..	..	..	..	..	..
MI353	do	296	680	640	680	696	408	396	808	1,080	>2,000	..	..	..	..	..
MI353	do	308	720	508	560	580	D	..	..	..	..	..	..	..	..	..
MI355	do	280	420	608	800	1,896	>2,000	..	..	..	..	..	..	..	..	..

*P = One parasite seen in 100 oil immersion fields.
†N = No parasite seen in 100 oil immersion fields.
‡D = Died.

TABLE VII.

Details showing course of infection in animals inoculated with the experimental HL strain treated with 15 mg./20 g. sulphadiazine intraperitoneally (inoculum—5 million parasites per animal).

Mouse Number.	Passage Number.	Parasites per 10,000 RBCs from the first day of drugging.															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
MIII7	25	194	1,032	212	16	*P	†N	N	N	N	4	52	384	1,032	>2,000	..	..
MIII8	do	598	1,288	1,418	256	10	N	N	N	N	N	N	2	32	152	384	>2,000
M1119	do	200	1,778	444	36	..	N	N	N	N	N	N	N	N	40	376	1,316
M1183	do	290	‡D	..	..	..	..	..	..	..	..	..	..	..	..	..	..
M1184	do	300	600	1,080	1,100	128	1,400	1,360	1,408	D	..	..	..	..	..	..	..
M1185	do	208	D	..	..	..	..	..	..	..	..	..	..	..	..	..	..
M1186	do	208	D	..	..	..	..	..	..	..	..	..	..	..	..	..	..
M1249	29	290	328	260	P	N	N	N	32	50	80	190	360	380	1,100	D	..
M1250	do	328	490	D	..	..	..	..	..	..	..	..	..		..	..	..
M1251	do	296	480	88		N	N	N	N	N	P	18	96	108	208	928	1,960
M1252	do	288	520	68	P	N	N	N	N	N	P	12	80	180	292	808	1,808
M1253	do	380	508	48	N	N	N	N	N	8	20	60	180	360	988	>2,000	..
M1362	33	284	328	D	..	..	..	..	..	..	..	..	..	..	..	..	..
M1363	do	360	D	..	..	..	..		..	..	..	..	..	..	..	..	..
M1364	do	266	528	108	p	N	N	N	N	16	280	960	1,808	..	..	..	..
M1365	do	372	680	82	N	N	N	N	N	N	P	128	1,280	..	..	..	..
M1366	do	296	D	..	..	..	..	..	..	..	..	..	..	..	..	..	..

*P = One parasite seen in 100 oil immersion fields.

†N = No parasite seen in 100 oil immersion fields.

‡D = Died.

TABLE VIII.

Details showing course of infection in animals inoculated with the experimental HH strain treated with 15 mg./20 g. sulphadiazine intraperitoneally (inoculum—5 million parasites per animal).

Mouse number	Passage number.	Parasites per 10,000 RBCs from the first day of drugging.														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
M1120	20	248	672	608	248	*D	..	..	..	..	..	..	..	..	..	..
M1121	20	272	678	324	12	†N	N	‡P	12	144	432	1,264	>2,000	..	..	..
M1122	20	416	584	628	220	132	3	6	8	42	192	878	>2,000	..	..	..
M1175	20	220	480	320	120	90	60	48	40	28	16	8	D	..	..	..
M1176	20	180	600	408	108	D	..	..	..	..	..	..	..	..	..	..
M1177	20	196	680	D	..	..	..	..	..	..	..	..	..	..	..	..
M1178	20	208	D	..	..	..	..	..	..	..	..	..	..	..	..	..
M1254	24	260	680	460	268	108	96	28	10	12	208	320	1,200	>2,000	..	..
M1255	24	248	560	D	..	..	..	..	..	..	..	..	..	..	..	..
M1256	24	232	608	328	200	120	88	12	16	8	10	8	380	1,200	1,960	>2,000
M1257	24	392	648	308	338	96	64	8	4	9	12	28	408	1,700	>2,000	..
M1258	24	296	528	D	..	..	..	..	..	..	..	..	..	..	..	..
M1357	28	296	1,080	960	720	800	460	88	52	84	580	1,890	>2,000	..	..	..
M1358	28	308	D	..	..	..	..	..	..	..	..	..	..	..	..	..
M1359	28	208	996	808	608	400	208	60	32	28	660	1,840	>2,000	..	..	..
M1360	28	240	1,280	796	500	360	160	52	D	..	..	..	..	..	..	..
M1361	28	260	1,160	420	380	190	48	36	120	880	1,960	>2,000	..	..	..	..

*D = Died.
†N = No parasite seen in 100 oil immersion fields.
‡P = One parasite seen in 100 oil immersion fields.

The selection of LL strain followed the usual happenings as in previous similar experiments by others and the present authors (Ramakrishnan, Satya Prakash *et al.*, 1956, 1957 ; Krishnaswami, Satya Prakash *et al.*, 1954).

DISCUSSION.

Mice were specifically chosen for these studies, as their innate immunity, if any, to *P. berghei* infections is insignificant. Nor have they the capacity to acquire immunity to untreated infections. Therefore, the drug (sulphadiazine) exerted the only significant selection pressure.

A limitation to the study in retrospect, however, was that the evaluation of the degree of resistance was not made at regular intervals during the course of the investigations. If they had been carried out, the speed of selection of resistance in the three strains could have been accurately determined. Nevertheless, the available data indicated that the size of the parasite population was a factor in the speed of selection for resistance to sulphadiazine.

Of the twelve mice treated with a single dose of 0·5 mg./20 g. (Table II), nine became free from parasites in the peripheral blood for 3 to 6 days. The infection in the remaining three, however, persisted and the peripheral blood was not negative even for a single day. All the mice were not inoculated from the same donor. Under such circumstances, the variation indicated that a larger proportion of the inherently resistant parasites were distributed in the inocula used for the three animals. Another possibility was that the three animals differed in some way from the others in their own response to the drug. The former seemed more probable.

Protocols of the LL strain (Table III) and HL strain (Table IV) revealed that the selection of the resistant strain was gradual in both, but was slower in the former than the latter. A possible explanation was that a given parasite population consisted of individuals of varying degrees of susceptibility to the drug and their distribution possibly conformed to a normal curve. When the population was small as in the LL strain and the dose of the drug was small, the relative proportion of the survivors to the sub-minimal exposure was large. Whereas, when the population was large as in the HL strain and the dose of drug the same as in the case of the LL strain, the proportion of survivors was small.

In the protocols of the HH strain where the population as well as the dose of drug was large, susceptibles to different dosages, except to the highest, were wiped off and the survivors multiplied successively. This seemed possible on the basis of the hypothesis of Yudkin (1953) mentioned earlier.

Bishop's (1958) approach to the problem was different. The experimental strain arose from a single parasite, and was maintained as two separate clones in the two groups of experimental birds. In order to eliminate the variabilities, caused by inherent as well as acquired immunity of the host, the exposure to drug was commenced soon after inoculation. She concluded that the number of courses of treatment to produce an enhancement of resistance, was not always related to the

size of the inoculum. With the largest inoculum, however, an enhancement of resistance was observed after the minimum number (3) of the courses of treatment, whereas, with the smallest inoculum no enhancement of resistance was obtained.

In our series the speed of selection is related to both the size of population as well as the drug dose. The speed with which resistance manifested itself in the presence of a large parasite population in an environment of high sulphadiazine concentration, tends to show that individuals naturally resistant to sulphadiazine, exist in greater frequency amongst a large population. Probably, such individuals have a metabolic pathway other than the para-aminobenzoic acid enzyme system. If this contention is correct we do not have to invoke any mutagenic action in the selection of resistant strain, the only process involved being the elimination of susceptible individuals by high concentration of drug.

SUMMARY.

1. The speed of selection of a resistant strain of *P. berghei* is quicker when parasites in high density are exposed to large doses of sulphadiazine.

2. Such selection is much retarded when parasites in high density are exposed to small doses of sulphadiazine. The retardation is more marked than when parasites in low density are exposed to low doses of sulphadiazine.

3. It is suggested that in a large parasite population, greater number of individuals occur with natural resistance to sulphadiazine.

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Part XXX.

EFFECTS OF SPLENECTOMY ON THE COURSE OF BLOOD-INDUCED INFECTION IN RATS.†

BY

SATYA PRAKASH.

(Malaria Institute of India, Delhi.)

(July 13, 1960.)

SPLENECTOMY has long been a routine procedure in experimental malariology, Krishnan and Ghosh (1935) and Mulligan, Somerville and Swaminath (1940) studied the effect of splenectomy on monkey malaria. In respect of malaria caused by *P. berghei*, Rodhian (1949) observed that splenectomy of cotton rats with latent infection, resulted in a parasitic relapse which was not intense and did not end fatally. On the contrary, he found that if previously splenectomised cotton rats were inoculated, the resultant infection was very intense and similar to that in albino mice. Galliard and Lapierre (1950) splenectomised albino rats with latent infection and found that the post-splenectomy course of parasitaemia was as severe as that of the primary infection, and became latent once again in 23 to 25 days after splenectomy.

This paper reports prolonged observations on the course of infection in albino rats, splenectomised before and after inoculation. Certain changes were also observed in the morphology of parasites in the splenectomised animals.

METHODS AND MATERIAL.

Adult albino rats, the strain of parasites and the techniques of inoculation were the same as already described by Ramakrishnan *et al.* (1951).

Parasites were enumerated against 10,000 erythrocytes in stained thin films. All inoculations were made by the intraperitoneal route and the dose of infection was 80,000 parasitised erythrocytes in some, and 1 million in other inoculations. Splenectomy was done under Nembutal anaesthesia and no sulpha-drug was used in dressing the wound.

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*The strain of *P. berghei*, used in the experiment, is being maintained by blood passage in albino rats at the Malaria Institute of India, Delhi, since 1952. It was originally obtained from London through the courtesy of Brig. J.S.K. Boyd of the Burroughs Wellcome Laboratories.

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

HAEMATOLOGY.

The haematology of normal and infected rats (with their spleens intact) has already been reported by Ramakrishnan *et al.* (1953). Table I records the haematology of splenectomised uninfected and infected rats.

It is seen from Table I that splenectomy of normal animals lowered the total erythrocyte and leucocyte counts. The polychromatophilic cell (reticulocyte) counts were not appreciably altered. It indicated that in the uninfected splenectomised animals there was no increased erythropoisis. The increase in leucocytes in the splenectomised animals appeared to be due to an increase in polymorphonuclear leucocytes.

A comparison of the data of animals during parasitic relapse showed that while the total red cell counts were the same in both splenectomised as well as those whose spleens were intact, there was a considerable increase of the reticulocytes in the former than in the latter. Apparently, the haemopoitic centres responded better in the splenectomised animals than in intact animals.

The total leucocyte count was found to be highest in the animals which showed parasitic relapse due to splenectomy. These latter animals also showed high neutrophil count. In the uninfected splenectomised animals, however, it was still higher.

COURSE OF INFECTION.

The course of infection was observed in three sets of animals, *viz.*, intact, splenectomised and inoculated, and the animals which were splenectomised during latency of infection. The data are shown in Table II.

Death occurred between 53 and 340 days. Except one (R578), all had patent parasitaemia till death. In a few cases the blood was negative on some days.

The observations were restricted to seventeen days. It is seen from Table II that the average daily parasitaemia was of the highest order in the intact animals. The average daily parasitaemia in animals splenectomised during latency of the infection, was higher during the first 5 days than that of intact animals. It was lower during the subsequent 10 days after which there was a tendency to increase. In the animals which were inoculated after splenectomy, the parasitaemia was lower than in intact animals throughout the period of observation, except towards the end when there was a tendency to increase.

The data presented in Table II pertain to the 14 intact animals, chosen at random from the large series of animals used for routine strain maintenance during the same period of the current investigation. The daily parasitaemia of individual animals was not appreciably different from the average, presented in Table II. Of the 14 animals, 12 died after acute infection on 6th to 13th day of infection. Two survived the primary parasitaemia and the infection in them became latent.

TABLE I.

Effect of splenectomy on the haematology of rats.

	Number of observations.	Erythrocytes in millions per c.mm.	Polychromatophils per 10,000 erythrocytes.	Parasitised cells per 10,000 erythrocytes.	Leucocytes per c.mm.	Leucocyte count per cent.				
						Neutrophils.	Lymphocytes.	Monocytes.	Eosinophils.	Basophils.
Normal splenectomised	18	5·91	214	Nil	11,530	60·2	31·4	6·7	2·7	..
Normal intact	60	6·40	262	Nil	12,055	48·0	41·0	9·0	2·0	..
Splenectomised latent	11	5·3	1,039	34	13,788	50·0	45·0	4·0	1·0	..
Relapse intact	6	5·53	163	5·3	9,297	42·0	45·0	10·0	2·0	1
Acute intact	52	4·58	691	331·0	13,281	49·0	39·0	9·0	2·0	1

TABLE II.

Average daily parasitaemia per 10,000 erythrocytes in intact and splenectomised rats.

	Number of animals.	Average daily parasitaemia from the first day of patency.															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Intact animals	14	2	14	130	308	457	812 D1	868 D3	1,131 D4	2,626 D1	776	1,090 D1	931 D1	804 D1	550	337	4	1
Splenectomy during latency	10*	5	63	348	689	628	362	184	27	26	47	129	171	80	26	41	83	100
Infected after splenectomy	5	0·8	37	89	102	214	309	182 D1	228	193	296	239	241 D1	150	150 D1	465	150	250 D1

D=Death figure, i.e., Number of animals died.
*Death occurred between 53 and 340 days. Except one (R-578), all had patent parasitaemia till death. In a few cases there were a few days on which blood was negative.

There was considerable individual variation of the daily parasitaemia of the different animals which were splenectomised during latency of the infection. Only one animal (R-575) died on the 76th day after splenectomy. The blood of the animal did not show patent parasitaemia during the last 26 days of its life. All the others showed continuous patent parasitaemia, except for occasional short negative intervals, and lived for different periods ranging from 53 to 340 days from the first day of patent parasitaemia following splenectomy. In two animals the daily parasitaemia reached 2,464 and 3,600 counts per 10,000 erythrocytes which were higher than usual counts in normal animals. But such high counts were seen only for one or two days and were soon reduced. But the total parasitaemia load in these animals, splenectomised during latency of infection, was much higher than the primary parasitaemia, because of the longer duration of the former as compared to the latter.

The parasitaemia in four, out of 5 animals that were first splenectomised, ran a course similar to that in intact animals and all the four animals died of the infection between the 7th to the 17th day of infection. The fifth animal continued to live up to the 37th day with patent parasitaemia on all days and was sacrificed. In not one of the animals was the daily parasitaemia higher than that of any of the intact animals. It appeared, therefore, that the innate natural immunity of albino rats was not in any way diminished by removal of spleen prior to infection. Indeed in the case of one splenectomised animal the total parasitic load that the animal tolerated during its life of 32 days of patent parasitaemia, was much higher than any of the intact controls. At the same time it was evident that in the small series of animals splenectomised prior to the infection, the capacity to acquire immunity was interfered with. The single animal that lived as long as 37 days after the inoculation, was not able to overcome its primary parasitaemia.

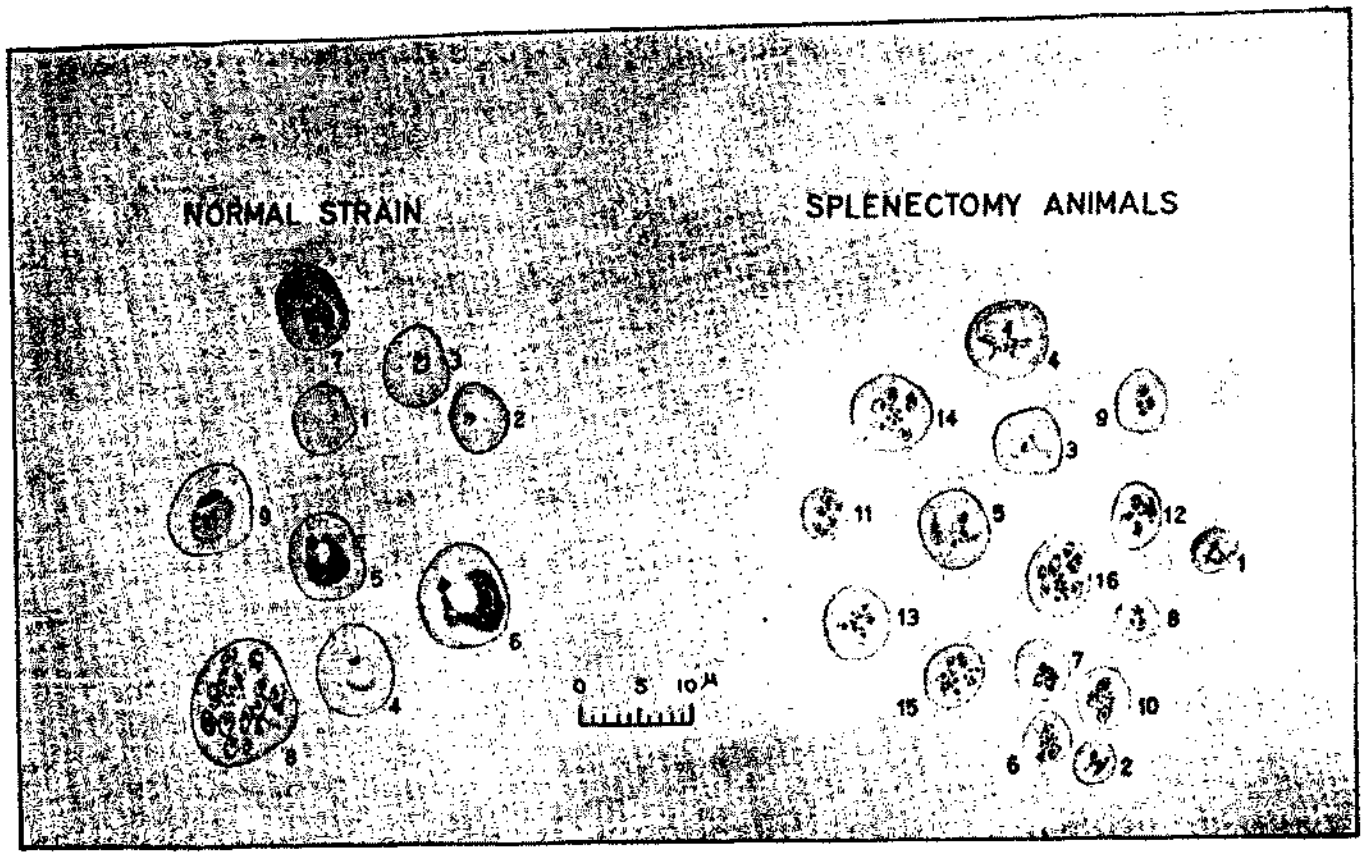
CHANGES IN THE MORPHOLOGY OF PARASITES.

It was observed that parasites in the animals that were splenectomised during latency of infection, appeared different in certain respects from those in intact animals. In general the younger parasites seemed to have a greater degree of amoebiodicity (Plate I) and assumed highly irregular shapes. The pigment seemed to be more abundant in parasites of splenectomised animals. The mature schizonts were smaller in size and occupied less of the erythrocyte than parasites in intact animals. The average number of merozoites per schizont was less than that of parasites in intact animals.

Table III summarises the data with reference to the average number of merozoites per mature schizont.

Ramakrishnan and Satya Prakash (1950) showed that normally the number of merozoites varies from 10 to 22, usually about 12, arranged in the form of a rosette and less frequently scattered irregularly. From Table III it will be seen that 77 per cent of the mature schizonts, observed in splenectomised animals, had only between 4 and 6 merozoites each.

PLATE 3
Parasites.



Normal strain. 1. Normal R.B.C. 2. Early ring. 3. Mature ring. 4. Early trophozoite. 5 and 6. Mature trophozoite. 7. Early schizont. 8. Mature schizont. 9. Early gametocyte.

Splenectomy strain. 1, 2, and 3. Rings. 4, 5, and 7 Trophozoite (Pigment seen). 6, 8 and 9. Schizonts (Two merozoits). 10, 11, 12 and 13. Schizonts with three to four merozoites. 14, 15 and 16. Five to six merozoites.

TABLE III.

Number of merozoites per mature schizont in splenectomised rats.

Number of mature schizonts examined.	NUMBER OF MATURE SCHIZONTS WITH MEROZOITE NUMBERS.				
	Two	Four.	Six.	Eight.	More than eight.
28	0	13	9	5	1
12	1	4	6	1	0
84	1	29	42	8	4
10	0	3	4	2	1
23	0	10	9	3	1
70	9	32	24	5	0
95	0	39	41	13	2
70	4	85	14	17	0
77	9	27	25	12	4
Total 469	24	192	174	66	13

Ramakrishnan *et al.* (1951) showed that adult albino rats compared to mice, possess a greater degree of natural resistance to *P. berghei* infection or, in other words, are less susceptible than mice. Present work shows that the course of infection in adult splenectomised rats is not appreciably intensified when infected after the removal of the spleen. Four out of five such animals died between the 7th to 17th day after experimental inoculation. The fifth animal, however, survived with parasites continuously patent in its peripheral blood for 37 days when it was sacrificed. Taking the four animals that died of infection, it appeared that splenectomy, prior to infection, did not in any way alter the natural innate immunity of rats. It was the capacity to acquire immunity after infection that was interfered with.

The above inference is in confirmation of a similar conclusion arrived at by Mulligan, Somerville and Swaminath (1940) from their experiments in *P. cynomolgi* infection in *sinicus* monkeys. They observed that splenectomy of *sinicus* monkeys, prior to experimental inoculation with *P. cynomolgi*, did not appreciably decrease the high degree of natural resistance of intact monkeys to the infection. It was, therefore, argued that the natural resistance in this case was dependent *more* upon inherent unsuitability of the body tissues or fluids for the development of the infection. They inferred that "the greater the degree of natural resistance to malarial infection possessed by an intact animal the more it is dependent upon *non*-specific agencies, which operate independently of the lymphoid macrophage system." The present findings in rodent malaria are in agreement with this inference. In this connection it may be recalled that Satya Prakash *et al.* (1952) reported that the bandicoot, a close relative of the rat, is not susceptible to *P. berghei* infection. The natural innate resistance of the bandicoot was found by them not to decrease, even after removal of the spleen from the animal.

It has been customary to use the term "non-specific agencies" in the past with reference to immunity, particularly of the innate, natural variety, when the factor

or factors concerned were known to be independent of the humoral or cellular mechanisms. It is relevant to note that there is considerable scope for the spectrum of "non-specific agencies" to be narrowed, as more and more of them become known to be specific. An example is the finding of Hawking (1954) that suckling baby rats, which exhibited an innate immunity to *P. berghei* infection, developed intense infection by merely increasing the *para-minobenzoic* acid available to them.

The role of the integrity of the lymphoid macrophage system is, however, different in the case of acquired immunity. The one animal that was infected after splenectomy and survived for 37 days till it was sacrificed and all the 9 animals that were splenectomised when the infections were latent, behaved in a totally different way from infected intact animals. With the exception of one animal (R-575), all the splenectomised ones showed continuous patent parasitaemia till death (periods ranging from 53 to 340 days). In two of them, however, occasionally, on a few days the peripheral blood was negative to parasites. Rat-575 was the only one whose peripheral blood was negative to parasites continuously, during the last 26 days of its post-splenectomy life of 76 days. These results showed that the removal of the spleen resulted in a considerably reduced capacity on the part of the host to acquire sufficient immunity to overcome the patent parasitaemia.

There seemed to be one other aspect of splenectomy with reference to acquired immunity. Ramakrishnan and Satya Prakash (1950) showed that infection can last in intact rats in a latent form up to at least 6 months and probably longer. It is seen that animals splenectomised during latency, lived with continuous patent parasitaemia for periods of 2 to 3 months and one actually lived for nearly 12 months. Such periods of patent parasitaemia were very much prolonged in every one of the animals splenectomised during latency than in any of the intact animals as well as thousands of other animals, observed in the course of routine strain maintenance. This may be interpreted in the light of the possibility of there being an anti-toxic immunity separate from an anti-parasitic immunity (Viswanathan, 1951). If such a possibility is conceded, one may interpret the findings, that the spleen is more intimately related to the development of anti-parasitic immunity than anti-toxic immunity.

Some of the changes in morphology of the parasite as already stated, i.e., the greater amoebiodicity and pigment in the early forms, the decreased number of merozoites in the mature schizonts occupying less of the erythrocytes, have never been encountered in thousands of normal strain animals, examined by the author. It is not possible to explain the exact significance of these observations in the present studies. It would not be incorrect to infer from these observations that absence of spleen is responsible for these morphological differences.

CONCLUSIONS.

Studies were conducted to investigate the role of spleen in building up the immunity status in albino rats with *P. berghei* infections.

It was observed that splenectomy resulted in a considerably reduced capacity on the part of the host to acquire sufficient immunity to overcome the patent parasitaemia. It appeared that spleen may be more intimately related to the development of anti-parasitic immunity than anti-toxic immunity.

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

Part XXXI.

SELECTION OF A PRIMAQUINE* RESISTANT STRAIN.

BY

SATYA PRAKASH,

A.K. CHAKRABARTI

AND

D.S. CHOUDHURY.

(*Malaria Institute of India, Delhi.*)

[July 19, 1960.]

SELECTION of strains of Plasmodia sp. with lowered sensitivity to various antimalarials by their prolonged administration in sub-minimal doses has been reported from time to time by a number of workers in the field, and has been reviewed by Thurston (1953) and Krishnaswami *et al.* (1954). Nauck (1934), Fulton and Yorke (1941), Bishop and Birkett (1948) and Bishop and McConnachie (1952) have reported a low-grade resistance of *P. knowlesi* and *P. gallinaceum* to Pamaquine. Literature on the subject has so far not furnished any evidence of resistance of any species of *Plasmodium* to Primaquine, except the work by Ray *et al.* (1956) at the Malaria Institute of India, Delhi, showing about eight-fold resistance of *P. knowlesi* against this drug. The present work reports the selection of Primaquine resistant strain of *P. berghei*.

MATERIAL AND METHODS.

Adult mice were used irrespective of sex from the colony maintained at the Malaria Institute of India. The normal strain of the parasite† used in the study has been maintained by rat-to-rat passage in the Institute since 1952, and it had undergone 392 passages prior to inoculation into mice for the present experiment. The technique followed to select the resistant strain and determine the degree of resistance was that as described by Krishnaswami *et al.* (1954).

* The proprietary preparation used is primaquine diphosphate (Imperial Chemical Industries). Each tablet contains 7·5 mg. of base.

† *P. berghei* obtained through the courtesy of Brig. J. S. K. Boyd from Burroughs Wellcome Laboratories Ltd., London.

The minimum effective dose of primaquine to produce a class II* effect was determined according to the technique described by Ramakrishnan *et al.* (1951). It was found to be 0·15 mg. per 20 gram mouse-weight twice a day by mouth for three consecutive days (total dose 45 mg./kg.).

At the commencement of the selection of the resistant strain the dose of drug administered to mice was 0·15 mg. per 20 gm./wt., twice a day by mouth for one day (i.e., 1/3 of the total dose of the drug required to produce a class II effect). The dose was gradually increased in subsequent passages till one of 2·25 mg./20 gm. body weight twice a day by mouth was reached in the 41st passage. The above dose was maintained in the remaining 53 passages. Higher strength of the solution became too viscid and was difficult to administer orally. A total of 94 passages was made during a period of 1 year and 4 months. Table I shows the serial number of passages and the progressively increasing drug schedule adopted for the selection of the resistant strain.

In some of the passages the administration of the drug was withheld to ensure that strain was not lost by repeated exposure to higher doses of the drug. For every passage more than one animal was used in order to safeguard the strain from being lost due to the death of animals.

RESULTS.

In order to determine the degree of resistance of the strain, six normal mice were inoculated with 5×10^6 parasites each, obtained from one of the experimental animals at the end of the 87th passage. Primaquine 1·875 mg. per 20 gram body weight was administered to each of the inoculated mice by mouth twice daily for three consecutive days, commencing from the first day when the density of parasitaemia was in the region of 200 parasites per 10,000 erythrocytes. At the end of 90 passages, four other normal animals were inoculated with 5×10^6 parasites each, obtained from the experimental animals. The animals thus inoculated were treated with 2·25 mg. of primaquine per 20 gram body weight twice daily by mouth for three consecutive days. Two other groups of normal mice were similarly inoculated with parasites from the experimental animals after the ninety-first and ninety-fourth passages, respectively, and similarly treated with primaquine. The course of infection in the animals inoculated, as above, was noted and the relevant data have been furnished in Table II.

It is seen from Table II that the treatment with a dose of 1·875 mg. of the drug per 20 gm. body weight gave rise to a Class II effect on the infection in three, out of six animals. The infection in the remaining three did not respond to the dose, indicating thereby a twelve-fold tolerance of the strain of parasites to the drug.

* *Minimum effective dose (Class II effect).*—The minimum effective dose of a drug was considered to be that dose which cleared the peripheral blood for a minimum period of three consecutive days when administered orally on three consecutive days commencing from a day on which the parasites were patent.

TABLE I.

Protocols for the selection of primaquine resistant strain of P. berghei

Serial number of passage.	Mouse number.	Date of passage.	Details of drug administration.		Time taken (Days).
			Dose administered in mg./20 gm. twice a day by mouth.	Number of times.	
0	M-2051	May 27, 1958	0·15 mg.	One	..
1	M-2066	May 31, 1958	0·15 ,,	One	4
2	M-2091	Jun. 6, 1958	0·225 ,,	One	6
3	M-2101	Jun. 10, 1958	0·225 ,,	One	4
4	M-2140	Jun. 17, 1958	0·25 ,,	One	7
5	M-2166	Jun. 24, 1958	0·25 ,,	One	7
6	M-2179	Jun. 28, 1958	0·5 ,,	One	4
7	M-2194	Jul. 2, 1958	0·5 ,,	One	4
8	M-2207	Jul. 6, 1958	0·5 ,,	One	4
9	M-2224	Jul. 11, 1958	0·75 ,,	One	5
10	M-2243	Jul. 15, 1958	0·75 ,,	One	4
11	M-2263	Jul. 20, 1958	0·75 ,,	One	5
12	M-2278	Jul. 24, 1958	0·75 ,,	One	4
13	M-2300	Jul. 30, 1958	0·75 ,,	One	6
14	M-2341	Aug. 5, 1958	0·75 ,,	One	6
15	M 2359	Aug. 10, 1958	1 ,,	One	5
16	M-2382	Aug. 14, 1958	1·5 ,,	One	4
17	M-2401	Aug. 19, 1958	1·5 ,,	One	5
18	M-2441	Aug. 26, 1958	1·5 ,,	One	7
19	M-2463	Aug. 31, 1958	1·5 ,,	One	5
20	M-2482	Sep. 4, 1958	1·5 ,,	One	4
21	M-2504	Sep. 9, 1958	1·5 ,,	One	5
22	M-2540	Sep. 14, 1958	1·5 ,,	One	5
23	M-2557	Sep. 19, 1958	1·5 ,,	One	5
24	M-2584	Sep. 24, 1958	1·5 ,,	One	5
25	M-2604	Sep. 30, 1958	1·5 ,,	One	6
26	M-2628	Oct. 7, 1958	1·5 ,,	One	8
27	M-2659	Oct. 14, 1958	1·5 ,,	One	7
28	M 2675	Oct. 18, 1958	1·5 ,,	One	4
29	M-2704	Oct. 24, 1958	1·5 ,,	One	6
30	M-2760	Oct. 30, 1958	..	..	6
31	M-2785	Nov. 6, 1958	..	..	7
32	M-2805	Nov. 12 1958	1·5 ,,	One	6
33	M-2849	Nov. 18, 1958	1·5 ,,	One	6
34	M-2866	Nov. 22, 1958	..	..	4
35	M-2890	Nov. 28, 1958	1·5 ,,	One	6
36	M-2912	Dec. 2, 1958	1·5 ,,	One	5
37	M-2952	Dec. 9, 1958	1·5 ,,	One	7
38	M-3014	Dec. 16, 1958	1·5 ,,	One	7
39	M-3044	Dec. 23, 1958	1·5 ,,	One	7
40	M-3072	Dec. 30, 1958	..	..	7
41	M-9	Jan. 3, 1959	2·25 ,,	One	4
42	M-26	Jan. 8, 1959	2·25 ,,	One	5
43	M-42	Jan 13, 1959	2·25 ,,	One	5
44	M-92	Jan 20, 1959	2·25 ,,	One	7
45	M-121	Jan. 24, 1959	2·25 ,,	One	4
46	M 150	Jan. 31, 1959	2·2 ,,	One	7
47	M-195	Feb. 7, 1959	2·25 ,,	One	7
48	M-235	Feb. 13, 1959	2·25 ,,	One	6
49	M-253	Feb. 19, 1959	2·25 ,,	One	6

(*Contd.*)

TABLE I.—*(concld.)*

Serial number of passage.	Mouse number.	Date of passage.	Details of drug administration.		Time taken (Days).
			Dose administered in mg./20 gm. twice a day by mouth.	Number of times.	
50	M-275	Feb. 25, 1959	2·25 mg.	One	6
51	M-302	Mar. 5, 1959	2·25 ,,	One	8
52	M-319	Mar. 10, 1959	2·25 ,,	One	5
53	M-339	Mar. 17, 1959	2·25 ,,	One	7
54	M-357	Mar. 23, 1959	2·25 ,,	One	6
55	M-383	Mar. 26, 1959	..	..	3
56	M-391	Mar. 30, 1959	..	..	4
57	M-410	Apr. 2, 1959	..	..	3
58	M-428	Apr. 4, 1959	..	..	2
59	M-436	Apr. 7, 1959	..	..	3
60	M-465	Apr. 11, 1959	..	..	4
61	M-484	Apr. 14, 1959	..	..	3
62	M-504	Apr. 20, 1959	2·25 ,,	One	7
63	M-528	Apr. 24, 1959	2·25 ,,	One	3
64	M-554	Apr. 30, 1959	2·25 ,,	One	6
65	M-639	May 9, 1959	2·25 ,,	One	9
66	M-675	May 16, 1959	..	..	7
67	M-686	May 19, 1959	2·25 ,,	One	3
68	M-715	May 24, 1959	2·25 ,,	One	5
69	M-746	May 30, 1959	2·25 ,,	One	6
70	M-779	Jun. 6, 1959	..	..	7
71	M-801	Jun. 10, 1959	..	..	4
72	M-820	Jun. 12, 1959	..	..	2
73	M-858	Jun. 17, 1959	..	..	5
74	M-882	Jun. 22, 1959	2·25 ,,	One	5
75	M-913	Jun. 27, 1959	2·25 ,,	One	5
76	M-934	Jul. 1, 1959	2·25 ,,	One	4
77	M-951	Jul. 6, 1959	2·25 ,,	One	5
78	M-976	Jul. 12, 1959	2·25 ,,	One	6
79	M-997	Jul. 16, 1959	2·25 ,,	One	4
80	M-1028	Jul. 21, 1959	2·25 ,,	One	5
81	M-1062	Jul. 28, 1959	2·25 ,,	One	7
82	M-1080	Aug. 1, 1959	2·25 ,,	One	4
83	M-1115	Aug. 8, 1959	2·25 ,,	One	7
84	M-1164	Aug. 13, 1959	2·25 ,,	One	5
85	M-1197	Aug. 18, 1959	..	..	5
86	M-1215	Aug. 22, 1959	2·25 ,,	One	4
87	M-1242	Aug. 26, 1959	2·25 ,,	One	4
88	M-1293	Sep. 2, 1959	..	..	5
89	M-1329	Sep. 8, 1959	..	..	6
90	M-1349	Sep. 14, 1959	..	..	6
91	M-1365	Sep. 17, 1959	2·25 ,,	One	3
92	M-1400	Sep. 22, 1959	..	..	5
93	M-1432	Sep. 26, 1959	2·25 ,,	One	4
94	M-1471	Oct. 1, 1959	2·25 ,,	One	5
				Total	492

Table II.

Course of infection in animals inoculated with experimental strain and treated with primaquine.

Mouse number.	Dose of drug administered.	Number of parasites found per 10,000 R.B.C. from the 1st day of drugging.												
		1st day.	2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	11th day.	12th day.	13th day.
M-1312	A	522	136	46	N	12	D	..	..	..	..	..	..	..
M-1313	A	274	152	3	P	6	336	>2,000	>2,000	>2,000	D	..	..	..
M-1314	A	172	40	36	P	N	N	N	P	254	1,612	624	>2,000	>2,000
M-1315	A	286	144	42	2	N	N	62	>2,000	>2,000	D	..	..	..
M-1316	A	348	48	4	P	N	N	N	32	1,292	936	510	D	..
M-1317	A	320	96	6	N	N	N	N	12	328	610	316	368	918
M-1379	B	424	106	5	P	N	P	72	630	>2,000	>2,000	D	..	..
M-1380	B	256	84	P	N	N	N	P	4	512	>2,000	>2,000	>2,000	D
M-1381	B	252	48 D	..	..	..	..	..	..	..	..	..	..	..
M-1382	B	482	212	3	N	N	P	P	162	610	352	1,134	1,532	>2,000
M-1440	B	256	66	6	N	N	N	N	N	N	P	28	412	436
M-1441	B	192	72	P	N	N	N	N	N	N	6	212	>2,000	>2,000
M-1442	B	210	24	N	N	N	N	N	N	N	N	N	N	N
M-1519	B	236	92	P	N	N	N	N	N	N	P	2	42	406
M-1520	B	240	48	2	N	N	N	N	6	18	176	184	634	1,078
M-1521	B	216	62	P	N	N	N	N	N	3	10	516	476	324
M-1522	B	242	42	4	N	N	N	N	N	P	4	240	1,632	1,034

A = 1·875 mg. per 20 gram body weight twice a day by mouth for three days (Total dose 562·5 mg./kg.).

B = 2·25 mg. per 20 gram body weight twice a day by mouth for three days (Total dose 675 mg./kg.).

D = Dead.

N = No parasite found in 100 oil immersion fields.

P = 1 parasite in 100 oil immersion fields.

>2,000 = More than 2,000 parasites per 10,000 erythrocytes.

Table III.

Course of infection in animals inoculated with experimental strain (6 months after stoppage of exposure to drug) and treated with primaquine.

Mouse number.	Dose of drug administered.	Number of parasites found per 10,000 R.B.C. from the 1st day of drugging.											
		1st day.	2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	11th day.	12th day.
M-422	A	296	264	192	D	..	..	..	..	..	..	..	..
M-423	A	312	382	D	D	..	..	..	..	..	..	..	..
M-424	A	384	146	34	D	..	..	..	..	..	..	..	..
M-425	A	49	402	202	D	..	..	..	..	..	..	..	..
M-426	A	316	310	82	D	..	..	..	..	..	..	..	..
M-427	A	244	62	D	..	..	..	..	..	..	..	..	..
M-506	A	404	84	3	N	N	N	P	12	164	>2,000	>2,000	>2,000
M-507	A	450	326	2	N	N	N	2	194	>2,000	>2,000	>2,000	D
M-508	A	216	146	2	N	N	N	N	P	26	618	1,184	1,264
M-509	A	178	184	3	N	N	N	N	P	14	142	488	1,154

A = 1·875 mg. per 20 gram body weight twice a day by mouth for three days (total dose 562·5 mg./kg.)
D = Died.
N = No parasite found in 100 oil emmersion fields.
P = 1 parasite in 100 oil emmersion fields.
>2,000 = More than 2,000 parasites per 10,000 erythrocytes.

The infection in seven animals, out of the eleven, treated with a dose of 2·25 mg. of the drug per 20 gm. body weight showed a clear Class II effect. One animal died, and in three there was no response to the treatment, indicating a fifteen-fold tolerance of the parasites to the drug.

The response of the parasites to the two different doses of the drug, one higher than the other, indicated the presence of a strain of the parasite resistant to primaquine at least twelve times the parent strain.

In order to study the stability of resistance, the drug was stopped at 94th passage and the resistant strain was maintained by mouse-to-mouse passage for five months. Ten albino mice, inoculated with this resistant strain, were exposed to a dose of 1·875 mg. per 20 gm. body weight by mouth twice a day for three consecutive days. The details are presented in Table III. It was observed that six animals died and the parasites in the surviving four demonstrated twelve-fold resistance as in the earlier experiments. It was further noticed that the dose appeared to be toxic to the animals.

DISCUSSION.

From the results of the present work it is clear that it has been possible experimentally to detect the presence of a strain of *P. berghei* twelve times more resistant to primaquine than the parent strain. In each of the two replicates used to determine the degree of resistance, the infection in a proportion of the animals responded to the treatment. The infection in the remaining animals, however, did not respond to the treatment. At this stage, it would appear that the selection of the resistant strain was not complete. The parasite population consisted of a proportion susceptible to 2·25 mg. of the drug per 20 gm. body weight of the host and another proportion resistant to the same dose. As already mentioned, this dose was found to be toxic to the hosts. Further, more concentrated solutions of the drug were too viscid for oral administration to the animals. Nevertheless, it is of interest to note that during the five months of passage of the experimental strain without exposure to drug, the infection in 4 out of 10 mice, did not respond to twelve times the dose of the drug required to cure an infection of the parent strain.

It has been noted from a review of the literature on the subject, as also observed from the results of the present experiments, that the selection of a resistant strain of *P. berghei* to 8-aminoquinolines, occurs in a comparatively much shorter period of time than in the case of either simian or the avian species of the parasite. This phenomenon of early selection of a resistant strain of *P. berghei* may perhaps be attributable either to certain intrinsic difference in the physiology of *P. berghei* from that of other species of malaria parasites or to some difference in the metabolism of the drug in mice as compared with other animal hosts. Ramakrishnan *et al.* (1957) gave ample indication of the above two aspects in course of their work with chloroquine. It has not, however, been possible within the scope of the present experiments to precisely define the factor or factors responsible

for the early development of resistance in *P. berghei* against primaquine which has to be further investigated.

An attempt to select a strain with a higher degree of resistance than reported at present, appears to be difficult, because the drug became very viscid in higher concentrations and was, therefore, unsuitable for oral administration.

SUMMARY.

The selection of a strain of *P. berghei* tolerant to primaquine has been evidenced by the results of the investigations undertaken. It has been estimated that it requires at least twelve times the scheduled minimal dose of the drug (for the parent strain) to clear the tolerant strain of the parasite from the peripheral blood of infected mice. The selection of the strain has been achieved by the administration of gradually increasing doses of the drug to the experimental animals through series of passages of the parasites.

The resistance was unaltered during the thirty-five passages without any exposure to drug over a period of five months.

The basis of development of resistance has been discussed with an indication of a further scope of investigation.

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Indian Journal of Malariology, **15**, *2, June 1961.*

A NOTE ON THE SUSCEPTIBILITY OF RAT-FLEAS OF DELHI TO DDT., DIELDRIN AND GAMMA BHC.

BY

M.I.D. SHARMA

AND

G.C. JOSHI.

(Malaria Institute of India, Delhi.)

[December 26, 1960.]

Due to the large scale use of insecticides, particularly in the field of malaria eradication, there is every possibility of insects, other than mosquitoes, becoming insecticide resistant. Recently in India, Patel *et al.* (1960) and Mohan (1960) observed DDT-resistant rat-fleas in villages of Poona District of Maharashtra State and in Gundlupet Town of Mysore State, respectively. In both the areas, DDT has been sprayed for several years under the National Malaria Control/Eradication Programme. These findings have highlighted the urgent need of maintaining vigilance on the insecticide susceptibility of insects of public health importance in general, and rat-fleas, being the vector of plague, in particular. For such studies it is of paramount importance to get data on the insecticide susceptibility for the normal population of the insect species concerned. Collection of such data is not easy after the extensive use of insecticides in the country. Keeping this objective in view, susceptibility tests on rat-fleas collected from unsprayed localities of Delhi City* were conducted against DDT, dieldrin and gamma BHC during the period August to November, 1960. The results obtained are presented in this publication so that these may serve as base line data for purposes of comparison by other workers engaged in similar studies.

For conducting these studies, fleas were collected from rats obtained from those areas of the city only which had never been sprayed with any insecticide†. The rats were killed by strangulation in a clean white enamelled basin and as the fleas left their dead bodies, they were gently picked up by means of a sucking tube and transferred into a clean glass jar. Strips of plain paper were put inside the glass jar to provide resting place for fleas. The insects so collected were brought to the

*In the urban area of Delhi, except for the belt of houses along the River Jamuna which are sprayed with DDT, antilarval measures are enforced for malaria eradication. Fleas for the investigations were collected from rats obtained from those areas of the city only which had never been sprayed with any insecticide.

†The rat-flea population, during the period of observations from August to November, 1960, comprised 63 per cent *X. cheopis* and 37 per cent *X. astia*. The ratio of the two sexes in *X. cheopis* was 1·6 females to one male and in *X. astia* 3 females for every male.

laboratory and kept there for 2-3 hours after which any dead or injured specimens were separated.

Insecticide susceptibility tests were carried out by utilizing the insecticide impregnated filter papers as recommenced by the World Health Organization in their provisional technique. Whatman filter papers No. I were impregnated with different concentrations of the insecticides following Busvine and Nash (1954) method. From each of the treated filter papers a strip, 5 cm. × 2·5 cm., was cut, folded twice lengthwise in the form of 'Z' and put in a clean glass tube (15 cm. long and 1·5 cm. in diameter). The tubes so prepared, with filter papers impregnated with different concentrations of each insecticide, were used as exposure tubes. With the help of a sucking tube, about ten fleas were introduced into each exposure tube, the opening of which was secured with a piece of fine muslin and rubber ring. Only active fleas and as far as possible fed, were used in the tests. For each of the three insecticides, 8 to 14 replicate tests were carried out with 327 to 797 fleas. At the end of exposure period of one hour, the fleas of each exposure tube were transferred to a clean holding tube* (15 cm. long and 1·5 cm. in diameter) which, like the exposure tube, contained a similar folded strip of clean (untreated) filter paper. Appropriate controls were observed for each test. Mortality counts in insects were made 24 hours after the exposure period. After this, dead and alive insects were suitably mounted and identified species-wise and sex-wise, separately for each insecticidal concentration and test.

The data obtained were analysed by the probit analysis method (Finney, 1952) and median lethal concentration (*MLC*) values of insecticides for the different categories of insects were estimated from the graphs by the eye estimation method† (Tables I, II and III).

MLC values of DDT, dieldrin and gamma BHC in the case of *X. cheopis* females were found to be 0·51, 0·04 and 0·013 per cent respectively as against values of 0·25, 0·02 and 0·005 for the males of the species. This indicated that the males were more susceptible to the insecticides than the females. In field studies, usually the susceptibility data for both the sexes are analysed together. By doing so the MLC values for *X. cheopis* (males and females combined) of DDT, dieldrin and gamma BHC were 0·4, 0·035 and 0·01 per cent respectively.

In the case of *X. astia*, due to the very limited number of the males being available, only the susceptibility to DDT of the males of the species could be determined. The MLCs of DDT, dieldrin and gamma BHC for the females were 0·1, 0·01 and 0·001 per cent respectively. The MLC of DDT for males of the species was 0·07 and the MLC for both the sexes was 0·1 per cent.

*A pinch of moist saw dust was put at the bottom of each holding tube. This practice reduced insect mortality in control tests to a considerable extent.

†Data of the experiments with high insect mortality in controls were not included for the statistical analysis.

TABLE I.

Susceptibility of rat-fleas to DDT.

Species.	Concentration of DDT.																								MLC (per cent.)
	0·03125			0·0625			0·125			0·25			0·5			1·0			2·0			Control.			
	n	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	
X. cheopis (females).	36	2	..	46	8	12·7	36	7	14·6	31	9	24·7	41	19	43·8	25	17	66·1	18	16	86·1	53	3	5·6	0·51
X. cheopis (males).	25	10	20·0	33	13	19·0	35	15	23·7	34	20	45·0	36	26	62·9	22	20	88·0	9	9	100·0	12	3	25·0	0·25
X. cheopis (Both sexes combined).	61	12	11·6	79	21	19·1	71	22	24·2	65	29	38·9	77	45	54·1	47	37	76·5	27	25	91·7	65	6	9·2	0·40
X. astia (females).	25	8	24·1	41	16	33·1	32	20	58·1	35	24	64·8	25	22	86·7	19	19	100·0	7	7	100·0	29	3	10·3	0·1
X. astia (males).	11	5	27·2	16	10	50·0	16	12	66·6	10	8	73·3	9	9	100·0	17	17	100·0	6	6	100·0	8	2	25·0	0·07
X. astia (Both sexes combined).	36	13	26·1	56	26	38·0	48	32	61·3	45	32	66·6	34	31	89·7	36	36	100·0	13	13	100·0	37	5	13·5	0·1

n=number of fleas exposed. *r*=number of fleas dead. *p*=corrected mortality.

TABLE II.

Susceptibility of rat-fleas to dieldrin.

Species.	Concentration of dieldrin.																								MLC (per cent.)
	0·00312			0·00625			0·0125			0·025			0·05			0·10			0·20			Control.			
	n	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	
X. cheopis (females).	18	4	15·4	21		22·2	12	4	27·5	17	7	36·0	9	5	51·6	14	10	68·9	14	12	84·4	25	2	8·0	0·04
X. cheopis (males).	19	7	19·5	10	4	23·6	12	7	46·9	19	12	53·0	8	6	68·1	6	5	78·7	10	9	87·2	14	3	21·4	0·02
X. cheopis (Both sexes combined).	37	11	19·4	31	10	22·2	24	11	37·8	36	19	45·7	17	11	59·5	20	15	71·3	24	21	85·6	39	5	12·8	0·035
X. astia (females).	9	3	13·2	20	12	47·9	17	11	54·1	14	11	72·0	..	..	..	4	4	100·0	..	..	..	13	3	23·1	0·01

n=number of fleas exposed. *r*=number of fleas dead. *p*=corrected mortality.

TABLE III.

Susceptibility of rat-fleas to gamma BHC.

Species.	Concentration of gamma BHC.														
	0·0003125			0·000625			0·00125			0·0025			0·005		
	n	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*
X. cheopis (female).	25	4	..	24	4	0·7	29	5	1·5	48	12	10·7	32	12	25·5
X. cheopis (male).	9	3	7·6	15	7	26·1	12	6	30·7	21	12	40·5	24	15	48·0
X. cheopis (Both sexes combined).	34	7	..	39	11	9·2	41	11	7·5	69	24	17·6	56	27	34·5
X. astia (female).	10	4	13·9	28	18	48·6	31	23	62·8	30	25	76·0	33	26	78·1

TABLE III.—(*Concld.*)

Species.	Concentration of gamma BHC—(*Contd.*).												MLC (per cent.)
	0·01			0·02			0·04			Control.			
	n	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	
X. cheopis (female).	34	16	36·9	18	15	80·1	10	10	100·0	25	4	16·0	0·013
X. cheopis (male).	18	13	61·4	8	7	82·6	6	6	100·0	18	5	27·8	0·005
X. cheopis (Both sexes combined).	52	29	43·9	26	22	80·6	16	16	100·0	43	9	20·9	0·01
X. astia (female).	20	18	85·6	9	9	100·0	9	9	100·0	33	10	30·3	0·001

n = number of fleas exposed. *r* = number of fleas dead. *p* = corrected mortality rate.

From these studies it may be concluded that *X. astia* was more susceptible to the three insecticides than *X. cheopis* and that the males* of both the species were more so than the females.

SUMMARY.

Insecticide susceptibility studies on the rat-fleas from unsprayed urban areas of Delhi have been described. Base line data on the susceptibility of *X. cheopis* and *X. astia* to DDT, dieldrin and gamma BHC, have been presented. *X. astia* was found to be more susceptible to these insecticides than *X. cheopis*.

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*In the case of *X. astia* males, susceptibility to DDT only could be determined.

Indian Journal of Malariology, **15**, 2, June 1961.

REPORT ON AN EPIDEMIC OCCURRING DURING ERADICATION OF MALARIA IN GIR FOREST, GUJARAT STATE, INDIA.

BY

T.B. PATEL

AND

G.J. AMBWANI.

(Directorate of Public Health, Gujarat State, Ahmedabad.)

[August 18, 1960.]

THERE is a very meagre information about epidemics which might occur during or after eradication of malaria. Such epidemics should, therefore, be reported in order to enable malaria workers to foresee the order of events and to realise their importance.

HISTORY OF OUTBREAK.

The outbreak under report occurred in two National Malaria Eradication Units of Amreli and Junagadh and was detected in the month of April, 1960.

On April 26, 1960, the Unit Officer, Amreli Unit, during his routine tours came across 53 fever cases in village Vadli of Jafrabad Taluka. Examination of blood smears confirmed the diagnosis of malaria. History of patients revealed that all the affected persons were labourers by occupation and had contacted infection while working on road construction works in the Gir forest area. It was also clear from the history that labourers from different villages, who had been employed for road construction work in the Gir forest area, had suffered from similar type of fever and as a result thereof had been forced to leave for their respective villages.

Detailed investigations were, therefore, immediately carried out with a view to ascertain the number of labour camps established in the forest area, the period when they were first established and the villages from which the labourers had come. These investigations revealed that road construction work in Gir forest area was started in January and February, 1960, and that labourers had come from 14 villages of Jafrabad and Dhari Talukas of Amreli Unit and from two villages of Una Taluka of Junagadh Unit. The total population of the affected villages is 16,000. From May 1 to May 15, 1960, all these villages were visited and an enquiry was made from each house. In all 342 blood smears were collected from 370 fever cases and examined. Two hundred forty-six were found to be positive for malaria parasites of which 174 were for *P. falciparum*, 67 for *P. vivax* and 5 for mixed infection of *P. falciparum* and *P. vivax*. Enquiry also revealed that the villagers

MAP 1.

Gir forest area and the affected villages of Jafrabad, Una and Dhari Talukas.

who had remained in their villages, had not suffered from malaria. Details of blood smears collected from the affected villages have been tabulated in Table I.

TABLE I.

Details of examination of blood smears collected from fever cases detected in affected villages.

Taluka.	Serial number.	Name of the village.	Population.	Number of fever cases.	Number of blood smears taken.	Results :			
						P. falciparum.	*P. vivax.*	Mixed.	Total.
Jafrabad	1	Chitrasan	566	85	80	53	14	0	67
	2	Vadli	624	70	70	28	14	0	42
	3	Timbi	2,078	40	40	11	11	1	23
	4	Rohisa	1,426	33	27	23	1	1	25
	5	Wadhera	1,089	30	29	19	4	0	23
	6	Kadiali	710	8	4	2	2	0	4
	7	Sokhada	88	20	17	6	8	1	15
	8	Bakaria Mota	85	12	7	3	3	0	6
	9	Dadhala	373	4	4	1	0	0	1
	10	Dharabandar	377	8	6	4	2	0	6
	11	Dholadri	296	2	2	0	0	0	0
	12	Mithapur	659	1	1	0	0	0	0
	13	Khodiar Dam	...	6	6	2	3	0	5
Dhari	1	Dalkania	1,392	19	17	1	3	0	4
Una	1	Kob	1,248	3	3	3	0	0	3
	2	Khatriwada	902	29	29	18	2	2	22
		Total		370	342	174	67	5	246

GIR FOREST.

Since infection was contacted by villagers inside Gir forest, it will be necessary to describe the forest area in some details.

It extends from Una Taluka of Junagadh Unit to Dhari Taluka of Amreli Unit in the North and in the West up to Talala and Mendara Talukas of Junagadh Unit (Map 1). It is a scrub forest spread over an area of 500 square miles and is well-known all over the world as an abode for the famous Kathiawad Lions. There is only one small hillock situated roughly in the centre of the forest (known as Sassan hill), but terrain on the whole is undulated and is traversed by small rivulets and streams which flow almost throughout the year the Average annual rainfall is about 1,500 mm. It is inhabited by persons whose main occupation is cattle grazing. To a little extent some area has been deforested for agricultural purposes. People live clustered together in a small area popularly known as '*Nes*'. Dwellings are small, one-room huts, made of wood and mud. Since people have to shift from one place to another inside the forest area in search of grazing ground for their cattle, many huts are built for only temporary residence. Such temporary huts are built out of tree-branches and leaves.

The cattle is kept in fenced area at some distance so as to prevent entry of lions in the huts. Fence is usually made up of cactus and thorny bushes. Some of the

'*Neses*' which are permanently inhabited are known as 'villages'. The total number of '*Neses*' and villages is 123 and they have an aggregate population of 10,000.

This area was first surveyed by Major J.A. Sinton in April, 1929. The survey of Sassan village then revealed a spleen rate of 100 per cent and child parasite rate of 65 per cent. *A. culicifacies* and *A. stephensi* were incriminated as primary vector species, and transmission season was found to extend from June to December.

SPRAYING HISTORY OF GIR FOREST.

The spraying was first started in this forest area in 1949, by the then Public Health Department of Saurashtra. The details of the spraying are as follows :—

1949—One round of 50 per cent DDT wettable powder at the rate of 56 mg. per sq.ft. in the month of July.

1950— —do—

1951—Two rounds of insecticidal spraying, one in June and another in August, but at the same dosage schedule.

1952— —do—

1953 to 1957 — Two rounds of 75 per cent DDT wettable powder in June and September at the rate of 112 mg. per sq. ft.

1958 to 1959 — Three rounds at the same dosage schedule, third round starting from the middle of December and lasting for a month.

These measures have, however, not given us satisfactory results as can be seen from the study of the survey results of Sassan village. Unfortunately, Sassan village has not been surveyed regularly. The available data tabulated in Table II indicate that malaria transmission has not been intercepted in this area for over a number of years. The study of the malaria morbidity statistics of Sassan Dispensary (*vide* Table III) also reveals the same fact.

TABLE II.

Results of survey of Sassan village.

	1929.	1958-59.	1959-60.
Spleen rate.	100·0	15·0	3·0
Child parasite rate.	65·0	Not done	0·0
Infant parasite rate.	Not done	Not done	Not done

TABLE III.

Monthwise malaria morbidity figures shown as percentage of malaria cases to all cases as reported by the Medical Officer, Sassan Dispensary, from 1955-1960.

	1955.	1956.	1957.	1958.	1959.	1960.
January	Not reported.	Not reported.	26·0	18·8	Not reported.	13·4
February	22·3	36·4	30·4	27·2	-do-	15·9
March	22·6	19·6	23·1	18·8	18·2	14·5
April	19·1	18·8	26·4	18·8	21·7	7·2
May	20·7	26·0	21·3	26·2	23·8	14·0
June	23·2	23·7	31·1	19·6	23·8	
July	44·9	Not reported.	40·4	20·8	16·3	
August	23·1	12·7	32·6	20·4	9·1	
September	29·5	17·5	26·3	57·9	11·5	
October	30·5	24·0	16·1	Not reported.	3·7	
November	34·0	2[illegible]·3	34·7	do	12·7	
December	24·7	39·5	40·0	26·7	17·0	

ROAD CONSTRUCTION WORKS AND LABOUR CAMPS IN GIR FOREST.

A few road construction works were started in the Gir Forest area in the beginning of the year 1960 :—

(1) One such work was the construction of a road, about seven miles long, between Sassan and Kapuriya *Nes*. To start with, 70 to 80 labourers were employed by a private contractor on or about February 1, 1960. Within 10 days, the strength of the labour rose to 300 persons. These labourers used to camp either in Sassan village or at the work-site where temporary sheds had been erected by the contractor. The occurrence of fever cases was first noticed in the last week of February, and by about March 25, 1960, the whole camp was deserted by the labourers. On April 4, 1960, the contractor brought a fresh batch of 70 labourers from other villages, but all came down with malaria by about April 15, 1960. Therefore, broadly speaking, malaria attacks appeared after the completion of intrinsic incubation period of 10 to 12 days. Temporary sheds erected for labourers had escaped spraying. Sassan village itself was last sprayed in the second week of December, 1959.

(2) The second work was the construction of a road, about 19 miles long, from Jamvala *Nes* to Sap *Nes*. Actual construction work was, however, commenced from Jamvala and Sap *Nes*. It seems the work was started in the beginning of January and by about middle of January the number of labourers employed was 250. It is not clear as to when the first case occurred but great number of fever cases began to be reported in the beginning of February, 1960, and by the end of February, majority of the labourers, having suffered from malaria attacks, began to leave the labour colony. In order to maintain the progress of the work, the labour

reinforcements were regularly brought in by the contractor. But within a fortnight, majority of them used to come down with malaria.

(3) A third labour colony was established at Panikota *Nes* near Prachi Road. A few labourers were first employed in the beginning of May, 1960, for breaking the metal to be used for road construction work. Their strength, however, rose to 40 by the end of the first week of May, 1960. By about May 16, 1960, fever cases began to be reported and many labourers deserted the work-site. As usual, reinforcements were brought in by the contractor regularly and when this camp site was visited on May 21, 1960, out of 17 blood smears examined 4 were found to be positive for malaria parasites (*P. falciparum*). History revealed that labourers had suffered after a stay for a fortnight in that area.

Investigations carried out in the nearby *Neses*, i.e., Sassan, Jamvala, Sapnes, Kapuriya, and in other 8 *Neses*, did not reveal any outbreak of malaria. Detection of few malaria cases in some of these *Neses* during the first week of May, 1960, was not a new occurrence as a few sporadic cases have always been seen in forest areas in the past years. Actually 67 blood smears were collected from these 12 *Neses* between May 1 and May 30, 1960. On examination, only four were found to be positive for malaria parasites (*P. falciparum*), (Table IV).

TABLE IV.

Details of blood smears collected during May, 1960, from Neses *of Gir Forest.*

Taluka.	Serial number.	Name of the village.	Population.	Number of fever cases.	Number of blood smears taken.	Results.			
						P. falciparum.	*P. vivax.*	Mixed.	Total.
Visavadar	1	Limadhera	692	8	8	1	0	0	1
Talala	1	Sassan	515	4	4	1	0	0	1
	2	Kapuriya	21	10	10	0	0	0	0
	3	Bhojde	319	5	5	0	0	0	0
	4	Javantri	428	8	8	1	0	0	1
	5	Babira	90	4	4	0	0	0	0
	6	Dabhoda	50	4	4	0	0	0	0
Una	1	Vankia	748	2	2	0	0	0	0
	2	Kasaria	460	11	11	..	..	..	..
	3	Dodhi	51	2	2	0	0	0	0
	4	Asundvali	18	1	1	0	0	0	0
	5	Gir Mandir	18	8	8	1	0	0	1
				67	67	4	0	0	4

The fact that no undue increase in malaria cases had occurred in the Gir Forest area, is also supported by malaria morbidity figures of Sassan Dispensary reproduced in Table III.

DISCUSSION.

It will be seen that all the malaria patients encountered in the affected villages were labourers by occuption and had contacted infection while working inside the Gir Forest area. Malaria transmission in the Gir Forest has never been completely intercepted. This is amply clear from the study of survey results of Sassan *Nes*. It has, therefore, been always known that residual infection persists in this area which we call as "problem area". The problem of persistence of malaria in this area is being studied, but the fact remains that here was an area where, so to say, a fire was smouldering.

On the other hand the labourers came from an area where DDT spraying since 1953 had successfully brought the reproduction rate below unity and the infection was on its way out. This conclusion has been surmised from the cumulative malariometric indices of the affected talukas of Jafrabad and Dhari, (Tables V*a*, V*b* and V*c*).

TABLE V*a*.

Cumulative spleen rate.

	1952-53	1953-54	1954-55	1955-56	1956-57	1957-58	1958-59	1959-60
Dhari Taluka.	20·6	13·14	10·14	6·34	1·34	1·76	1·77	1·18
Jafrabad Taluka.	..	..	..	..	..	..	0·60	0·00

TABLE V*b*.

Cumulative child parasite rate.

	1952-53	1953-54	1954-55	1955-56	1956-57	1957-58	1958-59	1959-60
Dhari Taluka.	17·8	4·55	4·88	2·22	3·16	0·75	0·0	0·0
Jafrabad Taluka.	..	..	..	..	..	..	0·0	0·0

TABLE V*c*.

Cumulative infant parasite rate.

	1952-53	1953-54	1954-55	1955-56	1956-57	1957-58	1958-59	1959-60
Dhari Taluka.	..	6·25	8·33	0·0	0·0	0·0	0·0	0·0
Jafrabad Taluka.	..	..	..	..	..	..	0·0	0·0

In such a community, immunity will be at a very low ebb and majority of persons will be more or less non-immune.

Introduction of such non-immunes into an area, where transmission of malaria has not been interrupted, may be likened to the introduction of dry wood into smouldering fire (dry wood will catch fire in no time).

Such an epidemic is not a new occurrence. The association of malaria epidemic with migrant labour is sufficiently well-known to be honoured with a name of its own "malaria of tropical aggregation of labour" and is known to occur in areas where malaria is unstable. But such a localised epidemic, during eradication of malaria, poses different questions. Two inherent features of localised epidemic are :—

(1) its great intensity, and

(2) its diffusability.

Because of its intensity, many great works and enterprises have been abandoned in the past. Its diffusability will cause in each village, in the eradication phase, an appearance of parasitic reservoir of a considerable extent. In the outbreak under report, labourers returned to their villages as parasite positive cases. Such dispersal will cause epidemics arising from big origins as against epidemic arising out of small origin postulated by Macdonald (1957).

In such a type of epidemic the source, as in an epidemic of small origin, will neither be a chronic case nor sporadic case or persons with measurable immunity and, therefore, of relatively poor infective value, but large number of cases freshly infected without measurable immunity and of high infective value due to large number of highly infective gametocytes. Whereas in epidemics of small origin the true growth of the epidemic would be from a few number of secondaries, arising out of solitary chronic cases, the true growth of the type of epidemic postulated would be directly from the primaries imported into the villages and as such will give rise to a considerable number of secondary cases. Thus during the second incubation interval there will be a geometrical increase in the daily increase of new cases as against the arithemetical increase in the daily increment of new cases during the second incubation interval in epidemics of small origin.

This order of events in an epidemic of large origin, therefore, points out to the necessity of not only early detection of malaria cases during surveillance phase but also to the necessity of devising ways and means to control it during the first incubation interval of the outbreak.

Luckily, this order of events did not occur in the affected villages under report because the return of parasite positive labourers to villages occurred at a time when seasonal conditions were unfavourable to transmission of malaria. The result was that no introduced cases could be detected and the epidemic died a natural death. But for such a conclusion, in the absence of a surveillance organisation, the end results would have been disastrous.

Outbreaks of such epidemics during eradication phase need to be given special attention because it may lead to multiple foci of infection in several places and thus may create grave consequences. As such wherever there is likely to be congregation of labour force on several construction projects, the malaria organisation should watch the situation very carefully. Luckily, in the epidemic described,

the labour force came only from the nearby villages and their return to their villages occurred during the period when malaria transmission was not possible. This may not happen every time and may lead to extensive outbreaks of malaria with dire consequences to the eradication campaign when a number of large development projects are being executed both by the Government and by the private sector in many parts of the country throughout the year.

During eradication phase, the following three types of epidemics can be envisaged :—

(1) Epidemics arising out of small origin,

(2) Epidemics which are recrudescent epidemics, e.g., "An outbreak of malaria in parts of Thana District, Bombay State, India, after severel years of successful control" (Patel, Ramachandra Rao and Ambwani 1961), and

(3) Epidemics (of a type described above) arising out of big origins.

SUMMARY AND CONCLUSION.

1. A malaria outbreak amongst a changing labour force of approximately 600, working in the Gir Forest area on road construction works during early months of 1960, has been described.

2. Three hundred seventy fever cases were investigated and in all 246 blood smears were found positive for malaria parasites.

3. In the Gir Forest areas, the outbreak occurred amongst introduced labour population which was rendered more or less non-immune as a result of continuous and effective control measures taken for a period of preceding seven years in the villages from which the labourers came.

4. As a result of dispersal of labour to their own villages, 14 villages with a total population of 16,000 were exposed to the risk of malaria outbreak.

5. The order of events in an epidemic, arising from these multiple foci of infection, has been described.

6. Luckily extensive outbreak in the affected villages did not occur because dispersal of labour occurred during the non-transmission season.

7. According to mathematical epidemiology, grave consequences can occur if timely action is not taken to curb the outbreak arising from big origins during the first incubation interval.

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Indian Journal of Malariology, **15**, 2, *June 1961*.

STUDIES ON THE SUSCEPTIBILITY OF *PEDICULUS HUMANUS CORPORIS DE G.* TO DDT, GAMMA BHC AND PYRETHRINS.

BY

M.L.D. SHARMA,

B.N. MOHAN

AND

N.N. SINGH.

(*Malaria Institute of India.*)

[December 27, 1960.]

INTRODUCTION.

THE dramatic success of DDT in controlling *Pediculus humanus corporis* and thereby the incipient typhus epidemic in Naples during 1943 resulted in its wide-spread use throughout the world for controlling body lice (Brown, 1958). However, this initial success of DDT in the control of body lice did not last long. In 1950-51, the application of DDT resulted in the precipitation of resistance in body lice, infesting a large group of military personnel stationed in Korea (Hurlbut *et al.*, 1952). Since then insecticide resistence in body lice has been reported from many places. From the available data, it would be seen that the number of countries reporting resistance in body lice to DDT, gamma BHC or pyrethrins are 22 up to date against two up to 1952.

In their review of insecticide susceptibility data of body lice up to 1956, Wright and Brown (1957) observed that resistance in a population of lice varied from one individual host to another. They also recorded a few cases of high resistance in lice collected from individuals never exposed to any insecticide. However, they observed generally lowered susceptibility to DDT in lice collected from regularly dusted areas than those from areas where the population was either never or occasionally treated with insecticidal dusts.

In India, very little work has been done to study the insecticide susceptibility of lice. Systematic studies on the susceptibility of body lice to DDT, gamma BHC and pyrethrins were, therefore, undertaken in three widely separated areas of the country. These investigations were conducted in the villages of Meerut District of Uttar Pradesh during March, 1960, Gundlupet Taluk of Mysore State during June-July, 1960 and in Baroda City of Gujarat State during September, 1960. The results obtained are presented in this communication.

MATERIALS AND METHODS.

In the experimental areas, no insecticide was ever used for anti-louse purposes, though the houses there had been regularly sprayed with DDT or gamma BHC for about five to ten years under the National Malaria Control/Eradication programme. In each area, adult blood-fed body lice of both sexes were collected from the clothings of infested persons and kept in test tubes containing small pieces of lint. The insects so collected from the field were brought to the laboratory and kept for two hours for conditioning. The active adult lice were then exposed to cloth pieces treated with different concentrations of DDT, gamma BHC and pyrethrin powders supplied in the W.H.O. test kit for body lice*. The number of lice exposed to each concentration of different insecticides varied from five to twenty-six depending upon the number of insects available. Two to six replicate tests were performed with each concentration of the three insecticides. The period of exposure of insects to insecticide impregnated cloth pieces was 24 hours in most of the tests. Lice found dead or moribund† at the end of exposure period were counted as dead. The mortality rates, in all the concentrations of the three insecticides tested were found to vary from 80·0 to 100·0 per cent.

With a view to getting the critical range of mortality in the test insects, the exposure period was reduced to 12 hours in some tests carried out with the lice collected from the villages of Meerut District of Uttar Pradesh. The mortalities in insects were recorded at the end of the exposure period. Similarly, for the same reason, in some tests carried out in Gundlupet Taluk of Mysore State, and Baroda City of Gujarat State, body lice were also exposed to DDT for four hours; to gamma BHC for two hours and to pyrethrins for half an hour, one hour and two hours. In these tests, mortalities in insects were recorded after 24 hours of holding.

The mortality rates in the different concentrations of DDT, gamma BHC and pyrethrins, with continuous exposure period of 24 hours, were very high and no correlation was obtained between the different concentrations of the insecticides and mortality rates in the insects exposed. Even with the shorter period of exposure (four hours for DDT, two hours for gamma BHC and half-an-hour, one hour and two hours for pyrethrins) the response of body lice to the insecticides was erratic. Therefore, the data could not be analysed for the quantitative estimation of the means (MLC values) and the variance of tolerance distribution for different insecticides. However, for purposes of comparison the average mortality rates for each insecticide were estimated from the insect mortality data obtained with 24 hours exposure in different concentrations.

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* In control tests, body lice were exposed to clean cloth pieces in accordance with the W.H.O. technique. It is, however, desirable that for proper comparison, control tests are carried out on cloth pieces treated with the diluent powder only (without any insecticide).

† Insects found incapable of co-ordinated movement were considered as moribund.

RESULTS AND DISCUSSION.

The data of susceptibility tests obtained with 24 hours exposure of body lice to DDT, gamma BHC and pyrethrins are depicted in Tables I, II and III, respectively. It would be seen that the mortality figures of body lice in different replicate tests and with different concentrations of insecticides were almost the same. It may, therefore, be concluded that the susceptibility to the three insecticides of body lice, collected from different persons in the villages of Meerut District of Uttar Pradesh, Gundlupet Taluk of Mysore State and Baroda City of Gujarat State, was almost identical. The avearge mortality rates of body lice with 24 hours of exposure to four concentrations of DDT (5·0, 1·0, 0·2 and 0·04 per cent), gamma BHC (2·5, 0·5, 0·1 and 0·02 per cent) and pyrethrins (0·2, 0·04, 0·008 and 0·0016 per cent) were found to be 98·3, 100·0 and 99·6 per cent , respectively, in the case of lice collected from the villages of Meerut district of Uttar Pradesh. Values obtained for lice collected from Gundlupet Taluk of Mysore State and Baroda City of Gujarat State were also similar (Table IV). The average mortality rates in insects from the latter two localities for DDT, gamma BHC and pyrethrins, were 86·5, 99·1 and 96·5 per cent and 95·0, 100·0 and 98·3 per cent, respectively. The mortality rates in body lice collected from the villages of Meerut District of Uttar Pradesh for DDT, gamma BHC and pyrethrins after 12 hours of continuous exposure were found to be 85·8, 99·3 and 98·3 per cent., respectively (Table IV). A comparison of these two sets of results obtained with 12 hours and 24 hours of continuous exposure periods suggested that the reaction of the insects to each of the insecticide was completed in this strain within 12 hours, resulting in similar mortality rates in the insects.

For comparison the base line data of average mortality rates, with their fiducial limits at P =0·01, for the three insecticides were also estimated. For this purpose, the average mortality values* of body lice, of colony strains and field populations never subjected to insecticide dusting, reported by Wright and Brown (1957) and Narasimhan (1959), were used. The estimated values in abridged form are given in Table V. The base line data regarding the average mortality rates (with their fiducial limits) of body lice, when exposed to four concentrations of DDT (5·0, 1·0, 0·5 and 0·1 per cent), two concentrations of gamma BHC (0·25 and 0·5 per cent) and pyrethrins (0·02 and 0·04 per cent), were estimated from the results of susceptibility tests carried out with more than eight thousand body lice in over 30 countries of Europe, Asia, Africa and America. The estimated average mortality rates in insects of 92, 97 and 97 per cent for DDT, gamma BHC and pyrethrins, respectively, may be considered as population values of insecticide susceptibility for the susceptible strains (normal) of body lice. In the tests carried out in the villages of Meerut District of Uttar Pradrsh, 349, 315 and 290 body lice were exposed to

* The mean values, and the range of variation of the mean at P=0·01, were estimated after transforming the mortality rates to angular values with the help of Table XII of Fisher and Yates (1948).

TABLE I.

Results of susceptibility tests of P. humanus corporis *to DDT by the W.H.O. technique. Continuous exposure for 24 hours, response recorded at the end of exposure period.*

Locality.	Number of persons from whom lice were collected.	5·0 PER CENT DDT.		1·0 PER CENT DDT.		0·2 PER CENT DDT.		0·04 PER CENT DDT.		CONTROL.	
		Number exposed.	Moribund /Dead.	Number exposed.	Moribund /Dead.	Number exposed.	Moribund /Dead.	Number exposed.	Moribund /Dead.	Number exposed.	Moribund /Dead.
(Meerut, U.P.)											
Arthala	1	10	10	10	10	10	10	10	10	10	..
Arthala	3	11	11	10	10	10	10	10	7	9	..
Arthala	3	10	10	10	10	10	10	10	9	10	..
Arthala	5	14	12	14	14	14	14	5	4	8	..
Karara	4	25	25	26	26	25	25	25	25	25	..
Karara	4	20	20	20	20	20	20	20	20	20	..
	Total	90	88	90	90	89	89	80	75	82	..
Corrected mortality rate (per cent).		98·9		100·0		100·0		97·7		C=0·0	
Gundlupet Taluk, Mysore State.	7	10	10	10	10	10	6	10	3	10	1
	8	20	20	20	19	20	20	20	18	20	1
	Total ;	30	30	30	29	30	26	30	21	30	2
Corrected mortality rate (per cent).		100·0		96·5		85·7		67·8		C=6·7	
Baroda City, Gujarat State.	1	20	20	20	20	20	20	20	15	90	..
	2	10	10	10	10	10	10	10	9	..	..
	Total :	30	30	30	30	30	30	30	24	90	..
Corrected mortality rate (per cent).		100·0		100·0		100·0		80·0		C=0·0	

TABLE II.

Results of susceptibility tests of P. humanus corporis *to gamma BHC by the W.H.O. technique. Continuous exposure for 24 hours, response recorded at the end of exposure period.*

Locality.	Number of persons from whom the lice were collected.	2·5 PER CENT GAMMA BHC.		0·5 PER CENT GAMMA BHC.		0·1 PER CENT GAMMA BHC.		0·02 PER CENT GAMMA BHC.		CONTROL.	
		Number exposed.	Moribund /Dead.	Number exposed.	Moribund /Dead.	Number exposed.	Moribund /Dead.	Number exposed.	Moribund /Dead.	Number exposed.	Moribund /Dead.
(Meerut, U.P.)											
Arthala	1	10	10	10	10	10	10	10	10	10	..
Arthala	4	10	10	10	10	21	21	8	8	10	2
Arthala	5	14	14	14	14	16	16	11	11	8	..
Karara	4	25	25	25	25	25	25	26	26	25	..
Karara	3	10	10	20	20	20	20	20	20	20	..
	Total :	69	69	79	79	92	92	75	75	73	2
Corrected mortality rate(per cent).		100·0		100·0		100·0		100·0		C=2·7	
Gundlupet Taluk, Mysore State.	7	10	10	10	10	10	10	10	10	10	1
	3	20	20	20	20	20	20	20	19	20	2
	Total :	30	30	30	30	30	30	30	29	30	3
Corrected mortality rate (per cent).		100·0		100·0		100·0		96·3		C=10·0	
Baroda City, Gujarat State.	1	20	20	20	20	20	20	20	20	Same as for DDT (Table I)	
	2	10	10	10	10	10	10	10	10		
	Total :	30	30	30	30	30	30	30	30		
Corrected mortality rate (per cent).		100·0		100·0		100·0		100·0			

TABLE III.

Results of susceptibility tests of P. humanus corporis *to pyrethrins by the W.H.O. technique. Continuous exposure for 24 hours, response recorded at the end of exposure period.*

Locality.	Number of persons from whom the lice were collected.	0·2 PER CENT PYRETHRINS.		0·04 PER CENT PYRETHRINS.		0·008 PER CENT PYRETHRINS.		0·0016 PER CENT PYRETHRINS.		CONTROL.	
		Number exposed.	Moribund /Dead.	Number exposed.	Moribund /Dead.	Number exposed.	Moribund /Dead.	Number exposed.	Moribund /Dead.	Number exposed.	Moribund /Dead.
(Meerut, U.P.)											
Arthala	1	10	10	10	10	10	10	10	10	20	1
Arthala	4	10	10	10	10	10	10	9	9	10	2
Arthala	3	10	10	10	10	10	10	10	9	10	..
Karara	4	25	25	25	25	25	25	26	26	25	..
Karara	3	10	10	20	20	20	20	30	20	20	..
	Total :	65	65	75	75	75	75	75	74	85	3
Corrected mortality rate (per cent).		100·0		100·0		100·0		98·6		C=3·5	
Gundlupet Taluk, Mysore State.	7	10	10	10	10	10	10	10	10	10	2
	3	20	20	20	20	20	20	20	17	20	..
	Total :	30	30	30	30	30	30	31	27	30	2
Corrected mortality rate (per cent).		100·0		100·0		100·0		86·2			
Baroda City, Gujarat State.	1	20	20	20	20	20	20	20	20	Same as for DDT (Table I)	
	2	10	10	10	10	10	10	10	8		
	Total :	30	30	30	30	30	30	30	28		
Corrected mortality rate (per cent).		100·0		100·0		100·0		93·3		C=0·0	

TABLE IV.

Average mortality rate (per cent) of body lice when continuously exposed to varying periods of time on four concentrations of DDT, gamma BHC and pyrethrins.

Locality.	Insecticide.	AVERAGE MORTALITY RATE (PER CENT) AT THE VARYING FOUR CONCENTRATIONS OF DIFFERENT INSECTICIDES*.	
		24 hours of continuous exposure*	12 hours of continuous exposure*
Meerut District, Uttar Pradesh.	DDT	98·3 (349)	85·82 (268)
	Gamma BHC	100·0 (315)	99·3 (275)
	Pyrethrins	99·6 (290)	98·3 (250)
Gundlupet, Mysore State.	DDT	86·5 (120)	..
	Gamma BHC	99·1 (120)	..
	Pyrethrins	96·5 (121)	..
Baroda City, Gujarat State	DDT	95·0 (120)	..
	Gamma BHC	100·0 (120)	..
	Pyrethrins	98·3 (120)	..

*Mortalities in body lice were recorded at the end of the specified periods of continuous exposure.

N.B: Figures in parenthesis are the total number of body lice exposed in the four concentrations of each insecticide tested.

DDT, gamma BHC and pyrethrins and average mortality rates of 98·3 (99·6)†, 100·0 (100·0)† and 99·6 (100·0)† per cent., respectively, were observed. The average mortality rates of the susceptible strain of body lice may range between 86-96, 92-99 and 94-100 per cent for DDT, gamma BHC and pyrethrins, respectively (values derived from Table V). The average mortality rates for DDT, gamma BHC and pyrethrins, estimated from the results of the tests carried out on body lice obtained from villages of Meerut District of Uttar Pradesh, lie either outside the upper limit of the respective range or well within it. From this it may be concluded that the strain tested was susceptible to all the three insecticides. Similarly, in the tests carried out in Gundlupet Taluk of Mysore State average mortality rates of 86·5 (93·9)†, 99·1 (100·0)† and 96·5 (100·0)† per cent were observed when 120, 120 and 121 body lice were exposed to the varying concentrations of DDT, gamma BHC and pyrethrins, respectively. The average mortality rates of 95·9 (100·0)†, 100·0 (100·0)† and 98·3 (100·0)† per cent in body lice collected from Baroda City of Gujarat State were obtained with DDT, gamma BHC and pyrethrins, respectively, when 120 insects were exposed to each of the three insecticides. These mortality rates, when referred to Table V, indicate that the strains of body lice from the above mentioned two localities were also susceptible to the insecticide.

† Figures in parenthesis were estimated from the data for the insecticidal concentrations falling within the respective range of concentrations specified in Table V. As the difference in the two sets of figures for each insecticide being not significant, Table V could be consulted for any one set of figures.

TABLE V.

An abridged table showing 99 per cent fiducial limits of the average mortality rates for different numbers of standard insects of the susceptible population of body lice, exposed to the specific concentrations of DDT, gamma BHC and pyrethrins following the W.H.O. technique.

[Based on the data reported by Wright and Brown (1957) and Narasimhan (1959)]

AVERAGE MORTALITY RATE AT ALL CONCENTRATIONS OF DDT*-92·0 PER CENT :				AVERAGE MORTALITY RATE AT ALL CONCENTRATIONS† OF GAMMA BHC-97·0 PER CENT.				AVERAGE MORTALITY RATE AT ALL CONCENTRATIONS‡ OF PYRETHRINS-97·0 PER CENT.			
DDT.				Gamma BHC.				Pyrethrins.			
n	Per cent mortality lower limit.	*n*	Per cent mortality upper limit.	*n*	Per cent mortality lower limit.	*n*	Per cent mortality upper limit.	*n*	Per cent mortality lower limit.	*n*	Per cent mortality upper limit.
20	66	20–50	100	20	75	20–60	100	20	78	20–600	100
30	71	60–80	99	30	80	80–200	99	30	88	1200	99
40	75	90–120	98	40	82	240–480	99	40	86	..	..
50	77	140–150	97	50	85	600–1200	98	50	87	..	..
60	78	160–360	96	60	86	..	..	60	88	..	..
80–100	81	400 600	95	80	87	..	..	80	90	..	..
120–140	82–83	1200	94	90–100	88	..	..	90–100	91	..	..
150–200	85	..	..	120–140	86–89	..	..	120–140	92	..	..
240–280	86	..	..	150–200	91	..	..	150–200	93	..	..
350–480	87	..	..	240–350	92	..	..	240–280	94	..	..
600–1200	88	..	..	360–600	93	..	..	350–480	95	..	..
..	..	..	..	1200	95	..	..	600–1200	96	..	..

99 per cent (at P=0·01) fiducial limits for varying numbers of insects exposed (*n*) to DDT, gamma BHC and pyrethrins.

Lower limit $= M - 3\sqrt{\frac{820{\cdot}7}{n}}$ and upper limit $= M + 3\sqrt{\frac{820{\cdot}7}{n}}$ where M = Angular value of average mortality rates of the susceptible population of body lice and $\frac{820{\cdot}7}{n} = V\phi$ where ϕ is measured in degrees. After calculating the fiducial limits, these values were converted to percentages with the help of Table XII of Fisher and Yates (1948). In certain cases where the upper limits were more than 90 in angular values, on practical ground the mortality rates for such cases were taken as 100 per cent. In all cases mortality rates were expressed to the nearest whole numbers.

If the number of lice exposed, in some experiments, are not found in the table, the fiducial limits may be estimated by interpolation.

N.B. This table can only be used for interpretation of the data obtained by the W.H.O. technique for measuring the susceptibility of body lice with the specified concentration (s) of the three insecticides.

* Specified concentrations (in per cent) of DDT : =0·1, 0·5, 1·0, 5·0
† Specified concentrations (in per cent) of gamma BHC : =0·25, 0·5
‡ Specified concentrations (in per cent) of pyrethrins : =0·02, 0·04

SUMMARY.

Insecticide susceptibility tests were carried out on body lice obtained from the villages of Meerut District of Uttar Pradesh, Gundlupet Taluk of Mysore State and Baroda City of Gujarat State, during the period March-September 1960, using the W.H.O. test kit for body lice. The strains of lice obtained in the three localities were found to be susceptible to DDT, gamma BHC and pyrethrins.

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Indian Journal of Malariology, **15**, 2, *June 1961*.

OBSERVATIONS ON MASS THERAPY WITH DIETHYL-CARBAMAZINE IN FILARIA CONTROL UNIT, FAIZABAD, UTTAR PRADESH.

BY

DIWAN CHAND,

M.V. SINGH

AND

V.K. BHASKAR.

(*Malaria and Filariasis Organization, Uttar Pradesh, Lucknow.*)

[September 2, 1960]

UNDER the auspices of the National Filaria Control Programme, a Filaria Control Unit was established in Faizabad in January, 1957, with the object of carrying out control measures in a compact block having a population of about 1,30,000 in the urban and 1,70,000 in rural areas of the district. An area having a population of about 30,000 was selected as "Special Study Area" for the purpose of assessment of results (Map 1). This paper summarises the parasitological findings after the completion of mass therapy with diethylcarbamazine which is one of the control measures.

Pre-control survey.—A preliminary survey was carried out in the Special Study Area. The infection, disease and endemicity rates are depicted in Table I according to the various age-groups. The infection is due to *W. bancrofti*.

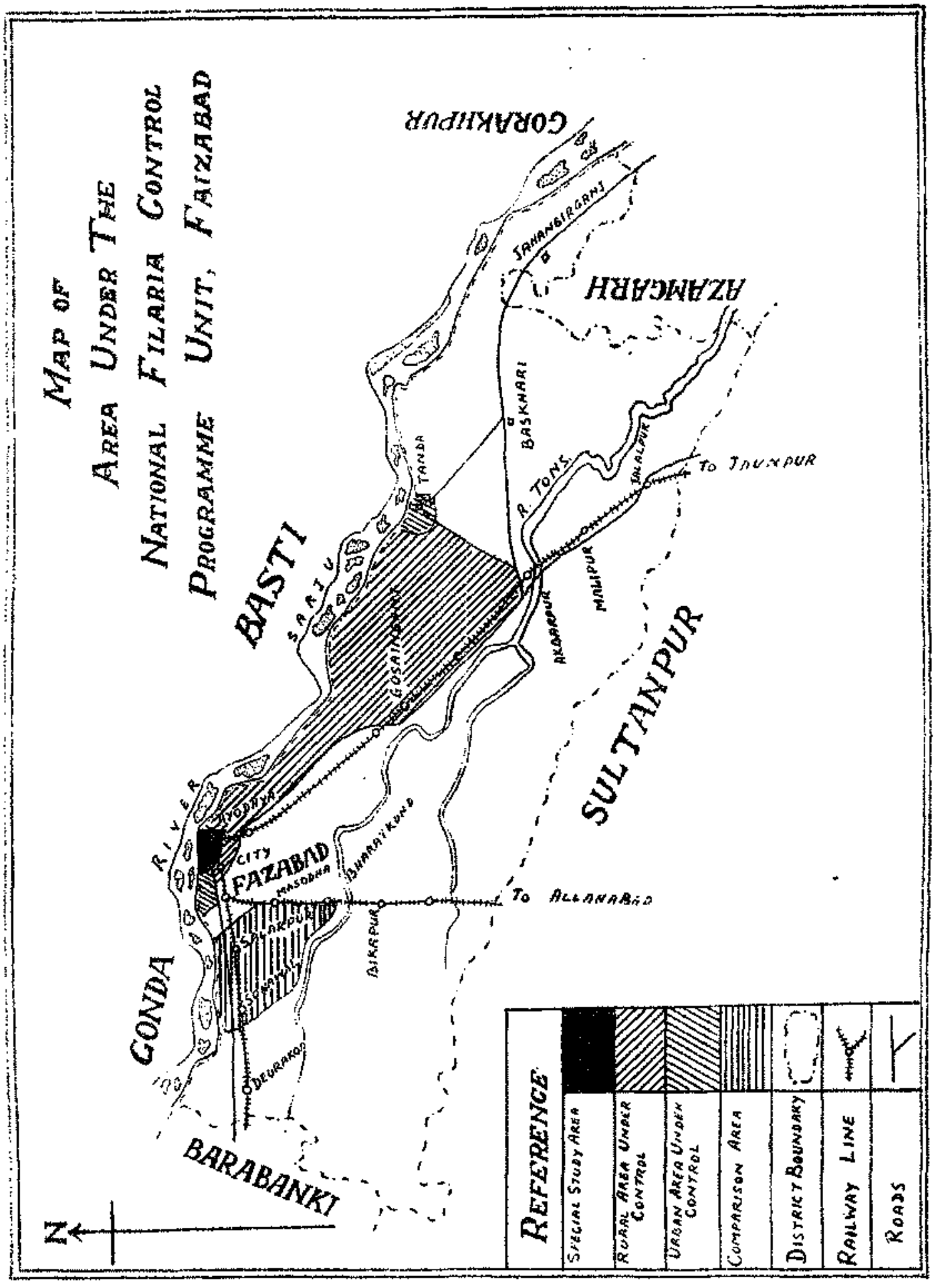
TABLE I.

Infection, disease, endemicity rates and average infection in the Special Study Area according to the age-group before starting mass therapy.

Age-group in years.	Total examined.	Infection (per cent).	Disease (per cent).	Endemicity (per cent).	Average infestation per 20 c.mm. of blood.
0– 5	318	4·4	0·31	4·6	16
6–10	545	9·3	3·6	12·2	14
11–20	1,113	12·3	5·3	16·5	15
21–30	1,113	13·8	5·3	18·2	16
31–40	659	14·3	7·8	20·1	14
41–50	410	14·0	8·4	21·0	17
Above 50	392	14·3	4·3	17·0	15
	4,550	12·5	5·3	16·6	16

ENTOMOLOGICAL OBSERVATIONS.

The entomological observations were carried out from January to December, 1958. The details of the dissection of *C. fatigans* are shown in Table II.


Map of
Area Under The
National Filaria Control
Programme Unit, Faizabad
BASTI
GORAKHPUR
AZAMGARH
SULTANPUR
GONDA
BARABANKI
River
Tanda
Baskhari
R. Tons
Akbarpur
Malipur
To Jaunpur
To Allahabad
City
Masodha
Bharatkund
Bikapur
N
Reference
Special Study Area
Rural Area Under Control
Urban Area Under Control
Comparison Area
District Boundary
Railway Line
Roads

Map I.

TABLE II.

Result of dissection of C. fatigans *from the Special Study Area prior to drug administration.*

Area.	Number of *C. fatigans* dissected.	Number found positive.	STAGES OF DEVELOPMENT OF LARVA :			
			I	II	III	IV
Special Study	2,402	68	..	12	35	21

MASS THERAPY.

Mass therapy with Diethylcarbamazine was started in the month of May, 1958, and was completed in the last week of December, 1958, taking about 8 months to cover the population.

Drug distributing teams were organized and each team consisted of two Field Workers. The teams were supplied with the drugs for distribution, and the enumeration registers bearing the details of the population. The drug was distributed to all the available persons, with the exception of children below two years, pregnant women and persons with chronic ailments, for five consecutive days in the following doses :—

Above 18 years	2 tablets (100 mg. each)
12 to 18 years	1·5 tablets
6 to 12 years	1 tablet
2 to 6 years	0·5 tablet

It was ensured that every person swallowed the drug with the help of water in the presence of distributors. The drug distributing teams worked directly under the supervision of medical men.

Publicity and press propaganda was carried out simultaneously. The details of mass therapy are given in Table III.

Out of a population of 3,00,093 enumerated, 23,543 or 7·8 per cent were either exempted or were absent during the therapy. Thus total persons available for mass therapy were 2,76,550, representing 92·2 per cent of the population under control. Out of these persons available for mass therapy, 66·3 per cent took the drug for varying number of days. The percentage of persons taking the drug for all the 5 days was 61·1.

REACTIONS OBSERVED IN THE COURSE OF MASS THERAPY.

Various reactions, such as headache, body pain, nausea, fever (varying from 99° to 102°F.), diarrhoea, constipation and lymphangitis were noted in a population of 21,301, representing 10·6 per cent of those who took the drug. Maximum reactions (10·69 per cent) were noted on the second and third day of therapy. Such reactions were symptomatically treated by the teams under the supervision of medical men. Table IV shows various reactions noted during mass therapy.

TABLE III.

Details of mass therapy.

Age-group (in years).	Population enumerated.	Number of persons naturally exempted (including absentees) from taking the drug.	Persons avilable for mass therapy.	Number of persons who took the drug.	Number of persons who refused the drug.	NUMBER OF PERSONS WHO RECEIVED THE DRUG FOR :					Number of persons who discontinued.
						5 days.	4 days.	3 days.	2 days.	1 day.	
Above 18	1,76,514	15,085	1,61,429	1,18,449 (67·6)	42,980 (26·6)	1,09,319 (62·4)	3,225 (1·8)	2,603 (1·4)	1,784 (1·41)	1,525 (0·89)	12,666
12–18	31,692	2,407	29,225	21,652 (68·3)	7,573 (25·5)	19,826 (62·2)	618 (1·9)	516 (1·6)	348 (1·09)	344 (1·08)	3,090
6–12	46,340	3,012	43,328	32,813 (70·8)	10,515 (24·3)	30,277 (65·3)	925 (1·9)	715 (1·5)	494 (1·6)	402 (0·8)	4,154
2–6	45,547	2,979	42,568	26,239 (57·6)	16,329 (38·3)	24,085 (52·8)	670 (1·4)	673 (1·4)	441 (0·9)	370 (0·8)	3,346
Total	3,00,093	23,543 (7·8)	2,76,550 (92·2)	1,99,153 (66·3)	77,397 (28·0)	1,83,500 (61·1)	5,438 (1·1)	4,507 (1·0)	3,067 (1·02)	2,641 (0·9)	23,256

The figures in parenthesis give the percentage.

TABLE IV.

Persons showing reactions in mass therapy.

Total number of persons who took the drug.	Diarrhoea.	Fever.	Body pain.	Headache.	Constipation.
1,99,153	6,174 (3·1)	8,143 (4·09)	3,541 (1·78)	3,375 (1·70)	68 (0·04)

Figures in brackets indicate percentage.

POST-MASS THERAPY SURVEY.

Follow-up survey was carried out in the Special Study Area from January, 1959, in order to assess the immediate result of the mass therapy. Only 2,936 persons were common to both the surveys. The results of the follow-up survey are given in Table V.

Table VI shows the percentage decrease or increase in the infection rate in different age-groups following the mass therapy as well as changes in the average infestation.

DISCUSSION.

The experience gained in carrying out the mass therapy amongst a large population has shown that in the filarious areas majority of the population does cooperate towards the success of such operations provided the publicity is adequate. However, quite a large population (representing 33·7 per cent of the population) who may be harbouring infection, could not be covered by the mass therapy because they were either exempted or they refused to take the drug. Even amongst the persons who were available for mass therapy, some did not take the drug for all the 5 days.

The percentage of reactions, which works out to about 10·6 per cent, also limits the use of drug. The percentage of reactions is approximately the same as that of the infection rate which supports the close relationship between the drug reactions and presence of microfilaria in the blood.

The reduction in the infection rate (45·60 per cent *vide* Table VI) is not proportional to the percentage of the persons taking the drug for all the 5 days (61·1 per cent). It is further observed that 54·4 per cent of the infected persons are still left positive for microfilariae. In contrast, the reduction in average infestation by 62·50 per cent is noteworthy. The combined effect of the reduction in infection rate and microfilarial infestation on the dynamics of the transmission of filariasis and its effect on the community, are being studied.

SUMMARY.

1. The results of pre-control survey have shown that the area selected for control operation had moderate degree of endemicity, being 16·6 per cent.

2. The type of infection present in Faizabad District is *W. bancrofti* and the vector is *C. fatigans.*

3. The percentage of persons who took the drug for varying number of days is 66·3 per cent, while who took the drug for all the 5 days is 61·1 per cent.

4. The post-mass therapy survey was carried out which shows that the infection rate has been reduced from 12·5 to 6·8 per cent while the average infestation has been reduced from 16 to 6 per 20 c.mm. of blood in the Special Study Area.

TABLE V.

Infection, disease, endemicity rate and the average infestation amongst the persons in the Special Study Area examined before and after therapy.

Age-group (in years).	Number of persons examined.	Infection rate, per cent.	Disease rate, per cent.	Endemicity rate, per cent.	Average infestation per 20 c.mm. of blood.
0– 5	160	1·9	1·9	3·8	15
6–10	300	4·3	0·3	4·3	4
11–20	705	5·3	2·8	7·5	6
21–30	811	7·1	5·2	11·6	6
31–40	410	9·8	10·7	18·5	8
41–50	300	8·3	10·0	17·6	3
Above 50	250	9·6	7·2	16·0	7
Total	2,936	6·8	5·4	11·4	6

TABLE VI.

Infection rate, and average infestations in pre- and post-mass therapy surveys (all persons examined in the post-therapy were also examined in the pre-therapy survey).

Age-group (in years).	Total examined (initial survey).	Infection rate (initial survey).	Average infestation per 20 c.mm. of blood (initial survey).	Total examined (post-therapy survey).	Infection rate (post-therapy survey).	Average infestation per 20 c.mm. of blood (post-therapy survey).	Percentage decrease in infection rate.	Percentage decrease in average infestation.
0— 5	318	4·4	16	160	1·9	15	56·82	6·25
6—10	545	9·3	14	300	4·3	4	53·76	71·43
11—20	1,113	12·3	15	705	5·3	6	156·91	60·00
21—30	1,113	13·8	16	811	7·1	6	41·47	62·50
31—40	659	14·3	14	410	9·8	8	31·37	42·85
41—50	410	14·9	17	300	8·3	3	44·29	82·35
Above 50	392	14·3	15	250	9·6	7	32·87	53·33
Total	4,550	12·5	16	2,936	6·8	6	45·60	62·50

ACKNOWLEDGEMENT.

The authors are extremely grateful to Shri S.P. Srivastava, Assistant Entomologist, and the Filaria Inspectors and other staff of the Filaria Control Unit, Faizabad, for their wholehearted co-operation.

Indian Journal of Malariology, **15**, 2, *June 1961*.

NEW METHODS FOR THE MAINTENANCE OF A LABORATORY COLONY OF BED-BUG, *CIMEX HEMIPTERUS* FABRICIUS, WITH OBSERVATIONS ON ITS BIOLOGY.

BY

B.L. WATTAL

AND

N.L. KALRA.

(*Malaria Institute of India, Delhi.*)

[September 2, 1960.]

INTRODUCTION.

THE Indian bed-bug, *Cimex hemipterus* Fabricius (*C. rotundatus* Signoret), which is also referred to as tropical and sub-tropical bed-bug, is widely distributed in the plains of India. In the hilly regions, however, *C. lectularius* Linn. is the common species. Although bed-bugs have always been present in rural communities in India, their populations seemed to have been considerably reduced following the wide-scale use of residual insecticides under the National Malaria Control (now — Eradication) Programme. However, the relief from bed-bugs in certain areas, particularly in humid and warm districts of Bombay and Orissa States, seems to have been of a limited duration. The impression seems to be that bed-bug populations have increased over the populations prevalent in the pre-DDT era. The causes attributed to this increase were that the bed-bugs have developed resistance to insecticide (Rao and Halgeri, 1956) and that the residual insecticides have interfered with the role of natural predators/parasites in regulating the population of this insect (Wattal and Kalra, 1960).

A colony of the bed-bugs was raised at the Malaria Institute of India, Delhi, for insecticidal and biological studies. The methods adopted for successful maintenance of the colony, and the observations made on some aspects of the biology of *C. hemipterus*, are reported in the present paper.

LABORATORY REARING.

Among others, the techniques for laboratory rearing of bed-bugs have been described by Patton (1908), Girault (1910), Rendtorff (1938), Woodbury and Barnhart (1939), Davis (1956) and Adkins and Arant (1959). The methods followed by these workers involved maintenance of bed-bugs in glass containers, varying in size from specimen tubes of 3 inch × 1 inch size and petridishes to wide-mouth glass jars. In each case, pieces of paper (paper towelling or blotting paper) were provided for oviposition and shelter. The containers were provided with cotton wool plugs, fine cloth, 60-mesh gandy, or nylon net covers. Adequate precautions were taken to prevent bed-bugs from getting out of the

feeding or rearing chambers, which in some cases were separate. The bugs were fed by placing the mouth of the container directly on the host. The host varied from man, guinea-pig, to rabbit. The bugs were fed either daily or on alternate days or once a week*.

In the Malaria Institute of India, Delhi, attempts to colonise the bed-bug in the laboratory were made during the autumn of 1957 whan adult bed-bugs were obtained from the bedsteads used in the Police Barracks, Delhi. However, the colony got established only during early 1958 after more bugs from the Police Barracks were obtained.

The bed-bug colony was housed since its inception till January, 1960, in a basement room of 12 feet×12 feet with 8 feet height. The room had only one entrance and was provided with an exhaust fan and a ceiling fan. The temperature was controlled by putting electric heaters during the winter months and providing ice slabs during the summer. During the dry months, humidity was increased by spreading water-soaked gunny bags directly under the ceiling fan. The average temperature varied between 75° and 84°F. and the relative humidity between 74 and 85 per cent.

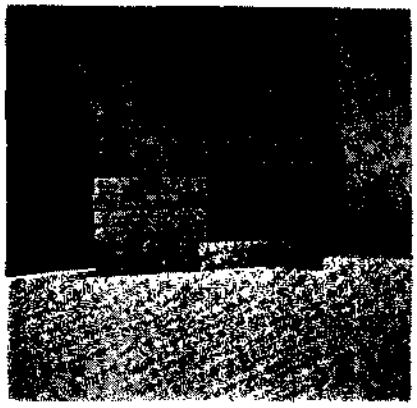
To start with, conventional methods for rearing the bed-bugs in specimen tubes (3 inch × 1 inch) and round glass jars (height 8 inch, diameter 6 inch) were followed. In case of bugs kept in specimen tubes, the feeding became cumbersome as each tube had to be handled separately while feeding on the shaven back of a rabbit. Only a few specimens could be kept in one tube and the supporting piece of coarse paper (folded alternately 2–4 times) had repeatedly to be changed due to faecal matter being deposited on it. Also, the first- and second-stage nymphs were sometimes lost in the process of feeding. The glass jar method was slightly better, in that 50 to 100 adults could be kept in each jar. Grease was applied to the rims of the jars to prevent bugs (all stages) from escaping. The specimen tubes were provided with cotton wool plugs and the jars were covered with glass panels supported at one end with plasticin. The bugs in the jars were fed on alternate days on hand by inserting the same between the coarse papers. Later a rat, in a wire net (9 mesh to an inch) sparrow-cage of 6×3×4 inch dimensions, was confined at each night in the jar. However, with this method also it became difficult to handle the bugs at will and, in spite of water barriers provided underneath the jars, some of the bugs did escape while handling. Eventually the following two methods were adopted successfully for mass rearing of the bed-bugs in the laboratory.

(A) WOODEN TRAP GLASS JAR METHOD (Plate I).

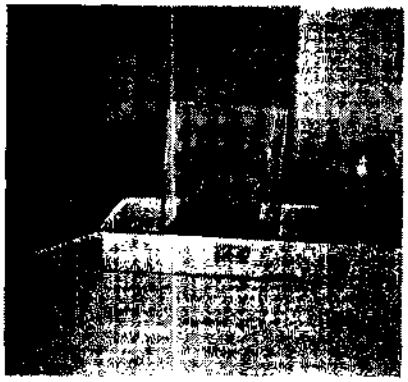
Two pieces of unseasoned wood (packing wood) of 6 inch length, 1½ inch width and 3/8 inch thickness are put together by a samll hinge on one side and a catch on the other. Each piece is provided with a central groove of 115 mm. (4½ inch)

* For review of these methods, *see* Adkins and Arant (1959).

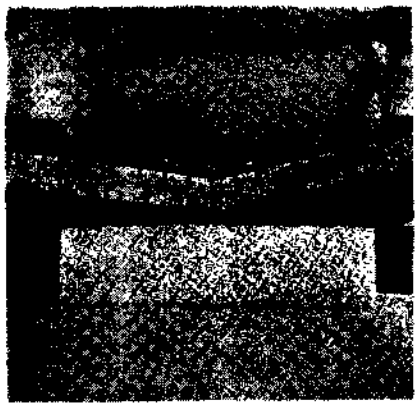
PLATE I.



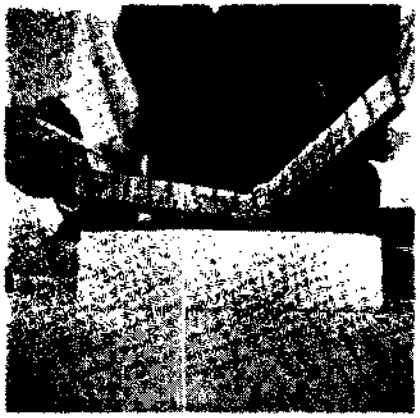
(a)



(b)



(c)



(d)

Laboratory testing of *Culex fatigans* by wooden trap-glass jar method.

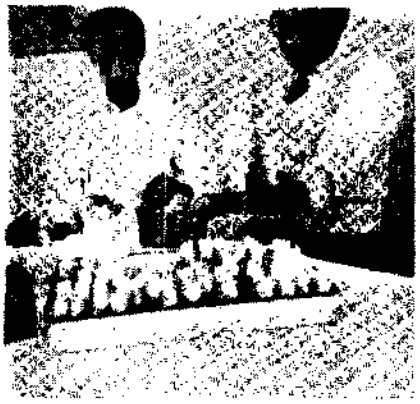
(a) Wooden traps. (b) Wooden traps with bed bugs in a glass jar. The glass jar is in a tray of water. A caged albino rat is within the colony jar. (c and d) Showing opened wooden trap for the removal of adult bugs.

PLATE II-A.

(*a*) (*b*)

PLATE II-B.

(*a*) (*b*)



(*c*)

PLATE II-A. Field collection of *Cimex hemipterus*.

(*a*) Getting the bugs out from a cot. (*b*) Wooden block showing holes for capture of bed-bugs.

PLATE II-B. (*a, b* and *c*) Feeding bugs on different hosts.

length, 8 mm. width and 3 mm. depth (approximately). Besides, six to seven side-grooves of about 15 mm. length, 3 mm. width and 2½ mm. depth are made, alternating on either side of the central groove, connecting the central groove with the rim of the trap. The two sides of the trap, when thus apposed, have 6 holes on one linear side and 7 on the other. Each hole has a diameter of about 5 mm. This trap thus provides entrance for the bugs through the holes and the bugs can rest in the side groove and mate in the central groove. No light can possibly pass within the trap as the side channels are not in a straight line.

Twelve such traps are placed one upon the other, in rectangular glass jars of 8 inch × 6 inch × 12 inch (length × breadth × height) size and fifth stage nymphs, at the rate of 20 per trap, are introduced in such a jar. The bugs immediately enter the traps.

For feeding, each day a caged rat, as mentioned above, is introduced in the evening and removed in the morning. The traps are subsequently inspected on each day for fifth-stage nymhps which are removed with the help of a camel hair brush to another such jar and eventually used in experiments. The open jars are placed in enamel trays containing water. No other precaution for the escape of bugs is necessary as none seems to leave the traps. The wooden traps are cleaned every 4 to 5 months after the removal of eggs with a hair brush. The colony can be augmented by increasing the number of jars as and when required.

(*B*) MOSQUITO COLONY CAGE METHOD.

Bed-bugs are also being reared in this laboratory in a wooden mosquito cage of 2 feet × 2 feet × 2 feet size, described by Russell and Mohan (1939). The front of the cage is provided with a glass plate of 1·4 foot height below which are provided two open windows (8 inch × 10 inch) with muslin sleeves. The cage is plastered with mud from inside and is kept in a metal tray containing water. Folded pieces of rough paper are introduced within the cage to provide for oviposition and resting of the bugs. A rabbit cage is permanently kept in the cage and a rabbit is introduced each evening and removed in the following morning. In this method, a constant supply of bed-bugs is ensured and the cage can hold a large population. However, handling of bugs is difficult and there is the possibility of nymphs escaping on the body of the rabbit. The rabbit cage is removed every 3 months for cleaning.

The papers from the cage are periodically removed and burnt when new set of papers is introduced.

FIELD COLLECTION (Plate II-A).

(1) In the Indian villages the furniture, commonly used, are cots which are used for both resting during the day and sleeping at night. Bed-bugs can be obtained from cots by spreading a white sheet of cloth on the ground, placing a cot over it and beating the cot with a stick. The bugs fall on the sheet of cloth and can be picked by hand or brush. Adult bugs can also be picked by gently

holding them with forceps. Bugs, hiding in the wooden frame of the cot, can be dislocated by tapping the frame with the stick, and picked as mentioned earlier.

(2) More convenient method, particularly in the semi-urbanised localities, is to keep at night an unseasoned wood block of 12 inch × 1½ inch × 3 inch (length × breadth × height) between the strings of the bedsteads. In the wooden block are bored small holes (50 on the upper and lower sides and 25 on either side) of 5 mm. diameter and 10 mm. depth. Next day or after a few days the bed-bugs can be collected from the wooden block by gently tapping it on a hard surface.

(3) The small wooden traps, used for rearing the bed-bugs, can also be used to trap these insects from the bedsteads or occupied quarters of a dwelling. These traps are placed in the corners of a room at night and removed early in the morning or after a few days. Such traps are also useful in collecting bed-bugs from bed-rolls and other clothings by keeping them in such articles for a few days and then removing them for inspection.

OBSERVATIONS ON BIOLOGY.

Considerable published data are available on the different aspects of the biology of bed-bug (Patton and Cragg, 1913; Cragg, 1923; Herms, 1943; Matheson, 1950; Busvine 1951; Roy and Brown, 1954).

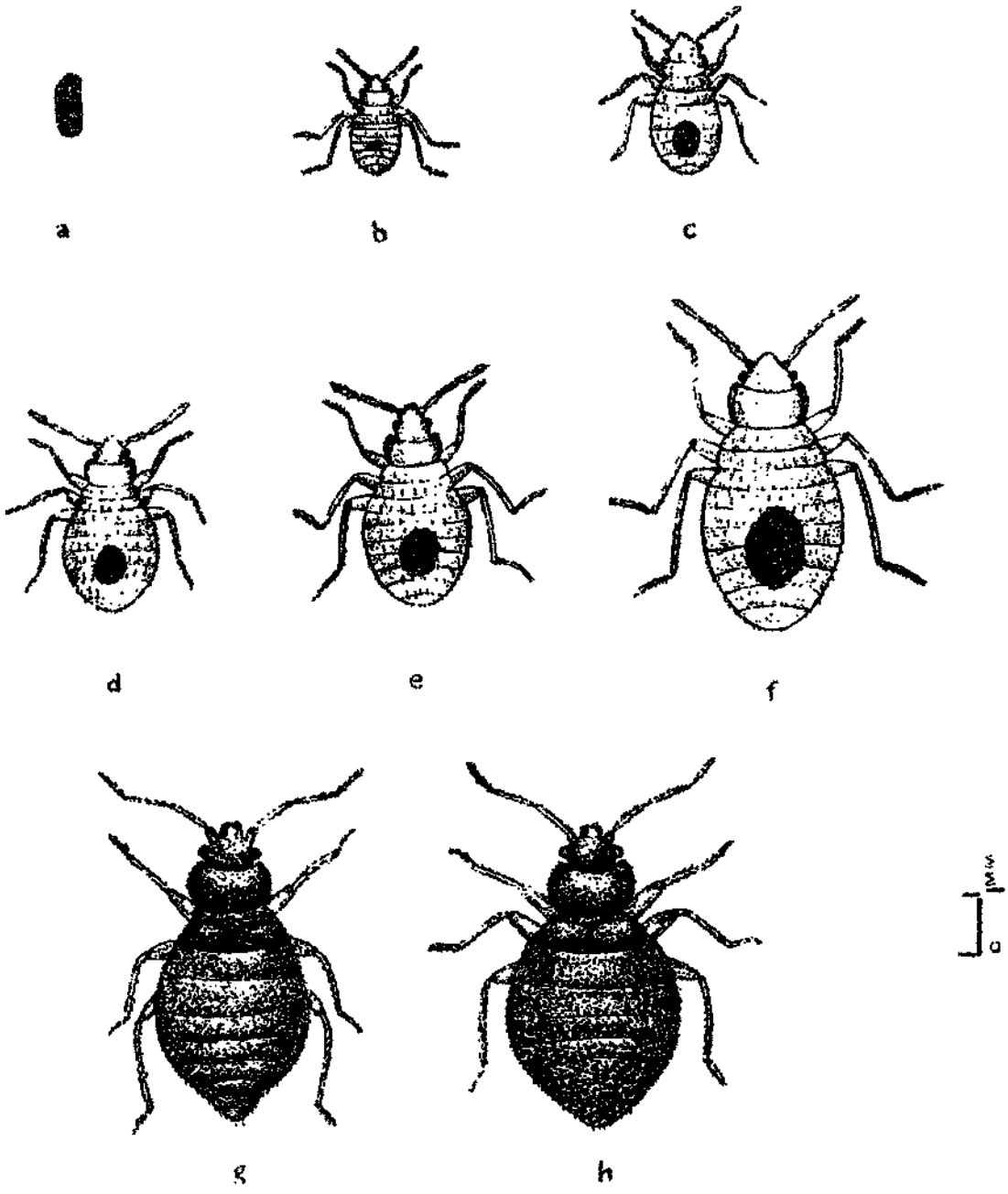
However, most of the data are conflicting and pertain largely to *Cimex lectularius*. There are very few observations available on the quantitative bionomics of *Cimex hemipterus*. Therefore during 1958-59, a series of observations were made on the common species of Indian bed-bug under the laboratory conditions described earlier.

In this series of experiments, the bugs were confined in glass specimen tubes (3 inch × 1 inch) plugged with cotton wool. A rectangular piece of filter paper with 4 to 5 alternate folds was introduced inside the tube to provide shelter and oviposition site. At the time of feeding, the paper was brought in contact with the skin of the host by placing the glass tube on to the surface of the host. The tubes were kept in photographic enamel dishes inside the insectary.

(i) LIFE HISTORY, (Plate III).

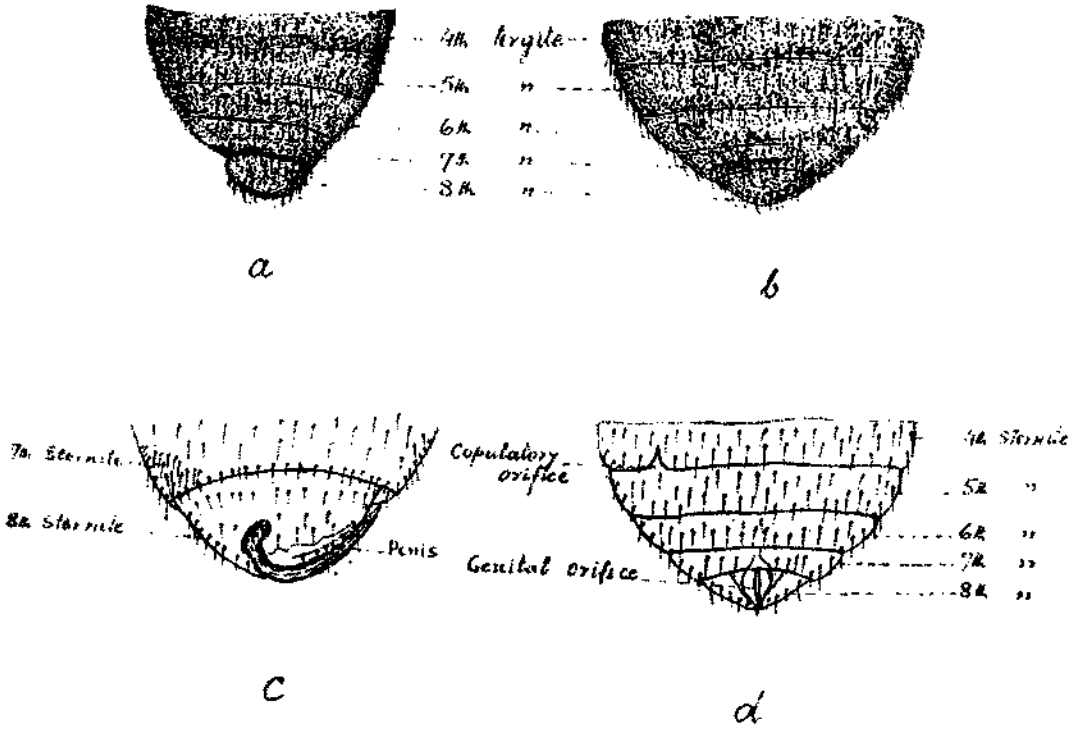
The bed-bug is characterised by paurometabolous development. There is an egg stage, 5 nymphal stages and the adult stage in the life cycle of *C. hemipterus* (as also in other members of the family *Cimicidae*). The viable eggs are creamy white, large and visible to the naked eye, whereas the imperfect (non-viable) eggs are yellowish, smaller and slightly crumpled. The egg has been observed to hatch after 3 days. In one-day-old eggs the eyes of the developing nymph appears as a red dot towards the operculum. The first stage nymph, as also the subsequent stages and the adult, take a blood meal after 24 hours after hatching/moulting. The first and the fourth nymphal stages last 4 days each, whereas the second and the third stages last for 3 days each. The fifth nymphal stage lasts for 6 to 7 days.

PLATE III.



Life history of *Cimex hemipterus*.
(*a*) Egg; (*b*—*f*) I to V nymphal stages; (*g*) Male; (*h*) Female.

PLATE IV.



Cimex hemipterus. (*a*, *b*) Dorsal aspect of the terminal segments of male (*a*) and female (*b*). (*c*, *d*) Ventral aspect of the terminal segments of male (*c*) and female (*d*).

The whole life cycle from egg to adult in case of either sex, under the laboratory conditions, is completed in 23 to 24 days. Each nymphal stage was observed to moult after a single blood feed taken to repletion. In case the bug got disturbed while feeding, it would take more than one blood meal for moulting and the life cycle consequently got delayed.

Table I indicates the average time taken for completion of the life cycle, the length and breadth of all stages in the life history and the weight of each stage immediately after hatching/moulting. As expected, there seems to be a gradual increase in the size and weight of the nymphs till the adult stage is reached. The first nymphal stage, however, weighs less than the egg. This difference is primarily due to the weight of the egg-shell. The males are slightly smaller than the females and also weigh slightly less. With experience, it was possible to differentiate between the various stages of the bed-bug with the naked eye on the basis of this size and the colour. The first-stage nymph is pale and the course of the blood can be followed within the body. The subsequent nymphs are light red to deep red in colour, whereas the adult is dark reddish-brown. The male is slightly smaller than the female. Males can be differentiated from the females by the curved penis observed on the ventral side and by the difference in the anal segment dorsally (Plate IV *a*). The anal segment in females runs continuously in the preceding segment, whereas in males it forms an obtuse angle with the preceding segment (Plate IV *b*).

TABLE I.

Average time taken for completion of the life cycle of Cimex hemipterus *(with size and weight of each stage) under laboratory conditions.*

Stage in life cycle.	Hatching*/ moulting† period (In days).	Size‡ (mm.):		Weight‡ (mg.)
		Length.	Breadth.	
Egg	3	1·01	0·38	0·19
(Nymphs)				
Stage first	4	1·29	0·6	0·1
Stage second	3	1·89	0·92	0·34
Stage third	3	2·09	1·26	0·76
Stage fourth	4	2·78	1·52	1·1
Stage fifth	6-7§ (6·4)§§	4·04	1·98	2·0
(Adults)				
Male	..	4·28	2·25	4·1
Female	..	4·5	2·38	4·32
Total	23-24			

* First nymphal stage. † Subsequent nymphal stages and adult. ‡ Unfed.
§ Range. §§ Average.

(ii) FEEDING AND MATING.

All the five nymphal stages and both sexes of adult bug require a blood meal, human or animal, for survival. In fact, as mentioned earlier, the moulting from one stage to another is dependent on the availability of a blood meal and moulting takes place only after the blood meal has been taken to repletion. Table II indicates the time taken by each stage for a full blood-meal and the amount (mg.) of blood ingested per meal. It was observed that the adults take less time in feeding than the nympal stages. The amount of blood ingested by adult was less than the preceding nymphal stage. The males ingest about half the amount of blood taken by the females. All the nymphs that eventually moulted into males or females, did not show any difference in the amount of blood ingested or the time taken for a blood-meal. That more time was taken in feeding in the nymphal stages as compared to the amount of blood ingested, needs elucidation by undertaking further experiments. However, the fact that more blood is ingested in nymphal stages as compared to their size, is probably due to the demands of rapid growth of various tissues, particularly in the last nymphal stage.

TABLE II.

Average time and the amount of blood taken per feed by Cimex hemipterus *under laboratory conditions.*

Stage in life cycle.	Time taken (Minutes).	Amount of blood taken per feed (mg.).
Nymphs		
Stage first	3·5—4* (3·7)†	0·4—0·6* (0·5)†
Stage second	5—6 (5·4)	1·2—1·4 (1·3)
Stage third	7—8 (7·2)	3—4 (3·1)
Stage fourth	9—10 (9·6)	3·6—4·8 (4·5)
Stage fifth	9—10 (9·6)	5·8—7·6 (6·8)
Adults		
Male	3	2·4—3·0 (2·6)
Female	4—5·5 (4·7)	5·2—6·2 (5·8)

* Range. † Average.

The copulation takes place immediately before or after feeding. It was observed that when males and females are isolated 24 hours after the last moult, the males immediately seek out the females and copulate with them. The copulation lasts for about 20 seconds. However, where the female is not stationary, the whole act may take up to 100 seconds before the male disengages. The first batch of eggs is laid 3 days after the blood meal. Mating was found to be essential for oviposition.

(iii) BIOTIC POTENTIAL AND LONGEVITY.

Observations on the biotic potential and the longevity of female *C. hemipterus* were made under three conditions :—

(*a*) When a single female was confined with a single male.
(*b*) When a single female was impregnated only once and then isolated.
(*c*) When a single female was isolated before its being impregnated.

The results of these observations are summarised in Table III. It was observed that frequent mating results in bed-bug laying more eggs than when impregnated only once. Also, the imperfect eggs, which are laid towards the last days of the egg-bearing period, were considerably few in the case of females frequently impregnated, as compared to the females mated only once. The females that were not mated did not yield any eggs.

TABLE III.

Biotic potential and longevity of adult Cimex hemipterus *under laboratory conditions**.

Serial number.	Mating.	Eggs per female.	Per cent imperfect eggs.	Longevity (days).	
				Female.	Male.
1	Frequent	92—125† (103·3)‡	1·3	40—46† (41·3)‡	115—136† (119)‡
2	Once	78—87 (83·3)	9·4	50—64 (56·6)	..
3	Nil	..	..	169—215 (197·6)	..

* The bugs in these observations were given human blood meals twice a week.
† Range. ‡ Average.

The average total number of eggs laid per female, when frequently mated, was much higher (103·3) than the eggs laid by females mated only once. However, under the two conditions, there was not much difference in the number of eggs laid by a female bug at each oviposition. The eggs laid in the case of frequently mated bugs varied from 1 to 10, whereas in the case of bugs mated only once it ranged from 1 to 9. Whereas the eggs were laid till the observation specimens died in case of the females frequently mated, the oviposition stopped after 29 to 36 days in case of the females mated only once, although the females lived up to 64 days. Therefore, it seems that a single fertilization may not be providing enough sperms or the stimulus for prolonged oviposition.

In case of the observation where males and females were kept together, it was found that males lived much longer than the females (Table III). However, the females showed different longevity under varied biological conditions. The unfertilized females lived much longer (159 to 215 days) as compared to the females once impregnated (50 to 64 days) and those impregnated frequently (40 to 46 days). Apparently, longevity of the female is dependent on the number of ovipositions under-gone by it.

(iv) SEX RATIO AT THE LAST MOULT.

Out of 100 nymphs kept for observation for the sex ratio in the adult, only 33 moulted into females and the remaining 67 turned out to be males.

(v) HOST PREFERENCE (Plate II *A*).

Bed-bugs are known to feed both on human and animal blood. During the observations reported here, experiments were set to determine the host preference, if any, of this pest by using easily available sources of blood. The host preference was to be determined by the number of eggs laid and the longevity of the female bug.

Single male and female *C. hemipterus* were isolated in specimen tubes, as described earlier, and allowed to feed on alternate days on the specified hosts. The observations were made simultaneously using man, bulbul*, chick, rabbit and albino rat as the blood source. Eggs were laid after three days after the first blood meal. Since the females could mate frequently, the eggs were laid every day till the females survived.

Table IV indicates the longevity of females and the number of eggs laid in their life time on different hosts. Human blood seemed to yield the highest number of eggs (104·3), followed by chick (90·7), rabbit (86·3), rat (83·7) and bulbul (59·0). The longevity of females maintained on different blood sources, did not show much variation.

TABLE IV.

Longevity of females and the number of eggs laid by Cimex hemipterus *when fed on different hosts.*

Serial Number.	Source of blood.	Eggs laid.	Longevity.	Order of host preference.
1	Human	99—108* (104·3)†	42—45 (44·0)	1
2	Bulbul	56—62 (59·0)	36—44 (40·0)	5
3	Chick	80—102 (90·7)	38—45 (41·7)	2
4	Rabbit	78—98 (86·3)	35—41 (37·3)	3
5	Rat	76—92 (83·7)	36—47 (41·0)	4

*Range. †Host preference.

The life range reported in Table IV is very close to that reported in Table III. For maintenance of regular colonies, however, rabbit and rat can be used to advantage because of relatively good number of eggs obtained, and their being readily available and easy to handle.

* *Molpastes haemorrhous haemorrhous.*

DIFFERENTIATION BETWEEN *CIMEX LECTULARIUS* AND *C. HEMIPTERUS*

(Plate V).

In India, in the hilly regions, both *Cimex lectularius* and *C. hemipterus* are encountered. These two species can be differentiated as follows :—

C. lectularius.—Sides of pronotum widely dilated, broader than width of an eye and densely fringed with curved hairs. Apical margins of hemelytra nearly straight, rounded towards inner angles.

C. hemipterus.—Sides of pronotum not widely dilated and not reflexed, fringed with sparse, nearly straight hairs. Hemelytra with apical margins distinctly rounded.

SUMMARY.

Two simple and convenient methods for the laboratory rearing of common Indian bed-bug, *Cimex hemipterus*, have been described. In the first method traps (6 inch × 1½ inch × 3/8 inch) of unseasoned wood, with side holes and inside grooves, are placed in a rectangular glass jar in which a caged albino rat, introduced every night, provides the blood meal. The bugs rest and mate inside the dark channels of the trap. The eggs are also laid in these grooves. This method provides for the nymphs and adults of known age and the number of such colonies can be increased as and when desired. The bugs are easy to handle and do not escape. The second method described is for maintenance of stock colonies of bed-bugs. The bugs are raised in a conventional wooden mosquito colony cage (2 feet × 2 feet × 2 feet) in which are placed a series of rough folded paper pieces. A caged rabbit, introduced each night, provides the blood meal.

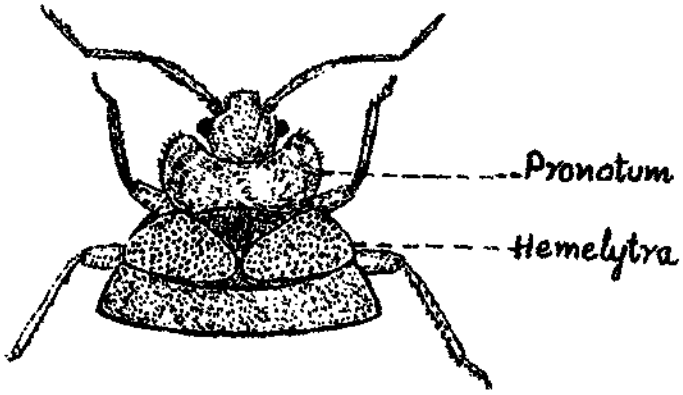
Methods of field collection of bed-bugs have been mentioned. It has been observed that the wooden traps, used in colony cages, can entrap bugs under natural conditions also.

Since very little published work is available on the quantitative bionomics of the Indian bed-bug, observations were made on the following aspects of the biology of *Cimex hemipterus* under the laboratory conditions. It was observed that the bugs complete their life cycle in 23-24 days. Each nymphal stage as well as the adult feeds after 24 hours after hatching/moulting. A single blood meal, taken to repletion, is required for moulting. Males are smaller than females and also weigh less. Adults were observed to take less time in feeding than the nymphal stages. The amount of blood ingested by adults was found to be less than the fifth nymphal stage. Males were observed to ingest about half the amount of blood taken by the females.

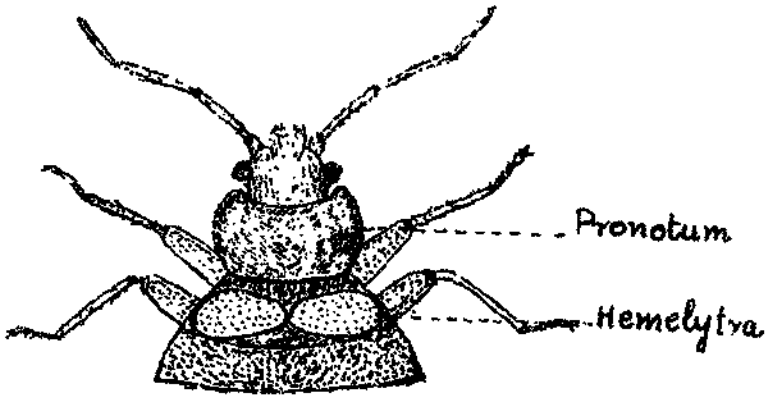
Copulation was observed to take place immediately before or after the blood meal and lasted for about 20 to 100 seconds.

The female bed-bug may lay one to ten eggs per oviposition. Frequent mating and blood feeds were found to yield the maximum number of eggs. The

Plate V.



Cimex lectularius



Cimex hemipterus

Head and thorax of *Cimex lectularius* and *C. hemipterus*.

females, which mated only once, stopped egg-laying after 36 days of their life, although they lived up to 64 days. Also single mating resulted in a higher proportion of imperfect (non-viable) eggs. Females not allowed to mate, did not lay any eggs, and lived up to 215 days. Frequently mated females lived less (up to 46 days) than those mated once (up to 64 days). Males lived much longer than females.

The ratio of males to females at the time of hatching was found to be 2 : 1.

Bed-bugs, when maintained on man and different animals, were found to lay more eggs when reared on man; followed by chick, rabbit, rat and bulbul.

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Review.

MALARIA ERADICATION—A BRIEF DISCUSSION OF ITS PRINCIPLES, PROCEDURES AND PROBLEMS. By V. Venkat Rao. (42 pages. *Price* Re. 1·00, Foreign 2 shillings). (Available from the author at "White House", Daba Gardens, Visakhapatnam-4, India).

The mode of transmission of malaria, the basic principles of the concept of malaria eradication, the techniques used and the problems likely to arise in the implementation, have been described briefly in this small booklet. Written mostly in simple non-technical language, the publication provides useful information on the subject for the lay public in general for whom, the author says, it is primarily intended. The importance of health education for the success of the malaria eradication programme, has been rightly emphasised. However, the manner in which the public should participate actively in this campaign, has not been highlighted. The booklet could have included a chapter to emphasize the specific objective of the programme, and served as a medium for enlightening the reader that Malaria Eradication is not Mosquito Eradication. These appear to be some of the important lacunae in a publication of this nature.

—A.K.K.

ANNOUNCEMENT.

PATRICK BUXTON MEMORIAL PRIZE.

THE Dean of the London School of Hygiene and Tropical Medicine has pleasure in announcing the results of the essay competition, announced in the spring of 1960 and closed in March 1961, for the Patrick Buxton Memorial Prize. The Prize of £150 has been awarded, on the recommendation of the Board of Adjudicators, to Dr. B.O.L. Duke of the Helminthiasis Research Unit, Kumba, Cameroons, West Africa, for his essay entitled "The natural history of loiasis". The essay will be bound and, appropriately inscribed, lodged in the Library of the London School of Hygiene and Tropical Medicine.

Indian Journal of Malariology, **15**, *3, September 1961.*

FILARIASIS IN BAHRAICH DISTRICT, UTTAR PRADESH.

BY

DIWAN CHAND,

M. V. SINGH

AND

R. N. SHRIVASTAVA.

(*Malaria and Filaria Organization, Uttar Pradesh*).

[May 15, 1960.]

INTRODUCTION.

BAHRAICH, one of the eastern districts of Uttar Pradesh, is bounded by Nepal Tarai in the north, Bara Banki District in the south, Gonda District in the east and the River Gogra in the west, separating it from Kheri-Lakhimpur and Sitapur districts, with an area of 2,654 sq. miles. The district is divided into three Tehsils, i.e., Bahraich Sadar, Kaiserganj and Nanpara (Map 1).

PHYSICAL FEATURES.

The three main regions of Bahraich are the basins of (1) Rapti in the north-east, (2) of Kauriala and Gogra rivers in the west, and (3) a long narrow plateau between the two rivers running its entire length from north-west to south-east. The table land is well defined, stands at a height of about 40 feet above the level of the country, has a uniform breadth and forms a water-shed between the two big rivers.

Additionally there is a Tarai region which is completely different from the rest of the areas and extends from the extreme Nepal border of Pargana Nanpara up to the north fringe of Bhinga (Sadar Tehsil) and whole of the Tulsipur Pargana. It is low and is flooded during rains.

The entire district is covered with a network of small rivers and rivulets as also many lakes.

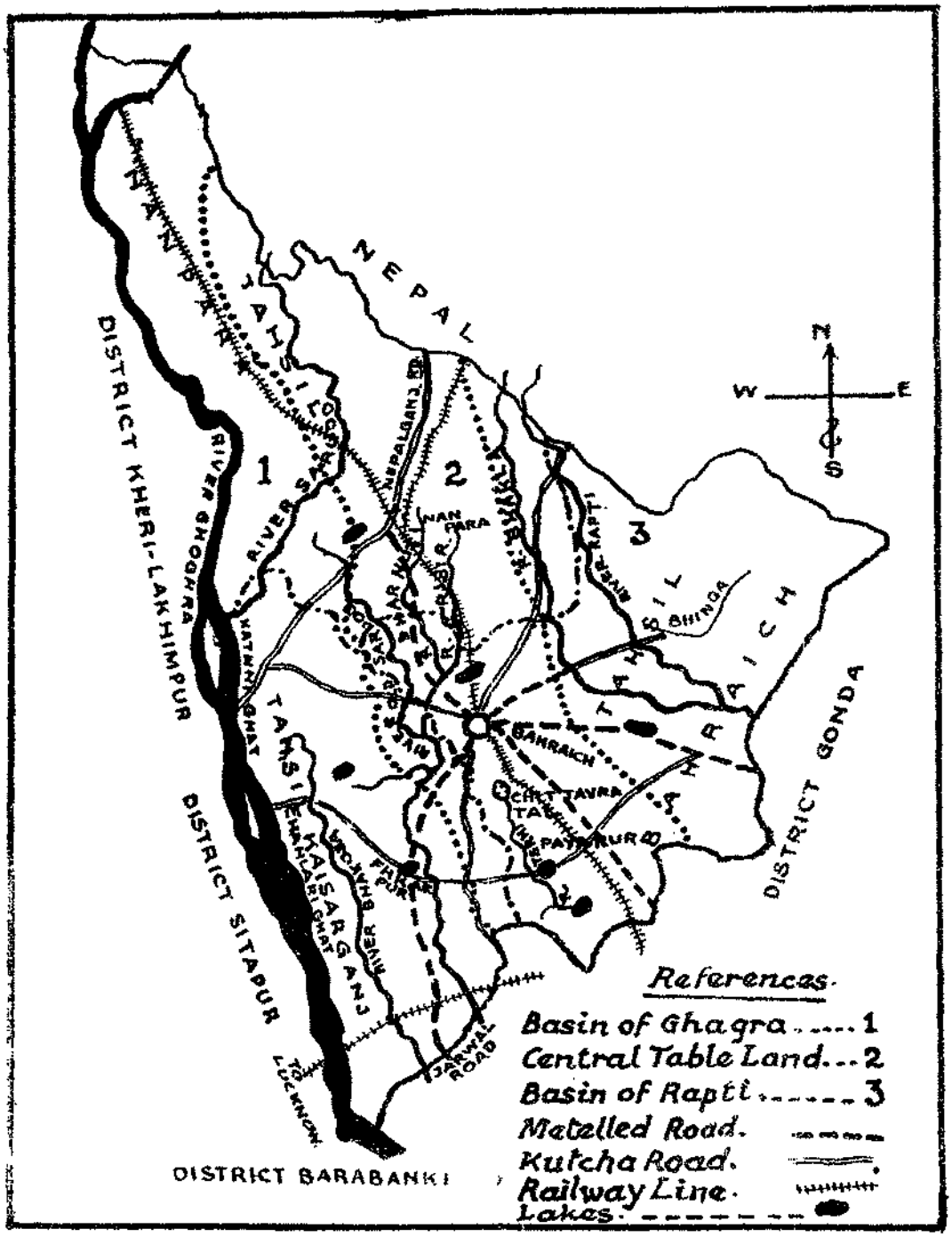
According to the local terminology, there are three distinct zones (Map 1) of the district :—

(1) *Uprahar* :—The upper portion of the district, mainly the table land referred to above, with the sub-soil water level varying from 20 to 30 feet.

(2) *Tarhar* or *Ganjar* :— The low-lying area of the district, mainly the basin of the two rivers referred to above, with the sub-soil water level varying from 10 to 15 feet.

MAP 1.

Map of District Bahraich, Uttar Pradesh, showing Physical Features.



(3) *Tarai* :—The low lying area of the district just south of the Nepal Hills, with a high sub-soil water level, i.e., less than 10 feet. During the rainy season, the area is continuously flooded.

It is commonly believed that filarial manifestations, including hydrocele (Gund), are characteristic of *Tarhar* and *Tarai* only and not of *Uprahar*.

POPULATION.

The population of the district is 13,47,116 (1951 census) consisting of 7,06,042 males and 6,41,074 females. The urban population is 72,380. The rural population of 12,74,736 live in 1927 villages.

CLIMATE.

The climate is hot and humid, the maximum and minimum temperatures being 110°F. and 48°F., while the relative humidity varies from 70 to 90 per cent.

RAINFALL.

The average annual rainfall is 45·65 inches, the maximum being in the Tarai area (Nanpara). During the last few years, there has been considerable variations in the annual rainfall.

SOCIO-ECONOMIC CONDITIONS.

The majority of the population are agriculturists. There are a few small-scale industries as well. The average living standard is low though the district produces surplus food grains. There is an annual big fair at the district headquarters called '*Sayed Sallar*' attended by many persons from the district and from neighbouring areas. Recently a small population from Deoria and Gorakhpur has been settled in the forest area of Nanpara Tehsil. Communications are poor, though connected with the neighbouring districts by road and rail, and with Nepal by road.

MEDICAL AND PUBLIC HEALTH ASPECTS.

At the district headquarters, there are a number of hospitals. A number of dispensaries—allopathic and indigenous systems—, maternity and child welfare centres, two primary health units, and a leprosy control unit, are scattered throughout the district.

Malaria has been a major problem, and a unit of the National Malaria Eradication Programme is functioning in the District. The district is also endemic for cholera and small pox. Goitre is prevalent in the Gogra basin.

The sources of drinking water are shallow wells, rivers and lakes, while in Bahraich Town there is a limited protected water supply.

There is no drainage system anywhere in the district, with the result that in all areas the sullage water stagnates in small pools, thus forming numerous mosquito breeding places.

DEVELOPMENTAL SCHEMES.

Twelve Stage I and two Stage II blocks are functioning, while nineteen more Stage I blocks are expected to be opened by the end of the Second Five-Year-Plan period.

FILARIASIS—A RETROSPECT.

Filariasis, it is learnt, has been recently introduced in this district. A few cases had been, it is reported, noted in Bahraich Town about 25 to 30 years back, and that from the last 10 to 15 years only there is an upward trend in the incidence of filariasis. No filaria surveys have been carried out in the past in this area.

The report of the "Eastern Districts Epidemic Enquiry Committee (1951)" of the Uttar Pradesh Government has not mentioned filariasis as an important disease, though stated to be prevalent in some places. In their recommendations, they have mentioned that the disease can be controlled by effective spraying of houses with DDT.

FILARIA SURVEY—METHODS AND MATERIAL.

A Filaria Survey Unit under the National Filaria Control Programme (Raghavan, 1955) carried out a survey to delimit the filarious areas in the district. The results of this survey are reported in this paper.

The areas were selected at random from the different urban and rural parts of the district. A door-to-door visit was made of the selected areas between 8 p.m. and midnight to examine as many persons as possible from all age-groups and from both sexes. The persons were exmined for disease manifestations like lymphadenitis, hydrocele, elephantiasis of the limbs, genitals and chyluria. Along with this, approximately 20 c.mm. of blood was obtained from each individual from a pricked finger and made into a thick smear. The air-dried smears were stained the next morning with J.S.B. I stain (Jaswant Singh and Bhattacharji, 1944) and later on examined for microfilaria. Their type and number in positive slides were determined.

RESULTS.

17,717 persons from all age-groups and both sexes were examined from the different urban and rural areas of the district.

The over-all infection, disease and endemicity rates were 5·65, 9·09 and 14·04 per cent., respectively. The average infestation was 33·1 Mf./20 c.mm. The minimum was one and maximum was 350 microfilariae in the positive slides. Only *Wuchereria bancrofti* was noted. The disease processes consisted of hydrocele, elephantiasis of the limbs and scrotum as also chyluria.

Three, out of 147 infants examined, were positive for infection, the youngest being 6 months old. Disease was noted even in an infant of about one year of age with hydrocele (not congenital) of 2 months' duration.

The infection and disease prevalence amongst different age-groups for the communities examined are set out in Tables I and II.

TABLE I.

Details of persons of different age-groups with infection,

Age-group (years).	NUMBER OF PERSONS :		Infection rate, per cent.
	Examined.	With infection.	
0 to 1	147	3	2·04
2 to 5	1,228	33	2·68
6 to 10	2,378	93	3·91
11 to 20	3,976	205	5·23
21 to 30	4,693	298	6·35
31 to 40	2,988	188	6·62
41 to 50	1,568	105	6·69
51 and above	739	51	6·90
Total	17,717	986	5·65

TABLE II.

Details of persons of different age-groups with filarial disease.

Age-groups (years).	NUMBER OF PERSONS :		Disease rate, per cent.
	Examined.	With disease.	
0 to 1	147	1	0·67
2 to 5	1,228	9	0·73
6 to 10	2,378	32	1·34
11 to 20	3,976	220	5·53
21 to 30	4,693	557	11·86
31 to 40	2,988	436	14·59
41 to 50	1,568	242	15·43
51 and above	739	114	15·42
Total	17,717	1,611	9·09

An analysis of Table I shows that the infection rate rises with the age till age-group 21—30, thereafter remaining more or less steady. The disease rate, it would be noted from Table II, is negligible in the earlier age-groups and increases thereafter with the age.

TYPES OF DISEASE PROCESSES.

Table III shows the incidence of various disease manifestations observed in the population surveyed.

It would be noted from Table III that hydrocele and genital lesions formed the bulk of the disease processes.

TABLE III.

Types of disease manifestation and their relative prevalence amongst persons surveyed.

Disease manifestation.	Number showing disease manifestation.	Per cent.
Hydrocele and other genital affections.	1,201	74·57
Right upper limb.	55	3·41
Left upper limb.	49	3·04
Right lower limb.	145	9·00
Left lower limb.	115	7·13
Chyluria.	22	1·36

SEX AND INFECTION/DISEASE.

Table IV sets out the microfilarial incidence amongst males and females in the population examined.

TABLE IV.

Microfilarial incidence in males and females of the population examined.

Sex.	NUMBER OF PERSONS :		Infection rate, per cent.
	Examined.	With infection.	
Male.	13,726	795	5·79
Female.	3,991	139	4·76
Total	17,719	986	5·65

It would be noted from Table IV that the rate of infection significantly differs in the two, the females having lesser infection than males. This applies equally to the incidence of disease, as shown in Table V.

TABLE V.

Incidence of filarial disease amongst males and females in the population examined.

Sex.	NUMBER OF PERSONS :		Infection rate, per cent.
	Examined.	With disease.	
Male.	13,726	1,472	10·72
Female.	3,991	139	3·48
Total	17,717	1,611	9·09

INCIDENCE IN PERSONS OF DIFFERENT COMMUNITIES.

The incidence of infection and disease in the Hindu and Muslim communities, in the areas surveyed, did not show any significant difference as is evident from Table VI.

TABLE VI.

Incidence of filarial infection and disease amongst Hindu and Muslims.

Community.	Number examined.	INFECTION :		DISEASE :	
		Number.	Rate, per cent.	Number.	Rate, per cent.
Hindus.	12,020	647	5·38	1,103	9·17
Muslims.	5,697	339	5·95	508	8·91
Total	17,717	986	5·65	1,611	9·09

FILARIASIS IN URBAN AND RURAL POPULATIONS.

The infection, disease and endemicity rates of the population in the urban and rural areas examined are set out in Table VII.

TABLE VII.

Filarial infection, disease and endemicity rates in urban and rural populations.

Terrain.	Number examined.	INFECTION :		DISEASE :		ENDEMICITY :	
		Number.	Rate, per cent.	Number.	Rate, per cent.	Number.	Rate, per cent.
Urban.	4,944	331	6·69	552	11·36	22	17·41
Rural.	12,773	655	5·12	1,059	8·29	87	12·73
Total	17,717	986	5·65	1,611	9·09	109	14·04

From Table VII, it would be noted that the infection is prevalent not only in the urban but also in the rural areas. Urbanisation of the rural areas would appear to have an influence in higher endemicity rate as noted in Ikauna (26·27 per cent), Payagpur (30·1 per cent), Risia (35·04 per cent) and Mihinpurwa (32·81 per cent). The municipal areas of Bahraich and the notified areas of Bhinga and Nanpara had a higher endemicity rate which could be explained due to the greater prevalence of favourable mosquito breeding sites for *Culex fatigans*, leading to greater quantum of transmission.

ENTOMOLOGICAL OBSERVATIONS.

Random adult mosquito collections were made in different areas — urban as well as rural — between 7·30 and 11·00 a.m. The mosquitoes collected were identified and the female mosquitoes dissected. The following species of mosquitoes were collected :—

A. subpictus.
A. fluviatilis.
A. pallidus.
A. annularis.
A. barbirostris.
C. fatigans.
C. gelidus.
C. bitaeniorhynchus.
Aedes sp.
Armegeres sp.

Culex fatigans was found to be universally present, though more prevalent in the urban than in the rural areas.

During the period August, 1957, to March, 1959, 12,281 mosquitoes (2,735 anophelines and 9,546 culicines) were collected. Out of this, 1,882 anophelines and 4,690 culicines were dissected for filarial infection; only 411 culicines were found infected with one or other stage of the developing larvae. Infective forms were noted only in *Culex fatigans*. The results of dissection are set out in Table VIII.

TABLE VIII.

Results of mosquito dissection for filarial infection.

Mosquito species dissected	NUMBER :		HEAD :		THORAX :				ABDOMEN :			
	Dissected.	Positive.	Stage III.	Stage IV.	Stage I.	Stage II.	Stage III.	Stage IV.	Stage I.	Stage II.	Stage III.	Stage IV.
Anophelines.	1,852	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Culicines.	4,690	411	28	23	Nil	52	41	Nil	54	113	Nil	Nil

BREEDING PLACES.

The most common types of breeding places for *Culex* according to terrain were as follows :—

1. *Urban* :—Houses, drains, ablution water near the service type of latrines, as also pits and pools.
2. *Rural* :—Soakage pits for sullage water, water collections from the cattle-sheds, tanks and small ditches.

Due to the lack of proper drainage system, numerous breeding places were present for round-the-year breeding of *C. fatigans*.

Culex fatigans was noted to breed in comparatively clean water collections as well.

CLIMATE AND ENDEMICITY.

This area is moderately endemic for filariasis, the definition of "*moderate*" being according to Iyengar (1938). From the meteorological data, the degree of endemicity noted and the types of disease processes, it would appear that this area fits in as an area with favourable climatic conditions for filarial transmission as an "intermediate" or 4-8 months according to the classification of Acton and Rao (1930).

TERRAIN AND FILARIA.

As mentioned earlier, Bahraich has three topographical divisions :

(1) the basin of two big rivers ; Gogra and Rapti or *Tarhar*,

(2) the table land between these two big rivers or *Uprahar*, and

(3) the *Tarai*.

As stated before, the local belief is that the *Uprahar* is free from filaria. The infection, disease and endemicity rates in these three divisions are analysed in Table IX.

TABLE IX.

Filarial infection, disease and endemicity rates in the Uprahar, Tarhar and Tarai regions of Bahraich District.

Division.	Number examined.	Infection:		Disease:		Endemicity rate, per cent.
		Number.	Rate per cent.	Number.	Rate, per cent.	
Uprahar.	10,377	472	4·57	914	8·8	12·83
Tarhar.	5,841	440	7·53	577	9·87	16·67
Tarai.	1,499	74	4·93	120	8·00	12·20
Total	17,717	986	5·65	1,611	9·09	14·04

It will be noted from Table IX that the local belief, that the *Uprahar* is free from filaria, is not supported. However, it would be seen from Table IX that the infection rate in the *Tarhar* is greater than that in the *Tarai*. This can be due to not only the relatively high sub-soil water but also the lesser degree of flushing and dilution of breeding places as compared to what happens in the *Tarai* though with a much higher water level than the *Tarhar*.

SUMMARY.

The physical features, climatic and socio-economic conditions, public health problems, and the connected activities of Bahraich District are set out.

The results of filariasis surveys, carried out for the first time between August, 1957 and March, 1959, of 17,717 persons from urban and rural areas of different age-groups and of both sexes, are set out. *W. bancrofti* was the only infection prevalent. The infection rate was 5·65 per cent. An infant of 6 months of age was positive for *W. bancrofti*. The average infestation rate was 33·1 Mf./20 c.mm., the maximum being 350 and the minimum of one microfilaria per positive slide.

The over-all disease rate was 9·09 per cent, consisting of elephantoid limbs, genito-urinary lesions like hydrocele, filarial scrotum and chyluria. A child of one year of age had a hydrocele (not congenital) of two months' duration.

The infection was prevalent in both urban and rural areas. *Culex fatigans* was the only vector for *W. bancrofti*.

In all these areas, numerous favourable breeding grounds existed for *Culex fatigans* which bred in dirty as well as in some clean water collections. The density of *C. fatigans* was more in the urban than in the rural areas.

The difference in the infection and disease in males and females was studied. In males the rates were significantly higher than in females.

No significant difference was noted in the filarial endemicity of the urban or the rural areas.

Amongst Hindus and Muslims, the filarial infection and disease rates were nearly the same.

Filariasis is prevalent in all the three topographical divisions of the district (*Terai, Tarhar* and *Uprahar*). The infection rate, however, is higher in *Tarhar* than in the other areas. The probable reasons for the same are analysed.

The district is moderately endemic for filariasis and the length of favourable period of transmission could be "intermediate", i.e., 4 - 8 months as defined by Acton and Rao (1930).

ACKNOWLEDGEMENT.

The authors are thankful to the staff of the Filaria Survey Unit, Bahraich, and specially Shri S. Wasi-ud-Din, Laboratory Assistant, for their active co-operation in these studies. They are also thankful to the Civil Surgeon, the District Planning Officer, the District Medical Officer of Health and the Anti-Malaria Officer, Bahraich, for general information referred to in this paper.

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Indian Journal of Malariology, **15**, *3*, *September 1961*.

STUDIES ON THE INCIDENCE AND TRANSMISSION OF FILARIASIS IN BHAGALPUR TOWN (BIHAR).

BY

B.K. VARMA,

N.L. DASS

AND

V.P. SINHA.

(*Department of Health Services, Bihar.*)

[August 17, 1960.]

INTRODUCTION.

STUDIES on the incidence and transmission of filariasis were carried out at Bhagalpur Town during the period December, 1957, to December, 1958, under the auspices of the National Filaria Control Programme (Raghavan, 1955). Bhagalpur, situated at a latitude 25° 15' North and longitude 87° 0' East, is an ancient city and includes Champa, the capital of Anga of Mahabharat fame. The Bhagalpur Municipality has an area of 11·09 sq. miles and a population 1,15,075 according to the 1951 census.

The town has a filtered water supply supported by tube-wells. It has a natural system of drainage. The drains are mostly kutcha and partly pucca and have open surfaces. Nearly all the drains flow northwards into the River Ganges.

METEOROLOGICAL CONDITIONS.

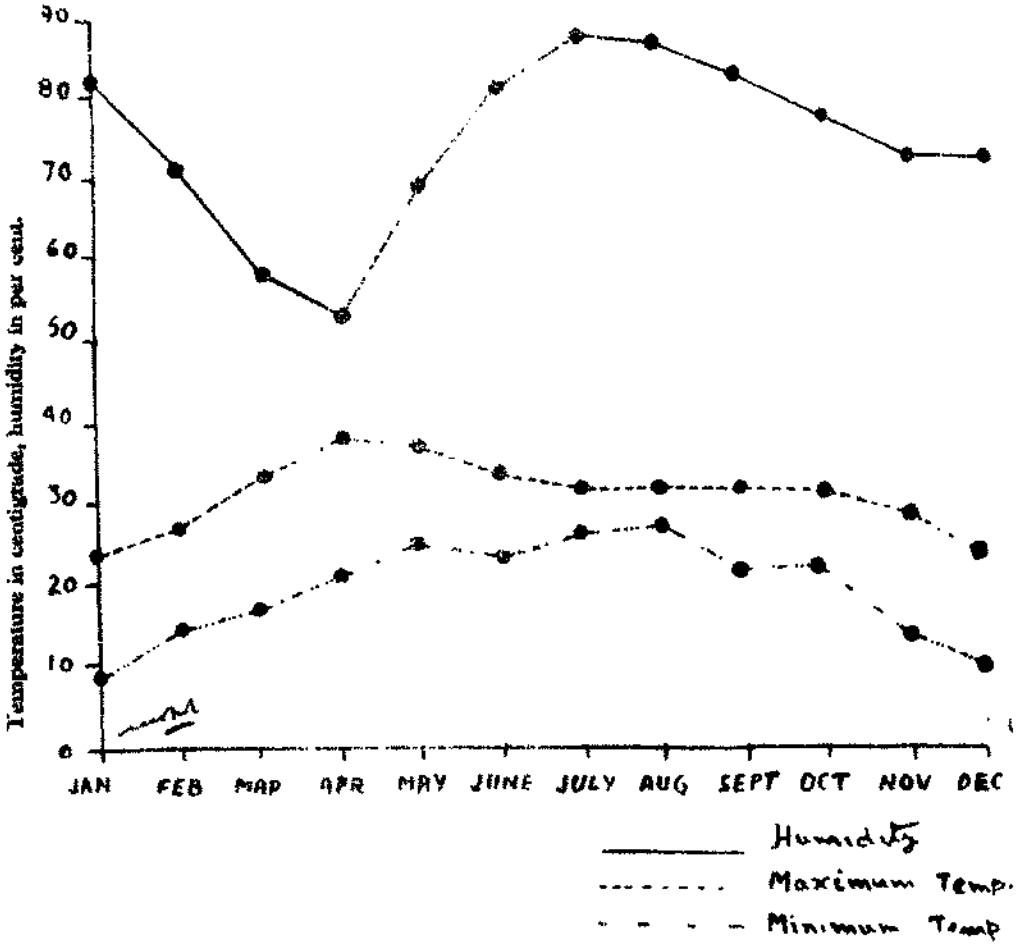
The temperature of the place varies from an average minimum of 11·7°C. in January, the coldest month, to an average maximum of 37·6°C. in May, the hottest month of the year (Graph 1).

The humidity varies considerably from 54·2 per cent in April to 81·6 per cent in September (Graph 1).

Except for the months of December and January, the maximum temperature is above 26·7°C. throughout the year—a condition which tends to support the transmission of filariasis during the ten remaining months. During the months of July, August and September the minimum temperature also remains at or above 26·7°C. and the associated humidity above 70 per cent makes these months most favourable for maximum transmission of filariasis. The period February to June is not so favourable for transmission of filariasis as the humidity is persistently below the optimum of 60 per cent.

GRAPH 1.

Average maximum and minimum temperature and relative humidity for the years 1952–56.



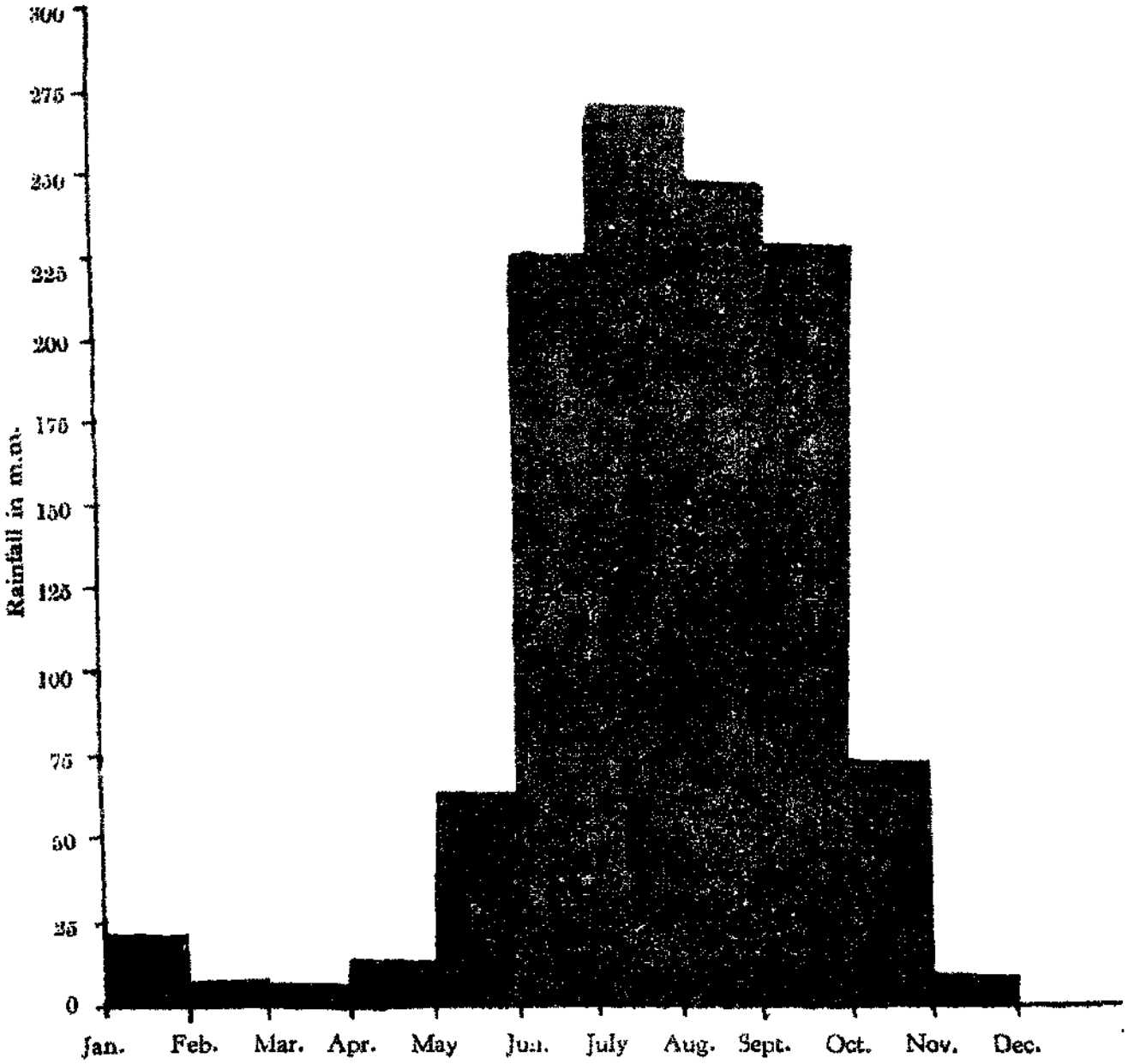
The average annual rainfall is 1183 mm. The monsoon usually starts in June and the rains reach the peak in July, with equitable distribution in August and September (Graph 2).

MATERIALS AND METHODS.

To assess the extent of infection and disease among the inhabitants of the town, thick blood smears (about 20 c.mm. from each person) were obtained at random between 8·30 p.m. and 11·30 p.m. A clinical record of the persons thus examined was maintained. The blood smears were stained with either Leishman or J.S.B. stain on the following day after dehaemoglobinisation and then examined for the presence or absence of microfilaria under the microscope. The number of microfilaria in each smear was finally counted and recorded.

GRAPH 2.

Average of monthly rainfall for the years 1952-56.



Further, to study the nature and degree of transmission, mosquitoes were collected from fixed and random catching stations which comprised human dwellings, mixed dwellings and cattle-sheds. The mosquitoes thus collected were identified, dissected and examined to know the vector species, its density and the extent of infection in them.

RESULTS.

In course of the present survey, 6,645 persons, representing 5·7 per cent of the population, were examined within the municipal area of Bhagalpur Town. The results of the investigation are summarised in Table I and II and in Graph 3.

TABLE I.

Incidence of filariasis in different ages and two sexes.

Age (years).	BOTH SEXES :				MALES :			FEMALES :		
	Number examined.	Infection rate.	Disease rate.	Average infestation. per 20 c.mm.	Number examined.	Infection rate.	Disease rate.	Number examined.	Infection rate.	Disease rate.
1 and below	104	0	0	0.	82	0	0	22	0	0
2	96	5·2	5·2	28·3	55	1·8	3·6	41	9·7	7·3
3	116	10·3	6·0	16·3	65	9·2	4·6	61	11·7	7·8
4	106	9·4	6·6	15·1	59	8·4	3·3	47	10·6	10·7
5	166	7·2	7·7	17·0	89	3·3	7·8	77	10·1	7·7
6	190	9·5	7·8	22·6	107	11·2	11·2	83	7·2	3·6
7	213	15·4	11·6	16·5	137	13·1	8·6	76	19·7	17·1
8	260	13·0	11·9	14·3	174	20·0	12·6	86	15·1	10·4
9	187	19·2	12·2	17·4	121	20·6	14·0	66	16·6	9·0
10	247	13·3	14·5	12·7	184	13·5	14·6	63	12·6	14·2
11 to 20	1,425	20·0	42·6	21·3	1,054	21·1	43·3	371	16·6	40·7
21 to 30	1,572	25·8	43·7	17·7	1,213	26·2	43·6	359	24·5	41·5
31 to 40	1,066	27·2	42·5	17·4	758	28·2	43·4	308	25·0	40·5
41 to 50	543	28·7	43·4	17·2	377	29·4	43·5	166	27·1	43·3
51 and above	334	25·7	56·7	22·5	190	26·3	64·6	144	25·0	46·5
Total all ages	6.625	21·4	35·1	18·4	4,665	22·1	36·5	1,960	19·6	31·7

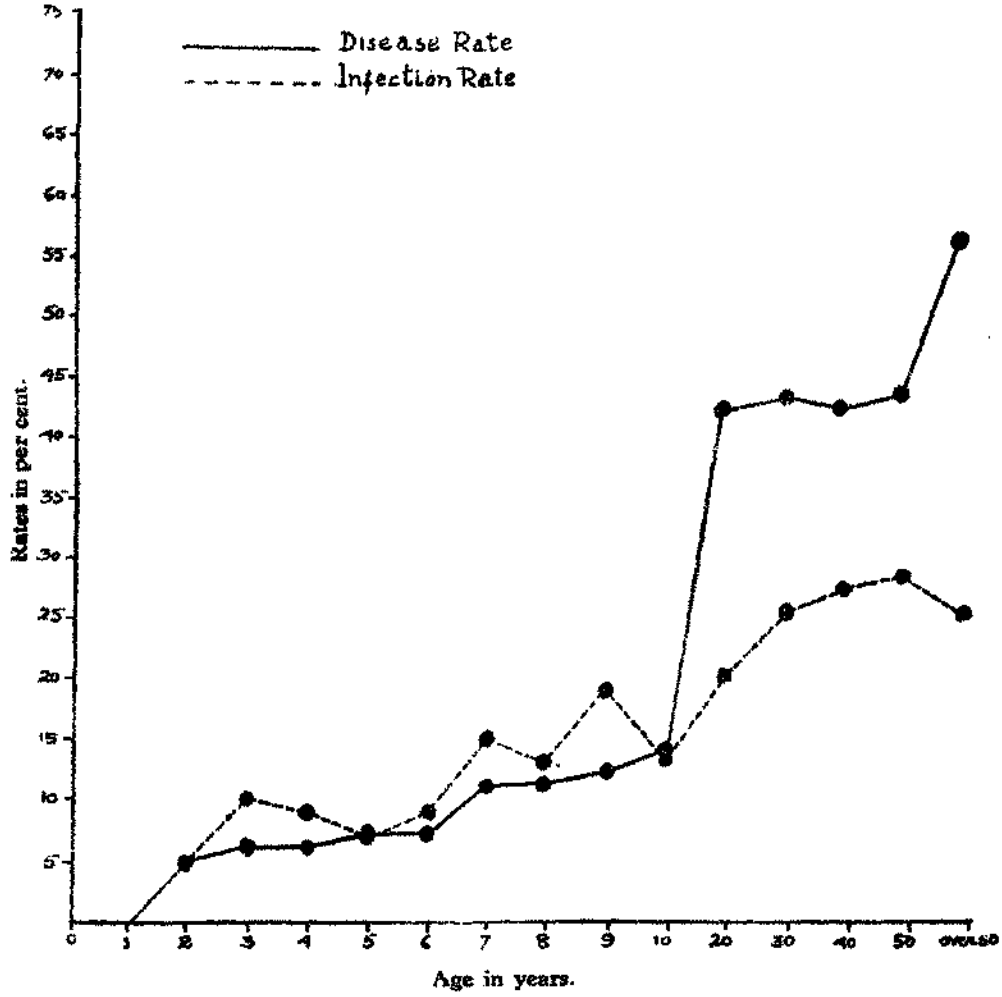
TABLE II.

Density and dissection of mosquitoes.

Months.	CULEX FATIGANS (WIED) :		
	Number Dissected.	Infection rate, per cent.	Infectivity rate, per cent.
December, 1957	182	9·3	8·2
January, 1958	227	5·2	4·8
February, 1958	401	9·9	8·4
March, 1958	470	14·4	10·8
May, 1958	331	7·9	3·2
June, 1958	124	14·5	7·2
July, 1958	96	23·9	17·7
August, 1958	89	11·2	8·2
September, 1958	54	12·9	9·2
October, 1958	134	5·9	2·2
November, 1958	334	5·08	3·5
December, 1958	128	8·6	6·2
Total	2,570	10·1	..

GRAPH 3.

Age-wise incidence of filarial disease and infection.



The examination of the data collected brings to light the following facts :—

The disease and infection rates among the people residing in Bhagalpur Town averaged 35·1 per cent and 21·4 per cent respectively. Further the clinical manifestation of the disease showed a rising incidence with age (Table I).

The types of the disease manifestations recorded during the survey, consisted of swelling of the extremities—upper, lower, or both (temporary or permanent), hydrocele, other genital manifestations (including elephantoid scrotum, epidimo-orchitis etc.). Hydrocele formed the commonest manifestation among the males, being present in 1,323 cases (77·1 per cent of males showing disease). In females the commonest symptom was the swelling of the lower limbs.

The entomological survey revealed the presence of the following species of mosquitoes in the area during the period of observation :—

Culex fatigans (Wied.)
Culex cornutus.
Anopheles annularis (Ven der Wulp).
Anopheles vagus (Donitz).
Anopheles subpictus.
Anopheles stephensi (Liston).
Armegeres obturbans (Walker).
Aedes aegypti.

Of these, *Culex fatigans* was the abundant species frequenting the human habitations. Domestic cesspools and stagnant drains with heavy organic pollution were found to be the chief breeding places.

The density of *Culex fatigans* (Wied.) ranged from 23·4 per ten-man-hours to 392·7 per ten-man-hours during the different months of the year (Graph 4).

The dissection of the mosquitoes revealed that *Culex fatigans* (Wied.) carried the natural infection of *Microfilaria bancrofti* in all its developmental stages. On an average, 10·1 per cent of them showed the infection, the monthwise range being from 5·2 per cent to 23·9 per cent (Table II). Similarly the infectivity, rate varied from 2·2 per cent to 17·7 per cent. No other species of mosquitoes showed any stage of the microfilaria.

DISCUSSION.

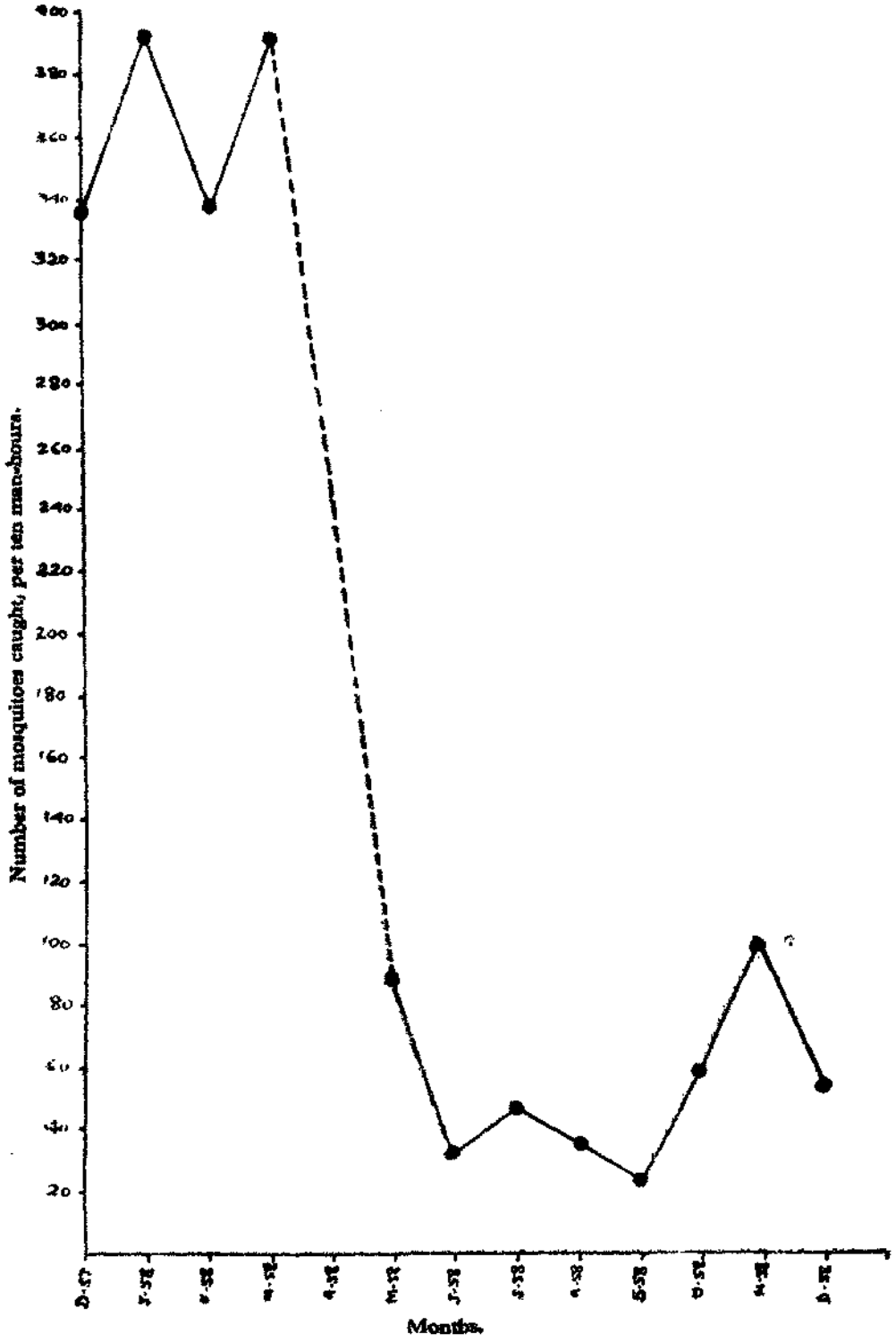
In the present investigation the authors noted that out of 4,665 males examined, 1,706 (36·5 per cent) showed clinical signs, and 1,032 (22·1 per cent) the microfilaria in blood, whereas for 1,960 females the corresponding figures were 622 (31·7 per cent) and 386 (19·6 per cent) respectively.

According to Meckenzie (1925) there was greater infection in men than in women. The greater incidence of clinical filariasis in men was reported also by King *et al.* (1929), who further observed that with increase of age the difference narrowed down and past 40 years the incidence was the same in both the sexes. Farooque and Qutubuddin (1946) found no predilection for either sex, which is similar in the present findings.

Considering the age factor, the youngest persons to show microfilaria in blood were boys and girls of 2 years of age ; also the disease was noted in the same children. The disease in them was characterised by lymphangitis and mild transient swellings of the limbs which exacerbated and reduced periodically. Cause *et al.* (1945) found the youngest age with infection and disease as 2 and 11 years respectively, both being males. According to them the incidence of disease increased with age. Farooque and Qutubuddin (1946), King *et al.* (1929) and Kanta *et al.* (1956) recorded similar findings.

GRAPH 4.

Density of Culex fatigans *per ten man-hours (month-wise).*



The present studies also confirmed the direct relationship of age and disease and the maximum incidence of disease was noted in the age-group 51 years and above (Table I).

The filarial infection, as determined by blood examination, showed a rising incidence with age up to 30 years only and thereafter it was almost stationary (Table I). Kant *et al.* (1956) had made similar observations. Krishnaswami (1955) reported progressive rise in infection rate up to 20 years and beyond that it was more or less constant.

The microfilaria infestation per 20 c.mm. of blood in positive persons ranged from 1 to 100. The average infestation per 20 c.mm. of blood was 18·4 in the community examined. Considering agewise, it did not show any relevance. It varied from 15·1 to 22·5 per 20 c.mm. of blood (Table I). However, among the adults, who formed the main source of infection (the infection rate ranging from 20 per cent to 28·2 per cent), it ranged from 17·2 to 22·5 per 20 c.mm., and so it is a pointer to the magnitude of reservoir.

TRANSMISSION.

The maximum filarial infection and infectivity in *Culex fatigans* (Wied.), the only vector species recorded here, were observed during the month of July, followed by a minor drop in August and September. During the rest of the year these were low (Table II).

The seasonal variation in the maturity of the microfilaria in the body of the mosquitoes was also studied by Acton and Sundar Rao (1927). They reported that the development of the adult larvae took place in the shortest time during July, August and September. Similarly Sundar Rao and Iyenger (1929) found that the monsoon months—July to September—were the most favourable for the rapid development of a large number of embryos of the *Wuchereria bancrofti* in the body of *Culex fatigans* in the laboratory as well. They further found that during the winter months, the development was very slow and in the dry months hardly any development took place. Ray (1957) reported the maximum filarial infection in *Culex fatigans* during 'wet' or 'moist' months and minimum during the 'dry' part of the year.

The density of the vector, *Culex fatigans*, was at the peak during the period December-March and thereafter it came down and was low during May-September, the monsoon months (Graph 4). This also agrees with the reports of Ray (1957). The reduced breeding, following heavy rains, may be ascribed to dilution of organic matter and flushing out drains. During dry season, though the extent of breeding areas may be less, the absence of or infrequent rains and comparatively more rapid evaporation of water would perhaps be the contributory factors for attaining the degree of pollution required for *Culex* breeding (Ray, 1957).

Lastly the geographical situation along the River Ganges may also contribute to the preponderance of the disease in Bhagalpur Town. Acton and Sundar Rao (1927) stressed this point and recorded the geographical distribution of the disease along the sea-board of Bay of Bengal, the Indian Ocean and also along the course of the River Ganges. They suggested that during wet months, with relatively high humidity, the larvae will penetrate the skin more readily and the excessive moisture occurring during monsoon in the sea-board areas and along the course of the great rivers contribute a great deal to the high incidence of filariasis.

Thus it is evident that of the factors that determine the transmission of filariasis, climate plays a vital role in the life-history of the vectors and infection in them. Similar conclusion has been arrived at by Raghavan (1958) as well.

SUMMARY.

The disease rate in Bhagalpur Town was found to be 35·1 per cent and the microfilarial infection rate, 21·4 per cent.

The average microfilarial infestation per 20 c.mm. of blood was 18·4 per cent.

The youngest age at which the blood was found positive was 2 years and at the same age the external signs were observed.

The disease incidence was found to bear direct relationship with age.

W. bancrofti was the species recorded and *Culex fatigans* (Wied.) was the vector.

The highest infection and infectivity of the vector was observed during the monsoon months.

The highest density of the vector, *Culex fatigans*, was noted during winter months, i.e., relatively dry part of the year.

ACKNOWLEDGEMENTS.

The authors are greatly indebted to Dr. P.A. Singh, Director of Health Services, Bihar, for his kind permission to publish this paper.

The authors express their gratitude to Dr. B.S. Lal, B.Sc. (Cornell), M.Sc., Ph.D. (Maryland), Professor of Entomology and Zoology, Bihar Agricultural College, Sabour, for reading the typescript and his suggestion.

The authors are thankful to the Director, Regional Meteorological Centre, Calcutta, who has kindly supplied the meteorological figures of Bhagalpur Town for the years 1952-56

Thanks are also due to the staff of the Filaria Control Unit, Bhagalpur, for their active co-operation during the investigation.

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Indian Journal of Malariology, **15**, *3*, *September 1961*.

RESULTS OBTAINED IN THE THIRD YEAR OF PILOT STUDY OF MALARIA SURVEILLANCE MEASURES IN MYSORE STATE, INDIA.

BY

H. SHAMA SASTRY.

(Bureau of Malariology, Mysore State, Bangalore.)

[June 28, 1960.]

THE first two years' results of the pilot study carried out in Mysore State have already been communicated (Patel *et al.*, 1957 ; Shama Sastry and Narayana Iyengar, 1959 ; Shama Sastry, 1960). The observations, continued during the third year of the programme (October, 1958 to September, 1959), are presented here. The entomological data, however, are furnished from 1956-1957 onwards. The data are set out area-wise on the same basis as in the previous communications referred to above.

THE *A. FLUVIATILIS* AREA.

In erstwhile Mysore State, the areas selected for maintenance work are of two types. The first area, consisting of Shikaripur, Sorab, Sagar, Hosanagar and Thirthahalli Taluks in Shimoga District, Saklespur Taluk of Hussan District and Koppa and Sringeri Taluks of Chickmagalur District, was under complete suspension of spraying. The results of the third year of the pilot study are described in Part I of the present paper. The second area, consisting of Mudigere Taluk in Chickmagalur District and Belur Taluk in Hassan District, was under one round of spraying (October—November) till the year 1959, after which the spraying was completely suspended as this area satisfied the criteria laid down for the total suspension of spraying. The results of the pilot study, during the third year in this area, are described in Part II. The area of the past *A. culicifacies* malaria, was also under one round of spraying (April—May) till the year 1958, after which the spraying was totally suspended as the area satisfied the criteria laid down for total suspension of spraying. The results obtained in this area during the third year, form Part III.

The observations in North Kanara District where the vector in the past was *A. fluviatilis*, now under complete suspension of spraying, are reported in Part IV.

Results obtained in Coorg District and in the District Malaria Scheme area, Puttur, in South Kanara District, are presented in Parts V and VI.

PART I

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

This area comprises eight Taluks, as already referred to, with a total population of 4,03,971 (1951 census). All these eight taluks are covered by a net work of Primary Health Units and modified Health Units. Each Primary Health Unit is provided with two Health Inspectors and each modified Health Unit is provided with one Health Inspector. Active surveillance work is being carried out by the Health Inspectors of the Health Units. Surveillance Workers, specially appointed for the purpose, supplement the active surveillance work in the areas not provided with Health Units, Municipal Towns and the areas with Health Units in difficult terrain and with defective communications. Eighteen Surveillance Workers have been allotted to work in this area in addition to 118 sanctioned posts of Health Inspectors. Surveillance work is supervised by the Assistant Medical Officer of Health at the Primary Health Unit level, and by the Medical Officer of Health who is in charge of the Secondary Centre, at the Taluk* level. The population of this area is covered for surveillance in a period of 10 days during each fortnight. The average population covered on each day by a Junior Health Inspector or a Surveillance Worker during the year was 323 as against 313 during the preceding year. No vehicle was provided for surveillance work. The population to be surveillanced by each worker is less than the targeted figure because the Health Inspectors of the Health Units have to attend to other Public Health activities and general sanitation, etc., in addition to the surveillance work.

Table I furnishes the details of active malaria surveillance work from October, 1958 to September, 1959. The population surveillanced during each month ranged from 0·30 millon to 0·38 million as against 0·34 to 0·39 million during the preceding year. This fluctuation, as already pointed out by Shama Sastry and Narayana Iyengar (1959 *loc. cit.*), is due to :—

(i) vacancies among the sanctioned posts, (ii) there being not enough substitutes to be posted for other urgent health work such as epidemic control or fair sanitation duty and, (iii) frequent resignations of surveillance workers after brief period of service. Out of the sanctioned posts of 118 Junior Health Inspectors and 18 Surveillance Workers, the number working in any single round varied from 54 to 88 and 11 to 18 respectively. Provision was made for leave reserves in the Health Units but the posts could not be filled due to dearth of suitably qualified persons. The situation is under critical examination.

FEVER MORBIDITY.

A total of 8,301 fever cases were investigated during the year under report, as against a total of 10,322 fever cases investigated during the preceding year. The average number of fever cases, per 1,000 population, ranged from 1·49 to 2·9 as against the range of 1·66 to 3·23 during the preceding year.

* Taluk means a Revenue Division of the District.

Out of 8,301 fever cases investigated, a total of 8,084 cases have been analysed age and sex-wise (Table II). The number of fever cases in the age-group '0 to 4 years', forms about 1/7th of the total as in the preceding year.

BLOOD SMEAR EXAMINATION.

Blood smears were examined after staining with J.S.B. Stain. A smear was declared negative only after examining at least 100 fields of thick smear in 3 to 5 minutes. The smears were examined at the laboratories attached to the Bureau of Malariology, Bangalore ; Malaria Investigation Centre, Mandya ; Malaria Field Station, Saklespur ; and the District Health Offices and Secondary Centres.

7,786 blood smears from fever cases were examined during the year. Three smears were found to be positive, 2 for *P. falciparum* and one for *P. vivax*. Of these, 2 cases were encountered in Koppa Taluk of Chickmagalur District and both of them were reported as imported from South Kanara District which has come under spraying since November, 1959. The third positive case was encountered among labour in Manigadde Estate of Saklespur Taluk in Hassan District and this was reported to have been imported from South Arcot District, Madras State. Particulars of parasite-positive cases are detailed in Table III. As against 3 parasite positive cases encountered in the fever surveillance during the preceding year, 3 positive cases were encountered during the year under report also.

ENTOMOLOGICAL DATA.

Table IV shows the collection of all anophelines and vectors since October, 1956 to September, 1969. In Sagar area, the per man-hour density of all anophelines has gone down to 0·71 during the year under report as against the per-man-hour density of 5·32 during the preceding year. The vector densities were as follows :—

The per man-hour density of *A. culicifacies* rose from 0·005 to 0·025 in human dwellings-cum-mixed dwellings and from 0·043 to 0·075 in cattle-sheds. The per man-hour density of *A. fluviatilis* declined from 0·003 in human dwellings-cum-mixed dwellings and 0·015 in cattle-sheds (preceding year) to 0·0007 and 0·011 in human dwellings cum-mixed dwellings and cattle-sheds respectively during the year under report.

In Shikaripur area, the per man-hour density for all anophelines was 6·9. *A. culicifacies* density was 0·029 and 0·056 in human dwellings (including mixed dwellings) and in cattle-sheds respectively. The per man-hour density for *A. fluviatilis* was 0·012 and 0·007 in human dwellings (including mixed dwelling) and cattle-sheds respectively. So, there was a slight rise in the per man-hour density of *A. culicifacies*, over what it was during the preceding year. As regards the per man-hour denesity of *A. fluviatilis*, there was an increase from 0·001 to 0·012 in human dwellings (including mixed dwellings) and a decline from 0·019 to 0·007 in cattle-sheds.

TABLE I.

Details of active malaria surveillance work during the period October, 1958, to September, 1959, in the Pilot Surveillance Study areas in Mysore State.

Area.	Population (In million).	Part of the text matter of the present paper in which described.	Spraying suspended or reduced.	Population surveillanced. Monthly average. (In million).	Average Number of Health Inspectors or Malaria Surveillance Workers who worked.		Number of fever cases investigated during the year.	Number of blood smears taken.	Results of blood smears.		Number of anti-malaria pills (4-amino-quinoline) issued.	Average range of fever cases, per 1,000 population, surveillanced during each month.	Species of parasites found in the positive slides.		
					Health Inspectors.	Malaria Surveillance Workers.			Number positive.	Number negative.			*P. vivax.*	*P. falciparum.*	Mixed.
(i) Erstwhile Mysore :															
A. fluviatilis area	0·40	I	Suspended since October, 1956.	0·31 to 0·38	75	15·5	8,801	7,786	3	7,783	26,433	1·49 to 2·9	1	2	0
A. fluviatilis area	0·12	II	Reduced round since October, 1956. Suspended since the first round (May-June, 1959) in Belur and since the second round (October-November, 1959) in Mudigere Taluk.	0·09 to 0·12	19·6	3·9	2,193	1,692	1	1,691	6,654	0·98 to 2·2	0	0	1
A. culicifacies area	0·10	III	Reduced round since October, 1956. Suspended since the first round in May-June, 1959.	0·06 to 0·11	4·7	2·0	2,089	1,927	3	1,924	5,873	0·25 to 3·0	3	0	0

(ii) Integrated areas :															
North Kanara District (*A. fluviatilis* area).	0·51	IV	Suspended since June, 1956.	0·12 to 0·5	..	41·0	7,524	7,354	4	7,350	22,472	1·29 to 2·46	3	1	0
Coorg District. (*A. fluviatilis* area).	0·19	V	Suspended since June, 1957.	0·11 to 0·17	11·6	6·7	5,257	4,134	55	4,079	16,169	1·9 to 4·1	36	13	6
District Malaria Scheme, Puttur. (*A. fluviatilis* and *A. culicifacies* areas.)	0·22	VI	Suspended since June, 1957.	0·17 to 0·22	...	21·8	3,966	3,946	..	3,946	13,024	1·2 to 1·97	0	0	0
Total	1·54			..	..	..	29,330	26,839	66	26,773	90,625	..	..	..	..

TABLE II.

Analysis of fever cases investigated—Age and Sexwise.

Age-group (years).	Total number of fever cases.	Male.	Female.	Percentage in relation to total fever cases.	Percentage of population under each age-group.
0 — 4	1,137	584	553	14·06	13·5
5 — 14	1,903	1,034	869	23·54	24·8
15 and above.	5,044	3,036	2,008	62·40	61·7
Total	8,084	4,654	3,430	..	..

TABLE III.

Details of the positive blood smears collected under the malaria fever surveillance work during the period October, 1958 to September, 1959.

Serial number.	District.	Taluk.	Name of the Primary Health Unit or Circle.	Village.	PARTICULARS OF THE PATIENT.		Date of taking the blood smear.	Results.	History and movement.	Treatment.	Remarks.
					Age.	Sex.					
1	Chick-magalur.	Koppa.	Koppa.	Balamata.	20 Years.	Male	January 13, 1959	*P. falci-parum.*	Athur and Sannur, Karakala Taluk.	Avlochlor on February 21, 1959 and February 25, 1959.	Imported from South Kanara, which place has been included under spraying since June, 1959.
2	Chick-magalur.	Koppa.	Koppa.	Madala-koppa.	2 Years.	Male	January 29, 1959	*P. falci-parum* rings.	Puttur, South Kanara.	Avlochlor on February 20, 1959, and February 25, 1959.	Imported from South Kanara, which place has been included under spraying since June, 1959.
3	Hassan.	Sakleah-pur.	Sakleah-pur.	Mani-gadde Estate.	20 Years.	Female	January 21, 1959	*P. vivax*	Spleen 2 size. Imported from South Arcot, Madras State.	4 and 8-Amino-quinoline from August 24, 1959 to August 28, 1959.	Imported from South Arcot, Madras State.

Saklespur area (Table V).—There was a marked rise in all anopheline density in the high-rainfall area over those of the preceding years, while there was a marked fall in the low-rainfall and intermediate-rainfall areas. During the year under report, the per man-hour density of all anophelines has been 18·5, 1·7 and 5·1 in the high, low and intermediate rainfall areas respectively. *A. culicifacies* continued to appear in low densities, as during the preceding year, in cattlesheds. While *A. culicifacies* was absent in human dwellings and mixed dwellings in the precedings years, one *A. culicifacies* has been collected in the human dwellings-cum-mixed dwellings during the year under report. *A. fluviatilis* was available in the low and intermediate rainfall areas as during the preceding year but in lower densities than those during the preceding year. *A. fluviatilis* was completely absent during the year 1959 in the high-rainfall area, while they were collected in small numbers during the preceding years.

PART II.

A. FLUVIATILIS AREA OF ERSTWHILE MYSORE STATE.

(REDUCTION OF FREQUENCY OF SPRAYING TO ONE ROUND).

Mudigere Taluk of Chickmagalur District and Belur Taluk of Hassan District were selected for reduction in the number of spraying rounds. One round of spraying with 112 mg. dose per sq. ft., was carried out in October and November, 1958, in Mudigere Taluk and spraying was completely suspended in Belur Taluk since the first round of 1959-60 (May – June, 1959) as this area satisfied the criteria laid down for suspension of spraying. These taluks are also covered by a net work of Primary and Secondary Health Units. The surveillance work was commenced in these taluks in October, 1956. There are 16 Primary and 3 Modified Health Units in this area, with a sanctioned staff of 35 Junior Health Inspectors and 5 Malaria Surveillance Workers. The total population of the two Taluks is 0·12 million (1951 census) and the average population surveillanced per day by each worker was 478 as against 405 during the preceding year. The pattern of surveillance has been similar to the one described under Part I of this paper. Details of Malaria Surveillance work carried out since October, 1958 to September, 1958, are depicted in Table I.

FEVER MORBIDITY.

A total of 2,193 fever cases were investigated during the year, covering a population of 0·09 to 0·12 million during different rounds (Table VI). The average number of fever cases, per 1,000 population, during the year ranged from 0·98 to 2·2 as against the average of 1·00 to 1·96 during the preceding year.

Age and sexwise details in respect of 6 cases were not available. The percentage of fever cases, in the age-group of '0-4 years', forms about 1/6th of the total cases investigated as against 1/9th during the preceding year.

TABLE IV.

Collection of anophelines and vectors, and their densities in different shelters from which they were collected, in areas selected for Malaria Surveillanae Work. From 1958-59 to end of September, 1959.

Area.	Years.	Hours spent in:			Number of all anophelines collected.	Density, per man-hour.	*A. culicifacies*:				*A. fluviatilis*:			
							Human dwellings and mixed dwellings.		Cattle-sheds.		Human dwellings and mixed dwellings.		Cattle-sheds.	
		Human dwellings and mixed dwellings.	Cattle-sheds.	Total (all shelters).			Number collected.	Density, per man-hour.	Number collected.	Density, per man-hour.	Number collected.	Density, per man-hour.	Number collected.	Density, per man-hour.
		H. M.	H. M.	H. M.										
(i) Spraying suspended in the *A. fluviatilis* area (Part I of the present paper).														
Sagar	1956-57	1871 20	904 05	2775 25	13,988	5·03	26	0·13	65	0·701	6	0·003	17	0·018
	1957-58	1527 40	889 20	2417 00	12,865	5·32	8	0·005	39	0·043	6	0·003	14	0·015
	1958-59	1273 40	674 50	1948 30	14,001	0·71	33	0·025	51	0·075	1	0·0007	8	0·011
Shikaripur	1956-57	1453 30	771 00	2224 30	5,505	2·47	8	0·0055	16	0·02	13	0·008	6	0·008
	1957-58	997 40	579 40	1577 20	8,374	5·3	19	0·019	17	0·029	2	0·001	12	0·019
	1958-59	1237 50	667 50	1905 40	13,266	6·9	36	0·029	38	0·056	16	0·012	5	0·007
(ii) Reduced-round in the *A. fluviatilis* area (Part II of the present paper).														
Belur	1956-57	1031 50	446 30	1478 20	7,154	4·8	7	0·006	54	0·12	1	0·0009	14	0·031
	1957-58	1167 30	353 50	1521 20	9,349	6·14	10	0·008	79	0·223	9	0·007	84	0·237
	1958-59	1000 30	281 50	1282 20	3,691	2·8	2	0·002	39	0·13	0	0·0	15	0·05
(iii) Reduced-round in the *A. culicifacies* area (Part III of the present paper).														
Krishnarajanagar	1956-57	1438 00	587 20	2025 20	2,975	1·46	9	0·006	20	0·034	0	0·0	0	0·0
	1957-58	1453 40	616 20	2070 00	6,189	1·98	6	0·004	3	0·004	0	0·0	0	0·0
	1958-59	1320 50	604 10	1925 00	9,907	5·1	3	0·002	9	0·015	9	0·006	14	0·023

(iv) North Kanara District—*A. fluviatilis* area under suspension of spraying. (Part IV of the present paper)*.

1958-59*	5540	30	3359	50	8600	20	22,064	2·56	7	0·001	49	0·016	4	0·0007	14	0·004

(v) Coorg District—*A. fluviatilis* area. Spraying suspended. (Part V of the present paper).

1957-58	56	50	30	40	87	30	123	1·4	0	0·0	0	0·0	0	0·0	2	0·065
1958-59†	470	20	363	50	834	10	1,016	1·2	2	0·004	2	0·005	8	0·01	19	0·05†

(vi) District Malaria Scheme Area, Puttur. (Part VI of the present paper).

1956-57	1131	30	748	30	1880	00	5,254	2·7	42	0·03	110	0·14	0	0·0	0	0·0
1957-58	575	30	366	30	209	00	1,562	2·7	10	0·02	25	0·11	0	0·0	0	0·0
1958-59†	1049	10	579	20	1628	30	2,354	1·4	11	0·01	44	0·07	0	0·0	0	0·0†

H.=Hours. M.=Minutes.

*Figures, for the years prior to 1958-59, have not been furnished as the surveillance work was being carried out in different types of tracts, i.e., rice and garden tracts, alternately, depending on the transmission season. During the year under report, the entire district was covered. Hence, figures for the current year only are furnished.

†Up to end of September, 1959.

TABLE V.

Collection of anophelines and vectors in Saklespur Area for the calender years 1956, 1957, 1958 and 1959 (Up to end of September, 1959).

A. FLUVIATILIS AREA — (Part I of the present paper) SPRAYING SUSPENDED.

	1956			1957		
	High rainfall area.	Low rainfall area.	Intermediate rainfall area.	High rainfall area.	Low rainfall area.	Intermediate rainfall area.
All anophelines	80	185	349	44	161	853
A. culicifacies	0	0	6	1	7	226
A. fluviatilis	0	2	0	2	3	3
Man-hours spent	200·0	205·30	205·13	22·58	48·02	90·30
Per man-hour density for all anophelines.	0·4	0·9	1·7	1·91	3·35	9·42

	1958			1959		
	High rainfall area.	Low rainfall area.	Intermediate rainfall area.	High rainfall area.	Low rainfall area.	Intermediate rainfall area.
All anophelines	695	451	1672	3046	426	2533
A. culicifacies	2	3	254	5	1	256
A. fluviatilis	3	11	7	0	5	10
Man-hours spent	68·40	63·35	168·10	163·30	239·00	494·30
Per man-hour density for all anophelines.	10·12	16·55	9·94	18·5	1·7	5·1

(Contd.)

TABLE V (*Concld.*).

Shelters from which the vectors were collected in Saklaspur Area and their density for the calender years, 1956, 1957, 1958 and 1959 (Up to end of September, 1959).

	1956						1957					
	High rainfall area.		Low rainfall area.		Intermediate rainfall area.		High rainfall area.		Low rainfall area.		Intermediate rainfall area.	
	Human dwellings and mixed dwellings.	Cattle-sheds.	Human dwellings and mixed dwellings.	Cattle-sheds.	Human dwellings and mixed dwellings.	Cattle-sheds.	Human dwellings and mixed dwellings.	Cattle-sheds.	Human dwellings and mixed dwellings.	Cattle-sheds.	Human dwellings and mixed dwellings.	Cattle-sheds.
A. culicifacies	0	0	0	0	0	6	0	1	0	7	0	226
A. fluviatilis	0	0	0	0	0	0	0	2	0	3	0	3
Man-hours spent	200·00		205·30		205·15		22·53		48·02		90·30	
Per man-hour density of *A. culicifacies.*	0	0	0	0	0	0·03	0	0·043	0	0·145	0	2·91
Per man-hour density of *A. fluviatilis.*	0	0	0	0·0	0	0	0	0·0	0	0·062	0	0·038

	1958						1959					
	High rainfall area.		Low rainfall area.		Intermediate rainfall area.		High rainfall area.		Low rainfall area.		Intermediate rainfall area.	
	Human dwellings and mixed dwellings.	Cattle-sheds.	Human dwellings and mixed dwellings	Cattle-sheds.	Human dwellings and mixed dwellings.	Cattle-sheds.	Human dwellings and mixed dwellings.	Cattle-sheds.	Human dwellings and mixed dwellings.	Cattle-sheds.	Human dwellings and mixed dwellings	Cattle-sheds.
A. culicifacies	0	2	0	3	0	254	0	5	0	1	1	225
A. fluviatilis	0	3	0	11	0	7	0	0	0	5	0	10
Man-hours spent	..	4·30	64·10	63·35	6·30	161·40	99·0	64·30	129·0	110·0	238·0	256·36
Per man-hour density of *A. culicifacies.*	0	0·031	0	0·047	0	1·57	0	0·076	0	0·009	0·004	0·99
Per man-hour density of *A. fluviatilis.*	0	0·046	0	0·173	0	0·43	0	0	0	0·045	0	0·038

TABLE VI.

Analysis of fever cases—Age and sexwise—Belur and Mudigere Taluks.

Age-group (Years).	Total number of fever cases.	Male.	Female.	Percentage in relation to total cases.	Percentage of population under each age-group.
0 — 4	340	184	156	15·55	13·5
5 — 14	403	250	153	18·43	24·8
15 and above.	1,444	888	556	66·02	61·7
Total	2,187	1,322	865	..	..

BLOOD SMEAR EXAMINATION.

1,692 blood smears from fever cases were examined. Of these, one smear was found positive for mixed infection with *P. vivax* and *P. malariae*. 1,691 blood smears were found negative for malaria parasites. During the preceding year, 1,670 blood smears were collected, examined and found negative for malaria parasites.

ENTOMOLOGICAL DATA.

Table IV depicts the collection of all anophelines and vectors since October, 1956 to September, 1959. There is a decrease in all anopheline density from 4·8 and 6·14 in the preceding two years to 2·8 in the year under report. While the density of *A. fluviatilis* in human dwellings-cum-mixed dwellings was 0·0009 and 0·007 during the preceding two years, it was 0·0 during the year under report. In cattle-sheds, the per man-hour density for *A. fluviatilis* was 0·031 and 0·237 during the preceding two years as compared to 0·05 during the year under report. There was also a decrease in the per man-hour density of *A. culicifacies* both in human dwellings-cum-mixed dwellings and cattle-sheds. During the preceding two years, the density of *A. culicifacies* in human dwellings-cum-mixed dwellings was 0·006 and 0·008. During the year under report, it was 0·002. In cattle-sheds, the *A. culicifacies* density during the year under report was 0·013 while it was 0·223 and 0·12 during the preceding 2 years.

PART III.

THE PAST *A. CULICIFACIES* AREA OF ERSTWHILE MYSORE STATE.

(REDUCTION IN FREQUENCY OF SPRAYING TO ONE ROUND.)

In Krishnarajnagar Taluk of Mysore District, reduction to one round of spraying was introduced during the year 1956. D.D.T. spraying was being carried out in this area at the rate of 112 mg. per sq. ft. during May—June. As this area satisfied the criteria laid down for suspension of spraying, D.D.T. spraying, due in May—June, 1959, was not carried out.

Surveillance work was started in the area during October, 1956, by availing of the services of the Health Unit staff. There are 7 Primary Health Units with a Secondary Centre, and only one Senior Health Inspector is provided for each Primary Health Unit. The pattern of surveillance in this area was confined to one visit to all the villages during the month. No transport was provided. For a total population of 0·107 million, in addition to 7 Health Inspectors, 2 Malaria Surveillance Workers were posted during April, 1957. The average population covered per day by a Senior Health Inspector or a Surveillance Worker came to about 1,500. However, the daily performance per worker varied from unit to unit, depending on the density of population and terrain. Details of the work carried out are presented in Table I.

FEVER MORBIDITY.

A total of 2,089 fever cases were investigated during the year. The average number of fever cases per 1,000 population varied from 0·25 to 5·0 during the year under report, as against the average varying from 0·22 to 1·23 during the preceding year. There was a rise in the fever rate investigated during May to September, in contrast to the recorded rise during October to February during the preceding year.

ANALYSIS OF FEVER CASES—AGE AND SEXWISE.

Table VII depicts the analysis of fever cases—age and sexwise.

TABLE VII.

Analysis of fever cases—Age and sexwise.

Age-group (Years).	Total number of fever cases.	Male.	Female.	Percentage in relation to total cases.	Percentage of population under each age-group.
0 — 4	581	319	262	27·82	13·5
5 — 14	579	342	237	27·73	24·8
15 and above	928	487	441	44·45	61·7
Total	2,088	1,148	940		

Data in respect of one fever case were not readily available.

The percentage of fever cases in the age-group '0 - 4 years', forms about 1/4th of the total cases as against 1/6th and 1/5th during the preceding 2 years.

BLOOD SMEAR EXAMINATION.

1,927 blood smears from fever cases were collected and examined. Of these, 1,924 blood smears were found negative for malaria parasites and 3 blood smears were found positive for *P. vivax* infection. Of these three cases, the whereabouts of one are not known. The other case gave history of movement, and

the third case was sporadic. Investigations revealed that there was no local transmission.

Apart from these 3 positive cases, one more case with *P. vivax* infection was found in a blood smear collected at random. This case was reported as sporadic.

ENTOMOLOGICAL DATA.

A. culicifacies appeared in low densities during 1956-57, and during 1957-58 there was a decline from 0·006 to 0·004, and there was a further decline from 0·004 to 0·002 in human dwellings-cum-mixed dwellings during the year under report. In cattle-sheds, it declined from 0·034 in 1956-57 to 0·004 in 1957-58 but there was a slight rise during the year under report, i.e., the density was 0·015. *A. fluviatilis*, which was absent during the preceding two years, appeared in low densities, i.e., 0·006 and 0·023 in human dwellings-cum-mixed dwellings and cattle-sheds, respectively, during the year under report (Table IV).

Part IV.

NORTH KANARA DISTRICT.

(*A. FLUVIATILIS* AREA, SPRAYING SUSPENDED, POPULATION 0·51 MILLION).

After completion of spraying for the tenth year in succession, D.D.T.-spraying was suspended in North Kanara District in June, 1956. An organisation was simultaneously started to study the effects of suspension of spraying. D.D.T.-spraying with 112 mg. per sq. ft., is, however, being carried out once in the hypo-endemic villages in the coastal belt and twice in the villages situated in 10 mile belt adjacent to Goa border in Supa and Karwar taluks and in the Dandeli mining area, covering a total population of about a lakh, so as to create a "CORDON SANITAIRE".

Since August, 1956, a comprehensive surveillance programme has been taken up in the villages of North Kanara District. Surveillance work up to December, 1958, was restricted to the malaria transmission season. Since December, 1958, onwards, surveillance work has been re-organised in the entire district. Forty surveillance workers, sanctioned for the purpose, with 12 permanent Havildars (superior field workers) of the National Malaria Eradication Unit, trained in surveillance work, were detailed for malaria surveillance work in the entire district throughout the year instead of only during the malaria season.

The entire population of North Kanara District was divided into 48 surveillance divisions, each in charge of a surveillance workers, with a population of about 10,000, depending on density of population and communications. Kanara West Division, with a population of 3,28,906, was divided into 25 divisions and Kanara

East Division, with a population of 1,88,874, was divided into 23 divisions. In each division, 2 malaria surveillance workers were kept as reserve. The two Malaria Inspectors of the West and the two of the East Division supervised the surveillance work under the overall supervision of the Medical Officer of Health of the respective divisons, assisted by the Assistant Medical Officer of Health. The details of work carried out are presented in Table I. The pattern of malaria surveillance was as follows :—

(*a*) Only one visit was paid to each of the houses in the area during the month,

(*b*) the staff on malaria surveillance duty belonged to malaria organisation,

(*c*) transport was not provided to surveillance workers. Each worker resided in a centrally situated headquarter village, covering the villages allotted to him on foot or by bicycle,

(*d*) surveillance work was separated from the general public health work.

FEVER MORBIDITY.

7,524 fever cases were investigated during the year under report. The number of fever cases per 1,000 population surveillanced ranged from 1·29 to 2·46 as against the average range of 0·91 to 3·62 during the previous year. An analysis of the fever cases investigated, is depicted in Table VIII.

TABLE VIII.

Analysis of fever cases investigated—Age and sexwise.

Age-group (Years).	Total number of fever cases investigated.	Male.	Female.	Percentage in relation to total.	Percentage of population under each age-group.
0 — 4	902	479	423	13·47	13·5
5 — 14	1,922	1,165	757	28·73	24·4
15 and above	3,868	2,574	1,294	57·80	61·7
Total	6,692	4,218	2,474	..	

Age and sexwise details in respect of 832 fever cases were not readily available. It can be made out that the number of fever cases in the age-group '0 - 4 years', forms about 1/7th of the total and the incidence of fever is comparatively lower among females, as during the previous year.

BLOOD SMEAR EXAMINATION.

Out of 7,354 blood smears collected from fever cases and examined, 7,350 blood smears were found negative for malaria parasites and 4 were found positive ; 3 for *P. vivax* and 1 for *P. falciparum* (light) infection. On investigation it was found that 3 of these positives were sporadic cases, and one was imported. All the four positive cases were treated with scheduled doses of 4 and 8-aminoquinolines.

ENTOMOLOGICAL DATA.

During the year under report, only 4 *A. fluviatilis* in human dwellings-cum-mixed dwellings and 14 *A. fluviatilis* in cattle-sheds were collected, the per man-hour density being 0·0007 and 0·004 respectively (Table IV).

Part V.

COORG DISTRICT.

(*A. FLUVIATILIS* AREA, SPRAYING SUSPENDED, POPULATION 0·2 MILLION).

Malaria surveillance work in Coorg District was started with effect from July, 1957, with the assistance of Health Inspectors and Health Sub-Inspectors. General health work was integrated with malaria work. There are 8 sanctioned posts of Health Inspectors and 8 posts of Health Sub-Inspectors, each of whom is assigned a population of 10,000 on an average and is required to visit the assigned population once during the month in 20 work-days. A population of 30,000, concentrated around dispensaries and hospitals, is excluded from malaria surveillance work. In addition to 8 Health Inspectors and 8 Health Sub-Inspectors, eight malaria Surveillance Workers were on malaria surveillance work exclusively. Table I furnishes the details of malaria surveillance work since October, 1958, to September, 1959.

FEVER MORBIDITY.

A total of 5,257 fever cases were investigated during the period. The average number of fever cases per 1,000 population varied from 1·9 to 4·1 as against 1·1 to 3·08 during the previous year. The incidence of fever was maximum during the month of September, 1959. An analysis of the fever cases investigated, is depicted in Table IX.

TABLE IX.

Analysis of fever cases investigated—Age and sexwise.

Age-group (Years).	Total number of fever cases.	Male.	Female.	Percentage in relation to total cases.	Percentage of population under each age-group.
0 — 4	706	371	335	15·32	13·5
5 — 14	1,220	659	561	26·46	24·8
15 and above.	2,684	1,586	1,098	58·22	61·7
Total	4,610	2,616	1,994		

Age and sexwise data in respect of 647 fever cases were not readily available. The number of fever cases in the age group '0 - 4 years', forms about 1/7th of the total, and the incidence of fever among females was less than that among males, as in the case of other areas.

BLOOD SMEAR EXAMINATION.

Out of 4,134 blood smears collected from fever cases and examined, 55 were found positive ; 36 for *P. vivax*, 13 for *P. falciparum*, 6 for mixed infection.

Apart from these, 64 blood smears were found positive (35 for *P. vivax*, 13 for *P. falciparum*, 5 for *P. malaria* and 11 for mixed infection) among the blood smears collected at random in the infected areas. All these positive cases were traced to *Yerwars* (Nomadic tribal people without a permanent abode) who had come to live in villages and the forest labour camps.

With a view to eradicating the source of infection, spraying has been resumed in the infected areas of the Srimangala and Gonikoppal divisions and the positive cases are being treated with 4 and 8-aminoquinolines.*

ENTOMOLOGICAL DATA.

Six *A. fluviatilis* in human dwellings-cum-mixed dwellings and 19 *A. fluviatilis* in cattle-sheds were collected during the year, giving a per man-hour density of 0·01 and 0·05 respectively (Table IV).

Part VI.

PUTTUR, SOUTH KANARA DISTRICT.

The South Kanara District Malaria Scheme area, Puttur, comprises a population of 0·23 millions, living in the foot-hill areas and adjacent plains on the western slopes of the western ghats. This is the *A. fluviatilis* and *A. culicifacies* area, with transmission season extending from December to May. The surveillance work was started during the month of September, 1957. The staff consisted of 6 Health Inspectors, 26 Field Assistants, one Assistant Entomologist and 2 Laboratory Assistants under a Malaria Health Officer. Each of the Field Assistants was assigned a population of 10,000. He visited each house once a month. No transport was provided. Out of the 26 Field Assistants, 23 were detailed for surveillance work and 3 for entomological work. The work of every batch of 4 Field Assistants was supervised by one Health Inspector, and the entomological work of Field Assistants was supervised by the Assistant Entomologist by actual random test collection in the villages. The blood smears were examined at the Laboratory attached to the Health Office, Puttur, and test checks made at the Central Laboratory, Bangalore. The details of malaria surveillance work in the Puttur area are depicted in Table I.

FEVER MORBIDITY.

A total of 3,966 fever cases were investigated. The average number of cases per 1,000 population surveillanced ranged from 1·2 to 1·97 during the different months, as against the average of 1·22 to 3·17 during the preceding year. The incidence was maximum during the month of June, 1959.

An analysis of the fever cases investigated, is depicted in Table X.

* *See* Aiappa, M.T. (1961) *Ind. Jour. Mal.*, 15, 1, pp. 1-10. —Editor.

TABLE X.

Analysis of fever cases investigated—Age and sexwise.

Age-group (Years).	Total number of fever cases investigated.	Male.	Female.	Percentage in relation to total.	Percentage of population under each age-group.
0 — 4	301	151	150	7·59	13·5
5 — 14	1,242	814	428	31·32	24·8
15 and above	2,423	1,634	789	61·09	61·7
Total	3,966	2,599	1,367		

The number of fever cases in the age-group '0 - 4 years', formed about 1/12th of the total, and the incidence of fever was low among females than males.

BLOOD SMEAR EXAMINATION.

Out of 3,946 blood smears examined, none was found positive for malaria parasites. During the preceding year, 4,993 blood smears were collected and examined. Out of these, one was found positive for *P. vivax*, and the rest were found negative for malaria parasites.

ENTOMOLOGICAL DATA.

These are presented in Table IV. No specimen of *A. fluviatilis* was collected during the year as well as during the preceding 2 years. 11 specimens of *A. culicifacies* in human dwellings-cum-mixed dwellings and 44 *A. culicifacies* in cattle-sheds were collected. The per man-hour density in these two types of shelters worked out to 0·01 and 0·07 respectively.

DISCUSSION AND CONCLUSION.

Malaria surveillance was continued in several areas of the Mysore State on the same patterns as described by Sharma Sastry and Narayana Iyengar (1959 *loc. cit.*), except in North Kanara District. In North Kanara District, however, the pattern of surveillance was changed over to one of monthly visits throughout the year in the entire district since December, 1958, instead of seasonal surveillance in the rice and garden tracts during the transmission season.

In Tirthahalli, Hosanagar, Sagar, Sorab and Shikaripur taluks of Shimoga District; Koppa and Sringeri taluks of Chickmagalur District and in Sakalespur Taluk of Hassan District where spraying has been completely withdrawn, surveillance work was carried out through fortnightly visits to every house in the area. General public health work and malaria surveillance work were integrated in this area.

In Mudigere and Belur taluks of Chickmagalur and Hassan districts respectively, where spraying rounds were reduced to one round in the year (In Belur Taluk, the one round due in May-June, 1959, was not carried out as this area satisfied the criteria laid down for suspension of spraying), fortnightly visits were paid by the staff; and the public health work and malaria surveillance work were integrated in this area.

TABLE XI.

Summary of epidemiological surveillance data for the different areas under study.

Serial number.	Area.	Population (In million).	Surveillance Staff.				Average population (in million) covered, per round.			Average number of fever cases per 1,000 population during different months.			Number of fever cases investigated.			Number positive, and the results of their investi	
			Sanctioned.		Working.												
			Health Inspectors.	Malaria Surveillance Workers.	Health Inspectors.	Malaria Surveillance Workers.	1956-57 (First year).	1957-58 (Second year).	1958-59 (Third year).	1956-57 (First year).	1957-58 (Second year).	1958-59 (Third year).	1956-57 (First year).	1957-58 (Second year).	1958-59 (Third year).	1956-57 (First year).	1957-58 (Second year).
1	*A. fluviatilis* area. Spraying suspended.	0·40	118	18	54 — 88	11 — 18	0·097 to 0·35	0·344 to 0·399	0·31 to 0·38	0·97 to 3·6	1·66 to 3·23	1·49 to 2·9	10,889	10,322	8,301	6 (Of these, 4 positive cases were detected during the surveillance studies, while the remaining 2 positive cases were observed in the course of taking smears at random).	6 (Of these, 3 positive cases were detected during the surveillance studies, while the remaining 3 positive cases were observed in the course of taking smears at random. (3 cases are with history of movement. 2 cases imported from hypo-endemic area of South Kanara).
2	*A. fluviatilis* area. Spraying rounds reduced.	0·12	35	5	16 — 22	3 — 5	0·098 to 0·12	0·101 to 0·126	0·09 to 0·12	0·18 to 1·52	1·00 to 1·96	0·98 to 2·2	1,997	2,145	2,193	Nil	Nil
3	*A. culicifacies* area. Spraying rounds reduced, but suspended in the later part of the third year of study.	0·10	7	2	2 — 6	2	0·04 to 0·091	0·05 to 0·11	0·06 to 0·11	0·19 to 0·91	0·22 to 1·23	0·25 to 5·9	543	641	2,089	Nil	Nil
4	North Kanara District. Spraying suspended.	0·51	..	52	..	18 — 48	0·013 to 0·47	0·079 to 0·51	0·12 to 0·5	0·82 to 3·2	0·91 to 3·62	1·29 to 2·46	2,103	3,393	7,524	6 (Of these, 3 cases were imported ones).	6 (3 cases imported from the neighbouring States, 3 sporadic).
5	Coorg District. *A. fluviatilis* area— Spraying suspended.	0·19	16	8	10 — 15	5 — 8	0·09 to 0·12	0·06 to 0·15	0·11 to 0·17	0·4 to 1·3	1·1 to 3·08	1·9 to 4·1	309 (Studies carried out for three months only).	2,606	5,257	Nil	14 (Local problems. Transmission not totally interrupted).
6	The District Malaria Scheme area, Puttur. *A. fluviatilis* and *A. culicifacies* area, spraying suspended.	0·22	..	23 (3 for entomological work).	..	17 — 23	Studies carried out for one month only.	0·21 to 0·22	0·17 to 0·22	Studies carried out for one month only.	1·22 to 3·17	1·2 to 1·97	776	5,157	3,966	Nil	1 (Relapse).

TABLE XI

Summary of epidemiological surveillance data for the different areas under study.

Surveillance Staff.			Average population (in million) covered, per round.			Average number of fever cases per 1,000 population during different months.			Number of fever cases investigated.			Number positive, and the results of their investigation.			Remarks.
ioned.	Working.														
Malaria Surveillance Workers.	Health Inspectors.	Malaria Surveillance Workers.	1956-57 (First year).	1957-58 (Second year).	1958-59 (Third year).	1956-57 (First year).	1957-58 (Second year).	1958-59 (Third year).	1956-57 (First year).	1957-58 (Second year).	1958-59 (Third year).	1956-57 (First year).	1957-58 (Second year).	1958-59 (Third year).	
18	54 — 88	11 — 18	0·097 to 0·35	0·344 to 0·399	0·31 to 0·38	0·97 to 3·6	1·66 to 3·23	1·49 to 2·9	10,889	10,322	8,301	6 (Of these, 4 positive cases were detected during the surveillance studies, while the remaining 2 positive cases were observed in the course of taking smears at random).	6 (Of these, 3 positive cases were detected during the surveillance studies, while the remaining 3 positive cases were observed in the course of taking smears at random. (3 cases are with history of movement. 2 cases imported from hypo-endemic area of South Kanara).	3 (All the 3 cases were imported ones, i.e., two from the South Kanara hypoendemic area, and one from South Arcot, (Madras State).	
5	16 — 22	3 — 5	0·098 to 0·12	0·101 to 0·126	0·09 to 0·12	0·18 to 1·52	1·00 to 1·96	0·98 to 2·2	1,997	2,145	2,193	Nil	Nil	1 (Sporadic case).	
2	2 — 6	2	0·04 to 0·091	0·05 to 0·11	0·06 to 0·11	0·19 to 0·91	0·22 to 1·23	0·25 to 5·9	543	641	2,089	Nil	Nil	3 (The whereabouts of 1 case are not known; 1 was with history of movement; the source of infection of the third case is not known).	One more positive was detected in the course of taking smears at random, during the third year (1958-59).
52	..	18 — 48	0·013 to 0·47	0·079 to 0·51	0·12 to 0·5	0·82 to 3·2	0·91 to 3·62	1·29 to 2·46	2,103	3,393	7,524	6 (Of these, 3 cases were imported ones).	6 (3 cases imported from the neighbouring States, 3 sporadic).	4 (1 case is imported one; 3 cases are sporadic).	
8	10 — 15	5 — 8	0·09 to 0·12	0·06 to 0·15	0·11 to 0·17	0·4 to 1·3	1·1 to 3·08	1·9 to 4·1	309 (Studies carried out for three months only).	2,606	5,257	Nil	14 (Local problems. Transmission not totally interrupted).	55 (Local problems).	64 positive cases, from smears taken at random, were also found during the third year.
23 (3 for entomological work).	..	17 — 23	Studies carried out for one month only.	0·21 to 0·22	0·17 to 0·22	Studies carried out for one month only.	1·22 to 3·17	1·2 to 1·97	776	5,157	3,966	Nil	1 (Relapse).	Nil	

In Krishnarajnagar Taluk of Mysore District, where one round of spraying was carried out annually, the spraying due in May-June, 1959, was not carried out as this area satisfied the criteria laid down for suspension of spraying. In this area also, the general public health work and malaria surveillance work were integrated and houses were visited once every month.

In Coorg District, where spraying has been suspended, the general public health work and malaria surveillance work were integrated and houses were visited once every month.

In North Kanara District, seasonal surveillance during the transmission season in the rice and garden tracts was changed over to one visit during the month to every house all through the year in the entire district.

In the District Malaria Scheme area of South Kanara District, the surveillance staff belonged exclusively to malaria organisation and houses were visited once a month.

FEVER MORBIDITY.

There was an increase in the number of fever cases investigated over that of the preceding year in all the areas except in areas Numbers 1 and 6, i.e., the *A. fluviatilis* area of erstwhile Mysore where spraying has been suspended and the District Malaria Scheme area, Puttur, where the number of fever cases investigated recorded a decline from what it was during the preceding year (*vide* Table XI depicting *"Summary of the Epidemiological Surveillance data"*).

In the *A. fluviatilis* area of erstwhile Mysore, i.e., in areas Numbers 1 and 2, where each house was visited twice every month, the average number of fever cases per 1,000 population surveillanced ranged from 1·49 to 2·9 and 0·98 to 2·2 respectively. In the *A. culicifacies* area (Area Number 3), where each house was visited once a month, the average ranged from 0·25 to 5·0. Except during the months of August and September, 1959, when the average worked out to 4·7 and 5·0 respectively, the averages ranged from 0·25 to 2·4 during the other months in this area. During the preceding two years, the averages in the *A. culicifacies* area were comparatively less than those obtaining in the *A. fluviatilis* area where two visits to each house in a month were scheduled (*vide* Table XI).

In North Kanara District (Area Number 4), where each house was visited only once every month, the average number of fever cases per 1,000 population surveillanced ranged from 1·29 to 2·46 as against the range of 0·82 to 3·2 during the first year and 0·91 to 3·62 during the second year. During the first year and first half of the second year, each house was visited twice every month. During the year under report, as previously described, the entire district was covered all through the year with one visit a month, to each house, instead of seasonal surveillance alternately in the rice and garden tracts with two visits a month to each house.

In Coorg District and the District Malaria Scheme area, Puttur, where each house was visited only once every month, the averages ranged from 1·9 to 4·1 and

1·2 to 1·97 respectively. While in the former there was an increase over that of the preceding year in the number of fever cases investigated, in the latter there was decrease in the number of cases investigated from that of the preceding year.

The epidemiological surveillance data from the different areas is summarised in Table XI. It is observed that the performance of the surveillance staff was improved over the preceding years. A certain portion of the population still remained uncovered during some of the visits. The reasons of such deficiencies appear to be mainly administrative and are being attended to.

EXAMINATION OF BLOOD SMEARS.

As many as 26,839 blood smears from fever cases were examined during the year for a total population of 1·54 million (1951 census). Details of these blood smears, collected from areas which were visited twice a month or once a month, are given in Table XII.

TABLE XII.

Details of blood smears.

Pattern of surveillance.	Population (in millions.)	Number of blood smears collected.	Estimated number of slides per million population.
Each house visited twice a month.	0·52	9,478	18,227
Each house visited once a month.	1·02	7,361	17,020
Total	1·54	26,839	

There appears to be no difference in the number of slides collected in the two areas with monthly and bimonthly visits.

POSITIVE BLOOD SMEARS.

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

It can be seen from the data contained in Table XIII, that the number of positives, encountered during the year under report, did not increase over that of the preceding years except in Coorg District where the number of positives has increased over that of the preceding year and this has occurred in a localised area. The infection has still been lurking among the tribal population (*Yerwars*). Action has been taken to eradicate the infection by resumption of spraying in the affected areas and also by treating the positives radically. The investigation of these cases has been communicated elsewhere separately*. Some sporadic cases have occurred in the *A. fluviatilis* area where spraying rounds have been reduced, and also in the *A. culicifacies* area where spraying rounds have been reduced. But for these occurrences, positive cases tend to dwindle gradually.

* *See* Aiappa, M. T. (1961) *Ind. Jour. Mal.*, 15, 1, pp. 1-10. —Editor.

TABLE XIII.

Findings in regard to the positive blood smears.

Area.	First year.		Second year.		Third year (under report).	
	Surveillance smears.	Random smears.	Surveillance smears.	Random smears.	Surveillance smears.	Random smears.
1. *A. fluviatilis* area where spraying was completely withdrawn.	4	2	3* *(2 with history of movement).	3† †(2 imported, 1 with history of movement).	3 (All imported).	..
2. *A. fluviatilis* area where spraying was reduced to one round in the year.	Nil	Nil	Nil	Nil	1 (Sporadic).	..
3. *A. culicifacies* area where spraying was reduced to one round a year.	Nil	Nil	Nil	Nil	3* *(Whereabouts of one case are not known. One case is with history of movement. Source of infection of the 3rd case could not be established).	1 (sporadic)
4. North Kanara District.	6‡ ‡(3 imported, 3 sporadic).	Nil	6§ §(3 imported, 3 sporadic).	Nil	4§§ §§(1 imported, 3 sporadic).	..
5. Coorg District.	Nil	Nil	14	Nil	55 (Local problems).	64
6. The District Malaria Scheme area, Putur.	Nil	Nil	1	Nil	Nil	Nil

The analysis of fever cases, age and sexwise, shows that incidence of fever cases among the age-group '0-4 years' formed about 1/6th to 1/7th of the total cases in the areas described under Parts I, II, IV and V of this paper, while it formed 1/3rd of the total in the areas mentioned in Part III, and about 1/12th of the total in the area mentioned in Part VI of this paper. The incidence of fever cases among females has been less than that among males in all the area.

DRUG DISTRIBUTION.

4-amino-quinoline tablets (Avlochlor and Resochin tablets) were distributed to fever cases in the areas surveillanced twice a month, and in the areas surveillanced once a month. Details in regard to distribution of the tablets, are contained in Table XIV.

There appears to be not much difference in the quantity of the anti-malaria drug used, per million population, in the two areas.

TABLE XIV.

Details in regard to distribution of the 4-amino-quinoline tablets.

Pattern of surveillance.	Population (in millions).	Number of fever cases investigated.	Number of 4 amino-quinoline tablets distributed to fever cases.	Estimated number of pills, per million population.
Each house visited twice a month	0·52	10,494	33,087	63,629
Each house visited once a month	1·02	18,836	57,538	56,409
Total	1·54	29,330	90,625	

ENTOMOLOGICAL DATA

In general, there was a gradual build-up of the anopheline density though the density of the carrier species was still far below the assumed critical density.

SUMMARY.

The surveillance programme, already described, was continued during the third year (October, 1958, to September, 1959). 29,330 fever cases were encountered in all the areas, out of which 66 cases were found microscopically positive. In addition, 65 positive cases were encountered during the other surveys. Epidemiological investigations of these cases showed that, 4 were imported, 4 were sporadic, and in respect of 4 full investigations could not be made. The remaining 119 cases were among a particular section of the population (*Yerwars*—a tribal population), living in the interior forest areas confined to south-eastern portion of the Coorg District. The investigation of these cases has been communicated separately*.

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

Experiences, gained during the three years of the pilot surveillance studies, have been made use of in organising the malaria surveillance since April 1, 1960, all through the State, comprising an estimated population of 22·5 millions.

ACKNOWLEDGEMENTS.

The author wishes to express his thanks to all the officers and officials in the respective districts who have actively participated in the Pilot Studies and to Sri K. Sitaramaiya, Malaria Supervisor, Bureau of Malariology, Bangalore, for his assistance. The valuable criticisms offered by the Director, National Malaria Eradication Programme, Delhi, and the Director, Malaria Institue of India, Delhi, are gratefully acknowledged.

* *See* Aiappa, M. T. (1961) *Ind. Jour. Mal.*, **15**, 1, pp. 1-10. —Editor.

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

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Indian Journal of Malariology, **15**, *3*, *September 1961*.

A NOTE ON THE RESULTS OF SURVEILLANCE PROGRAMME IN SAKALESPUR TALUK*, HASSAN DISTRICT, MYSORE STATE, BETWEEN OCTOBER, 1956—FEBRUARY, 1960.

BY

N.L. SITARAMAN,

C. ACHUTHAN,

SETHURAMA RAO,

A.R. SUNDER RAO

(Malaria Investigation Centre, Mandya, Mysore State.)

AND

H. SHAMA SASTRY.

(Bureau of Malariology, Mysore State, Bangalore.)

[January 4, 1961.]

INTRODUCTION.

SAKALESPUR TALUK in Hassan District, Mysore State, was one of the areas selected in 1946 for preliminary observations in large-scale malaria control measures by the use of residual insecticides like DDT. Prior to the introduction of residual insecticidal spraying in 1946, the whole of Sakalespur Taluk was highly malarious, with spleen rates above 60·0 per cent. The percentage of malaria cases treated in several dispensaries was more than 40·0 per cent. Malaria control by the use of residual insecticides was started in November, 1946, in 40 villages of Sakalespur Taluk. Early in 1949, the Government sanctioned a scheme for opening a Malaria Investigation Centre. A Secondary Health Centre at Sakalespur, with seven Primary Centres, was also sanctioned to provide integrated curative and preventive services to a population of 38,750 (1941 Census).

During 1949, although the benefit of malaria control measures was extended to the entire Secondary Centre area, there were still certain 'pocket' areas which remained un-protected. With the implementation of the National Malaria Control Programme in November, 1953, these 'pocket' areas were also included in the spraying programme. The criteria† for interruption of spraying were fulfilled in the

*Taluk : Each district is divided into taluks or tahsils, for the purpose of revenue administration.

†The Expert Committee of Malariologists appointed by the Government of India, in 1956, adopted the following criteria for interruption of spraying :—

(1) Childhood spleen rate is less than 5 per cent,
(2) Childhood parasite rate is less than 1 per cent, and
(3) Infant parasite rate is zero,

for a consecutive period of two years.

entire Taluk in 1955. Residual insecticidal treatment was withdrawn with effect from November, 1956, after establishing a surveillance organisation during October, 1956.

Patel *et al.* (1957) have described one year's results of surveillance work in North Kanara District, a typical *A. fluviatilis* area, which was part of Bombay State before the Reorganisation of States in November, 1956. Shama Sastry and Iyengar (1959) reported the results of a pilot study of malaria surveillance measures carried out in Mysore State between October, 1956—September, 1957. This paper presents the results of surveillance work carried out in Sakalespur Taluk of Hassan District, Mysore State, between October, 1956—February, 1960.

EPIDEMIOLOGY OF MALARIA IN SAKALESPUR TALUK.

The Taluk (Map 1) is a typical example of an area where malaria was transmitted by *A. fluviatilis*. Out of the 24 species of anophelines which had been recorded prior to 1946, only *A. fluviatilis* had been incriminated as the vector (Nursing *et al.*, 1934). The available information before 1946 indicated that no part of the year was climatologically unfavourable for malaria transmission. But the actual seasons of transmission were determined mainly by the opportunities for continuous breeding of the vector species, *A. fluviatilis*, which, in turn, was influenced by topography and rainfall.

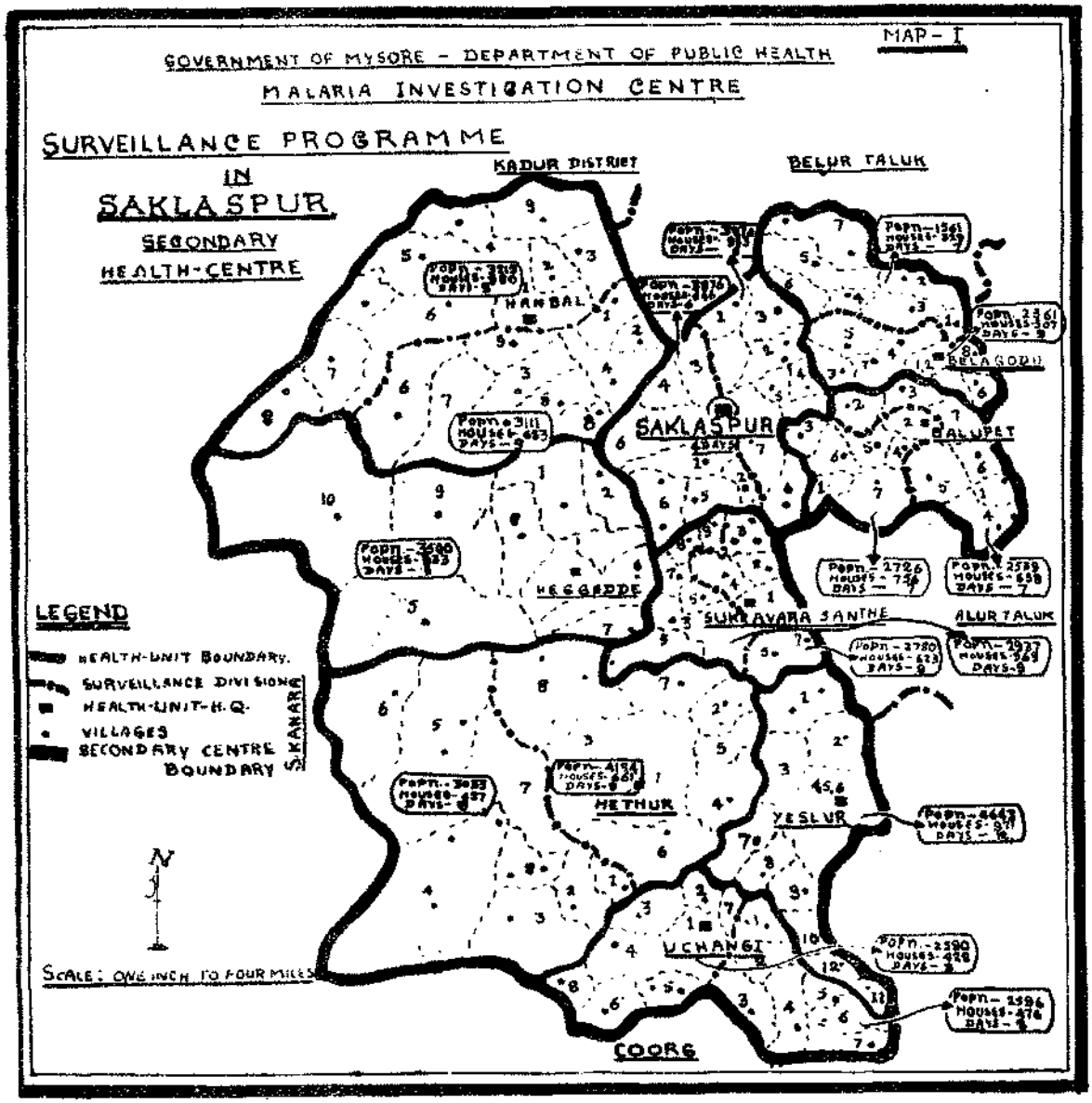
The taluk consists of three distinct zones with remarkable variations in rainfall. The western zone or the Ghat* Region consists of dense forests, steep and rugged valleys, the average annual rainfall extending up to 200 inches. There are a number of perennial streams, which, from November to May, afford ideal breeding places for *A. fluviatilis*. In this zone, no breeding of *A. fluviatilis* occurred during the monsoon months (June-October) because of the heavy precipitation. The eastern zone consists of gently rolling plains with a low range of hills. There is an abrupt diminution in rainfall to about 45 inches per annum. Although there are no perennial streams in the area, *A. fluviatilis* continued to breed throughout the year in the streams during the early and later parts of the monsoon and in the irrigation channels, taking off from tanks, during the monsoon. Between the western and eastern zones, there is an intermediate rainfall area with characteristics of both zones. Annual rainfall is about 85 inches. There are a few perennial streams and breeding of *A. fluviatilis* is confined to pre- and post-monsoon months.

The spleen rates during April and October, 1946, were 82·4 and 57·5 per cent respectively. Data regarding infant and childhood parasite rates for 1946 are not available. But in 1948, the infant parasite rate was 3·7 per cent.

During 1950-1953, detailed parasitological and entomological studies were conducted in three unsprayed 'pocket' areas in Sakalespur Taluk. These 'pockets' were surrounded by sprayed areas and exhibited low-to-moderate endemicity for malaria. All the three species of human plasmodia were present, though the

* Ghat=Hilly.

Map I.


MAP - I
GOVERNMENT OF MYSORE - DEPARTMENT OF PUBLIC HEALTH
MALARIA INVESTIGATION CENTRE
SURVEILLANCE PROGRAMME
IN
SAKLASPUR
SECONDARY
HEALTH-CENTRE
KADUR DISTRICT
BELUR TALUK
HANBAL
SAKLASPUR
BELAGODU
ALUR TALUK
HEGGADDE
SUNKAVARA SANTHE
HETHUR
YESLUR
UCHANGI
COORG
S. KANARA
LEGEND
HEALTH-UNIT BOUNDARY.
SURVEILLANCE DIVISIONS
HEALTH-UNIT-H.Q.
VILLAGES
SECONDARY CENTRE BOUNDARY
SCALE: ONE INCH TO FOUR MILES

overall parasite rate was very low. The childhood parasite rate varied from 0·6 to 4·6 per cent and the infant parasite rate was zero. Although a large number of *A. fluviatilis* and *A. culicifacies* were dissected, no naturally infected specimens were encountered. The malario-metric indices indicated that there was a recession of malaria even in these three unsprayed 'pocket' areas. It is possible that several factors contributed for this recession even in the absence of any specific malaria control measures, the most important being the protection of adjacent areas by DDT-spraying, better medical facilities due to the starting of Health Units, and improved economic conditions.

Some interesting observations were also made on the habits of *A. fluviatilis* in the area. The bulk of *A. fluviatilis* were collected in the cattle-sheds and mixed dwellings, with a small fraction in human dwellings. The anthropophilic index of the species was only 3·3 per cent. It is possible that, due to the recession of malaria in the area, the anthropophilic population of *A. fluviatilis* was on the decline and the zoophilic proportion on the increase (Brooke Worth and Sitaraman, 1952; Bhombore *et al.*, 1956).

RESULTS OF MALARIA CONTROL.

As stated earlier, malaria control measures which were started on an experimental basis in 1946 in 40 villages in the taluk, were gradually extended to cover the entire Secondary Centre area by 1949. The number of malaria cases treated in the dispensaries came down from 20,983 in 1946 to 1,855 in 1953, i.e., from 20·1 per cent to 5·7 per cent of the total cases treated. The spleen rate, which was 32·4 per cent in 1946, had fallen to 39·9 per cent in 1949 and thereafter there was a progressive decline. In 1953 when the National Malaria Control Programme was instituted, the spleen rate had come down to 3·4 per cent. No figures are available regarding the infant and childhood parasite rates between 1949 and 1953. Between 1954-1956, 985 infant blood smears, examined from the Health Unit area, were negative for malaria parasites. Similarly 713 infant blood smears from the three 'pocket' areas, which came under spray with effect from 1953, were negative for malaria parasites. Though the childhood parasite rates are not available for this period, but since the spleen rate was less than 5·0 per cent and the infant parasite rates were also nil for the same period, it is permissible to presume that the childhood parasite rate would have been well below one per cent.

There were also very significant changes in the demographic indices. Birth rate, which was 22·5 in 1946, rose to 31·6 in 1953; death rate, which was 21·7 in 1946, declined to 12·1 in 1953. The infant and maternal mortality rates, which were respectively 158·1 and 40·9 in 1946, had declined to 103·1 and 9·0 by 1953. Population of the Secondary Centre area which was 33,750 in 1941, had risen to 45,020 by 1951.

The implementation of the National Malaria Control Programme in 1953 yielded more rapid results, and by 1955 the malario-metric indices in the entire

Secondary Centre area and the three 'pocket' areas indicated that all the criteria laid down for interruption of spraying were fully satisfied.

The effect of this large-scale insecticidal spraying on anopheline densities in general, and on vector species in particular, has been remarkable. *A. culicifacies* and *A. fluviatilis* completely disappeared from human and mixed dwellings, the few specimens collected being entirely from cattle-sheds. There has been a corresponding decline in the other anopheline densities also. Table I furnishes details of entomological data in the 'pocket' areas before and after DDT spraying and after interruption of spraying.

SURVEILLANCE ORGANISATION.

When interruption of spraying was recommended in 1955, the setting up of a surveillance organisation was contemplated. On account of the existence of a network of Primary Health Units in the area, it was easily possible to mobilize the available staff and integrate surveillance work with the Health Unit activities. There are eight Primary Health Units in the Taluk, each Unit having a population varying from 3,000 to 6,000 and divided into two convenient divisions based on terrain and population (Map 1). Each of these two Divisions is provided with a Junior Health Inspector, who is required to visit all the villages in his respective division once during the month to collect and verify vital statistics and to attend to other health activities. The visits of these Inspectors to villages in their jurisdiction were utilised, with advantage, for surveillance work also. For the 'pocket' areas, with a population of 3,500, not covered by the Health Units, three Surveillance workers were appointed. Sakalespur Town, with a population of 5,578, was also included in the Surveillance Programme. Thus the total population, allotted for surveillance, works out to 54,107, comprising a population of 45,029 from the Secondary Health Centre area, 3,500 from the 'pocket' areas and 5,578 from the town.

The sanctioned staff consists of 18 Health Inspectors and 3 Surveillance workers. But, at no time the full complement of the sanctioned staff was working and the number actually on duty varied from 9 to 15 Health Inspectors and 0 to 3 Surveillance Workers. This has naturally resulted in the entire population allotted not being covered completely during both the rounds in any month. However, in some months it has actually been possible to cover the entire allotted population at least once in the month.

RESULTS OF SURVEILLANCE WORK.

Surveillance Programme has been implemented since October, 1956, and the results achieved are set out in Tables II, III and IV. In Table II, are presented the per cent population surveillanced, number of blood smears collected and examined and the fever cases per mille. Full details of the parasite positives, encountered during the surveillance programme, are presented in Table III, while Table IV depicts the annual averages, *viz.*, percentage population actually surveyed, fever case rate per mille and the average population surveyed by an individual worker.

TABLE I.

Anopheline densities in the Sakalespur area during the years 1952—1959.

HRA=High rainfall area. IRA=Intermediate rainfall area. LRA=Low rainfall area.

Species.	1952.			1953.			1954.			1955.		
	HRA.	IRA.	LRA.	HRA.	IRA.	LRA.	HRA.	IRA.	LRA.	HRA.	IRA.	LRA.
Numbers of *A. culicifacies*	28	466	228	9 (5)*	884	42 (4) *	Only random surveys were carried out.	153	Only random surveys were carried out.	0	1	
Numbers of *A. fluviatilis*	52	18	148	19 (2)*	24	30 (2) *		8		1	0	2
Numbers of other anophelines	5,000	3,703	7,488	949 (399)*	2,961	506 (90) *		723		48	71	94
Density per man-hour — *A. culicifacies*	0·13	0·7	0·8	0·2 (0·1) *	1·4	0·7 (0·1) *		0·24		0·0	0·007	0·0
Density per man-hour — *A. fluviatilis*	0·25	0·03	0·3	0·41 (0·05)*	0·04	0·5 (0·04)*		0·01		0·007	0·0	0·009
Density per man-hour — Others	23·8	5·8	27·3	20·6 (9·8) *	4·7	8·5 (2·0) *		1·1		0·34	0·1	2·1

*(Figures in brackets indicate data after spray).

(*Contd.*)

TABLE I (*Concld.*)

HRA=High rainfall area. IRA=Intermediate rainfall area. LRA=Low rainfall area.

Species.		1956.			1957.			1958.			1959.		
		HRA.	IRA.	LRA.	HRA.	IRA.	LRA.	HRA.	IRA.	LRA.	HRA.	IRA.	LRA.
Numbers of *A. culicifacies*		0	6	0	2	38	7	1	63	3	6	87	1
Numbers of *A. fluviatilis*		0	0	2	3	2	10	3	15	8	0	8	6
Numbers of other anophelines		80	343	183	338	640	254	2,882	2,002	404	935	,085	424
Density per man-hour	*A. culicifacies*	0·0	0·03	0·0	0·01	0·08	0·04	0·006	0·1	0·006	0·04	0·27	0·004
	A. fluviatilis	0·0	0·0	0·01	0·02	0·004	0·05	0·018	0·08	0·018	0·0	0·024	0·03
	Others	0·4	1·7	0·9	0·25	1·3	1·4	17·3	3·5	2·2	6·8	3·3	1·8

Explanatory Note :—The first round of spray in the high and in the low-rainfall-areas was carried out in April, 1953, at the rate of 200 mg./sq.ft. of DDT. After that, the areas were included under the National Malaria Control Programme. In all, six rounds of spray with residual insecticide till the end of December, 1955, were carried out, i.e., five rounds with DDT and the sixth round with DDT in some villages and Dieldrin in some other villages. The intermediate-rainfall-area has been under insecticidal treatment since 1946. During 1956, there was no spraying in all the three areas. During 1957 and 1958, only the low-rainfall-area received one round of spray with DDT at the rate of 112 mg./sq.ft. during May and August respectively. In 1959 there was no spraying even in the low-rainfall-area.

TABLE II.

Details of surveillance work carried out in the Sakalespur area during the period October, 1956 to February, 1960.

Total Population allotted = 54,107 (Secondary Centre Area = 45,029. Heggade Pocket Area = 3,500. Sakalespur Town = 5,578.)

Months.	1956			1957			1958			1959			1960		
	Population surveillanced. (per cent.)	Number of blood smears collected.	Fever cases per mille.	Population surveillanced. (per cent.)	Number of blood smears collected.	Fever cases per mille.	Population surveillanced. (per cent.)	Number of blood smears collected.	Fever cases per mille.	Population surveillanced. (per cent.)	Number of blood smears collected.	Fever cases per mille.	Population surveillanced. (per cent.)	Number of blood smears collected.	Fever cases per mille.
January	Surveillance work was organised with effect from October, 1956.			60·9	223	6·8	94·9	187	3·6	100·0	229	4·2	93·7	154	2·8
February				60·6	291	8·8	94·9	313	6·0	100·0	226	4·2	94·3	177	3·5
March				65·7	270	7·5	90·2	170	3·5	99·6	255	4·7	..	..	..
April				62·8	211 (1)*	6·2	100·0	145	2·6	89·4	199	4·1	..	..	..
May				78·3	171	4·0	95·6	144	2·7	93·0	208	4·1	..	..	..
June				74·5	225 (1)*	5·2	98·5	168	3·0	98·4	186	3·5	..	..	..
July				93·4	319 (1)*	6·3	98·6	159	2·9	98·4	148	3·1	..	..	..
August				100·0	298	5·5	98·5	176	3·2	84·6	156 (1)*	3·4	..	..	..
September				100·0	313	5·7	85·4	172	3·7	94·8	175	3·3	..	..	..
October	60·4	185	5·6	94·2	212	4·1	98·8	179	3 3	82·3	175	3·9	..	..	..
November	59·9	163	5·0	94·2	275	5·4	95·7	176	3·3	97·6	219	4·1	..	..	..
December	56·1	200	6·0	100·2	222	4·0	89·7	149	3·1	96·3	187	3·5	..	..	..
Total	..	548 (0)*	5·7	..	3,030 (3)*	5·7	..	2,138 (0)*	3·5	..	2,363 (1)*	3·9	..	..	..

*Figures in brackets indicate the malaria parasite positives

TABLE III.

Details of the parasite-positive cases encountered in the Sakalespur area during the surveillance programme between October, 1956—February, 1960.

Serial Number.	Primary Health Unit area or 'Pocket' area.	Village.	Case.		Date of taking blood smears.	Result.	History and movement	Treatment.	Remarks.
			Age in years.	Sex.					
1	Hethur	Kodarasthe	40	Male	April, 1957	*P. vivax*. Rings and trophozoites.	Frequent fever and rigor. Visiting Kateri village inth e Coorg District frequently.	6 tablets of Paludrin on April 25, 1957 and 4 tablets of Resochin on June 1, 1957.	Fever case.
2	Heggadde Pocket area	Kadmane Estate	19	Female	June, 1957	*P. vivax*. All stages.	Came from Malabar.	Not furnished.	Fever case.
3	Hethur	Kodarasthe	28	Male	July, 1957	*P. falciparum*. Crescents.	Frequent visits to Mangalore District.	Treatment with Primaquine.	Fever case.
4	Sakalespur	Manigadde	20	Female	August, 1959	*P. vivax*. Rings	Fever 2½ months previously. Before that, residing in her native place in South Arcot.	4 tablets of Aviochlor on August 21, 1959. Primaquine treatment from August 24, 1959.	Fever case. Blood smears taken on August 20, 1959, were found negative.

TABLE IV.

Annual averages of surveillance work in Sakalespur area.

Year.	Percentage of population surveillanced.	Fever cases per mille.	Number of parasite-positive cases.	Average population surveillanced by each Worker†.
1956 (From October).	58·8	5·7	0 (548)*	2,446
1957	82·1	5·7	3 (3,030)*	3,011
1958	95·2	3·5	0 (2,138)*	3,839
1959	93·7	3·9	1 (2,363)*	3,563
1960 (Up to March).	96·5	3·2	0 (331)*	4,016

†The average is based on the actual population surveillanced by each individual worker in one fortnight and not on the allotted average, since the number of workers actually engaged in surveillance work varied somewhat from month to month and since the full complement of the sanctioned staff were not working during any month.

*Figures in brackets denote the number of blood smears collected.

FEVER MORBIDITY AND THE BLOOD SMEARS EXAMINED.

During the last three months of 1956 (Table II), 548 fever cases were investigated and none of them proved positive for malaria parasites. The fever rate per 1,000 population surveyed worked out to 5·7. During 1957, 3,030 fever cases were detected, out of which 3 had malaria parasites in their blood. Detailed investigations about these three cases are presented in Table III. It will be seen that all the three cases gave histories of movement and migration into the area from outside. None of the cases was indigenous. The fever rate per mille in 1957 was again 5·7. In 1958, out of 2,138 fever cases investigated none proved to be positive for malaria parasites. The incidence of fever cases was generally lower than in 1957 and the rate per mille was 3·5. During the year 1959, out of 2,363 cases investigated, one proved to be positive for malaria parasites. The individual gave a history of migration from South Arcot in Madras State. The fever rate per mille was 3·9. During the year 1960, so far no parasite positive cases have been encountered.

From the fever rates during 1957-1959, it will be observed that, though there is no particular trend, the first half of the year generally shows a higher rate than the second half. The monthly incidence of fever cases has ranged from 2·6 to 8·8 per 1,000 population surveyed, while the annual averages vary from 3·2 to 5·7. The four parasite positive cases were recorded during April, June, July, and August, at a time when no *A. fluviatilis* was collected from the respective villages. Even otherwise, if these had been the indigenous cases, there should have been a progressive increase in the number of fever cases encountered.

No transport has been provided for the Health Inspectors or Surveillance Workers. The percentage of population surveillanced per round has varied from 56·1 to 100·0, since at no time was the full complement of the sanctioned staff working. The annual averages range from 58·8 to 96·5 per cent. The average

population surveillanced by each worker has varied from 2,446 to 4,016 per fortnight. If all the staff sanctioned had actually been on duty and if the entire population had been contacted, the average would have worked out to only 2,557 per worker per fortnight.

Considering the difficult nature of the terrain, the fact that most of the villages are situated in the interior and that many of them are virtually cut off from the outside communications during the monsoon months (June—October), the targets achieved so far are very encouraging and reveal that similar programmes can be carried out more easily and without much organizational difficulty in areas with better communications.

ENTOMOLOGICAL DATA.

The first half of Table I depicts a summary of the entomological collections before spraying and during the time the area was actually under spraying operations. The second half furnishes data after spraying was completely interrupted or there was a reduction in the number of rounds of spray. The annual average per man-hour densities of the vector species were low even before DDT operations, but in some months of the year, densities of vector species were high. These ranged from 0·0 to 17·9 in the case of *A. culicifacies* and from 0·0 to 1·1 in the case of *A. fluviatilis*. By the end of 1955, when spraying was interrupted in the area, the two species had completely disappeared from the human and mixed dwellings. The few specimens collected were from cattle-sheds. During the whole of 1956, *A. fluviatilis* was completely absent in the high and the intermediate rainfall areas, only 2 specimens being collected from cattle-sheds in the low-rainfall area. Only 6 *A. culicifacies* were collected from the intermediate rainfall area, the species being absent in the high and the low rainfall areas. During 1957 and 1958, both *A. culicifacies* and *A. fluviatilis* began to reappear in all the areas, but the numbers collected were very low and all the specimens were from cattle-sheds. During this period, however, there was a steady increase in the other anophelines collected, particularly *A. jeyporiensis* and *A. pallidus* which were the predominant species even during the pre-spraying period (Bhombore, *et al.*, 1954). In 1959, due to the rainfall being above average, there was a decline in the mosquito densities in the whole area, the vector species continuing to occur in numbers far below those during the pre-spraying period.

Larval searches also corroborate adult catches in all the three areas, there being a remarkable decline in the number of larvae collected.

ACKNOWLEDGEMENTS.

The authors are indebted to the various members of the malaria organisation in Mysore State who have participated in this work. Thanks are due to the

Director of Public Health in Mysore, Bangalore, for permission to publish this paper.

Special acknowledgements are due to Drs. B. Negeshwar and B. S. Nagaraj and to Shri S.A. Jeevendra Kumar.

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Indian Journal of Malariology, **15**, *3*, *September 1961*.

A NOTE ON THE INTERRUPTION OF SPRAYING OF RESIDUAL INSECTICIDES IN SOME VILLAGES OF VISVESVARAYA CANAL AREA, MANDYA DISTRICT, MYSORE STATE.

BY

H. SHAMA SASTRY,

A.R. SUNDER RAO,

T.S. RAMA RAO,

N.L. SITARAMAN

AND

C. ACHUTHAN.

(Bureau of Malariology, Mysore State, Bangalore.)

[March 30, 1961.]

INTRODUCTION.

THE Second Asian Malaria Conference held at Baguio, Philippines, in 1954, discussed the urgency of interrupting residual spraying of insecticides in areas which satisfied the required criteria for such interruption. The conference also suggested that, in each country, criteria for interruption of residual spraying should be set up by competent authorities fully conversant with the local epidemiology of malaria. Accordingly the Government of India set up in 1956 an Expert Committee to go into this problem and recommend areas ready for interruption. This Committee visited Mysore State in July, 1956, and selected certain areas as being ready for interruption of spraying. However, experimental interruption of spraying had already been instituted in a small group of villages in Mandya District in the beginning of 1956 itself.

Shama Sastry and Iyengar (1959) have already reported the first year's results of malaria surveillance measures in Mysore State, India. Sitaraman *et al.* (1961) have described in detail the results of three years' surveillance work in Sakalespur Taluk, Hassan District, which is a typical example of the *A. fluviatilis* area. This paper represents an account of observations carried out in a typically *A. culicifacies* area for a period of four years after interruption of spraying.

DESCRIPTION OF AREA.

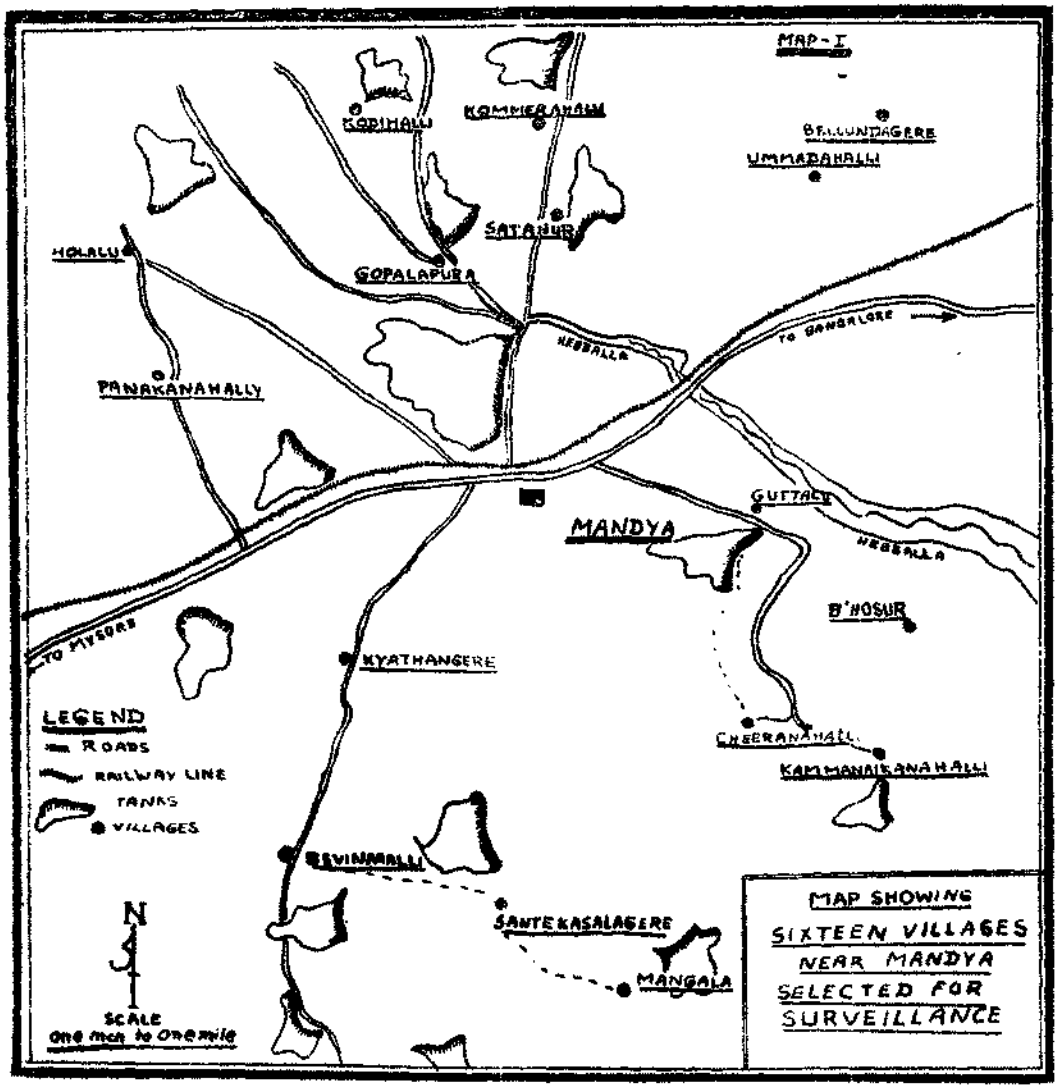
The Visvesvaraya Canal (formerly called Irwin Canal) is a high level canal, taking off from the Krishnarajasagar Reservoir, 12 miles from Mysore, designed to

irrigate 1,20,000 acres. The area commanded by the canal lies within the revenue jurisdiction of the Mandya and Mysore districts. A net-work of minor distributaries assures a perennial supply of water by gravity to the whole area. The population of Mandya District which was 5,81,710 in 1931 rose to 6,35,588 in 1941 and 7,17,545 in 1951.

HISTORY OF MALARIA.

Rao (1945) and Rao and Nassiruddin (1945) have discussed in detail the genesis of the malaria problem in the area. The villages in the area are situated in valleys and almost every village has a tank for conservation of rain water (Map 1.)

MAP 1



Prior to the advent of canal irrigation, the tanks used to hold water only during the rainy season. Though the area was economically backward, it was generally healthy, with only a few scattered foci of malaria. The average spleen rate was 15·3 per cent.

When the canal came into operation in January, 1932, the whole area was flooded and during the first few years no systematic control of water was possible. This uncontrolled irrigation led to an enormous rise in the subsoil water level and the tanks were always full. Within a period of 3 years (1932-35), the subsoil water level rose from 20-40 feet below the surface to practically ground level. An increase in the incidence of malaria in the freshly irrigated area was reported as early as 1932 and before the end of the year a widespread epidemic of malaria was in progress. Deaths from fevers increased twenty-fold. The average spleen rate, which was 15·3 per cent during the pre-irrigation period, rose to 50·00 to 90·00 per cent after the introduction of irrigation. By 1935 the conditions had settled down to a state of hyper-endemicity. Though relief measures by drug distribution and anti-larval measures by using paris green were started from the beginning, the malaria problem assumed serious proportions by 1941.

RESULTS OF MALARIA CONTROL MEASURES.

During 1941, malaria control by pyrethrum spraying was taken up along with permanent anti-malaria engineering works. In the new area (Malavalli Taluk), where these engineering works were adopted, malaria did not become a problem.

Till 1949, the main malaria control measure in the Visvesvaraya Canal area continued to be by pyrethrum spraying. In 67 villages, under observation, the spleen rate had come down to 47·7 per cent in October, 1950, from 81·5 per cent in April, 1942. With the availability of residual insecticides like DDT and BHC for large-scale use, the Visvesvaraya Canal area was taken up for residual insecticidal treatment since December, 1950.

In 16 study villages of Mandya Taluk, with a population of 13,604, the spleen rate in October, 1950, was 44·3 per cent and the Average Enlarged Spleen 1·9. By November, 1953, when the National Malaria Control Programme was initiated, these figures had come down to 12·12 per cent and 1·5 respectively. At the end of 1955, the former had further declined to 1·9 per cent. Dispensary Statistics during 1950 indicated that the percentage of malaria cases, in relation to total cases, was 41·03. It had come down to 4·09 per cent by 1953 and to 2·1 per cent in 1955.

Infant parasite rate, which was well above 5·0 per cent before 1950, came down to zero in October, 1951, after two rounds of residual insecticidal treatment and was maintained at that level till the end of 1955. The percentage of clinically diagnosed malaria cases, positive for malaria parasites, was 12·2 in 1951 ; and by 1954, it came down to 2·9 per cent. Infant and childhood parasite rates in the 16 study villages, during 1955, continued to be zero. Though childhood parasite rates are not available for the period 1954-55, inasmuch as the spleen

rate in these villages had come down to less than 5·0 per cent and the infant parasite rates were also nil for the same period, it is permissible to presume that the childhood parasite rate would have been well below one per cent.

Before control measures were adopted in the area, densities of *A. culicifacies* were high enough to maintain malaria transmission throughout the year (Rao and Nassiruddin, 1945 *loc. cit.*). During 1951, after three rounds of spray, the per man-hour densities of vector species had come down to levels far below the required level for malaria transmission. The per man-hour densities for *A. culicifacies*, *A. fluviatilis* and other anophelines during 1951 were respectively 0·5, 0·3 and 11·4. By 1953, they came down to 0·27, 0·03 and 3·7 per man-hour respectively. During 1955, there was a further decline to 0·0005, 0·0005 and 0·7 respectively. Larval densities also indicated a corresponding decline.

RESULTS AFTER INTERRUPTION OF SPRAYING.

It will be seen from the data presented above that by the end of 1955 the necessary criteria* for interruption of spraying were fully satisfied in these villages and consequently, as an experimental measure, spraying was withheld during the whole of 1956. Although a regular surveillance programme was instituted from only January, 1957, a very careful watch was maintained throughout 1956 by collection of infant blood smears every month, child blood smears once in six months, splenometric data biannually, and weekly entomological data.

645 infant blood smears were collected during 1956 from these study villages and all were negative for malaria parasities. 865 blood smears taken from children between 2—12 years of age, as also 611 blood smears collected from adults in these villages, were all negative for malaria parasites. The spleen rates in these villages during April and October, 1956, were 0·6 per cent and 0·08 per cent respectively.

For a total of 1856·0 man-hours spent during 1956, only one specimen each of *A. culicifacies* and *A. fluviatilis* were collected. *A. culicifacies* and *A. fluviatilis* formed only 0·02 per cent of the mosquitoes collected during the year, as against 0·07 during 1955, 0·3 and 0·7 during 1954, and 10·0 and 1·0 during 1953. No larvae of *A. fluviatilis* were collected during the entire year; only 5 *A. culicifacies* larvae being collected.

Since January, 1957, onwards, regular surveillance programme has been instituted in these villages. Entomological data are also being collected every week from these 16 villages. Other malario-metric indices like infant parasite rate, child parasite rate, and spleen rate have also been recorded. No transport has been

*The Expert Committee of Malariologists appointed by the Government of India, in 1956, adopted the following criteria for interruption of spraying :—

(1) Childhood spleen rate is less than 5 per cent.

(2) Childhood parasite rate is less than 1 per cent., and

(3) Infant parasite rate is zero,

for a consecutive period of two years.

provided as the villages are within a radius of five miles from the headquarters and as houses in the villages are compact and not scattered.

During 1957, 280 fever cases were detected and the blood smears drawn from all these cases were negative for malaria parasites. Spleen surveys conducted during April and October, 1957, revealed that the spleen rate remained zero. 951 child blood-smears examined were negative for malaria parasites, as also 151 infant blood-smears and 249 blood-smears collected at random from adults.

In 2011·0 man-hours spent in adult mosquito collections in these villages during 1957, 16,729 anophelines were collected, of which 3 (0·017 per cent) were *A. culicifacies* and 2 (0·014 per cent) were *A. fluviatilis*.

In 1958, 97 blood-smears, drawn from fever cases, were negative for malaria parasites. There was no rise in the spleen rates. Blood-smears, taken from 98 infants, 684 children and 363 adults, were also negative for malaria parasites.

38,973 anopheline adults were collected during a total of 2854·0 man-hours. Of these, 22 (0·008 per cent) were *A. culicifacies* and 72 (0·026 per cent) were *A. fluviatilis*.

91 fever cases detected during 1959 and the blood-smears drawn from these cases were negative for malaria parasites. Spleen surveys conducted during April and October, 1959, indicated that the spleen rate remained zero. 1,590 child blood-smears examined were found to be negative for malaria parasites ; as also 320 infant blood-smears, and 402 blood-smears obtained at random from adults.

In 2483·0 man-hours spent in adult mosquito collections in these study villages during 1959, 25,447 anophelines were collected, of which 14 (0·055 per cent) were *A. culicifacies* and 38 (0·15 per cent) were *A. fluviatilis*.

The data collected so far indicate that interruption of spraying in these villages has not resulted in any increase in or recurrence of malaria in the area. However, it would be too premature to draw any definite conclusions at this stage, as observations will have to be continued for some more years. Also in an adjoining Taluk (Nagamangala) no regular anti-malaria measures were in force till recently. A strict vigilence is being maintained and ample provision has been made for immediate resumption of spraying operations whenever the need arises.

ACKNOWLEDGEMENTS.

The authors are indebted to the late Dr. D.K. Viswanathan for helpful guidance in the beginning of these studies and to Dr. B.A. Rao for constant encouragement. Special thanks are due to Dr. K.S. Rajanna for assistance in the studies. This paper is being published with the permission of the Director of Public Health, Mysore State, Bangalore.

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Indian Journal of Malariology, **15**, *3*, *September 1961*.

AQUATIC VEGETATION OF DAMODAR VALLEY.

Part IV.

AQUATIC VEGETATION OF BOKARO RESERVOIR AND ITS CONTROL BY HERBICIDES.

BY

P. KACHROO*.

(Indian Council of Agricultural Research, New Delhi).

(March 23, 1961.)

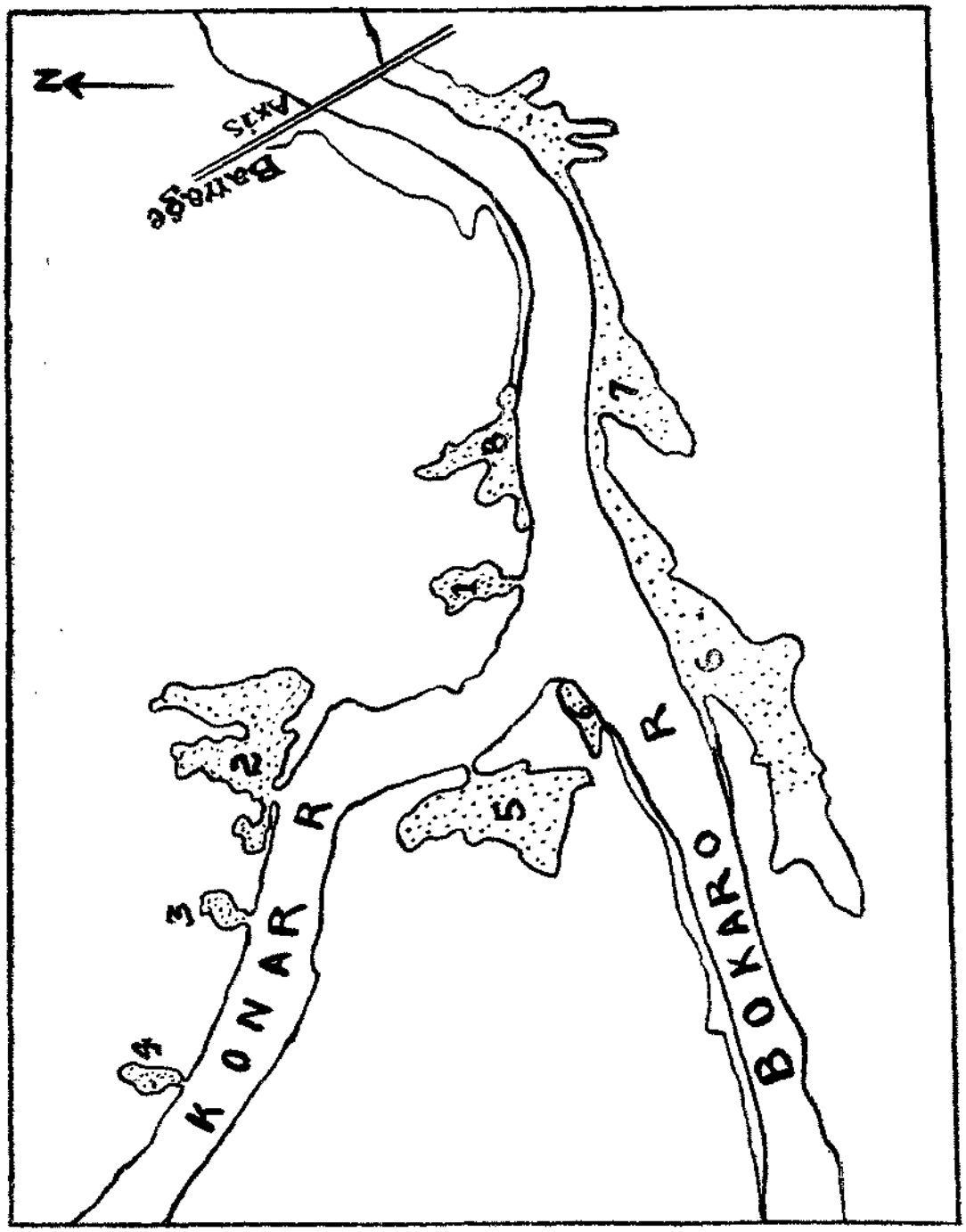
INTRODUCTION.

THE phanerogamic flora of the ponds and marshy lands, along with a detailed account of vegetation, pond contour in relation to marginal flora, associations and changes in the local pond flora and annual variation in the dominant marginal and central flora of some selected ponds in the Damodar Valley, and the association of aquatic vegetation with anopheline breeding within the same area, have been discussed in earlier publications (Kachroo, 1959*a* : 1959*b* : 1960). Preliminary herbicidal control of the aquatic weeds was experimentally conducted during 1955 to 1957 in selected areas, at the Station Nos. 5 and 1 (Map 1) only (Neogy *et al.*, 1956 ; Kachroo and Sharma, 1958). Thereafter the whole mass of vegetation at these stations as well as at other stations was sprayed. The present paper records the results of observations made during 1954-58 on the aquatic vegetation of Bokaro Reservoir (Map 1), its association with anopheline breeding and its control by application of herbicides.

The site.—Bokaro pond is situated in Hazaribagh District of Bihar at the confluence of Bokaro and Konar rivers (Map 1). The approximate latitude and longitude of the place are 23°, 47 min. *N.* and 85°, 53 min. *E.* It is a constant pool-level pond, with the barrage structure of gravity concrete, and it is integrally interrelated with the Konar Reservoir which supplies water to it throughout the dry season. The primary purpose of this pond is to supply cooling water to the Thermal Power Station. The hydrological and physiographical features of the pond are summarized below.

I	POND		
	1. Duration of the full pool-level	...	12 months.
	Stream elevation bed	...	725
	Submerged acreage	...	251 acres.
	Live storage fluctuation	...	Nil.
	Controlled flood storage	...	Nil.

* Formerly : Botanist, Damodar Valley Corporation.



Map 1.
Sketch map of Bokaro Reservoir, showing location of various breeding stations

(a) *Shore line :*

Length of shore line along the Bokaro River	...	6 miles (both side).
Total catchment area above barrage	...	700 sq. miles.
Area of the submerged paddy fields	..	29 acres.

(b) *Villages within half a mile of shore line (Population) :—*

RIGHT BANK : Garmuzara (p. 150), Hazari (p. 300), Bariabera (p. 300), Bhutkuria (p. 200), Mohlibund (p. 500).

LEFT BANK : Mahiriatand (p. 100).

II. BARRAGE

Number of gates	...	19
Concrete wall-level below gates	...	RL 731
Highest level of the gates	...	RL 744
Normal operating level	...	RL 743
Maximum flood level	...	RL 757
Fluctuation level	..	1·5 ft.

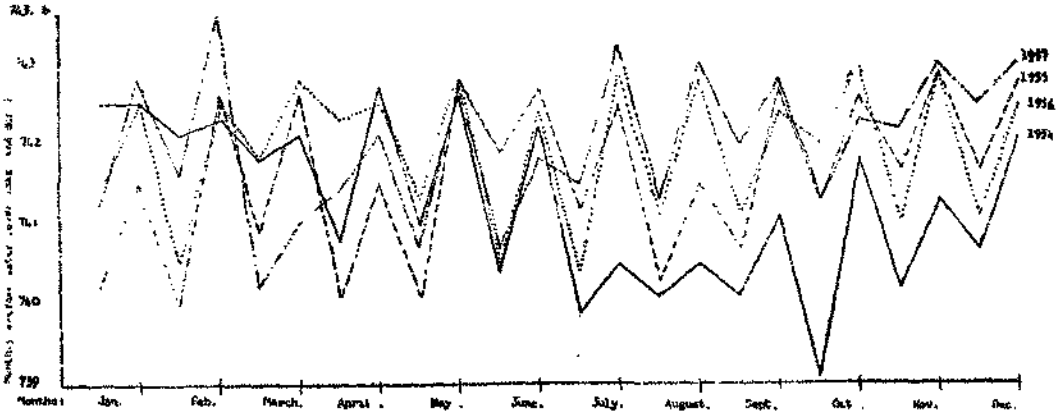
Being a constant pool-level pond, the reservoir showed very little fluctuation in the water level during 1954-57 (Graph 1). As a matter of fact, the maximum water level generally fluctuates between RL 741- RL 743 and the minimum between RL 739-RL 741 ; the difference between the two, each year or even each month, being practically negligible.

AQUATIC VEGETATION.

The constant pool-level of the Bokaro pond has caused water-level features which are most favourable for a luxuriant aquatic growth, resulting in favourable conditions for mosquito breeding. The entire fluctuation zone remains inundated for the most part of the year and is a repository of aquatic species. A few plants do occur in and along the reservoir itself. A major portion of the fluctuation zone used to be paddy fields in the pre-reservoir days.

GRAPH 1.

Monthly fluctuation of water level in the Bokaro pond during 1954-57.



Potamogeton crispus, Najas foveolata and masses of *Cladophora glomerata* usually float in the reservoir. The most common species in the fluctuation zone, according to their dominance, were : *Chara zeylanica, Hydrilla verticillata, Scirpus articulatus, Najas foveolata* and *minor, Potamogeton crispus, Polygonum tomentosum, (Typha angustata), Evolvulus nummularius, Aponogeton monostachyon, Monochoria hastasefolia, Alternanthera sessilis, Sagittaria guayanensis, Eragrostris brachyphylla, Oldenlandia heynei, Trapa bispinosa, Kyllinga monocephala, Polygonum hydropiper, Potamogeton indicus, Caesulia axillaris, Sphaeranthus indicus* and *Commelina salicifolia.*

Polygonum tomentosum formed forests during 1954-55 and *Typha angustata* during 1957-58. *Scirpus articulatus* was most dominant in fallow fields and was commonly associated with *Jussiaea repens* and *Sagittaria guayanensis. Monochoria hastaefolia* also formed thickets during 1957-58.

The following species were common in partially inundated areas. *Ammania baccifera ; Cyperus difformis, C. flavidus, C. iria, C. pumilus ; Emilia sonchifolia ; Eragrostis gangetica, E. tenella* var. *brevicaulis, E. uniloides, E. viscosa ; Eriocaulon quinquangulare ; Fimbristylis miliaceae, F. quinquangularis ; Grangea madaraspatna ; Lindernia crustacea ; L. pyxideria ; Ludwigia parviflora; Polygonum flaccidum; Rotala indica, R. laptopetala ; Scirpus supinus, S. squarrosus ;* and *Hygrophila spinosa.*

Croton sparsiflorus, Euphorbia thymifolia, Phyllanthus simplex, Ocimum canum, Desmodium triflorum and *Argemone mexicanum* grew along the shore-line and formed con-associations at a number of places with grasses, *Rotala indica* and *Ammania baccifera, ;* but normally some of them, e.g., *Croton, Ageratum* and *Ocimum* died on being submerged.

Wedelia calandulacea, Spilanthes acmella, Polygonum hydropiper, Cyperus haspan, C. tagetum, Phragmites karka, Panicum colonum, Crinum deflexum, and *Eriocaulon truncatum* were commonly encountered in the river bed beyond the fluctuation zone during the non-monsoon months.

Among the algae, the most common were *Chara zeylanica* (forming a submerged forest at Station No. 1), *Spirogyra maxima, Cladophora glomerata* and *Zygnema indica* var. *damodari.* The blue-green algae were mostly confined to fallow lands and *Lyngbya majascula* was one of the dominant algae collected during 1954-55. The following species of algae were collected during the present observations.

Chlorophyceae: *Eudorina elegans; Gonium pectorale; Pandorina morum; Sphaerella lacustris ; Chlorella vulgaris ; Quadrigula closterioides ; Pediastrum duplex, P. tetras ; Scenedesmus quadricauda ; S. acuminatus; Cladophora glomerata, C. sauteri ; Oedogonium decipiens ; Spirogyra maxima ; S. neglecta ; Zygnema indica* var. *damodari ; Penium polymorphum ; Closterium monoliforme, C. ralfsii ; Cosmarium granulatum, C. reniforme ; Desmedium swartzii ; Staurastrum dejectum ; Protosiphon botryoides.*

Characeae : *Chara zeylanica, Nitella acuminata* (epiphytic on *Potamogeton crispus).*

Bacillariophyceae : *Cyclotella comta., Melosira* sp., *Synedra ulna ; Ghomphronema ventricosum, Pinnularia viridis, Baccilaria paradoxa.*

MYXOPHYCEAE : *Microcystis aeruginosa, Chroococcus turgidus, Merismopedia tenuissima ; Chaemosiphon siderphilus* (epiphytic on *Lyngbya majascula*), *Nostoc linkiaea, Anabaena ambigua, A. unispora ; Spirulina major ; Oscillatoria sancta, O. claricentrosa ; Lyngbya majascula.*

ASSOCIATION WITH ANOPHELINE BREEDING.

Periodic water-level fluctuation, without seasonal recession, normally used in limiting the marginal growth invasions, was not of much value at Bokaro since a reasonable scope of recession is only one-and-a-half to two feet. The requirements of the Thermal Power Station do not permit recession to a point below the marginal invasion zones. Further, for the same reason seasonal recession could not be maintained. Thus, the fluctuation area became a permanent fixture in the form of 'small pockets', each with a small outlet into the reservoir. These small pockets formed breeding pools for mosquitoes (numbered as stations in Map 1).

The problematic mosquito breeding pools in the reservoir are Station Nos. 5, 6 and 7 and a part of Station No. 1. These are permanent and remain under water throughout the year. On the contrary, Station Nos. 2 to 4 and 8, which are slightly at higher elevation, dry up during the recession (March-May) and in autumn.

During the period under review, larvae of seven species of *Anopheles*, namely, *annularis, culicifacies, fluviatilis, hyrcanus, pallidus, subpictus* and *vagus* were collected at Bokaro. *Anopheles annularis, A. culicifacies* and *A. subpictus* were more commonly encountered ; whereas *A. fluviatilis* and *A. vagus* were rather rare. *A. culicifacies* is regarded as the chief vector of malaria in this region and it was nearly always associated with emergent flora.

Polygonum tomentosum was the main aquatic plant found to be associated with anopheline larvae, particularly those of *A. culicifacies* and *A. annularis*. The latter larvae were also collected in and around masses of floating *Spirogyra*. These larvae were observed to harbour externally large colonies of the blue-green alga *Microcystis aeruginosa* ; this gave the larvae a green appearance. *Jussiaea repens*, which formed small pockets within and without the marginal belt of *Polygonum*, was associated with *A. annularis, A. pallidus, A. hyrcanus* and to a lesser degree with *A. culicifacies*. Larvae of *A. hyrcanus* and *A. subpictus* were always associated with *Monochoria hastaefolia*, and that of the latter also with dense masses of floating *Cladophora*.

Scirpus articulatus (Station No. 1) was intimately associated with larvae of *A. culicifacies, A. hyrcanus* and *A. subpictus*, but to a lesser extent with *A. annularis* and *A. pallidus*. *Scirpus* was eliminated at Station No. 1 through application of herbicides during 1956, and thereafter *Chara zeylanica* and *Aponogeton crispus* were the dominant weeds there. However, the change in vegetation did not bring about any appreciable change in the composition of anopheline fauna, except that for a considerable decrease in the incidence of *A. culicifacies* and *A. annularis*. The instability of inundation at other Stations did not afford dependable data.

CONTROL OF VEGETATIVE GROWTH.

As already mentioned, the structure of the Bokaro pond is responsible for perpetual growth of aquatic plants and this poses a problem for mosquito control. Experience gained in other countries, notably in the U.S A., has shown that the control and eradication of aquatic weeds greatly reduces the mosquito breeding potential of a pond. It was, therefore, intended to study the effects of weedicides on the flora at Bokaro Reservoir to determine how far this could be profitably utilized for bringing down anopheline breeding at Bokaro.

The following herbicides were used : *dicotox*, ethyl ester of 2, 4-dichlorophenoxy-acetic acid ; *chloroxone*, 80 per cent sodium salt of 2, 4-d ; *fernoxone*, ± same as *chloroxone* ; *agroxone*, 27·5 per cent (approx.) wt./vol. of sodium 2-methyl 4-chloro-phenoxy-acetate ("Methoxone") ; *weedone LV-4*, butoxy-ethanol ester containing 4 lb. 2, 4-d acid equivalent per gallon ; *telvar DW*, active ingredient : 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea, 80 per cent and inert ingredient about 20 per cent ; *trioxone*, a mixed butyl ester of 2, 4, 5-trichlorophenoxyacetic acid ; *ACP-M-361*, a 2, 4-d formulation ; *atlacide*, a non-selective weed-killer based on sodium chlorate ; and *copper sulphate*. All these weed-killers are water-soluble and were sprayed at a rate of 2½ lb.* in 20 gallons of water per acre on aerial parts of plants. The results of the various trials are tabulated in Table I. The plants were sprayed in their natural habitat and the spraying was carried out in a zig-zag reversible manner so that each plant could be equally sprayed.

TABLE I.

Time for total kill of aquatic/semi-aquatic plants of the Bokaro pond after spraying with various aqueous solutions of herbicides during 1955-58.

Plants.	HERBICIDES USED (2½ lb. PER ACRE)*.						
	Chloroxone.	Agroxone.	Fernoxone	Copper sulphate.	Dicotox.	Weedone.	Atlacide.
Alternanthera sessilis	120 hrs.	96 hrs.	96 hrs.	(—)†	(—)	(—)	(—)
Caesulia axillaris	120 hrs.	120 hrs.	(—)	No effect	(—)	(—)	No effect
Cladophora glomerata	No action	No action		7 days	nil	(—)	nil
Eclipta alba	7 days	7 days	7 days	nil‡	14 days	21 days	nil
Grangea madaraspatna	144 hrs.	120 hrs.	120 hrs.	(—)	(—)	(—)	(—)
Jussiaea repens	120 hrs.	72 hrs.	48 hrs.	10 days	7 days	(—)	(—)
Kyllinga monocepahala	120 hrs.	96 hrs.	96 hrs.	nil	nil‡	nil	nil
Mazus rugosus	7 days	6 days	6 days	(—)	7 days	(—)	(—)
Monochoria hastaefolia	14 days	12 days	6 days	10 days	6 weeks	24 days	(—)
Polygonum tomentosum	22–30 days	36 days	24 days	nil	6 weeks	nil	(—)
Sagittaria guayanensis	48 hrs.	48 hrs.	48 hrs.	(—)	2 weeks	(—)	(—)
Sphaeranthus indicus	120 hrs.	(—)	(—)	(—)	3–4 weeks	(—)	(—)
Scirpus articulatus	22 days	(—)	(—)	4 weeks	4–5 weeks	(—)	nil
Typha angustata	Partial paralysis	(—)	nil‡	nil	nil	nil	(—)

* The quantity used refers to the amount of the technical compound and not the formulation.

† (—)=Herbicide was not applied.

‡ Initial effects marked but revived later, often within 24 hours.

It is seen from Table I that nearly all the weeds, treated with 2, 4-D formulations, were eliminated within a month, the delicate species (e.g., *Sagittaria*) was killed within 48 hours, but the hardy plants (e.g., *Polygonum*) took a month to die.

None of the herbicides applied could eliminate the grasses or the submerged weeds. Chloroxone partially paralyzed *Typha* and other grasses but the plants revived after 24 hours.

Copper sulphate, a non-hormonal weedicide, could only control growth of small annual weeds. It could not eliminate hardy species like *Polygonum*. However, it was effective against algae, eliminating masses of *Cladophora glomerata* within seven days.

DISCUSSION.

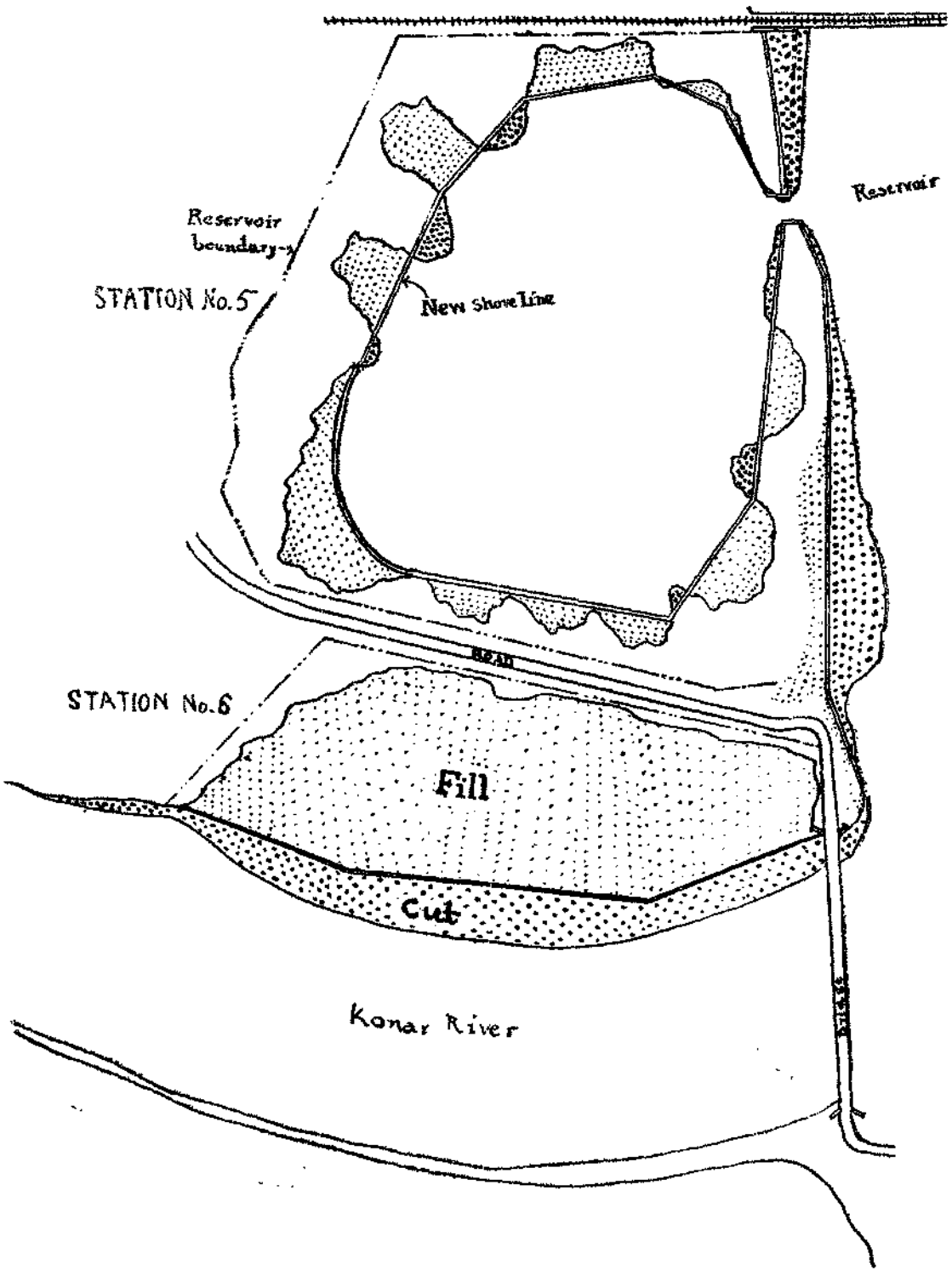
Bokaro Reservoir is a constant pool level pond and has, thus, greatly favoured a luxuriant aquatic growth. The uneven shoreline and the neglected fallow lands in and along the shallow fluctuation zone, as mentioned earlier, have created conditions for growth of a flexuous type of flora and provided optimum conditions for mosquito breeding.

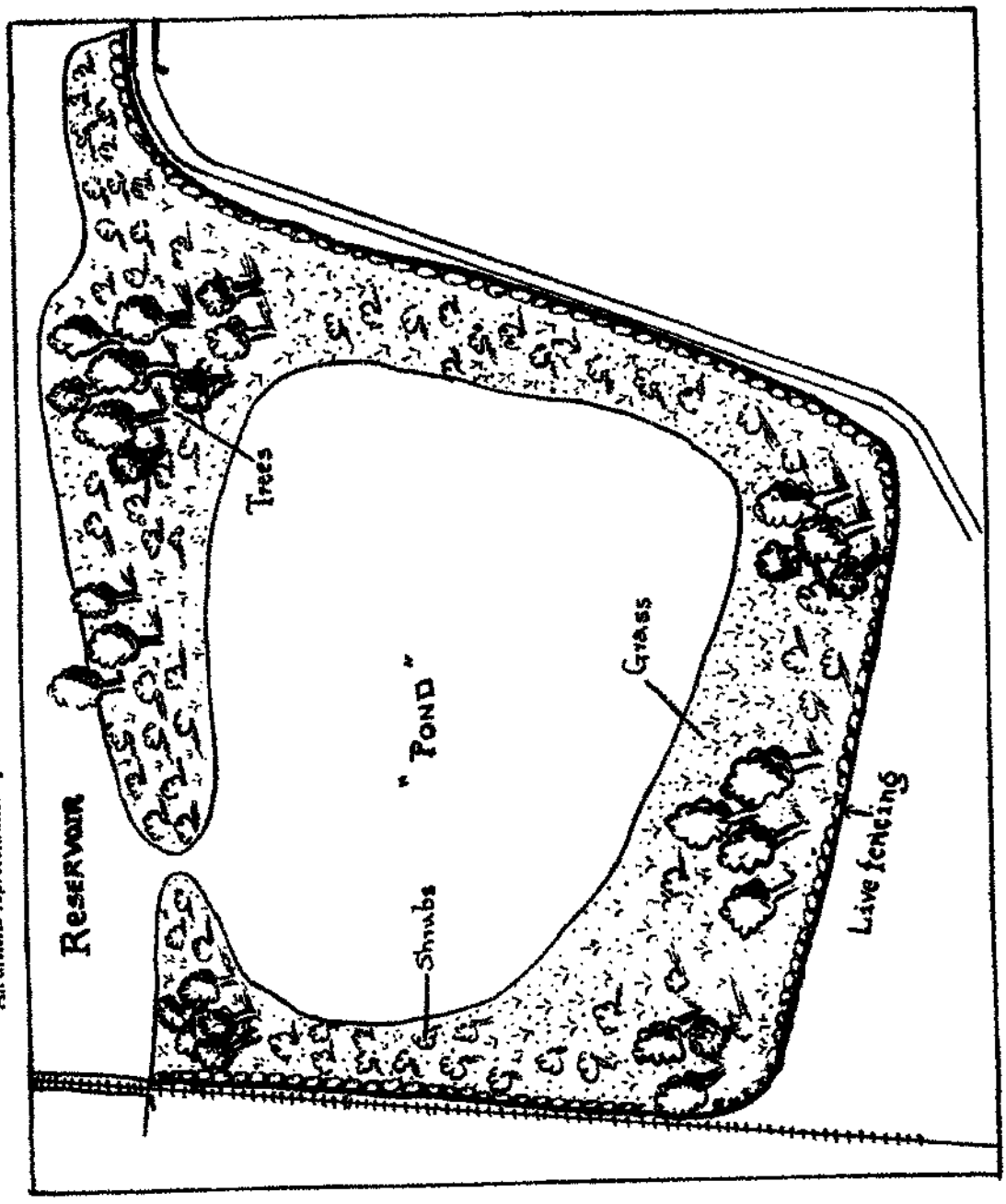
The common aquatic plants associated with *anopheline* breeding were *Polygonum tomentosum, Jussiaea repens, Scirpus articulatus, Monochoria hastaefolia,* and to a lesser extent *Hydrilla* and *Aponogeton*. Nearly all these plants form a dense cover and, thus, pose a problem for malaria control. Elimination of this flora has been seen to greatly reduce the incidence of mosquitoes. Both hormone and non-hormone types of weedicides were applied at Bokaro. The former included ethyl ester, sodium salt and butoxy ethanol ester formulation of 2, 4-D ; 2, 4, 5-trichlorophenoxy-acetic acid as well as 27·5 per cent wt./vol. of sodium 2-methyl 4-chlorophenoxy-acetate. Atlacide and copper sulphate were the only non-hormone type weedicides selected. Chloroxone (80 per cent sodium salt of 2, 4-D) proved very effective against the emergent broad-leaved weeds and was responsible for partial or complete disappearance of a majority of the species. But it could not control the growth of submersed species. Copper sulphate was only effective against the algae.

Initially it was thought that weedicide operations would be more conducive towards the solution of mosquito problem, but an assessment made at the end of the trials showed that such operations would be too expensive, as it would be necessary to spray the water surface, at least, twice a year. Further, it was noticed that the change in flora brought about by such sprays had also to be considered, e.g., when growth of *Polygonum* was controlled at Station No. 5, its place was immediately taken by an over-growth of *Typha angustifolia*, a grass difficult to control but definitely encouraging an undergrowth of weeds and filamentous algae. Due to these facts dewatering of the fluctuation zones, particularly at Station Nos. 5 and 7, would be greatly advantageous. The alternative arrangement for maintaining an interior drainage system or operation of pumping plant, would involve great annual expenditure. Besides, seepage from the earth bunds is considerable at these stations.

MAP 2.

Diagrammatic representation of the deepening and filling operations suggested for better maintenance of Station Nos. 5 and 6.





Map 8.

An artistic representation of Station No. 6 as it would look like after deepening and filling.

Thus, the necessity for *filling* and *deepening* would be unavoidable for reducing the vegetative growth-invasion in the fluctuation areas and in the main reservoir. Further, it would involve the elimination of the shallow problem areas by deepening the lower portion of the area and utilizing the earth removed earlier to fill the upper portion—this would result in a new and relatively steep shore-line where the growth invasion would be restricted ; and consequently left without any significant anopheline breeding. Maps 2 and 3 illustrate this principle at Station Nos. 5 and 6, and the same with slight local modifications could be conveniently applied to other areas.

The deepening and filling, by employing temporary diking and dewatering, would be useful. As moist ground conditions are a serious obstacle during deepening and filling, the frequent drying of the area could be envisaged by removing the aquatic vegetation and constructing temporary drains to lower the ground water level.

SUMMARY.

Bokaro Reservoir is a constant pool level pond. It is very rich in aquatic vegetation which is mostly of the flexuous type. *Polygonum*, *Typha* and *Chara* form dense cover over most of the fluctuation zones. *Polygonum tomentosum*, *Scirpus articulatus*, *Jussiae repens*, *Monochoria hastaefolia* and to a lesser extent *Chara*, *Hydrilla* and *Aponogeton* are intimately associated with anopheline breeding.

Various herbicides (formulations of 2, 4-D and copper sulphate), as aquatic spray, were applied to control the growth of these aquatic weeds. Of these, chloroxone (80 per cent sodium salt of 2, 4-D) proved effective against all the broad-leaved emergent flora. Copper sulphate eliminated algae. Since herbicides would have to be applied at least twice a year and would involve heavy expenditure, it is suggested that deepening and filling of the shore-line and provision for good drainage in the low-lying fluctuation zones might meet the requirements of malaria control.

ACKNOWLEDGEMENTS.

Thanks are due to Dr. B.P. Neogy for encouragement and to the Director, Malaria Institute of India, Delhi, for a number of suggestions incorporated in this paper.

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Indian Journal of Malariology, **15**, 3, September 1961.

STUDIES ON FILARIASIS IN THAILAND. PERIODICITY OF MICROFILARIA*.

BY

C.P. NAIR

(*Malaria Institute of India, Delhi.*)

AND

SOMBAT CHAYABEJARA.

(*Malaria and Filaria Control Division, Thailand.*)

[July 13, 1961.]

FILARIASIS is known to be endemic in Southern Thailand (Iyengar, 1953; Sombat Chayabejara, 1958). The endemic areas are stated to be confined to the thinly populated rural villages located in the low-lying lands surrounded by extensive marshy jungles. The infection prevalent is *B. malayi* (Buckley, 1960). No periodicity studies have so far been reported. Iyengar (1953 *loc. cit.*), however, believed that the microfilaria in the local population was nocturnally periodic. Since his report, periodic and semi-periodic forms of *B. malayi* have been recorded in human beings in Malaya by Turner and Edeson (1957). It seemed, therefore, desirable to study the periodicity of the microfilaria in Thailand. Results of such studies, carried out in three patients, are reported here.

MATERIAL AND METHODS.

A village in the Ronpibul District in Nakhon Srithammaraj Province in the north and another in Yaring District in Pattani Province in the south (both in Southern Thailand) were selected for this study. The villagers were collected in a central place in the village between 8.30 and 10.30 p.m. A drop of blood was obtained from a pricked finger from each person and examined. Of those detected with microfilariae in the blood (symptomless carriers), three adults—one from Nikhon Srithammaraj Province and two from Pattani Province—volunteered for these investigations.

Three samples of 20 c.mm. of measured blood, from a pricked finger, were obtained every two hours over a 24 hour period from each volunteer. Each 20 c.mm. sample was made into a thick oval smear about 1 inch × ⅝ inch on a clean glass slide. The smears were dried overnight and stained on the following morning in dilute Giemsa solution according to the technique of Wilson (1956) (40 drops of Giemsa stain to 100 c.c. of buffer solution pH 7·2). The dried stained smears were there-

*The investigation was carried out between 17th November and 3rd December, 1960. The senior author was on a United States Technical Cooperation Mission to India (ICA) fellowship.

after examined. The species of microfilariae was noted and their numbers counted and recorded.

RESULTS.

Only *B. malayi* microfilariae were observed in all the smears. A striking feature, noted during the examination, was the presence of many ex-sheathed microfilariae with their empty sheaths lying nearby. In a total of 744 microfilariae studied 244 were completely ex-sheathed (30·10 per cent), with the sheaths lying separately from the microfilariae. In many others, the sheaths were found partly detached from the microfilariae.

The microfilariae counts at the different hours, from the three volunteers, are set out in Table I.

TABLE I.

Microfilariae count in the blood smears obtained every two hours.

PARTICULARS OF THE VOLUNTEER.		*SERIAL NUMBER OF THE TWO HOURLY BLOOD SMEARS :											
Age (years).	Serial number of the volunteer.	1	2	3	4	5	6	7	8	9	10	11	12
		Average microfilarial count per 20 c.mm. of blood.											
50	I	0	0·66	0	0·33	8	16·6	30·66	28	21·3	43·6	26·6	12·3
27	II	0	0·33	0	0	1	4·33	15·33	7	8·33	4	2·33	1·66
29	III	0	0	0	0	3·66	27·33	24	25·66	21·33	14·33	13·66	2

Volunteer I—Male from Nikhon Srithammaraj. Volunteer II—Male from Pattani.

Volunteer III—Male from Pattani.

* 1 to 12 represent the two-hourly blood smears taken. They were taken at 9 a.m., 11 a.m., 1 p.m., 3 p.m., 5 p.m., 7 p.m., 9 p.m., 11 p.m., 1 a.m., 3 a.m., 5 a.m. and 7 a.m. from Volunteer I. In the other two, they were taken at 10 a.m., mid-day, 2 p.m., 4 p.m., 6 p.m., 8 p.m., 10 p.m., midnight, 2 a.m., 4 a.m., 6 a.m. and 8 a.m., respectively.

The data indicate :

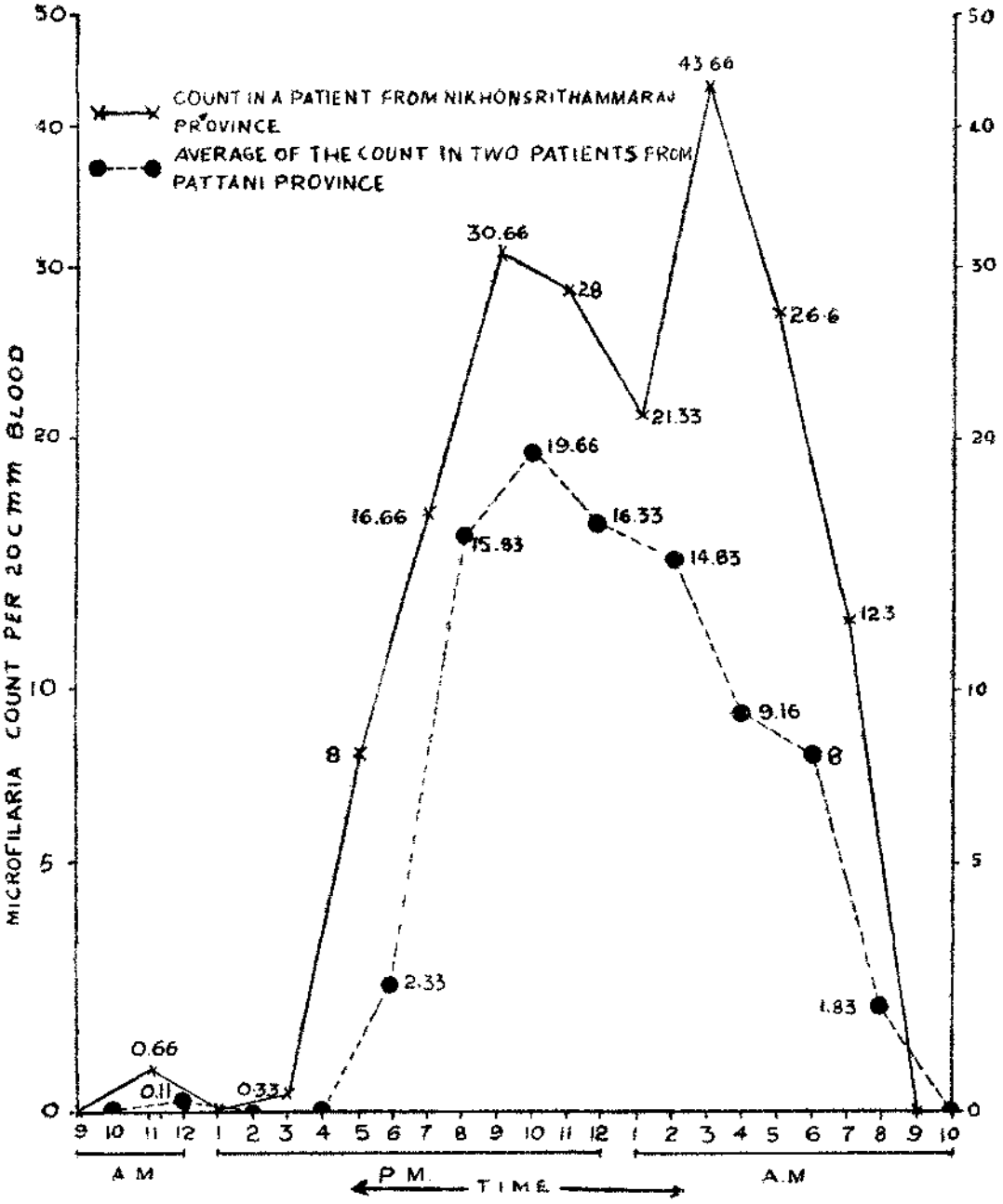
(*a*) Blood smears obtained by day (7 a.m. to 5 p.m.) in the case of volunteer I, and from 6 a.m. to 4 p.m. in the case of the other two, contained less numbers of microfilariae than those obtained during the night (*vide* Graph 1). Two, three and four smears obtained from volunteer I, II, and III, respectively, of the six smears examined during the day, contained no microfilariae even in 60 c.mm. samples. The ratio of counts by day : night (between 7 a.m. and 6 p.m. : between 7 p.m. and 6 a.m.) worked out to 21·30 : 166·76 or nearly 1 : 8 (Volunteer I); 4·33 : 40·00 or nearly 1 : 9 (Volunteer II) and 15·66 : 116 · 33 or nearly 1 : 7 in Volunteer III.

(*b*) In Volunteer I two peaks were observed in the microfilarial count, i.e. one at 9 p.m. and the other at 3 a.m. In the other two, only one peak was noted at 10 p.m. and 8 p.m. respectively.

(*c*) Generally there was a progressive increase of the microfilarial count from 5 p.m. or 6 p.m. till about 10 p.m. to midnight, whereafter it fluctuated, falling off by 9 a.m. to zero.

GRAPH 1.

Microfilaria counts at different hours of day and night.



DISCUSSION.

Raghavan (1949) observed ex-sheathed microfilariae of *B. malayi* in Giemsa stained blood smears obtained from the Medical Research Institute (Kuala Lumpur). Wilson (1956) considered this feature as a characteristic of the periodic form of *B. malayi* in that country where both periodic and semi-periodic forms were reported. The results of the present study show the nocturnal nature of periodicity. This fact, plus the 'ex-sheathing' noted, indicate the similarity of the observation made on the periodic form of the species in Malaya and Thailand.

In this connection, it may be stated that such an occurrence of "ex-sheathing" has not been observed in *B. malayi* infection in India or Ceylon. Raghavan (1957) drew attention to the lack of such "ex-sheathing" of *B. malayi* microfilariae in India, though the infection is periodic. Staining the smears of *B. malayi* microfilaria carriers in India with Giemsa stain, procured from Malaya or India, or subjecting the blood smears to "moist chambers" prior to staining, did not show the massive "ex-sheathing" as noted in the slides from Malaya (Raghavan, 1949). While more studies are needed, yet the significant difference has to be borne in mind.

It is interesting to compare the types of infection, their vectors and type of terrain in Malaya and Thailand. In the former place, the periodic form of *B. malayi* is reported from coastal rice-field areas, well populated and cultivated and without swamp forest. The vectors are the open swamp species of *Mansonia* such as *M. uniformis*, *M. indiana* and *M. annulifera* as well as the dark winged *A. barbirostis* and *hyrcanus* group (Laing, 1960). The semi-periodic form in Malaya, on the other hand, is confined to sparsely populated villages along the river banks in close proximity to extensive fresh water forest swamps. The vectors are *M. longipalpis* (*M. dives/bonneae*), and *M. uniformis* (Wharton, 1960). This form has been noted in a number of vertebrate hosts as well, other than man.

In Thailand, however, as stated earlier, the Surasthani Province that lies on the northern portion of the eastern coastal plain of Southern Thailand, is endemic for *B. malayi* (Iyengar, 1953). The terrain in this area can be compared to those areas in Malaya where the semi-periodic form of *B. malayi* has been recorded. The vectors in both the areas are *M. longipalpis* and *M. uniformis* (Wharton, 1960 *loc. cit.*; Iyengar, 1953 *loc. cit.*) Thus one could suspect the semi-periodic form of *B. malayi* in the Surasthani Province of Thailand.

The current studies in the Nikhon Srithammaraj Province (lying immediately adjacent to south of Surasthani), where both riverine swamp forest areas and also open swamps exist, have shown the presence of the periodic form only.

SUMMARY.

Periodicity studies of *B. malayi* were carried out in three volunteers from the Nakhon Srithammaraj and Pattani provinces in Southern Thailand.

The microfilariae were noted to be strictly nocturnal.

In the Giemsa stained blood-smears, many of these microfilariae were ex-sheathed, with their sheaths lying separately.

The importance and need for undertaking further investigations on the epidemiology of filariasis in Thailand is stressed, particularly in view of this country's close proximity to Malaya where semi-periodic and periodic forms of *B. malayi* have been detected, as also the adaptability of the former to a number of vertebrate hosts other than man.

ACKNOWLEDGEMENT.

The authors are indebted to Drs. Luang Ayurkithkosol (Advisor, Malaria and Filariasis, Central Thailand), Udaya Sandhinan (Director, Malaria and Filariasis Division, Thailand) and to Mr. Edgar A. Smith (Malaria Advisor to U.S.O.M., Thailand), for their stimulating encouragement. The authors are also very thankful to Dr. Bhavongvit Tanticharern, Medical Officer-in-charge, Southern Region of the Division of Malaria and Filariasis Control, for all his help and constant interest throughout this investigation.

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Indian Journal of Malariology, **15**, *4, December 1961.*

INFECTION OF THE ALBINO RAT WITH THE FILARIAL PARASITE, *LITOMOSOIDES CARINII*, OF COTTON RATS.

BY

S. P. RAMAKRISHNAN,

DALIP SINGH,

V. N. BHATNAGAR

AND

N. G. S. RAGHAVAN.

(Malaria Institute of India, Delhi.)

[October 31, 1960.]

INTRODUCTION.

LITOMOSOIDES CARINII, a natural filarial infection of the cotton rat (*Sigmodon hispidus*), has been widely used for laboratory studies, particularly for screening of antifilarial compounds (Hewitt *et al.*, 1947). The infection has been maintained by passage through the insect vector, the tropical mite, *Ornithonyssus bacoti*. The maintenance of the strain of the infection in the laboratory necessitates rearing and maintenance of an unfailing supply of both the vertebrate and invertebrate hosts of the parasite. The capricious breeding requirements and the wild nature of the cotton rats have made their rearing and handling difficult. Workers have repeatedly attempted, with inconsistent results, to see if the infection could be passaged and maintained in a more convenient laboratory animal like the white rat. Ramakrishnan *et al.* (1960), however, reported the preliminary results of successful infection of the white rat. One of the four white rats exposed to infective mites was positive for microfilariae in peripheral blood 66 days after exposure. Further studies, carried out on the susceptibility of the white rats to the cotton rat filarial infection, are reported in this paper.

MATERIAL AND METHOD.

The cotton rats*, white rats† and mites‡, used in these studies, were obtained from the colonies maintained at the Institute.

* The cotton rats are from a strain, obtained in 1956, through the courtesy of the Virus Research Centre, Poona, India.

† The white rats are from the colony maintained at the Malaria Institute of India, Delhi, from rats obtained from various sources in India.

‡ Mites were initially obtained from U.S.A. through the courtesy of Dr. J. Allen Scott, Deptt. of Preventive Medicine and Public Health, University of Texas, School of Medicine, Galveston, as well as from U.K. kindly made available by Dr. Frank Hawking, National Institute for Medical Research, Mill Hill, London. The colonies of mites are maintained in glass jars, 7 inches deep and having a diameter of 3½ inches. The open side of the jar is covered with nylon cloth with a mesh that will not allow the nymphs or adult mites to escape. This cloth is kept in position by rubber bands. A 2- or 3-day old baby mouse is kept in the jar once a week to feed the mites. The mouse is removed after 24 hours by which time it dies and the mites leave it.

The induction of the infection into the white rats was attempted by two different methods : (*a*) by transplantation of adult worms removed post-mortem from the infected cotton rats, and (*b*) by exposing the white rats to bites of infective mites.

The transplantation of adult worms was carried out with all aseptic precautions. The adult worms were lifted from the pleural cavities of a dead cotton rat (about 20 hours after death) with a sterile hooked needle and collected in a watch-glass containing warm normal saline. They were transplanted into the peritoneal cavity of an anaesthetised white rat through a subdiaphragmatic right-sided paramedian incision. The incision was sutured in separate layers and dressed with cibazol. The animal recovered normally in the course of about two hours, following the operation. The number of adult worms transplanted were not counted, to avoid too much handling of them.

In the experiments where the infection was induced through infective mites, the albino rats were directly exposed to the mites which had been given an infected blood meal 15 days earlier. These mites were distributed in the saw-dust which formed the bedding material of the nesting cages*.

In the first experiment, four normal healthy white rats (about 3 months old) were exposed to the infection, placing two of them in each of the two cages containing the infective mites for 8 days. They were then demitted and kept in clean cages for further observation. Similarly, more batches of white rats of different age-groups were exposed to bites of infective mites.

Blood smears from the rats were examined at weekly intervals for microfilariae of *L. carinii*, commencing from the fourth day in the rat which received the transplant of adult worms, and from the 40th day after exposure to the infective mites in the others.

RESULTS.

Microfilariae of *Litomosoides carinii* were detected in the peripheral blood of the rat with transplanted worms as well as many exposed to bites of infective mites. The results are mentioned in Table I.

The white rat, into which the adult worms were transplanted, was found to be positive on the first examination which was made on the 4th day following the operation. The microfilariae may have been in circulation even earlier.

One of the four white rats in the first batch became positive for microfilariae 66 days after exposure to infective mites. The cage companion of this white rat died after remaining negative for 60 days, following exposure. Post-mortem of that rat did not reveal any adult worms. The other two white rats did not show

* Cages are made of galvanized iron, measuring 18 inch × 12 inch × 12 inch with a lid on top and a water trough all around the inside of the cage towards the top. The lid is the only part where wire screen of ¼ inch mesh is used ; it is fixed to the cage by a pair of hinges. The lid can be easily lifted and rapidly closed while handling animals. Four such cages stand on steel-tables placed on a galvanized iron tray 24 inch deep. This tray contains dilute solution of cresol in water to take care of any mites that may escape the water in the troughs of the individual nesting cages.

TABLE I.

Protocol of white rats infected with L. Carinii.

Batch Number	Age of rats.	Mode of infection.	Number of white rats used.	Number of rats positive for microfilariae.	Day of first appearance of microfilariae in blood.	Duration of observation after appearance of microfilaria.
1	6 months	Adult worms transplantation.	1	1	4	218 days
I	3 months	Mite-induced	4	1	66	159 days
II	4 months	-do-	2	2	72	99 days
III	3 months	-do-	8	6	65 to 77	64 to 76 days
IV	25 days	-do-	20	17	57 to 77	36 to 55 days
V	20 days	-do-	20	20	63	19 days

microfilariae in the peripheral blood over an observation period of 86 days, following their exposure to infection. They were sacrificed on the 86th day. No adult worms were recovered from either of these animals.

It is interesting to note from Table I that all the 20 white rats (20 days old) in Batch V became positive for infection in the peripheral blood on the 63rd day of exposure to infected mites.

Out of the 20 animals in Batch V, six rats were exposed to infection in a cage containing infected mites previously fed on an infected white rat. The mites in this cage became infective after 15 days of blood meal on infected white rat as is the case with the mites fed on infected cotton rats. The pre-patent period for development of infection in the white rats, exposed to infective mites, was similar, irrespective of the fact whether the mites derived their infection from cotton rats or white rats. Table II shows the course of *L. carinii* infection in cotton rats and white rats.

DISCUSSION.

The first record of successful infection of the albino rat with *L. carinii* of the cotton rat was by Chandler (1931). The advantages of the white rat over the cotton rat, as an experimental animal, are obvious and attempts have been made by various workers to establish the infection in white rats. Some of the attempts were successful while others failed. In any case, the results have not been consistent in the past and, therefore, the recognised need for a suitable experimental model for filariasis in an animal, as suitable as the white rat, had up to now remained unfulfilled. The successful infection of the white rat in the present series of experiments has been consistent, indicating the practicability of using it for studies on experimental epidemiology of filariasis which have not been easily possible before. While all the factors contributing to the present success are not clear, yet a single outstanding factor seems to be that the younger rats (20 days old, *vide* Table I) are uniformly susceptible to the infection. Other factors, like the genetic strain of the rats and their diet, have also to be considered.

TABLE II.

Course of infection in white rats and cotton rats.

Rat Number.	Day of first appearance of microfilariae in blood after exposure to infection.	Number of microfilariae in 2 c.mm. blood at various intervals after the first appearance in peripheral blood.
	White rats	
2	66	4th week — 13 8th week — 70 12th week — 150 16th week — 245 19th week — 316
7	72	4th week — 2 8th week — 65 11th week — 53
8	72	4th week — 26 8th week — 30 11th week — 60
11	77	5th week — 70
12	66	5th week — 8
13	66	4th week — 25 7th week — 240
14	66	4th week — 61 7th week — 385
15	71	4th week — 45 6th week — 257
16	72	4th week — 191 7th week — 981
	Cotton rats	
32	86	4th week — 5 8th week — 1 14th week — neg.
34	61	4th week — 99 6th week — 490 Rat died.
35	92	4th week — 4 8th week — 30 12th week — 167
36	72	5th week — 96 10th week — 420 17th week — 470
38	67	4th week — 225 8th week — 540 12th week — 1607
39	48	3rd week — 305 5th week — 450

Wharton's (1946) attempts to transplant adult *L. carinii* into the pleural cavity of cotton rats were unsuccessful. Three days, following the transplantation, the worms were recorded dead. He succeeded, however, in the transplantation when the cotton rat had been previously splenectomised and its lymphoid macrophage system had been blocked by Indian ink. Bertram *et al.* (1946) transplanted adult *L. carinii* into the pleural cavities of white rats. Microfilariae appeared in the peripheral blood of these rats 14 to 18 days after the transfer, and in some cases persisted only for 22 to 23 days when they were sacrificed. On autopsy, the adult worms were found to be dead, fragmented and encapsulated.

Rohde (1959*a*), in a series of experiments, transplanted into the peritoneal cavity of 40 white rats adult filarial worms from recently killed cotton rats. In the blood of most of the rats, no microfilariae were demonstrable.

The single experiment, in the present series, to transplant adult *L. carinii* in the white rat was successful and was responsible for the rest of the work.

The note-worthy features are (*vide* Table I) : (*a*) that the cotton rat from which the worms were removed for transplantation had been dead at least 20 hours at the time, (*b*) the albino rat was an adult, (*c*) the microfilariae were found to be patent in the peripheral blood on its first examination on the fourth day after the transplantation, and (*d*) that the blood of the rat has been continuously showing microfilariae to date during an observation period of 218 days since the transplantation. Further, after the first 135 days of observation, the animal was splenectomised which has made no difference to the microfilariae count in the blood. In the cotton rat itself, the period of patency of microfilariae was found to be about a year (Bertram, 1957). The above mentioned findings indicate that the white rat can be a hospitable host to *L. carinii*.

Mite-induced infections have also been attempted in the past with only a few successful results. As mentioned earlier, Chandler (1931 *loc. cit.*) found that a white rat, housed with infected cotton rats and mites, was found to be infected. Hawking and Burrows in 1946 (quoted by Williams, 1948) successfully infected hamsters, white rats and mice and pre-bald rats through infective bites of mites. Williams and Brown (1946) showed that white rats can be experimentally infected by infective mites. In their series, the microfilariae appeared in the peripheral blood 80 days after exposure. Scott and Cross (1946) housed a single white rat along with two infected cotton rats and mites. The white rat was sacrificed 57 days later. Three immature *L. carinii* worms were found in the rat. Perhaps, the infection would have been found to be successful if only a longer period of observation had been undertaken. Bertram *et al.* (1946 *loc. cit.*) exposed 45 white rats to the bites of infective mites. They did not find microfilariae in the peripheral blood of any of the animals up to an observation period of 74 to 82 days. On autopsy after sacrifice, however, adult worms were found in the pleural cavities of the animals and microfilariae in the heart blood.

Later, Bertram (1947) exposed 39 white rats to bites of infective mites. The observation period extended up to 81 days, but no microfilariae were found in their peripheral blood. Williams (1948) found adult worms of *L. carinii* in white rats, 35 days after exposure to infective bites of mites. The findings, perhaps, would have been more significant if the observation period had been extended.

Olson *et al.* (1955) concluded that the white rat was partially immune to *L. carinii* infection and that such immunity was more pronounced in older rats. This is confirmed to a certain extent by the present experiments. Only a proportion of rats between the ages of 25 days to 3 months became infected (*vide* Table I) under the same conditions of exposure to infection. The proportion infected, however, was high and indeed both the rats 4 months old, exposed to the bites of infected mites, became infected. There would appear to be individual variations which may have to be investigated. It should also be borne in mind in this context that precise number of bites by infective mites have not been standardised in the experiments. Nevertheless it is note-worthy that all the 20 rats, which were 20 days old, became infected under identical conditions of exposure.

Rohde (1959*a*) successfully infected 17 white rats with *L. carinii* out of 19 exposed to the bites of tropical rat mites.

Rohde (1959*b*) reported successful mite-induced infection in 204 (70 per cent) out of 289 white rats. In fact, this report was responsible for our starting the work. In our series, if the four animals of the first batch (Table I) are excluded, 45 (90 per cent) out of the 50 exposed became infected. Indeed, if only the youngest age-group is considered, then one hundred per cent of the exposed rats (Batch V, Table I) became infected.

In our observations, the prepatent period of infection is not different in either of the two hosts, namely, the cotton rats and the white rats (Table II). The range in the former was 48 to 92 days (average 70·8 days) and in the latter, 66 to 77 days (average 69·7 days). The lack of precise standardisation of the number of infective bites mentioned earlier, is relevant here. The extrinsic incubation period for the parasites in mites has been observed to be identical whether the mites were infected by white rats or cotton rats.

The duration of patent microfilaraemia in white rats cannot be finally stated. The period of observation to date, in the animal into which the worms were transplanted, has been 218 days during which microfilariae have been continuously patent in the peripheral blood (Table I). This compares favourably with the duration of the infection in cotton rats which is stated to be about a year (Bertram, 1957). The maximum period of observation in rats with mite-induced infection has so far been only 159 days. During this period, microfilariae have remained patent in the peripheral blood. The duration of the infection in white rats is not yet known.

The only difference in the hospitality to *L. carinii* infection by white rats and cotton rats seems to be in the average number of microfilariae per 2 c.mm. of blood. From Table II, it is seen that it is greater in the latter than in former. Whether the difference is a function of the host or due to non-standardisation of the dose of infection, is yet to be determined.

Other studies, including the duration of infection in rats, are in progress. The observations so far made show that the albino rat in our laboratories is a suitable host for the permanent establishment of *L. carinii* for studies on experimental epidemiology, chemotherapy and to procure sufficient adult worms for preparation of antigen.

SUMMARY.

Adult worms of *L. carinii*, removed post-mortem from an infected cotton rat, were transferred to the abdominal cavity of a white rat. Microfilariae were present in the blood 4 days after the transfer. The blood has remained positive over an observation period of 218 days.

Out of the 54 white rats exposed to infective tropical mites, *Orinthonyssus bacoti*, 46 white rats showed microfilariae in the peripheral blood 66 to 77 days after exposure to infection. The animals have remained positive for microfilariae so far over an observation period varying from 19 days to 159 days following appearance of microfilariae.

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Indian Journal of Malariology, **15**, *4, December 1961.*

FILARIASIS IN CENTRALLY ADMINISTERED AREAS.

Part II. Survey of Laccadive, Minicoy and Aminidivi Islands.

BY

C. P. NAIR.

(*Malaria Institute of India, Delhi.*)

[November 16, 1960.]

INTRODUCTION.

A PRELIMINARY filaria survey of the Laccadive, Minicoy and Aminidivi Islands was carried out between December, 1954, and February, 1955, by Subramaniam *et al.* (1958). These islands were surveyed again in detail in April, 1958, to determine the current position with regard to the prevalence of filariasis. The results of this survey are recorded in this paper.

PHYSICAL FEATURES.

The Laccadive, Minicoy and Aminidivi Islands or the Laccadive "Archipelago" forms a long narrow belt, situated between 8 and 14 degree north latitude and 71 and 74 degree east longitude. The ten inhabited islands, Kiltan, Chetlet, Amini, Kadamath, Bitra, Agathi, Kavarathi, Androth, Kalpeni and Minicoy are shown in Map 1.

Chetlet, the northern most island, is about 145 miles south-west of Mangalore. On the extreme west, at a distance of 122 miles from Kozhikode (Calicut), is Agathi. The southern most island in the group is Minicoy, 213 miles south-west of Cochin.

For about six months in the year, during the monsoon, there is no communication between the mainland and the islands, as the sea is very rough. During the fair weather period, sailing vessels ply between the islands and the mainland, taking about one to three weeks for the journey each way.

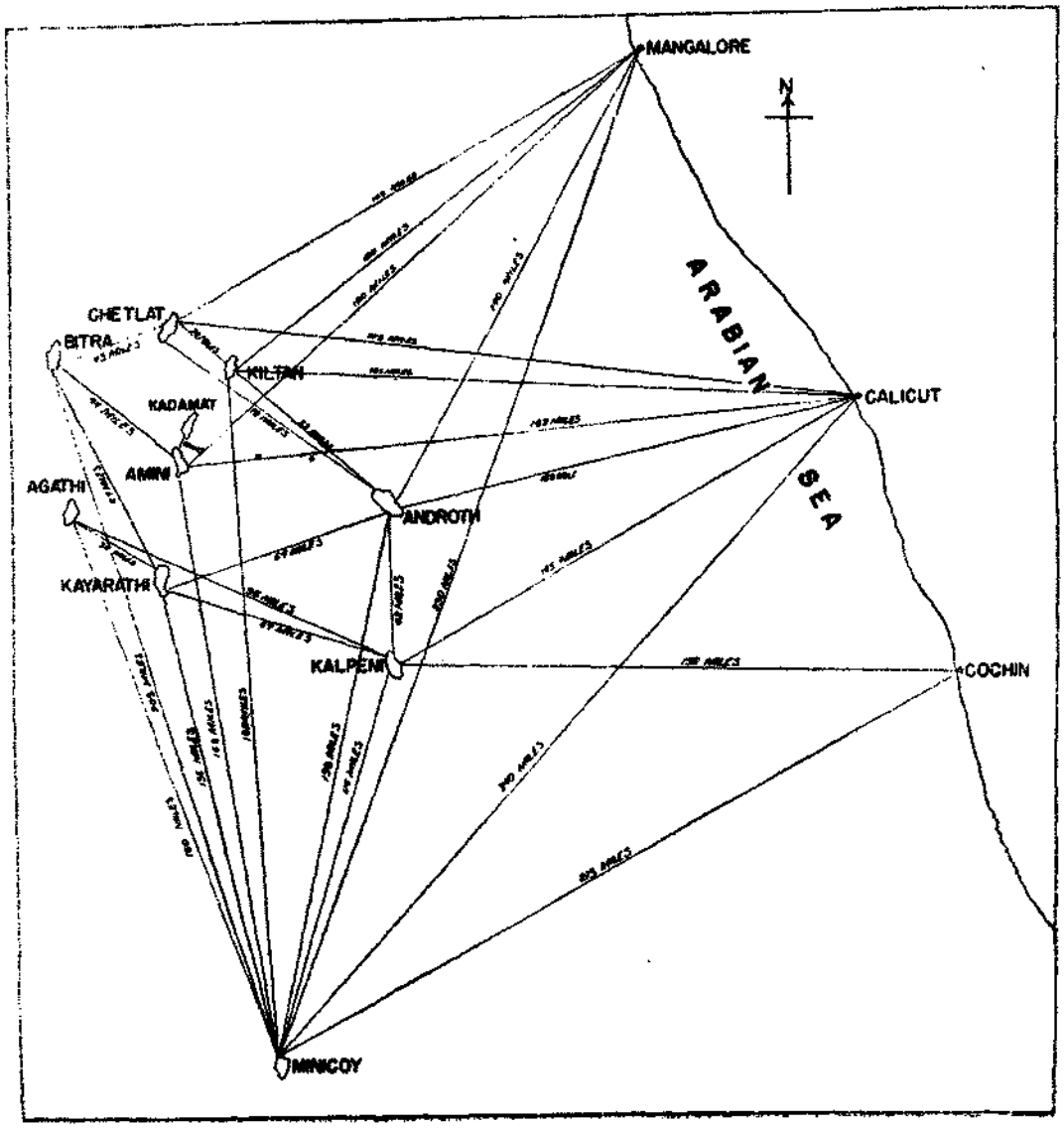
The islands are long and narrow, and appear as narrow banks of piled upsand in the sea. The area of the inhabited islands is about 10 square miles, ranging from 0·39 to 1·75 square miles.

The broadest part of the island is generally inhabited. On the western side of each island, excepting Androth, is a lagoon, which is a shallow semi-circular depression about 2 or 3 fathoms deep. The islands are mostly flat and are about 10 to 15 feet above the mean sea level.

The depth of sandy top layer varies from 3 feet (Minicoy) to 9 feet (Agathi). Below this, is the layer of lime-stone, ½ to 2 feet in thickness, with the water table and sandy layer again. Beneath the lime-stone and coral sand strata, is the coral rock layer at a depth of 9½ to 16 feet from ground level. The water table is about 6 feet (Androth) to 11½ feet (Agathi) from the ground.

MAP 1.

Laccadive, Minicoy and Aminidivi islands.



POPULATION.

The total population is 20,856 (1951 census), with a density of about 2,000 per square mile. The inhabitants in all the islands are Muslims.

CULTIVATION.

Coconut palms form the main cultivation. In Androth, and to a lesser extent in Kalpeni, in the cultivated portions of the land or *thottam*, sweet potato, pulses, and millets are grown. In Androth, paddy is also cultivated to a small extent. In Minicoy, barring the inhabited portions, the rest of the area has coconut palms and thick jungle, mostly of screw pine, growing to considerable heights. Grass is absent in many islands.

ANIMAL LIFE.

Animal life in the Islands is very much limited. Fowls have been recently introduced in all the islands. Birds are practically absent, except crows in Androth and Minicoy. Tree-rats are common and the domestic as well as garden lizards are found in varying densities in many of the islands.

OCCUPATION.

The chief occupation of the people is coconut farming, coir making and boat building. Some people are employed as crew in shipping concerns. Coconuts, copra, coir, country-made jaggery and vinegar are exported to the mainland. Fishing, for commercial purposes, is the chief occupation of the Minicoy people.

HOUSING CONDITIONS.

Except in Minicoy, the houses are scattered. The walls are made of coral or lime or sand-stone. The basement height is about two feet above ground level and the walls 8 to 9 feet thereafter. Buildings are invariably thatched, except a few with Mangalore tiles. Each house generally has a verandah and three to four rooms, with the kitchen separately housed. The surface area of the walls ranges between 1,500 and 4,000 square feet. The rooms are dark and poorly ventilated. In Minicoy, the houses are closely built, spacious and constructed with standard material, in rows facing in different directions. The surface area of the walls ranges between 3,000 and 4,000 square feet. The paths in between the rows of houses have the appearance of streets. Doors are big, but windows are relatively small. There are only foot-paths in these islands.

WATER SUPPLY.

Wells are the only source of sweet water, and are simple excavations reaching below the lime-stone stratum. Some of them are step-down wells with three sides protected by a layer of loosely packed coral stones, and one side provided with a

few steps. The depth of water is generally about two feet. Gradual conversion to draw-wells is being carried out in Minicoy and Androth and in some other islands.

For bathing purposes, there are separate tanks for males and females. Males bathe in tanks attached to the mosques. In Minicoy, the sides of all the tanks are mostly pucca, constructed as stone-steps. In other islands, only some tanks are well constructed. The rest are ill-built. In each island, there are a large number of bathing tanks for women. Except in Minicoy, the water in these tanks is polluted.

SEWAGE AND WASTE-WATER DISPOSAL.

In all the islands, people answer their calls of nature in open fields near the residential areas. Hermit-crabs and fowls help in the quick disposal of night soil. In Minicoy, however, the beach is used as the open latrine. The sandy soil of these islands is so porous that even during the rainy season there is no sullage problem, except some soakage pits in Minicoy.

POSSIBLE MOSQUITO-BREEDING PLACES.

The possible mosquito-breeding places are, (*a*) the coconut-husk soaking pits, (*b*) kutcha wells for watering the young coconuts, and (*c*) the trenches around the coconut nurseries. The coconut-husk soaking pits are numerous and usually scattered, except in Minicoy where they are located in selected areas outside the main village.

Numerous kutcha wells, for watering the young coconut palms or other gardens, are seen in many of the islands in a neglected state. The other water collections are the deep trenches in the coconut nursery plots.

METEOROLOGICAL CONDITIONS.

There are two monsoon periods, (*i*) south-west, from end of May to September, and (*ii*) north-east, from November to March ; the maximum precipitation being during the former period. The average annual rainfall is between 55 and 60 inches with about 90 rainy days. In some islands, it reaches 100 inches.

Relative humidity is high in all the islands, ranging from 74·3 to 85·1 per cent. During the premonsoon months, March to May, the climate is very oppressive with high humidity.

ADMINISTRATION.

Before 1957, these islands were under the Madras State and thereafter these have been under the Central Government.

EDUCATION.

In recent years, elementary schools have been started in each island, which are being upgraded to primary schools.

MEDICAL AND PUBLIC HEALTH ORGANISATION.

One dispensary each is functioning in Kiltan, Amini, Agathi, Kavarathi, Androth, Kalpeni and Minicoy. One medical officer for each dispensary is deputed annually, depending on the administrative convenience, either by the Kerala or the Madras State. For organising public health measures, sanitary inspectors are being posted for the first time.

HOSPITAL STATISTICS.

Round worm, filaria and asthma cases, treated for five consecutive years (1953 to 1957) in Amini, are depicted in Table I.

TABLE I.

Hospital figures.

Year.	Total cases treated (Number).	Number of cases with					
		Round worm.		Filaria.		Asthma.	
		Number.	Percentage in relation to total.	Number.	Percentage in relation to total.	Number.	Percentage in relation to total.
1953	6,180	413	6·7	43	0·7	64	1·0
1954	6,546	668	10·1	43	0·7	92	1·4
1955	9,561	1,253	13·1	45	0·5	188	1·9
1956	13,765	2,358	17·1	64	0·5	185	1·3
1957	5,410	586	10·8	11	0·2	135	2·2

PREVIOUS SURVEYS.

Filaria survey of the islands was carried out by a team of the Government of Madras in 1954-55 (Subramaniam *et al.*, 1955). Their findings indicated that the only islands that were non-endemic for filariasis, were Aminidivi, Kavarathi and Minicoy. The parasitological and entomological data noted by them are depicted in Tables II and III.

TABLE II.

Results of the filaria survey carried out in 1954-55 : Parasitological data.

Name of the island.	Percentage of the total population examined	Infection rate (Per cent).	Disease rate (Per cent).	Endemicity rate (Per cent).
Kiltan	37	8·4	5·5	13·9
Chetlet	30	13	13·40	26·4
Kadamath	20	14	7	21
Aminidivi	10	0	0	0
Agathi	25	4·2	0·8	5
Kavarathi	10	0	0	0
Androth	26·1	18·19	29·7	47·8
Kalpeni	10	9·7	16·29	25·9
Minicoy	9·5	0	0	0

TABLE III.

Results of the filaria survey carried out in 1954-55 : Entomological data.

Species.	Kiltan.	Chetlet.	Amini.	Kadamath.	Agathi.	Kavarathi.	Androth.	Kalpeni.	Minicoy.
C. fatigans	+	+	–	+	+	–	+	+	–
C. vishnui	–	+	+	–	+	+	+	–	+
C. tritaeniorhyncus	–	–	–	–	+*	–	–	+	+*
C. cornutus	–	–	–	–	–	+	–	–	–
Armegeres obturbans	+†	–	+†	–	–	+	+	+	+*
Aedes albopictus	–	–	–	–	+	–	–	+	+*
Aedes edwardsi	–	–	–	+†	–	–	–	–	+*
A. varuna	+†	+†	–	+	+†	+	+†	–	–
A. subpictus	+†	–	+†	–	–	+	+†	+	–
A. vagus	–	–	+	–	–	+	+†	+	–
A. hyrcanus	–	–	–	–	–	–	–	+	–
A. barbirostris	–	–	–	–	–	–	–	+	–

\+ = Positive.
– = Negative.
* Collection from the day-time resting places.
† Larval collections.

MATERIALS AND METHOD OF THE PRESENT SURVEY.

Except Bitra, all the inhabited islands were surveyed. The time spent in each island, except in Androth and Minicoy, was invariably less than two days. Night survey was conducted usually between 21·00 and 01·00 hours. A house-to-house survey of the different representative areas of the inhabited portions of the islands was carried out, examining as far as possible all the inmates of the houses (of all ages and both sexes).

From each person examined, about 20 c.mm. of blood, obtained from a pricked finger, was made into a smear. The air-dried smears were stained with J.S.B. stain on the following day and examined.

In addition, a clinical enquiry was made to elicit the presence or absence of various disease manifestations and in houses, selected at random, people were examined for evidence of lymphadenopathy.

During the day (between 7.30 a.m. and mid-day), mosquitoes were collected, breeding places surveyed and the topography of the island and the filariogenic conditions were studied. In Androth Island, the mosquito population was studied between 6 p.m. and 6 a.m. by half-an-hour catches every hour. The resting places of the mosquitoes were also recorded. The mosquitoes thus caught were later brought to the field laboratory, identified and dissected for evidence of filarial infection in them.

In addition, two-hourly blood smears of 5 microfilaria carriers were collected for a period of 24 hours from Androth Island to study the periodicity of microfilariae.

TABLE IV.

Prevalence of filarial infection, disease, endemicity and the average infestation in the different islands.

(A) Particulars in regard to the known endemic group of islands.

Name of the island examined.	Population.	Number examined.	Percentage of population examined.	Infection.		Average infestation. (Number per 20 c.mm.)	Disease.		Number with both disease and infection.	Endemicity rate (Per cent).
				Number.	Rate (Per cent).		Number.	Rate (Per cent).		
Kiltan	1,252	143	11·4	31	21·7	82	68	47·6	11	61·5
Chetlet	992	188	18·9	30	15·9	23	26	13·8	...	29·8
Kadamath	1,642	256	15·6	37	14·5	35	47	18·4	5	30·9
Agathy	2,038	679	33·3	82	12·1	26	103	15·2	10	25·8
Androth	3,651	1,378	37·7	258	18·7	39	356	25·8	49	41·0
Kalpeni	2,269	842	37·1	99	11·8	28	100	11·9	5	23·0
Total	11,844	3,486	29·4	537	15·4	39	700	20·1	80	33·2

(B) Particulars in regard to the islands not known so far to be endemic for filariasis (non/low endemic group).

Amini	3,184	239	7·6	1	0·4	1	13	5·4	..	5·9
Kavarathi	2,404	480	19·9	1*	0·2	12	2	0·4	..	0·6
Minicoy	3,447	650	18·8	..	..	..	3	0·5	..	0·5
Total	9,005	1·369	15·2	2	0·1	7	18	1·3	..	1·4

(C) Particulars in regard to all the islands.

Grand Total	20,849	4,855	23·3	539	11·1	36	718	14·8	80	24·2

*Belongs to Agathi Island.

Samples of water from two of the islands (Amini and Agathi) were collected for estimating the fluorine content.

At Kavarathi, Androth and Kalpeni, some garden lizards and cats were examined during the day-time for filarial infection.

RESULTS OF THE SURVEY.

(A) PARASITOLOGICAL AND CLINICAL OBSERVATIONS.

Altogether, 4,855 persons out of 20,849 (23·3 per cent of the total population) were examined. The infection, disease (obvious swellings only) and endemicity rates, as also the average infestation pertaining to the different islands, are depicted in Table IV. The endemicity rate was lowest in Kavarathi and Minicoy, being below 1 per cent. In Amini, it was 5·9 per cent. In Kalpeni, Agathi and Chetlet, it varied between 23 and 29·8 per cent. In other islands, *viz.*, Kadamath, Androth and Kiltan, the rates were 30, 41 and 61·5 per cent, respectively. Average number of microfilariae per carrier in the six heavily infected islands varied between 23 and 82. The infection in all cases was *W. bancrofti*.

Data regarding infection, disease and endemicity rates, as well as the average infestation in the various age-groups of the persons studied, in respect of the highly endemic islands of Kiltan, Chetlet, Kadamath, Agathy, Androth and Kalpeni, are depicted in Table V. It would be seen from Table V that a marked rise occurred in the infection rate with the age up to 20 years, and at the same time a steep rise in this incidence was obvious in the age-group of "3 - 5 years". Of 64 infants (below one year) surveyed in the different islands, no infection or disease was noted. The average number of microfilariae per positive slide, which

TABLE V.

Prevalence of filarial infection, disease, endemicity and the average infestation among different age-groups (both sexes) in the highly endemic group of islands (Kiltan, Chetlet, Kadamath, Agathi, Androth and Kalpeni Islands).

Age-group (Years).	Number examined.	Infection.		Average infestation (Number per 20 c.mm.).	Disease.		Number with both disease and infection.		Endemicity rate (Per cent).
		Number.	Rate (Per cent).		Number.	Rate (Per cent).	Number.	Rate (Per cent).	
1— 2	136	5	3·7	9	1	0·7	..	..	4·4
3— 5	235	42	17·9	17	8	3·4	..	..	21·3
6—10	489	69	14·1	34	23	4·7	3	0·6	18·2
11—20	855	164	19·2	35	129	15·1	22	2·6	31·7
21—30	787	113	14·4	43	233	29·6	24	3·1	40·9
31—40	465	63	13·5	46	145	31·2	22	4·7	40·0
41—50	308	53	17·2	45	89	28·9	5	1·6	44·5
Above 50	211	23	13·3	68	72	34·1	4	1·9	45·5
Total	3,486	537	15·4	237	700	20·1	80	2·3	33·2

was 9 in the age-group of "1 - 2 years", showed a steady rise in the successive age-groups and amounted to 68 in the oldest age-group "50 years and above". Beyond the age of 10, but up to 30 years, a marked and rapid rise in the disease rate occurred. On the whole, it can be generalised that the disease as well as the endemicity had a progressive rise with advancing age. 2·3 per cent of the people surveyed, had both infection as well as disease.

Data with regard to these details in respect of the Amini, Kavarathi and Minicoy islands, which have low endemicity for filariasis, are depicted in Tables VI to VIII.

TABLE VI.

Prevalence of filarial infection, disease, endemicity and the average infestation in different age-groups, in Amini Island.

Age-group (Years).	Number examined.	Infection.		Average infestation (Number per 20 c.mm. of blood).	Disease.		Number with both disease and infection.		Endemicity rate (Per cent).
		Number.	Rate (Per cent).		Number.	Rate (Per cent).	Number.	Rate (Per cent).	
1— 2	..	..	..	..	..	..	..	..	..
3— 5	1	..	..	..	..	..	..	..	..
6—10	20	..	..	..	1	5·0	..	..	5·0
11—20	79	..	..	..	1	1·3	..	..	1·3
21—30	69	..	..	..	8	11·6	..	..	11·6
31—40	46	..	..	..	1	2·2	..	..	2·2
41—50	18	1	5·6	1	..	..	..	..	5·6
Above 50	6	..	..	..	2	33·3	..	..	33·3
Total	239	1	0·4	1	13	5·1	..	..	5·8

Type of disease ... ... Hydrocele.
Number ... ... 13.
Percentage in relation to the total with disease ... 100.

TABLE VII.

Prevalence of filarial infection, disease, endemicity and the average infestation in different age-groups, in Kavarathi Island.

Age-group (Years).	Number examined.	Infection.		Average infestation (Number per 20 c.mm. of blood).	Disease.		Number with both disease and infecton.		Endemicity rate (Per cent).
		Number.	Rate (Per cent).		Number.	Rate (Per cent).	Number.	Rate (Per cent).	
1— 2	22	..	..	..	..	..	..	..	..
3— 5	37	..	..	..	..	..	..	..	..
6—10	62	..	..	..	..	..	..	..	..
11—20	98	1*	1·0	12	..	..	..	..	1·0
21—30	114	..	..	..	..	..	..	..	..
31—40	66	..	..	..	..	..	..	..	..
41—50	49	..	..	..	1†	2·0	..	..	2·0
Above 50	32	..	..	..	1†	3·1	..	..	3·1
Total	480	1	0·2	12	2	0·4	..	..	0·6

* Belongs to Agathi.
Type of disease ... ... Right and the left lower limb affection.
Number ... ... 2.
Percentage in relation to the total with disease ... 100.

TABLE VIII.

Prevalence of filarial infection, disease, endemicity and the average infestation in different age-groups, in Minicoy Island.

Age-group (Years).	Number examined.	Infection.		Average infestation (Number per 20 c.mm. of blood).	Disease.		Number with both disease and infection.		Endemicity rate (Per cent).
		Number.	Rate (Per cent).		Number.	Rate (Per cent).	Number	Rate (Per cent).	
1— 2	1	..	..	..	.	..	..	..	..
3— 5	6	..	..	..	..	..	..	..	..
6—10	48	..	..	..	..	..	..	..	..
11—20	217	..	..	..	1*	0·5	..	..	0·5
21—30	157	..	..	..	..	..	..	..	..
31—40	88	..	..	..	..	..	..	..	..
41—50	61	..	..	..	1*	1·6	..	..	1·6
Above 50	72	..	..	..	1†	1·4	.	..	1·4
Total	650	..	..	..	3	0·5	..	..	0·5

Type of disease.	Number.	Percentage in relation to the total with disease.
(i) *Hydrocele	2	66·7
(ii) †Left lower limb affected	1	33·3

The relative prevalence of various disease manifestations in different age-groups in the highly endemic islands, is depicted in Table IX which shows that elephantiasis of the lower extremity/extremities formed the major proportion of the disease manifestations, with hydroceles coming next. Many males in these islands had elephantiasis of limbs and scrotum developed to huge sizes.

In the highly endemic group of islands, 11·43 per cent of the persons, having one or the other disease manifestation (Table X), and 15·4 per cent of the persons examined (Table V), harboured microfilariae.

Prevalence of lymphatic gland enlargement, detected on examination during the night surveys, showed that in these highly endemic islands, about 45·1 per cent of the persons surveyed had either groin or supra-trochlear glands enlarged, whereas only 15·6 per cent had similar affection in the non/low-endemic islands. No apparent difference could be found in the infection and disease rates among persons with and without glandular enlargements in the highly endemic group of islands.

The youngest age at which infection was detected was 'one year', in two children, one at Agathi and the other at Androth. Corresponding to this, the earliest onset of disease manifestation (swelling) was observed in a male child, aged 2 years, in Agathi island.

Table IX.

Relative prevalence of various disease manifestations among different age-groups (both sexes) in the highly endemic group of islands.

Age-group (Years).	Number examined.	All manifestations.		Affection of the upper extremity.		Affection of the lower extremity.		Affection of both the extremities.		Hydrocele.		Hydrocele and other swellings.		Genital swelling.		Chyluria.	
		Number.	(Per cent).	Number.	(Per cent).	Number.	(Per cent).	Number.	(Per cent).	Number.	(Per cent).	Number.	(Per cent).	Number.	(Per cent).	Number.	(Per cent).
1— 2	136	1	0·7	..	..	1	0·7	..	..	..	..	..	..	..	..	..	..
3— 5	235	8	3·4	1	0·4	5	2·1	..	..	..	2	0·9	..	..	..	..	..
6—10	489	23	4·7	..	..	14	2·9	1	0·2	8	1·6	..	..	..	..	..	..
11—20	855	129	15·1	11	1·3	73	8·5	7	0·8	28	3·3	9	1·1	1	0·1	..	..
21—30	787	233	29·6	13	1·7	112	14·2	11	1·4	74	9·4	18	2·3	3	0·4	2	0·1
31—40	465	145	31·2	7	1·5	55	11·8	12	2·6	57	12·3	11	2·4	3	0·6	..	..
41—50	308	89	28·9	2	0·5	39	12·7	14	4·5	23	7·5	6	1·9	4	1·3	1	0·3
Above 50	211	72	34·1	3	1·4	29	13·7	7	3·3	22	10·4	10	4·7	1	0·5	..	..
Total	3,486	700	20·1	37	1·1	328	9·4	52	1·5	214	6·1	54	1·5	12	0·3	3	0·1

TABLE X.

Relative prevalence of the individual disease manifestations and the extent of concomitant infection in the highly endemic islands.

Disease manifestation.	BOTH SEXES :			
	Incidence of disease.		Cases showing microfilaria.	
	Number.	Percentage in relation to the total with disease.	Number.	Percentage in relation to the total with disease.
Upper extremity	37	5·3	7	8·9
Lower extremity	328	46·9	26	7·92
Both extremities	52	7·4	3	5·8
Hydrocele	214	30·6	37	17·3
Hydrocele and other swellings	54	7·7	4	7·42
Genital swelling	12	1·7	3	25·0
Chyluria	3	0·4	..	..
Total	700	100	80	11·4

During the survey, the time when the houses of 3,384 persons were visited for study, was recorded. Infection rate and the average infestation among these persons, analysed according to the different hours of the night when the blood smears were taken, are recorded in Table XI. No significant difference, either in the infection or the average infestation, could be observed in the results obtained at the different hours of the night up to 1 a. m., but during the next one hour there was a slight but definite reduction in these figures, followed immediately (*i. e.*, between 2 and 3 a. m.) by a sudden rise.

TABLE XI.

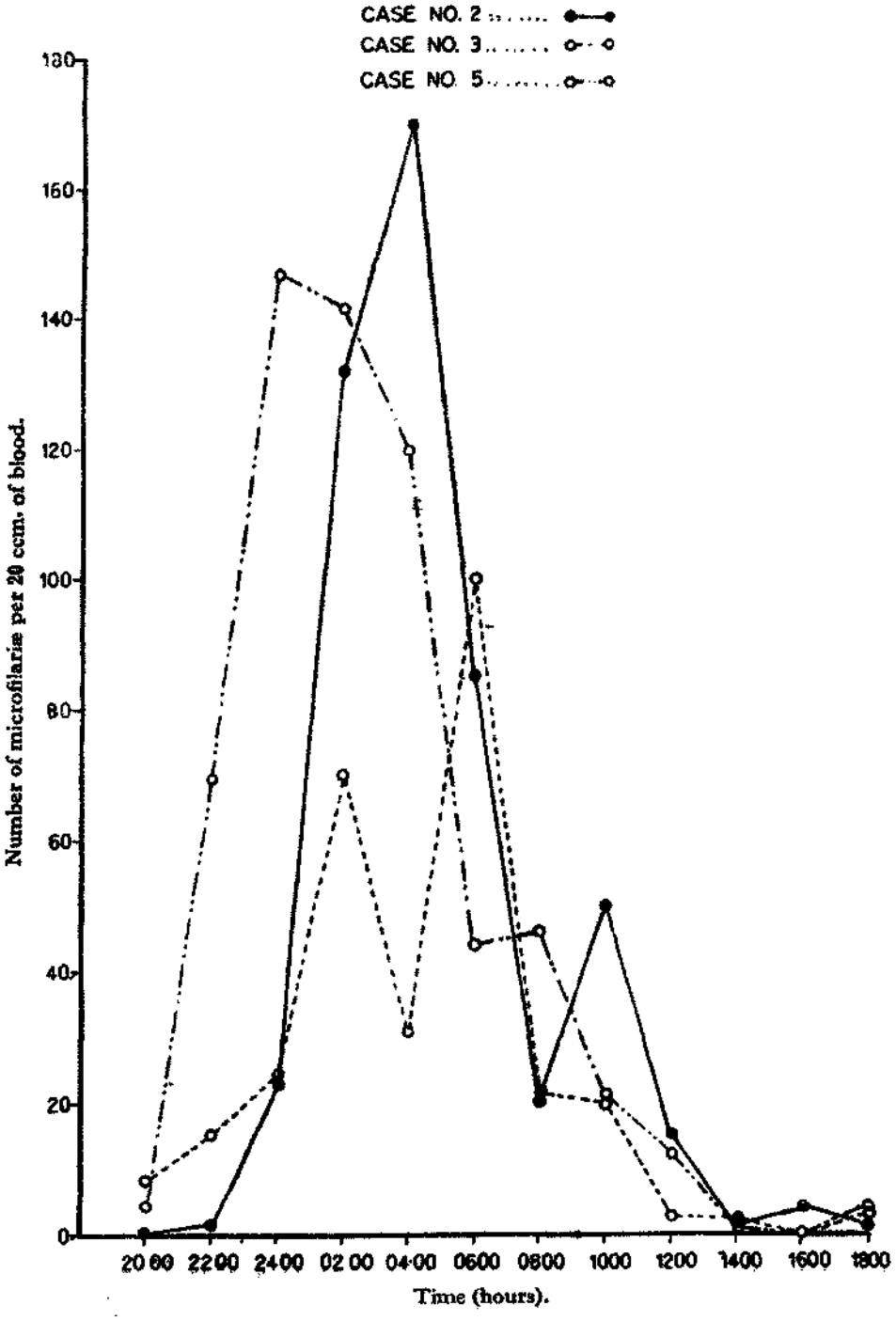
Prevalence of filarial injection and the average infestation (per 20 c.mm. of blood) at different hours of the night.

Time.	Number examined.	FILARIAL INFECTION.		Average infestation.
		Number.	Rate (Per cent).	
8— 9 p.m.	225	37	16·4	39
9—10 p.m.	745	112	15·0	39
10—11 p.m.	797	128	16·1	38
11—12 p.m.	790	122	15·4	37
12 p.m.— 1 a.m.	554	92	16·6	42
1— 2 a.m.	212	25	11·8	24
2— 3 a.m.	61	12	19·7	48
Total	3,384	528	15·6	38

The results of examination of blood smears, collected after every two hours, for a period of 24 hours, from the five microfilaria carriers (*W. bancrofti*) from Androth Island, are depicted in Table XII and in Graphs 1 and 2.

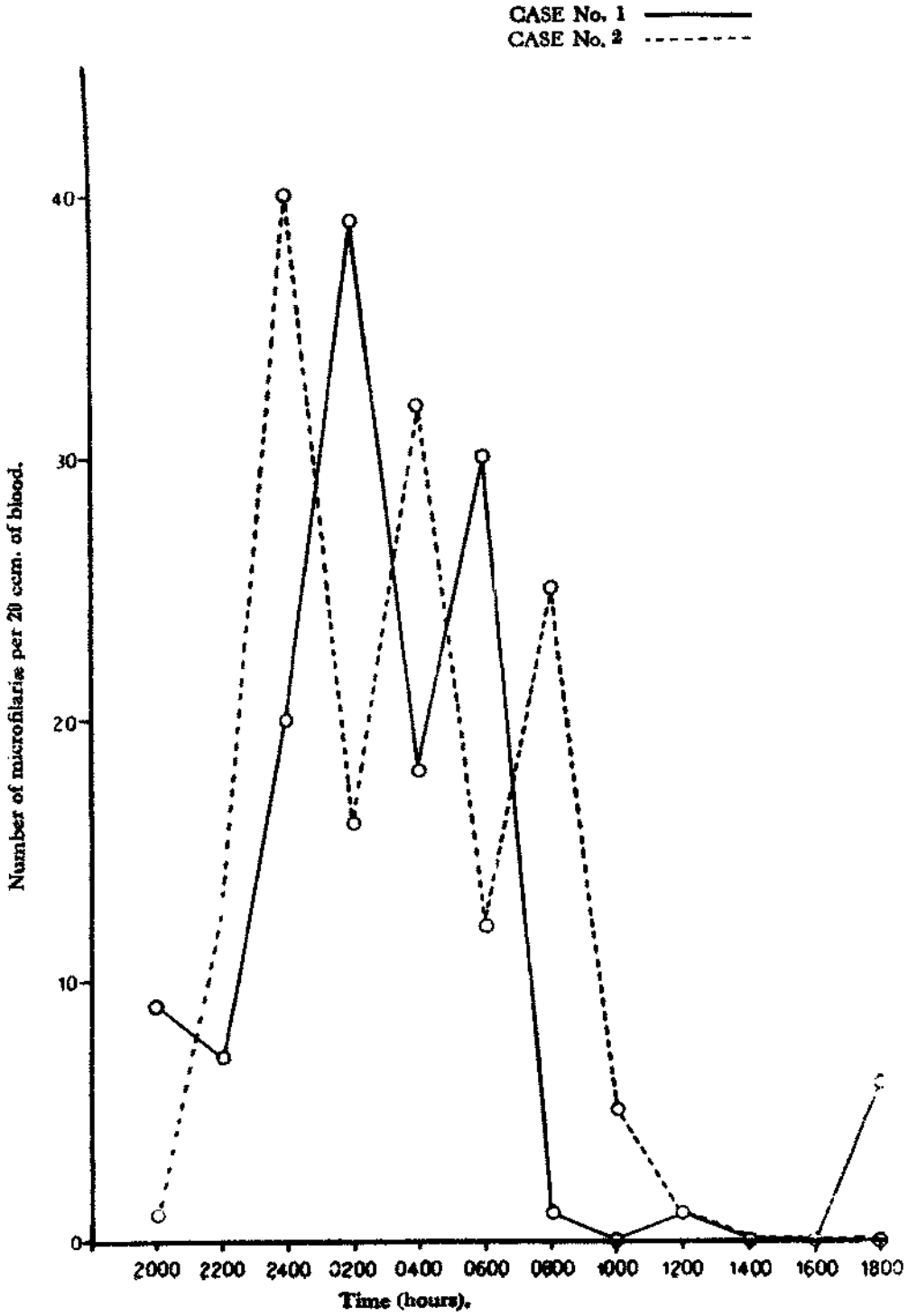
GRAPH 1.

Periodicity studies of W. bancrofti *microfilariae carried out at Androth Island (high counts).*



GRAPH 2.

Periodicity studies of W. bancrofti *microfilariae carried out at Androth Island (low counts).*



In all the five persons, nocturnal periodicity of *W. bancrofti* was observed. In three cases two peaks in microfilarial count were observed, and in two cases, only one peak. The first peak occurred between 12 mid-night and 2 a.m., and the second between 4 and 6 a.m. In three persons, microfilariae in small numbers could be seen even during day-time. It may, however, be mentioned that the investigation was made towards the end of *Ramzan* when the Muslims, for a period of about one month, keep awake for most of the time in the night, and starve and sleep for varying periods during day-time.

TABLE XII.

Microfilaria count of five microfilaria carriers, belonging to Androth Island, taken at different hours of the day and night.

Time.	Case 1.	Case 2.	Case 3.	Case 4.	Case 5
	Mf. count per 20 c.mm. blood.	Mf. count per 20 c.mm. blood.	Mf. count per 20 c.mm. blood.	Mf. count per 20 c.mm. blood.	Mf. count per 20 c.mm. blood
8·00 p.m.	9	Negative	8	1	4
10·00 p.m.	7	1	15	13	69
12·00 mid-night	20	23	24	40	147
2·00 a.m.	39	132	70	16	142
4·00 a.m.	18	170	31	32	120
6·00 a.m.	30	85	100	12	44
8·00 a.m.	1	20	21	25	46
10·00 a.m.	0	50	20	5	21
12·00 noon	1	15	3	1	12
2·00 p.m.	0	1	2	0	1
4·00 p.m.	0	4	0	0	0
6·00 p.m.	0	1	3	0	4

(*B*) ENTOMOLOGICAL OBSERVATIONS.

The species of mosquitoes collected from the various islands are depicted in Table XIII.

The per man-hour density of various mosquitoes caught from the different islands, and the results of dissection of *C. fatigans* are depicted in Table XIV. The over-all infection rate in *C. fatigans* in the different islands ranged from 8·7 to 24·3 per cent. The average infection rate in *C. fatigans* for all the infected islands was 16·7 per cent.

The total number of *C. fatigans* caught during the different hours of the night from three houses in the Androth Island, and the exact places from where they were caught, are shown in Table XV. The maximum number of mosquitoes caught, was between 2 a.m. and 4 a.m. About 50 per cent or even above 50 per cent of the total catch between 12 mid-night and 6 a.m., was at the time when the mosquitoes were actually feeding on persons sleeping indoor. Next to that, they were caught from hanging materials (clothes and ropes) and from walls up to 2 feet height.

Except in Amini, Kavarathi and Minicoy, the bathing tanks for women were the most favoured breeding places of *C. fatigans*. Breeding in the shallow step-wells was mostly *Anophelines*. Breeding was seldom observed in the deep draw-wells. *Culicine*, *Aedes* as well as *Anopheline* breeding occurred in coir soaking pits. In Amini, Kavarathi and Minicoy islands, no breeding of *C. fatigans* could be detected during the present survey.

TABLE XIII.

Adult mosquitoes collected from the various islands.

Species.	Kiltan.	Chetlet.	Amini.	Kadamath.	Agathi.	Kavarathi.	Androth.	Kalpeni.	Minicoy.
C. fatigans.	+	+	—	+	+	+	+	+	—
C. vishnui.	—	—	+	—	+	+	—	—	—
C. sitiens.	—	—	—	—	—	—	—	—	+
C. tritaeniorhyncus.	—	—	—	—	—	+	—	+	—
Armigeres obturbans.	—	—	—	—	—	+	—	+	—
Lutzia.	—	—	+	—	—	+	—	—	—
A. varuna.	—	—	+	—	—	+	—	—	—
A. subpictus.	+	+	+	+	—	+	+	+	—

\+ = Positive.
— = Negative.

(C) WATER ANALYSIS.

A sample, each of the drinking water collected from shallow wells at Agathi and Amini islands, was tested at the Provincial Hygiene Institute, Uttar Pradesh, Lucknow, for fluorine content, and was found to contain fluorine at the rate of 0·7 and 0·8 per cent., respectively.

(D) ANIMAL SURVEY.

The results of animal survey carried out in Kavarathi, Androth and Kalpeni islands are depicted in Table XVI.

Calotes versicolor was found infected in all the three islands, with a species resembling *C. guindiensis*.

(E) SOCIO-ECONOMIC ASPECTS.

Particulars about the socio-economic aspects of the disease were collected from a few filarial cases in Androth Island. Almost all the families had more than one person affected with elephantiasis. All the patients complained about constant worry and mental depression on account of illness. The number of days lost due to illness, varied; depending upon the severity and duration of the illness,

TABLE XIV.

Per man-hour density, infection rate and the average number of filarial larvae per infected mosquito, in different islands.

Island.	*C. fatigans.*					*C. vishnui**	*A. subpictus.**	*Armigeres**	*A. varuna.**	*C. tritaeniorhyncus.**
	Per man-hour density.	Infection rate (Per cent).		Average number of larvae per infected mosquito.		Per man-hour density.	Per man-hour density.	Per man-hour density.	Per man-hour density.	Per man-hour density.
		All stages	Infective larvae.	All stages.	Infective larvae.					
Kiltan	40·3	24·3	2·6	7·3	2·0	..	..	..	..	..
Chetlet	102·5	8·7	3·1	3·6	1·6	..	2·0	..	..	..
Kadamath	90·5	22·3	3·1	7·9	2·0	7·0	2·0	..	..	..
Agathy	58·0	16·9	..	3·0	..	24·0	..	.	..	..
Androth	89·5	15·8	4·1	4·8	3·2	..	0·5	..	..	..
Kalpeni	68·5	12·0	2·0	14·2	1·0	..	16·3	0·9	..	..
Amini	..	..	..	..	..	20·0	..	..	..	..
Kavarathi	..	..	..	..	..	11·5	6·0	3·0	1	1
Minicoy	..	..	..	..	..	..	..	..	..	35·0

*None of these species was found infected on dissection.

Table XV.

Particulars of the whole-night collection of C. fatigans *from three residential quarters in Androth Island.*

Time of collection.	Total collection (Number).	Objects from which C. *fatigans* were caught.													
		Hanging clothes.		Ropes.		Cobwebs.		Walls up to 2 feet height.*		Walls up to 4 feet height.		Roof.		While feeding.	
		Number.	Percentage in relation to total.	Number.	Percentage in relation to total.	Number.	Percentage in relation to total.	Number.	Percentage in relation to total.	Number.	Percentage in relation to total.	Number.	Percentage in relation to total.	Number.	Percentage in relation to total.
6 — 8 p.m.	54	16	29·6	36	66·7	..	..	2	3·7	..	..	..	..	..	..
8 —10 p.m.	43	20	46·5	12	27·9	2	4·7	5	11·6	4	4·7	..	..	2	4·7
10 —12 p.m.	53	14	26·4	..	..	15	28·3	1	1·9	2	7·5	5	9·4	14	26·4
12 p.m.— 2 a.m.	64	2	3·1	2	3·1	..	..	24	37·5	..	..	..	.	36	56·3
2 — 4 a.m.	118	27	22·9	..	..	..	..	35	29·7	..	..	..	..	56	47·5
4 — 6 a.m.	48	3	6·3	..	..	..	..	8	16·5	10	20·	2	4·2	25	52·1
Total	380	82	21·6	50	13·2	17	4·5	75	19·7	16	4·2	7	1·9	133	35·0

* Close to the people sleeping.

Table XVI.

Results of animal survey carried out in different islands.

Island.	Species of the Animal Surveyed.	Number Examined.	Number Positive.	Average infestation per 20 c.mm.
Kavarathi.	*Calotes versicolor.*	3	3	1,594
Androth.	*Calotes versicolor.*	3	2	327
Androth.	*Mabuya carinata.*	7	—	—
Kalpeni.	*Calotes versicolor.*	13	9	2,056
Kalpeni.	*Mabuya carinata.*	4	—	—
Kalpeni.	Fowl.	4	—	—
Kalpeni.	Cat.	2	—	—

In one case, as many as 168 days in a year were reported to be lost. Capacity for work during periods, even when acute attacks were absent, was stated to be about 20 per cent only in some of the cases. Due to the fact that the disease is chronic and widely prevalent among the islanders, they have become greatly apathetic. Since many islanders are victims of the disease, no serious social stigma is attached to the disease in this area. There was no evidence to show that the disease has caused sterility. The mechanical obstruction to the sex life, as a result of elephantoid swelling of scrotum and penis, was complained by some.

DISCUSSION.

Filaria is very highly endemic in six islands, i.e., Chetlet, Kadamath, Agathi, Androth, Kalpeni and Kiltan, out of the nine islands surveyed. A comparison of the results of the present survey with those recorded in the year 1954-55 (Subramaniam *et al.*, 1958) indicates that the prevalence of the disease is on the increase in all the islands. Subramaniam *et al.* (1958) considered the Aminidivi Island to be free from filariasis. The present survey indicates that within a period of about four years thereafter, this island too has become filarious.

The bathing tanks for women and the coconut-husk soaking pits form the main source of vector breeding in these islands.

It would appear from the present as well as the past survey that the Kavarathi and Minicoy islands have so far remained free from this disease.

Minicoy Island is the most distant one, with little communication and mixing up of the population with the population in the other islands. *C. fatigans* has not yet been introduced into this island. With favourable mosquito breeding places available, and with the frequent boat service introduced by the present administration between this and other islands with the mainland would, it is felt, facilitate the early introduction of the vector and filaria infection in this island also.

In the Amini Island, no *C. fatigans* was found during the present survey. As there is already the indication that filaria is just establishing itself in this island, it is likely that there are in all probability some untraced breeding grounds of *C. fatigans*. The coconut-husk soaking pits in Amini and Minicoy were found to breed other culicines. Whether in this water collection *C. fatigans* also is breeding or will eventually breed, is a thing that should be watched.

As was observed in the epidemiological investigations undertaken previously in the *W. bancrofti* areas (Iyenger, 1938 ; Krishnaswami, 1955 ; Nair, 1960), the findings of the present survey also showed a steady and progressive rise in the infection rate up to the age-group "20-30 years." A distinct feature in this survey, however, is the rise in the incidence of the infection rate from the age group "1-2 years" to the age-group "3-5 years", which is quite rapid and pronounced.

In Agathi Island, the prevalence of filaria in 1954-55 (Subramaniam *et al.*, 1958) was far less than what was recorded in the course of the present survey.

In this island, according to the present survey, 62·2 per cent of the people with disease manifestations, suffered from hydrocele of recent origin. It tends to indicate, therefore, that very active state of transmission of the disease has been occurring in the island since recent years.

Night surveys, for epidemiological investigations, are generally carried out in India and many other parts of the world between 8 p.m. and 12 midnight, and thus the examination of the different individuals in the community is spread over a period of 4 to 5 hours. At the same time it is to be remembered that due to the nocturnal periodicity of *W. bancrofti*, a gradual rise in the microfilaria count in peripheral blood is to be expected with advance of time in the night. Doubts are likely to arise whether the results of surveys conducted in different areas within this wide range of time are comparable or not, but the analysis made of the results applicable to the specific hours within this range of time has not shown any definite difference as compared to overall findings, and hence it is to be inferred that the standard procedure followed in the time of survey in the night is quite practical and dependable. However, more investigations of this nature are to be carried out before arriving at a final conclusion.

Collection of mosquitoes, spread over one whole night, gave rise to a higher catch of *Culex fatigans* after midnight and most of them invariably were collected when they were actually feeding on human beings. The periodicity studies indicated also an increase in microfilaria count more or less at this time.

The prolific breeding of *C. fatigans* in the highly endemic islands, the absence of animal population to provide alternative host for their blood feed and the recent origin of the disease in these islands, are considered to be some of the factors responsible for the high degree of endemicity of this disease, observed during the course of the present survey.

Microfilariae, resembling *C. guindiensis*, was detected from *Calotes versicolor* from the Kavarathi Island. *Culex fatigans* is the main vector of this infection. But in this particular island, no *C. fatigans* was found. It will be of interest to carry out a detailed investigation to know about the actual vector species of the infection.

There is a general belief that the low fluorine content of the local water supply favours the incidence of filariasis (Subramaniam and Srinivasan, 1955). In Aminidivi Island, during the previous survey (Subramaniam *et al.*, 1958), there was no filarial incidence. Samples of water from this island, as well as from the Agathi Island, a known filarial place, were collected and chemically analysed and no appreciable difference in the fluorine content was found. This, therefore, does not seem to lend support to the above mentioned theory.

SUMMARY.

Kiltan, Chetlet, Kadamath, Agathi, Androth, Kalpeni, Amini, Kavarathi and Minicoy, situated in the Laccadive, Minicoy and Aminidivi group of islands, were surveyed for filaria in the month of April, 1958. 4,855 persons, comprising 23·3 per cent of the total population, were surveyed. A very high incidence of

filarial infection was recorded in the first six islands. In these six islands, infection, disease and endemicity rates were on the average 15·4, 20·1 and 33·2 per cent, respectively. The indices for the Amini Island were 0·4, 5·4 and 5·8 per cent, for infection, disease and endemicity, respectively. The other two islands, *i.e.*, Kavarathy and Minicoy were found to be almost non-endemic for filariasis.

The prevailing species of infection was *W. bancrofti*, with *C. fatigans* as the vector which were noted profusely breeding in tanks used by women for bathing and in the coconut-husk soaking pits.

The epidemiology of filaria in these islands has been brought out. The periodicity of microfilariae was worked out at Androth Island and was found to be nocturnal. Preferential feeding time of *C. fatigans* in the night was investigated. No significant difference could be observed in the chemical nature of water with regard to fluorine content in the low endemic island (Amini), and the highly endemic island (Agathi). *Calotes versicolor* was examined from three islands and found to be infected in nature with a species resembling *Conspicuum guindiensis*. In the Kavarathi Island, animal infection prevailed even though human infection was practically absent.

ACKNOWLEDGEMENT.

The author wishes to express his thanks to the Administrator, Laccadive, Minicoy and Aminidivi group of islands, Kozhikode, for the facilities made available to the survey party , and the Assistant Director, Provincial Hygiene Institure, Lucknow, Uttar Pradesh, for chemical analysis of the water samples.

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Indian Journal of Malariology, **15**, *4, December 1961.*

FILARIASIS IN THE RURAL POPULATION AROUND BHAGALPUR TOWN.

PART I.

BY

B.K. VARMA*

N.L. DASS†

AND

V.P. SINHA‡

[December 22, 1960.]

INTRODUCTION.

UNDER the auspices of the National Filaria Control Programme (Raghavan, 1955), the Filaria Control Unit, Bhagalpur, conducted a random filaria survey of ten villages outside the Bhagalpur Municipal Area during the month of March, 1958.

The area undertaken for this investigation is a rural one, situated outside Bhagalpur Town, on the south-western side on the bank of River Ganges and comprises approximately 4,626 acres in ten villages with a population of 7,725. Houses are mostly mud-huts, with thatched or tiled roof. The sanitation of the area is very poor. Sullage water, containing organic materials from houses, flows out through small uncovered drains which end blindly. People defaecate in open fields near ponds, whereas the children defaecate on the sides of the drains or cesspools. Socio-economically the area is poor.

HISTORY OF FILARIASIS.

Enquiries from persons, above 60 years of age, revealed that they had not seen any elephantoid swelling before the World War II, though some cases, positive for both infection and disease, had been recorded (Korke, 1926) in this area.

THE SURVEY.

Random representative samples of the population, covering all age-groups and both sexes, were examined in the ten villages. Records of daily visits were maintained by trained survey party. The survey was conducted between 8·30 p.m. and 11·30 p.m. Filarial disease manifestations were recorded by enquiry and

*Chief Malaria Officer, Bihar, Patna.
†Filaria Research Officer, Filaria Control Unit, Bhagalpur (Bihar).
‡Assistant Entomologist, Filaria Control Unit, Bhagalpur (Bihar).

examination of the affected part. Three to four drops of blood (about 20 c.mm.) were obtained from the finger-tips of each individual and a thick blood smear was prepared. The thick blood smears were dehaemoglobinised the following day by tap water and stained by Leishman stain. The entire field of the smear was examined, the species of microfilaria was identified in each, and the number present in the positive smear was counted.

In each of the ten villages surveyed in this area, adult mosquito collections were made from 7.00 a.m. to 10.30 a.m. from fixed catching stations which consisted of human dwellings, cattle-sheds and mixed dwellings. Cesspools, tanks, ponds, unused wells, pots and tree-holes and the clay-pots for domestic animal feeding, in the area, were examined to detect mosquito breeding. The female mosquitoes were identified and dissected for possible filarial larvae.

RESULTS.

Altogether 1,940 persons, forming about 25·1 per cent of the population, were examined (Table I). The disease manifestations were noted in the form of swellings of the limbs (upper or lower), hydrocele and genital swellings.

The microfilaria rate varied from 5·3 to 17·6 per cent. The average infestation of microfilariae per 20 c.mm. of the positive blood smears, in all age-groups and sexes, was 11·7. The number of microfilariae per smear ranged from 1 to 58.

The data presented in Table II, and Graph 1, revealed a progressive rise of infection rate and disease rate up to the age of 20 and 40 years, respectively. The infection and the disease were low in children below 10 years. The infection rate and disease rate were higher in males than in females. The youngest age at which disease and infection were noted was 3 years in both sexes, and the symptoms were in the form of periodical swelling of the lower limbs.

ENTOMOLOGICAL SURVEY.

The species of the mosquitoes collected and identified were :—

(*i*) *Culex fatigans* (Wied.).
(*ii*) *A. annularis* (Van der Wulp).
(*iii*) *A. vagus* (Donitz).
(*iv*) *A. stephensi* (Liston).
(*v*) *A. subpictus* (Grassi).
(*vi*) *Armegeres obturbans* (Walker).

The females of all these mosquitoes were dissected and examined to locate the presence of microfilariae. *Culex fatigans* (Wied.) only exhibited the presence of all the four developing stages of the microfilariae in the abdomen, thorax and head/proboscis (*vide* details in Table III). Thus, *Culex fatigans* was the vector of microfilaria here.

TABLE 1.

Incidence of filariasis in different villages around Bhagalpur Town.

Name of village.	Approximate distance from Bhagalpur Town.	Persons examined.			With disease.				With microfilaria.				Both sexes.			
													With disease.		With microfilariae.	
		Male.	Female.	Total examined.	Male.	Percentage.	Female.	Percentage.	Male.	Percentage.	Female.	Percentage.	Number.	Percentage.	Number.	Percentage.
1. Srirampur	2·5 miles	106	98	204	30	28·3	19	19·3	18	16·9	18	18·3	49	24·01	36	17·6
2. Basantpur	5 miles	34	29	63	7	20·5	6	20·6	5	14·7	4	13·7	13	20·6	9	14·2
3. Ranichak	4 miles	178	170	348	46	25·8	36	21·1	28	15·7	21	12·3	82	23·5	49	14·08
4. Bhavnathpur	7 miles	165	155	320	42	25·4	29	19·7	25	15·1	19	12·2	71	22·1	44	13·7
5. Akbarnagar	10 miles	209	197	406	66	31·5	46	23·3	30	14·3	22	11·1	112	27·5	52	12·8
6. Gouripur	7 miles	90	83	173	27	30·0	24	28·9	14	15·9	8	9·4	51	29·5	22	12·7
7. Makandpur	6·5 miles	102	95	197	32	31·7	20	21·04	12	11·7	8	8·4	52	21·3	20	10·1
8. Rasidpur	12·5 miles	23	20	43	5	21·7	3	15·0	2	8·6	2	10·0	8	18·6	4	9·3
9. Khutcha	7 miles	60	51	111	9	15·0	7	13·7	6	10·0	4	7·8	16	14·4	10	9·09
10. Kharai	7·5 miles	41	34	75	5	12·1	5	14·7	3	7·3	1	2·9	10	13·3	4	5·3
Total		1,008	932	1,940	269	26·6	194	20·8	145	14·3	107	11·4	463	23·8	252	12·8

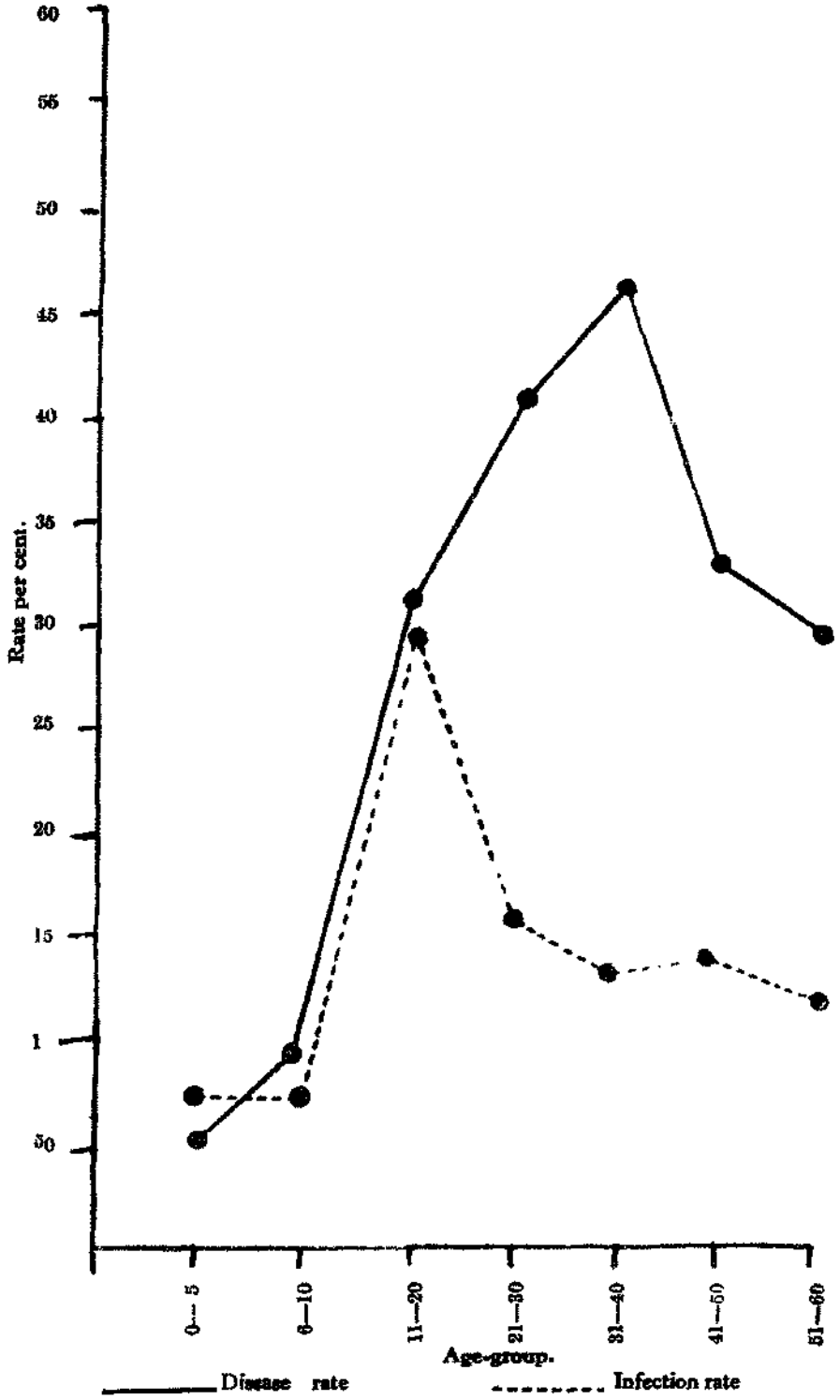
TABLE II.

Incidence of filariasis in different age-groups and two sexes.

Age-group (Years).	MALES					FEMALES					BOTH SEXES				
	Number examined.	With disease.		With microfilaria.		Number examined.	With disease.		With microfilaria.		Number examined.	With disease.		With microfilaria.	
		Number.	Percentage.	Number.	Percentage.		Number.	Percentage.	Number.	Percentage.		Number.	Percentage.	Number.	Percentage.
0 — 5	182	12	6·5	12	6·5	163	6	3·6	14	8·6	345	18	5·2	26	7·7
6 — 10	315	36	11·4	26	8·2	286	24	8·3	19	6·6	601	60	9·9	45	7·4
11 — 20	106	30	28·3	41	38·6	98	34	34·6	21	21·4	204	64	31·3	62	30·3
21 — 30	177	84	47·4	29	16·3	157	56	35·6	27	17·1	334	140	41·9	56	16·7
31 — 40	112	64	57·1	18	16·07	122	45	36·8	14	11·4	234	109	46·5	32	13·7
41 — 50	69	28	40·5	14	20·2	75	20	26·6	7	9·3	144	48	33·3	21	14·5
51 and above	47	15	31·9	5	10·6	31	9	29·03	5	16·1	78	24	30·7	10	12·9
Total	1,008	269	26·6	145	14·3	932	194	20·8	107	11·4	1,940	463	23·8	252	12·8

GRAPH 1.

Incidence of filaria disease and infection, among both sexes, in the rural population around Bhagalpur Town.



GRAPH 2.

Incidence of filariasis in the rural population around Bhagalpur Town and distances from Bhagalpur Town.

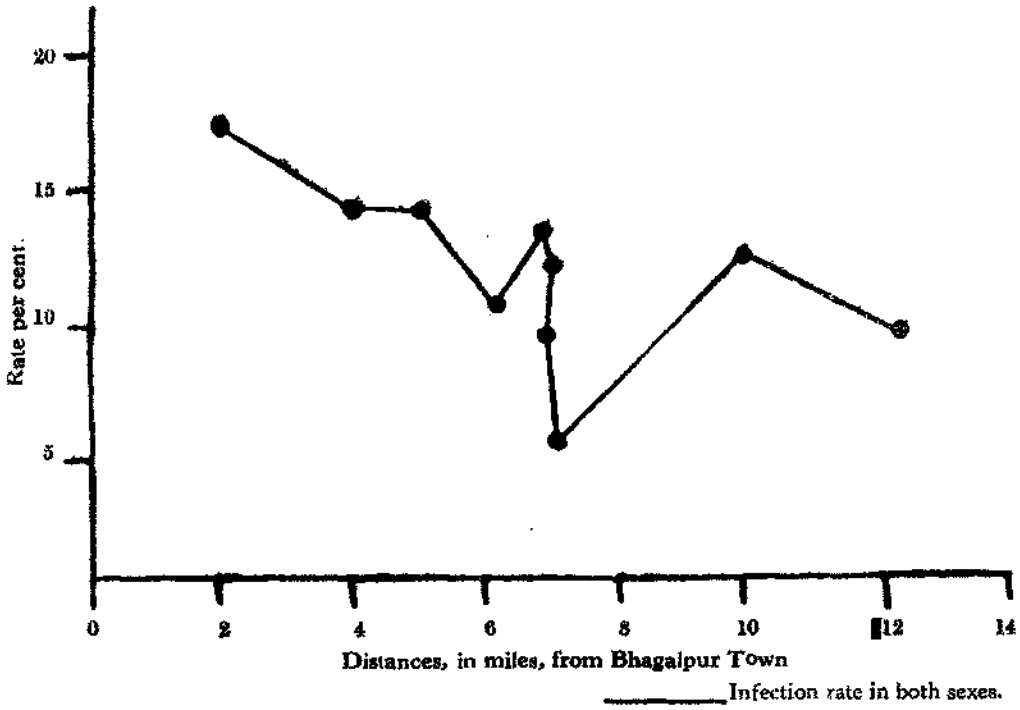


TABLE III.

Infection in C. fatigans *mosquitoes.*

Total number of *Culex fatigans* dissected.	Total number of *Culex fatigans* positive for all forms of microfilaria.	Infection rate for all forms of microfilaria (per cent).
224	11	4·9

The examination of the breeding places of mosquitoes revealed that *Culex fatigans* was found to breed in domestic cesspools, uncovered stagnant drains, unused wells, stagnant ponds and unused pots meant for feeding the domestic animals.

DISCUSSION.

Filariasis is believed to be of recent origin in the area. The disease had been brought from the town and has gradually spread in these villages by intercommunication. The history of the first case with elephantoid swellings can be traced back to a decade.

The authors noted that none of the ten villages surveyed, was free from either filarial infection or disease. The infection ranged between 5·3 and 17·6 per cent, and the disease manifestation between 13·3 and 29·5 per cent (Table I). Further, they observed a progressive increase in the incidence of infection in villages nearer the town (Table I, Graph 2). This observation showed that the filarial endemicity decreased progressively as the distances increased from the centre of Bhagalpur Town. But places like Akbarnagar and Rasidpur, situated 10 and 12·5 miles away from the town, showed higher endemicity as they were nearer to filarial foci other than Bhagalpur Town, e.g., Akbarpur is situated only 6 miles to the east of Sultanganj, a township with high filarial endemicity (Verma *et al.*, 1960). Thus the spread of bancroftian filariasis from the urban focus of Bhagalpur Town in the peripheral rural areas was readily discernible. That the filarious towns form the chief sources from which peri-urban areas slowly derive their complement of reservoirs of infection (by movement), as also adoption of urban ways of life add to the mosquito-genic conditions, and such rural areas, in turn, become filarious, should stimulate control measures primarily in the affected towns where bancroftian filariasis is prevalent.

The infection rate increased progressively up to the age of 20 years, and later on it was constant with minor fluctuations. Krishnaswami (1955) had similar observations in Mangalore.

The filarial disease rate was observed to rise progressively up to the age of 40 years and thereafter it was on a decline (Graph 1). Farooque and Qutubuddin (1946), Krishnaswami (1955) and Kant *et al.* (1956) reported a progressive rise of this disease up to the age of 50 years.

The disease rate (20·6 per cent) and the infection rate (14·3 per cent) in males were slightly higher than in females* (20·8 per cent and 11·4 per cent, respectively). MacKenzie (1925), Kant *et al.* (1956) and Baisas (1958) reported similar observations. Farooque and Qutubuddin (1946) reported that microfilarial incidence and disease rate showed no predilection for either sex.

SUMMARY.

This paper brings to light the following significant facts regarding the incidence of filarial infection and disease among the rural population around Bhagalpur Town.

Filarial infection and disease were present in all the villages surveyed.

The cumulative filarial disease rate was 23·8 per cent.

The cumulative filarial infection rate was 12·8 per cent.

The filarial endemicity rate was 28·1 per cent.

Of the total number of *Culex fatigans* dissected, 4·9 per cent showed natural infection with microfilariae.

*The disease manifestation, particularly in the genitals, is not always declared by women.

The lowest age, at which filarial disease and infection were noted, was 3 years, in both sexes.

The natural infection of *Wuchereria bancrofti*, the only species recorded, was observed in *Culex fatigans*.

The microfilaria rates were observed to decline gradually from the urban area towards the peripheral villages as the distances increased from the town, suggesting thereby a spread from the urban focus to the rural areas.

ACKNOWLEDGEMENTS.

The authors feel grateful to Dr. P.A. Singh, Director of Health Services, Bihar, for his kind permission to publish this paper.

The authors wish to express their gratitude to (1) Dr. B.S. Lall, B.Sc. (Cornell), M.Sc., Ph.D. (Maryland), Professor of Entomology and Zoology, Bihar Agricultural College, Sabour, (2) Dr. P.C. Basu, M.B. (Cal.), M.D. (Bonn), Assistant Director, Malaria Institute of India, Delhi, and (3) Dr. S.B. Lal, Deputy Director of Health Services, Bihar, for scrutinising the manuscript and their valuable suggestions for its improvements.

Thanks are also due to all the staff of the Filaria Control Unit, Bhagalpur, who actively co-operated in the survey.

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Indian Journal of Malariology, **15**, *4*, *December 1961*.

FILARIASIS IN THE RURAL POPULATION AROUND BHAGALPUR TOWN.

Part II.

BY

B.K. VARMA,*

N.L. DASS,†

AND

V.P. SINHA.‡

[December 22, 1960.]

INTRODUCTION.

UNDER the auspices of the National Filaria Control Programme (Raghavan 1955), the Filaria Control Unit, Bhagalpur, conducted a random filaria survey of eight villages and the surroundings of Bhagalpur Town, outside the Bhagalpur Municipality, during the months of July and August, 1958.

The area undertaken for this investigation is a rural one, situated at distances of 2 to 5 miles from Bhagalpur Railway Station on its south-eastern and south-western sides. The total population of these villages is 8,809. Socio-economically the area is poor, and agriculture and cottage industries form the main source of livelihood. Of these villages, Sabour only has semi-urban facilities and the Bihar Agriculture College is located here. The sanitation of the area is very poor, and the waste-water drains flow through open drains which end blindly.

METHODS AND MATERIALS.

A parasitological, clinical and entomological survey of eight villages (Table I) was undertaken. Random representative samples of the population, covering all ages and both sexes, were examined in these villages. Three to four drops of blood (about 20 c.mm.) were obtained from the finger-tips of each individual, between 8·30 p.m. and 11 p.m. and thick blood smears were prepared. The clinical records of the persons thus examined were maintained by the survey party. The thick blood smears were dehaemoglobinised the following morning and stained with either of the J.S.B. and Leishman stains. The smears were then examined under microscope for the presence or absence of microfilaria. The species of the microfilaria was identified and its number in each smear was finally counted and recorded.

* Chief Malaria Officer, Bihar, Patna.
† Filaria Research Officer, Filaria Control Unit, Bhagalpur (Bihar).
‡ Assistant Entomologist, Filaria Control Unit, Bhagalpur (Bihar).

In each of the eight villages surveyed, mosquitoes were collected from random catching stations—from human dwellings, cattle-sheds, and mixed dwelling from 7 a.m. to 10·30 a.m. The mosquitoes thus collected were identified, and the females dissected and examined to determine the vector species and the extent of natural infection in them.

RESULTS.

In the course of present survey, 1,342 persons, forming about 15·2 per cent of the population of the eight villages, were examined (Table I). None of the villages was free from disease and filarial infection among the inhabitants. The disease was manifested in the form of hydrocele, swelling of limbs (upper or lower), and genital parts and chyluria.

The data presented in Table II and Graph 1, revealed a progressive rise of the infection and disease rates up to the age-groups "31-40 years" and "51 years and above", respectively. The infection and disease rates were comparatively higher in males than in females. The youngest person to show the infection and disease was of 8 years and 7 years, respectively, both being girls. The average microfilarial infestation per 20 c.mm. of positive blood, in the community examined, was 19. The microfilarial number per positive smear ranged between 1 and 46.

ENTOMOLOGICAL SURVEY.

An investigation into the mosquito fauna, in the villages under observation, resulted in the identification of the following species :—

(*i*) *Culex fatigans* (Wied.).

(*ii*) *Anopheles annularis* (Van der Wulp.)

(*iii*) *A. Stephensi* (Liston).

(*iv*) *A. Subpictus* (Grassi).

(*v*) *A. Vagus* (Donitz).

The females of all the mosquitoes were dissected and examined for the presence of microfilaria in their bodies. *Culex fatigans* only exhibited the natural infection with *Wuchereria bancrofti* in all its developmental stages (*vide* details given below). No others species of mosquitoes showed any stages of the microfilaria.

INFECTION IN *C. FATIGANS* MOSQUITOES.

Number of *Culex fatigans* dissected=185.

Number of *Culex fatigans* positive for all forms of microfilaria=9.

Infection rate for all forms of microfilaria=4·8 per cent.

TABLE I.

Incidence of filariasis in different villages around Bhagalpur Town.

Name of village.	Approximate distance from Bhagalpur Town.	Persons examined.			With disease.				With microfilaria.				Both sexes.			
													With disease.		With microfilariae.	
		Male.	Female.	Total examined.	Male.	Percentage.	Female.	Percentage.	Male.	Percentage.	Female.	Percentage.	Number.	Percentage.	Number.	Percentage.
1. Sabour	5 miles	212	173	385	78	36·7	38	21·9	7	3·3	10	5·7	116	30·1	17	4·4
2. Fatehpur	4·5 miles	89	94	183	26	29·2	22	23·3	3	3·3	6	6·3	48	26·2	9	4·9
3. Gourachaki	4 miles	120	66	186	37	30·8	10	15·1	4	3·3	1	1·5	47	25·8	5	2·6
4. Mansarpur	4·5 miles	128	74	202	36	28·1	12	16·2	6	4·6	1	1·3	48	23·7	7	3·4
5. Babupur, Kishanpur	7 miles	70	32	102	19	27·1	9	28·1	5	7·1	2	6·2	28	27·4	7	6·8
6. Mancharpur Bhawanipur	2 miles	63	18	81	11	17·4	3	16·6	4	6·3	3	16·6	14	17·2	7	8·6
7. Raghopur	2 miles	71	13	84	18	25·3	6	46·1	6	8·4	3	23·01	24	28·8	9	10·7
8. Mahamadpur	4 miles	67	52	119	8	11·9	5	9·6	3	4·4	2	3·8	13	10·2	5	4·2
Total		820	522	1,342	233	28·4	105	20·01	38	4·6	28	5·3	338	25·2	66	4·1

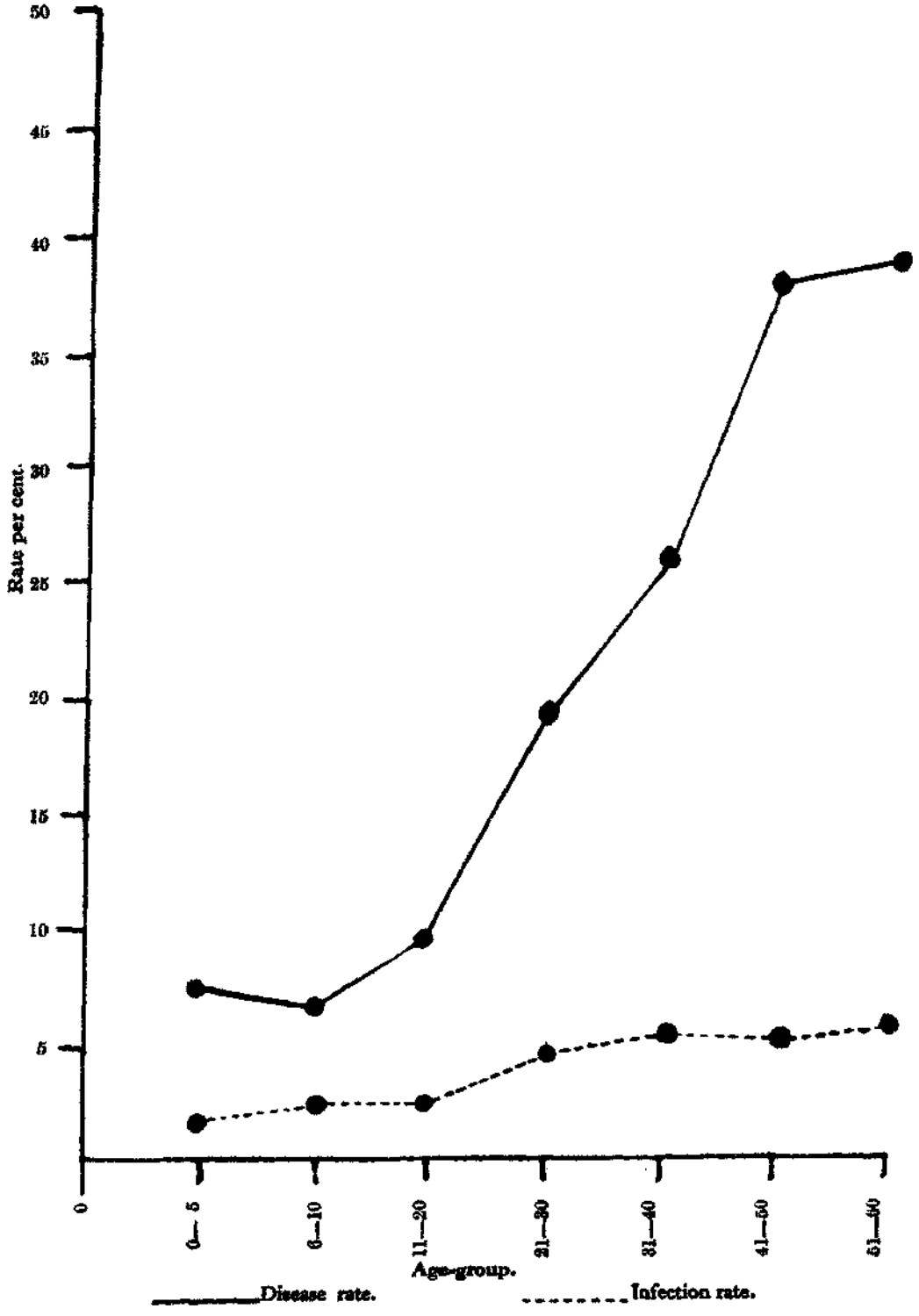
TABLE II.

Incidence of filariasis in different age-groups and two sexes.

Age-group (Years).	MALES					FEMALES					BOTH SEXES				
	Number examined.	With disease.		With microfilaria.		Number examined.	With disease.		With microfilaria.		Number examined.	With disease.		With microfilaria.	
		Number.	Percentage.	Number.	Percentage.		Number.	Percentage.	Number.	Percentage.		Number.	Percentage.	Number.	Percentage.
0 — 5	41	4	9·7	1	2·4	26	1	3·8	Nil	Nil	67	5	7·4	1	1·4
6 — 10	74	6	8·1	2	2·7	41	2	4·8	1	2·4	115	8	6·9	3	2·6
11 — 20	109	10	9·1	3	2·7	47	5	10·6	1	2·1	156	15	9·6	4	2·5
21 — 30	138	28	20·2	7	5·07	67	11	16·4	3	4·4	205	39	19·02	10	4·8
31 — 40	141	44	31·2	13	9·2	113	21	18·5	7	6·2	254	65	25·5	20	7·8
41 — 50	160	78	48·7	7	7·3	144	35	24·8	8	5·6	301	113	37·5	15	4·9
51 and above	157	63	40·1	5	3·1	87	30	34·4	8	9·1	244	93	38·1	13	5·3
Total	820	233	28·4	38	4·8	522	105	20·1	28	5·3	1,342	338	25·1	66	4·9

GRAPH 1.

Incidence of filarial disease and infection among both sexes in the rural population around Bhagalpur Town

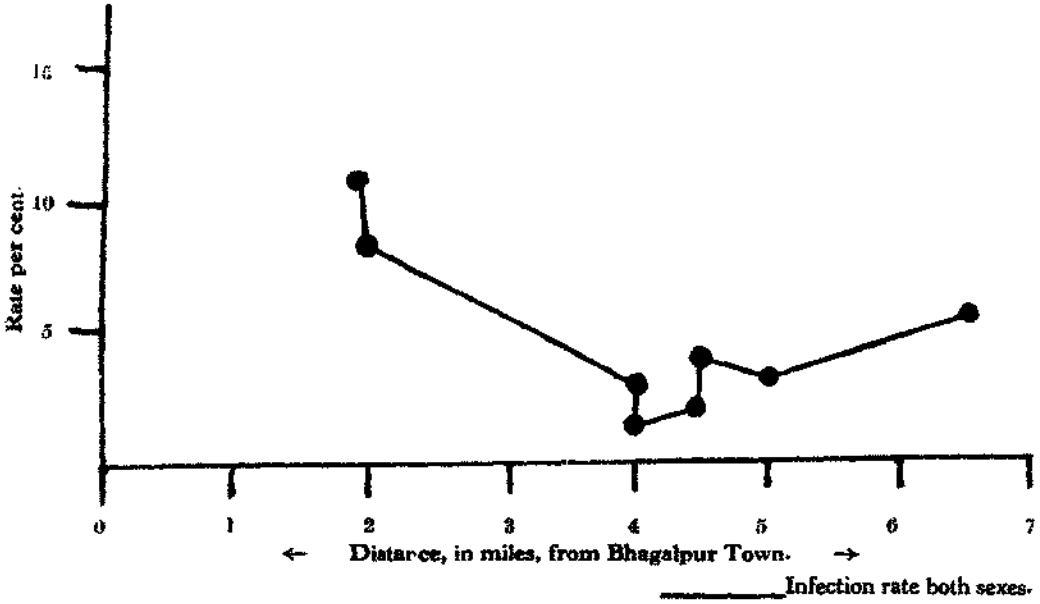


DISCUSSION.

In the present survey, the authors have noted that all the eight villages surveyed had the filarial infection and disease among their residents (Table I). The infection and disease rates in these villages ranged between 2·6 per cent to 10·7 per cent., and 10·9 per cent to 30·1 per cent., respectively (Table I). Further, they observed a higher incidence of infection in the villages comparatively nearer to the town, with slight fluctuations (Table I, Graph 2). Similar observation have been recorded in a previous paper of the present authors (Verma *et al.*, 1961)

GRAPH 2.

Incidence of filariasis in the rural population around Bhagalpur Town and distances from Bhagalpur Town.



The infection rate was observed to rise progressively up to the age 40 years and thereafter it was almost identical (Table II, Graph 1).

Age-wise analysis showed a progressive rise in the disease rate up to the age of "50 years and above". Farooque and Qutubuddin (1946), Krishnaswami (1955), Kant *et al.* (1956) and Varma *et al.* (1961) had similar observations (Table II, Graph 1).

SUMMARY.

Filariasis was found to be prevalent among the residents of all the eight villages surveyed.

The cumulative filarial disease rate was 25·2 per cent.

The cumulative filarial infection rate was 4·1 per cent.

The filarial endemicity rate was 26·4 per cent.

Culex fatigans only was observed as the vector of filariasis in the villages under investigation, and 4·8 per cent of them showed infection with *Wuchereria bancrofti*.

The lowest age, at which filarial infection and disease manifestations were noted, was 8 and 7 years, respectively.

W. bancrofti was the only species of filaria recorded.

ACKNOWLEDGEMENTS.

The authors are grateful to Dr. S.M. Hassan, Director of Health Services, Bihar, Patna, for his kind permission to publish this paper.

The authors are thankful to Dr. B.S. Lal, B.Sc. (Cornell) M.Sc., Ph.D. (Maryland), Professor of Entomology, Bihar Agricultural College, Sabour, for going through the type-scripts and his suggestions.

Thanks are also due to all the staff of the Filaria Control unit, Bhagalpur, for active co-operation in the survey.

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Indian Journal of Malariology, **15**, *4, December 1961.*

PRELIMINARY STUDIES ON THE *IN VITRO* ACTION OF POTASSIUM PERMANGANATE ON THE ADULT WORMS AND MICROFILARIAE OF *LITOMOSOIDES CARINII.*

BY

G.S. AHLUWALIA

AND

DALIP SINGH.

(*Malaria Institute of India, Delhi.*)

[April 15, 1961.]

INTRODUCTION.

CHEMOTHERAPY of filariasis is still in its infancy. Organometallic compounds, cyanine dyes, and even the latest in the series, Diethylcarbamazine, have all got their limitations. An ideal filaricide is yet to be found.

Anaerobic micro-organisms were reported to be adversely affected by a high concentration of oxygen in their environment, and compounds releasing oxygen had bacteriostatic effect on bacteria of this type (Goodman and Gilman, 1941). In the U.S.S.R., intragastric oxygen is a recognised method for the treatment of human ascariasis (Kravetz, 1954; Zaitseva *et al.*, 1954). Action of potassium permanganate on *Ascaris lumbricoides* was described by Vora (1960).

The effect of raised and lowered oxygen and carbon dioxide tension, on the periodic migration of human and animal microfilariae, has been extensively studied: "In patients infected with *W. bancrofti* the microfilarial count was rapidly reduced by breathing of oxygen, by muscular exercise and by hyper-ventilation" (McFadzean and Hawking, 1956). In the case of *L. carinii* in cotton rats, high oxygen pressure had no effect on microfilarial level, whereas with *D. immitis* and *D. repens* in dogs a rise in the microfilarial count was caused by increase or decrease of the oxygen pressure (Hawking, 1956). Edeson *et al.* (1957) have reported a fall in the number of microfilariae of *B. malayi* in man with the increase of oxygen tension whereas no marked change appeared with increased carbon dioxide tension. Preliminary results of *in vitro* studies on the action of potassium permanganate on adult filarial worms and microfilariae of *L. carinii* are reported here.

MATERIALS AND METHOD.

The experiments were carried out under aseptic conditions. Living adult worms were removed, after autopsy, from the pleural cavities of cotton rats infected with *L. carinii* in the laboratory. The worms were collected in sterilized physiological saline at 27°C., and relative humidity of 69 per cent. For testing the effect on microfilariae, infected blood drawn from the tail of a positive cotton rat was used.

Solutions of potassium permanganate in sterilized normal saline were prepared fresh every time and used in the dilutions of 1 in 1,000, 1 in 2,000, 1 in 4,000, 1 in 10,000, 1 in 20,000, 1 in 40,000, 1 in 100,000, 1 in 200,000 and 1 in 10,00,000. Normal saline (20 c.c.) was used for the controls.

(*a*) EXPERIMENTS WITH ADULT *L. CARINII.*

For each exposure, 20 c.c. of each of the solutions was used in petri dishes. The worms were transferred to these petri dishes by dissecting needles.

In one of the experiments (Table I), twelve adult worms, both males and females, were exposed to each of the following strengths of potassium permanganate : 1 in 1,000, 1 in 2,000 and 1 in 4,000, and also in the control. In the rest of the dilutions, the number of worms varied from twelve to twenty. In the second experiment (Table II), the number of worms exposed was seven in each strength of the solution. The adult worms that appeared dead, due to loss of all motility after exposure, were washed in normal saline repeatedly and were observed in normal saline separately for a period of three hours to see whether the worms regained motility.

TABLE I.

Results of exposure of living adult filarial worms (Litomosoides carinii) *to different dilutions of potassium permanganate.*

Serial number.	Concentration of potassium permanganate in normal saline.	Number of adult worms exposed.	Lethal period of exposure, in minutes.
1	1 : 1,000 ...	12	1
2	1 : 2,000 ...	12	5
3	1 : 4,000 ...	12	5
4	1 : 10,000 ...	12 to 20	10
5	1 : 20,000 ...	12 to 20	32
6	1 : 40,000 ...	12 to 20	35
7	1 : 1,00,000 ...	12 to 20	111
8	1 : 2,00,000 ...	12 to 20	All alive, and left overnight and found dead next morning.
9	1 : 10,00,000 ...	12 to 20	
10	Control in normal saline	12	

TABLE II.

Results of exposure of living adult filarial worms (Litomosoides carinii) *to different dilutions of potassium permanganate.*

Serial number.	Concentration of potassium permanganate in normal saline.	Number of adult worms exposed.	Lethal period of exposure, in minutes.
1	1 : 1,000 ...	7	1
2	1 : 2,000 ...	7	2
3	1 : 4,000 ...	7	3
4	1 : 10,000 ...	7	6
5	1 : 20,000 ...	7	17
6	1 : 40,000 ...	7	22
7	1 : 1,00,000 ...	7	112
8	Control in normal saline ...	7	581

(*b*) EXPERIMENTS WITH MICROFILARIAE OF *L. CARINII*.

The experiment was carried out at 37°C. Blood was taken in citrated saline (2 per cent) from the tail of an infected cotton rat. All solutions were incubated at 37°C. for half an hour before use. The control was kept in one c.c. Tyrode solution to which one c.c. normal saline was added whereas all the other solutions contained one c.c. of potassium permanganate solution in normal saline and one c.c. Tyrode solution so that the total volume in each case remained two c.c. (Table III).

TABLE III.

Results of exposure of microfilariae of L. carinii *to different dilutions of potassium permanganate in Tyrode solution.*

Serial number.	Concentration of potassium permanganate in Tyrode solution.	Lethal period of exposure to potassium permanganate solutions.
1	1 : 1,000 ...	5 minutes.
2	1 : 2,000 ...	10 minutes.
3	1 : 4,000 ...	30 minutes.
4	1 : 10,000 ...	5 hours and 45 minutes.
5	1 : 20,000 ...	10 hours and 8 minutes.
6	1 : 40,000 ...	27 hours and 35 minutes.
7	1 : 1,00,000 ...	27 hours and 15 minutes.
8	1 : 2,00,000 ...	26 hours and 5 minutes.
9	The control was very active even after 28 hours.	

Equal quantity of infected citrated blood, having sufficient number of microfilariae, was added to the control as well as to the solutions of potassium permanganate. One drop, each from the control as well as the different dilutions of potassium permanganate, was examined separately under the microscope at different intervals of time. The time at which all microfilariae died, indicated by loss of all motility in each batch, was recorded (Table III).

The time at which death of all the adult worms or the microfilariae, as the case may be, occurred, was taken as the lethal period of exposure for the particular batch of worms or the microfilariae.

RESULTS.

In the dilutions from 1 : 1,000 to 1 : 4,000, the adult worms became totally inert and appeared dead within five minutes of exposure (Tables I, II).

Worms exposed to the concentration of 1 : 200,000 and 1 : 1,000,000 were observed for a period of 105 and 101 minutes respectively (Table I) and then they were left overnight along with the control. On the next morning, all worms were found dead, including the control. From Tables I and II it is evident that the lethal period of exposure varied with changing the concentration of potassium permanganate and it increased with the increasing dilution.

In the second experiment, seven adult worms were exposed to the dilutions of 1 : 200,000 and 1 : 1,000,000 each. They appeared dead after 581 minutes of exposure, indicating thereby that potassium permanganate was ineffective in these

dilutions during an observation period of 581 minutes because the control also died in the same period.

The depth of colour, taken up by the worms, was proportional to the concentration of potassium permanganate used. None of the worms regained motility when kept in normal saline after exposure to potassium permanganate, showing that lethal effect of potassium permanganate was irreversible.

In the case of the microfilariae also (Table III), it was observed that the lethal period of exposure varied with the concentration of potassium permanganate used. In the dilution 1 : 200,000 the microfilariae were found dead one hour earlier than in the higher concentrations of 1 : 40,000 and 1 : 100,000. In the 1 : 1,000 and 1 : 2,000 dilutions, the action of potassium permanganate was most pronounced as was also observed in the case of adult worms.

DISCUSSION.

The results recorded (Tables I, II and III) indicate that potassium permanganate was lethal to adult filarial worms and microfilariae of *L. carinii* of the cotton rat. The lethal period of exposure was proportional to the concentration of potassium permanganate used. Although potassium permanganate had *in vitro* lethal action on the adult worms and microfilariae of *L. carinii*, further studies will have to be carried out to elucidate whether the lethal effect of potassium permanganate was due to the oxygen liberated by it or the toxicity of high concentration of potassium and manganese ions furnished by potassium permanganate in solution, or both. Potassium ions were found to effect the motility of adult worms of *L. carinii* (Bueding, 1949) The effect of manganese could not possibly be ignored in these studies because it has a lot to do in general with the activity of various enzymes such as phosphatases, cozymase, carboxylase and cholinesterase. Effect of manganese on the activity of arginase is of interest (Dei, 1941*a* : 1941*b*). Although Bueding (1949) reported that low concentration of manganese had no effect on the metabolic activity of *L. carinii*, production of AMC (acetylmethylcarbinol) by homogenates of the filarial nematodes of *L. carinii* was stimulated by manganese (Berl and Bueding, 1951).

Investigations may also be extended to the use and trial *in vivo* of other oxidising agents and non-oxidising salts of potassium and manganese to find out whether the adult filarial nematodes and microfilariae are vulnerable to such salts. In case the salts are found to liberate oxygen, potassium and manganese ions respectively at the site of the worms, their effect on the host cells would have to be studied. It would be worthwhile to study the effect of injection of potassium permanganate into the site of the normal habitat of the adult worms, such as the pleural cavity in the case of cotton rat. Further studies *in vivo* and also *in vitro* on the trials of other oxidising agents, non-oxidising compounds of potassium, manganese and allied organic compounds, are in progress.

SUMMARY.

Action of potassium permanganate solution *in vitro* on adult filarial worms and microfilariae of *L. carinii* of the cotton rat was studied.

The lethal effect of potassium permanganate was quite pronounced. The lethal period of exposure was proportional to the strength of potassium permanganate solution. The *in vitro* action of this drug was more pronounced on the adult worms than on their embroys.

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Indian Journal of Malariology, **15**, *4, December 1961.*

STUDIES ON THE *IN VITRO* ACTION OF POTASSIUM PERMANGANATE ON THE ADULT WORMS OF *DIROFILARIA IMMITIS*, MICROFILARIAE OF *DIROFILARIA REPENS* AND *DIROFILARIA IMMITIS* AND OF HYDROGEN PEROXIDE ON THE ADULT WORMS OF *LITOMOSOIDES CARINII.*

BY

DALIP SINGH

AND

G.S. AHLUWALIA.

(Malaria Institute of India, Delhi.)

[June 6, 1961.

INTRODUCTION.

In an earlier communication (Ahluwalia and Dalip Singh, 1961), the action of potassium permanganate *in vitro* on *L. carinii*, the filarial parasite of the cotton rat, was described. The lethal effect, produced on the parasites by potassium permanganate, seemed primarily attributable to the oxygen liberated by potassium permanganate in solution. The studies were extended to observe the action of potassium permanganate on the heart worms, *D. immitis*, of dog and also on the microfilariae of *D. repens* and *D. immitis*.

In order to determine whether the lethal action of potassium permanganate on *L. carinii* was due to the oxygen liberated by it in solution, *in vitro* studies were extended to the adult worms of *L. carinii* using hydrogen peroxide as the oxidising agent.

MATERIALS AND METHODS.

(a) *IN VITRO* EXPERIMENT WITH *D. IMMITIS*, THE ADULT FILARIAL HEART WORM OF DOG.

Living adult worms (*D. immitis*) were removed from the heart and superior vena cava of an infected dog at autopsy. The worms were collected in sterile physiological saline at room temperature.

Solutions of potassium permanganate were prepared fresh in sterile normal saline and used in the dilutions of 1 : 1,000 ; 1 : 2,000 and 1 : 4,000. Three worms were transferred by dissecting needles to petri dishes, each containing 20 c.c. of each of the dilutions of the solution. The control was set up with 20 c.c. of normal saline (Table I).

TABLE I.

Results of exposure of living adult filarial worms (D. immitis) *to different dilutions of potassium permanganate.*

Serial number.	Concentration of potassium permanganate in normal saline.	Number of adult worms exposed.	Lethal period of exposure.
1	1 : 1,000	3	7 minutes
2	1 : 2,000	3	15 minutes
3	1 : 4,000	3	35 minutes
4	Control in normal saline.	3	9 hours and 50 minutes.

The adult worms, which appeared dead after exposure to various solutions of potassium permanganate, were washed in normal saline repeatedly and in each case were kept separately in sterile physiological saline for observation over a period of twenty-four hours to see whether the worms regained motility.

(*b*) *IN VITRO* EXPERIMENT WITH MICROFILARIAE OF *D. REPENS* AND *D. IMMITIS.*

The experiment was carried out at 37°C. under aseptic conditions. Solutions of potassium permanganate were prepared fresh in sterile normal saline and used in the dilutions of 1 : 1,000 ; 1 : 2,000 ; 1 : 4,000 ; 1 : 10,000 ; 1 : 20,000 ; 1 : 40,000 ; 1 : 1,00,000 ; 1 : 2,00,000 and 1 : 10,00,000.

Blood was taken separately in 2 per cent citrate saline from the ear lobe of a dog infected only with *D. repens*, and a second having mixed infection of *D. repens* and *D. immitis*. Infected blood, as well as the other solutions, was incubated at 37°C. for forty-five minutes before use. The solution for the control experiment was one c.c. of Tyrode solution to which one c.c. normal saline was added whereas all the other solutions contained one c.c. potassium permanganate solution in normal saline and one c.c. Tyrode solution so that total volume in each case remained two c.c. Equal quantity of infected citrated blood, having sufficient number of microfilariae, was added to the control as well as to the different strengths of potassium permanganate solution. One drop from the control, and also from the rest of the dilutions of potassium permanganate, was examined separately under the microscope at different intervals of time.

(*c*) *IN VITRO* EXPERIMENT WITH HYDROGEN-PEROXIDE ON ADULT WORMS OF *L. CARINII.*

Living adult worms of *L. carinii* were taken out of the pleural cavities of albino rats with mite-induced infection (Ramakrishnan, Dalip Singh *et al.*, 1960). After washing in physiological saline, the worms were transferred to different dilutions of hydrogen-peroxide in normal saline. For each exposure, 40 c.c. of each of the solutions was used (Table II). The controls were observed in normal saline. The worms that appeared dead, were taken out, washed and kept in normal saline in order to see if they regained motility.

TABLE II.

Results of action in vitro of different dilutions of hydrogen-peroxide on living adult worms of L. carinii.

Serial number	Concentration of hydrogen peroxide in normal saline.	Number of adult worms exposed.	Lethal period of exposure, in minutes.
1	1·0 per cent	5	3
2	0·5 per cent	5	5
3	0·25 per cent	5	6
4	0·125 per cent	8	13
5	0·062 per cent	15	18

The time at which death of all the adult worms or the microfilariae, as the case may be, occurred, was taken as the lethal period of exposure for that particular batch of worms or the microfilariae.

RESULTS.

In the dilutions of 1 : 1,000 ; 1 : 2,000 and 1 : 4,000 of potassium permanganate, the adult worms died after 7, 15, and 35 minutes of exposure, respectively, and the control batch lived up to a period of 9 hours and 50 minutes (Table I).

From Table I, it is evident that the lethal period of exposure varied with the concentration of potassium permanganate, *i.e.*, it increased with the increasing dilution.

The worms became deeply coloured and appeared to be charred after exposure to potassium permanganate. None of the worms regained motility when kept in normal saline, and their death was confirmed.

In the case of the microfilariae (mixed infection of *D. repen* and *D. immitis*), potassium permanganate had no effect in any of the dilutions up to an observation period of three hours after exposure. In the case of the microfilariae of *D. repens* (single infection) also, the microfilariae remained active till 23 hours of exposure. Those in the control set up were found to be active even up to a period of twenty-seven hours ; observations were not continued after this time. It would appear that potassium permanganate is ineffective on the microfilariae of *D. repens* and *D. immitis*. Had potassium permanganate been effective, if not in the lower dilutions, at least in the higher concentrations such as 1 : 1,000 ; 1 : 2,000 and 1 : 4,000 the lethal time period would have been within reasonable limits, *i.e.*, 30 to 40 minutes or so as observed in the case of microfilariae of *L. carinii* (Ahluwalia and Dalip Singh, 1961 *loc. cit.*).

From Table II, it is clear that the lethal period of exposure increased with the increase in dilution of hydrogen-peroxide. None of the worms regained motility after washing and remaining in normal saline

Worms in the control observations (in normal saline), however, were active during an observation period of six hours.

DISCUSSION.

The results recorded (Table I) indicate that *in vitro*, potassium permanganate was lethal to living adult worms of *D. immitis* of the dog. The lethal period of exposure varied with different concentrations of potassium permanganate, *i.e.*, it increased with the increasing dilutions. It was found to be ineffective on the microfilariae of *D. immitis* as well as on the microfilariae of a mixed infection of *D. repens* and *D. immitis*.

The inefficacy of potassium permanganate *in vitro* towards the microfilariae of *D. immitis* and *D. repens*, and its effectiveness towards the microfilariae of *L. carinii*, may be due to the difference in the physiology of the microfilariae. This will have to be elaborated by further work.

However, the present work was not undertaken to establish potassium permanganate or hydrogen-peroxide as an antifilarial drug by itself, but it was carried out to provide a line of search for oxygen and oxidising agents in their different forms to be tried as potential antifilarials.

It is evident from the present studies that potassium permanganate is lethal to the adult worms, *D. immitis*, of dog and adult worms of *L. carinii* (Ahluwalia and Dalip Singh, 1961 *loc. cit.*). It was in view of this that the *in vitro* action of hydrogen peroxide, on the living adult worms of *L. carinii*, was also tried. It is evident from the results in Table II that hydrogen-peroxide, like potassium permanganate, also killed the adult worms. This showed that oxygen, liberated by either of these two compounds, when in contact *in vitro* with adult filarial worms, has definite lethal action on the latter.

It would also appear that the concentration of oxygen in the heart blood of dogs is too low to damage the adult worms (*D. immitis*) and, therefore, the extra oxygen supplied by potassium permanganate had lethal effect on the worms. It is also possible that one of the causes of the mortality of the adult worms of dog may be the interference by oxygen with the carbohydrate metabolism of the parasite. To our knowledge, carbohydrate metabolism of *D. immitis* has not been studied so far whereas it has been in the case of *L. carinii* (Bueding, 1949). Further studies *in vivo* as well as *in vitro* on the action of other oxidising agents, allied organic compounds and non-oxidising compounds of manganese and potassium, on worms and host cells, are in progress.

SUMMARY.

In vitro action of potassium permanganate solution on adult filarial worms of *D. immitis*, and on microfilariae of *D. immitis* and *D. repens* of dog, was studied.

In the case of the adult worms of *D. immitis*, the effect was quite pronounced. The lethal period of exposure was proportional to the strength of potassium

permanganate solution used. Potassium permanganate was inactive against the microfilariae of *D. immitis* and *D. repens* of dog.

Hydrogen-peroxide was found to have pronounced *in vitro* lethal action on the adult filarial worm, *Litomosoides carinii*, of the albino rat.

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Indian Journal of Malariology, **15**, 4, December 1961.

CULICINE FAUNA OF GORAKHPUR DISTRICT (UTTAR PRADESH).

BY

DIWAN CHAND*

M.V. SINGH†

AND

B.B. N. SRIVASTAVA‡

(July 31, 1961.)

INTRODUCTION.

THE District of Gorakhpur occupies the north-east corner of Uttar Pradesh. The northern border of the district is a moist and unhealthy tract, known as Tarai, on the south of which is a stretch of forest land. The open plain country forms an apparently level tract which, in reality, slopes gently from north-west to south-east. The Rapti, Rohin and Ami rivers run across the district while the Kuwan River cuts the south-western portion. There are large perennial lakes like Ramgarh Tal, Chillua Tal, etc., in the district. The subsoil level is high at places, being 8 to 10 feet.

Great extremes of heat and cold are rarely experienced in the Gorakhpur District. The maximum and minimum temperature is 88°F. and 66°F., respectively. The rainy season extends from the end of June to September. The average annual rainfall is 57·8 inches, and the relative humidity ranges from 55 to 96 per cent.

Presence of forests in a large measure is responsible for the abundance of rainfalls which, in combination with the effect of rivers and a remarkably high water level render the soil damp and the climate moist.

The population of the district is 2,378,077 (1951 census) while the area of the district is 2,437 sq. miles.

In the rural area, there is no drainage system, due to which there exist various types of water collections such as ponds, cesspools, kutchha drains, etc. In the urban area, the drainage system is imperfect, leading to the collection of sullage and the ablution water stagnating in cesspools and pits, forming ideal breeding place for culicine mosquitoes. Besides there are a number of big *nalahs*, and ponds with water hyacinth, which also provide breeding grounds for culicines mosquitoes.

* Deputy Director, Malariology, Uttar Pradesh.
† Assistant Director, Malariology, Uttar Pradesh.
‡ Assistant Entomologist, Malariology, Uttar Pradesh.

METHODOLOGY.

Routine and random collections of adult mosquitoes were carried out during the period January, 1958, to June, 1960, in the entire district (Map 1) to determine the prevalence of various species of culicine mosquitoes. Collections were made with the help of suction tube, aided by torch, between 7 a.m. and 9 a.m.

For routine collections, 60 catching stations, comprising 20 each of human dwellings, mixed dwellings and cattle sheds, were established. Random collections were made from different types of dwellings, outdoor places, forests, mango groves, etc. During indoor collections, special attention was paid to the dark corners of rooms, inside of dung-cakes, fire-wood, hanging objects, earthen pots, etc. During outdoor collections, attention was paid underneath leaves of grass, water hyacinth leaves, and particularly among bushes.

To find out the breeding places of different culicine mosquitoes, larval collections were made from ponds, cesspools, septic tanks, stagnant water around wells, unused wells, irrigation channels, river-beds and crevices of the side-walls of running drains, etc., with the help of larval net of ten inches diameter, laddles of three inches diameter and wellnets. Larval collections were also made from various ponds and water collections containing *Pistia stratiotes* and water hyacinth. Hatching of larvae was carried out in the laboratory to confirm the breeding places of different species.

OBSERVATIONS.

During the present study 1,10,791 mosquitoes were collected, out of which 77,607 were culicines and 33,184 anophelines. Out of 77,607 culicine mosquitoes collected, 48,114 were female and 29,493 male. Identification of the species has been confirmed by the Malaria Institute of India, Delhi. Table I gives the monthly collection of different culicine mosquitoes.

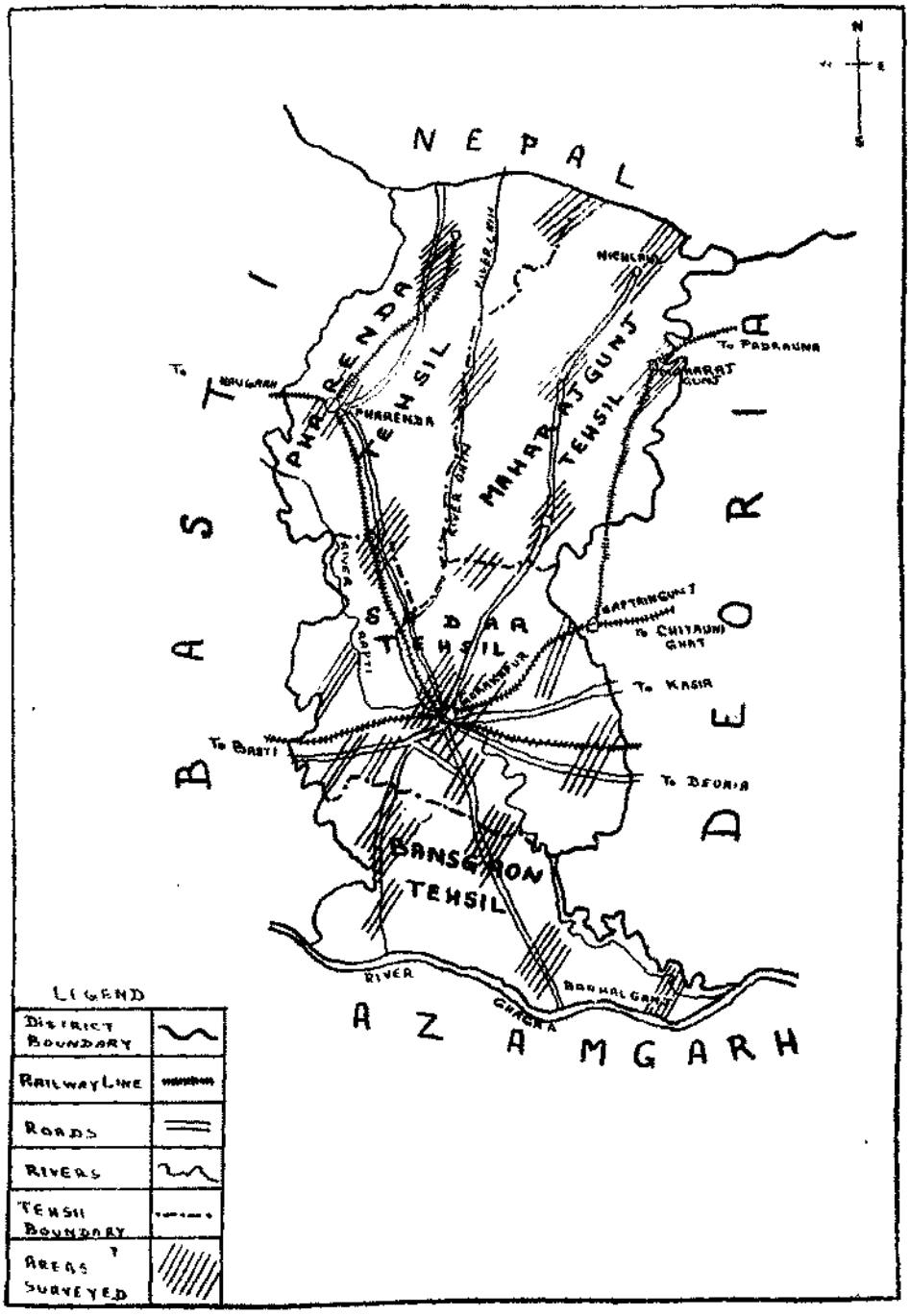
Table I shows the prevalence of *Culex fatigans, Culex vishnui, Culex bitaeniorhynchus, Culex gelidus, Culex (Lutzia) fuscanus, Mansonioides annulifera, Mansonioides uniformis, Mansonioides indiana, Mansonia crassipes, Aedes albopictus, Aedes aegypti, Aedes (stegomyia)-w-albus, Aedes (christophersomyia) thomsoni, Aedes pallidostriatus, Aedes lineatopennis and Armigeres obturbans* in the Gorakhpur District. It is also evident from Table I that the peak period of the *Culex* species is in the months of March-April and September-October, while those of the *Mansonioides* species and *Aedes* species in the months of November-December and July to September, respectively.

Various species of culicine mosquitoes collected were analysed for their extent of prevalence, which is set out in Table II.

It is seen from Table II that the most prevalent species in whole of the district is *Culex fatigans*, the percentage of which is 90·44, and next comes *Culex vishnui*.

MAP 1.

Map of Gorakhpur District, Uttar Pradesh, showing areas where mosquito collections were made.



TABLE

Monthly collection of culicine mosquitoes for

Name of species	January.		February.		March.		April.	
	M	F	M	F	M	F	M	F
1958								
Culex fatigans ...	1,204	1,790	599	1,382	1,323	1,845	2,403	2,050
Culex vishnui ..	45	80	70	50	100	120	125	175
Armigeres obturbans ...	..	2	..	3	2	6	3	7
Mansonicides annulifera ...	..	9	..	..	7	12	3	5
1959								
Culex fatigans ...	848	2,029	599	1,083	1,415	2,142	1,687	3,315
Culex vishnui ...	44	76	35	70	98	162	123	187
Culex (Lutzia) fuscanus ...	..	1	..	..	3	5	4	7
Culex bitaeniorhynchus ...	10	12	8	12	15	29	21	29
Culex gelidus ..	7	13	8	2	19	31	22	37
Aedes aegypti	4	11	..	..	..	..	..	..
Aedes albopictus	7	31	12	28	..	..	..	..
Aedes pallidostriatus .	2	8	..	3	..	..	..	.
Aedes (christophersomyia) thomsoni ..	..	4	..	3	..	..	..	..
Aedes-w-albus ...	..	..	..	..	..	..	..	..
Aedes lineatopennis ...	..	6	..	4	..	..	..	..
Mansonioides annulifera ...	7	8	..	..	..	..	8	12
Mansonioides uniformis ...	5	15	..	..	..	..	..	5
Mansonioides indiana ...	1	1	..	..	..	..	1	2
Mansonia crassipes ...	..	4	8	12	6	14	15	19
Armigeres obturbans ...	..	5	..	2	3	7	4	9
1960								
Culex fatigans ...	42	265	124	441	125	417	264	1,080
Cuelx vishnui ...	13	27	14	31	32	78	48	97
Culex (Lutzia) fuscanus ...	..	..	..	1	..	6	2	6
Mansonioides annulifera ...	4	16	..	4	1	9	..	9
Culex bitaeniorhynchus ...	..	4	3	4	7	18	14	16
Mansonioides uniformis ...	2	8	..	..	..	..	..	..
Mansonia crassipes ...	..	7	2	3	7	5	8	12
Aedes albopictus ...	..	5	2	4	..		..	..
Aedes-w-albus ...	..	2	..	..	..	..	..	..
Aedes pallidostriatus ...	..	..	..	..	..	..	..	..
Armigeres obturbans ...	..	..	..	..	2	6	2	8
Culex gelidus ...	..	5	..	2	8	12	4	24
Monthly total of all species during the observation period of January to June, 1961.	61	339	145	490	182	551	342	1,252

M=Male.
F=Female.

I.

the period January, 1958, to June, 1960.

Months															
May.		June.		July.		August.		September.		October.		November.		December.	
M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
1,051	1,632	874	1,738	943	1,685	684	1,462	1,212	2,007	3,234	1,306	600	2,112	462	1,811
35	80	24	76	25	69	35	48	119	196	184	246	70	55	46	84
..	2	..	..	..	..	..	..	2	7	2	9	..	3	..	2
..	..	..	..	..	..	..	..	..	..	..	2	15	27	21	29
767	1,705	655	1,247	350	788	322	753	607	1,720	1,215	1,099	480	951	339	1,780
34	66	25	58	15	47	15	35	114	96	126	174	60	90	24	66
..	..	..	..	..	..	..	..	1	2	..	2	1	..	..	..
7	11	8	9	2	4	..	4	3	22	8	27	12	8	7	8
..	..	..	..	..	..	..	..	17	13	12	16	..	9	2	10
..	..	..	4	5	15	15	35	7	11	..	4	..	..	..	..
..	..	16	65	39	81	115	135	21	59	15	45	..	5	..	..
..	..	..	..	14	26	7	18	4	11	..	7	..	..	..	..
..	..	..	..	18	24	12	16	7	8	..	5	..	..	..	..
	..	..	..	11	28	7	19	9	8	2	6	..	..	..	..
..	..	..	..	7	23	8	12	2	7	..	6	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	2	11	33	20	46
..	..	..	..	..	..	..	..	..	..	..	5	24	66	61	89
..	..	..	..	..	..	..	..	..	..	..	2	1	3	..	2
..	3	..	..	..	..	..	..	4	11	8	17	..	2	..	1
..	5	..	4	..	2	..	..	2	7	3	11	..	2	..	1
186	579	123	575	..	..	..	..	..	..	..	..	..	..	..	..
51	79	64	86	..	..	..	..	..	..	..	..	..	..	..	..
..	2	..	1	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	4	..	8	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	2	..	5	..	..	..	..	..	..	..	..	..	..	..	..
..	..	11	19	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	7	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	3	..	2	..	..	..	..	..	..	..	..	..	..	..	..
237	669	198	704	..	..	..	..	..	..	..	..	..	..	..	..

TABLE II.

Percentage of various species of culicines collected.

Name of the species.	Total number collected.	Percentage in relation to the total collection.
Culex fatigans ...	70,188	90·44
Culex vishnui ...	4,617	5·90
Culex bitaeniorhynchus ...	354	0·44
Culex gelidus ...	278	0·35
Culex (Lutzia) fuscanus ...	43	0·05
Mansonioides annulifera ...	326	0·42
Mansonioides uniformis ..	280	0·03
Mansonioides indiana ...	13	0·02
Mansonia crassipes ...	175	0·21
Aedes albopictus ...	714	0·92
Aedes aegypti ..	111	0·14
Aedes (christophersomyia) thomsoni ..	97	0·12
Aedes pallidostriatus ...	101	0·13
Aedes (stegomyia)-w-albus ...	99	0·12
Aedes lincatopennis ...	75	0·09
Armigeres obturbans ...	136	0·17
Total ...	77,607	..

RESTING HABITS.

During indoor collections of adult mosquitoes, the culicine species found were *Culex fatigans*, *Culex vishnui*, *Culex bitaeniorhynchus* and *Mansonioides annulifera*. During outdoor collections, the species found were *Culex gelidus*, *Culex vishnui*, *Culex bitaeniorhynchus*, *Mansonioides annulifera*, *Mansonioides uniformis*, *Mansonioides indiana*, *Mansonia crassipes*, *Aedes albopictus*, *Aedes aegypti*, *Aedes pallidostriatus*, *Aedes thomsoni*, *Aedes lincatopennis* and *Aedes-w-albus*.

The *Culex fatigans* has been found to be entirely indoor rester while *Mansonioides uniformis* and *Aedes* species as entirely outdoor resters.

FEEDING HABITS.

Precipitin tests of 460 blood meals from different species of Culicine mosquitoes were carried out at the Malaria Institute of India, Delhi. Table III depicts the result of precipitin test.

TABLE III.

Result of precipitin test.

Name of species.	Number of blood meals tested.	NUMBER POSITIVE FOR :				
		Human.	Bovine.	Dog.	Pig.	Goat.
Culex fatigans ...	439	258	85	2	..	3
Culex vishnui ...	8	1	4	..	..	..
Mansonioides annulifera ...	13	6	4	..	..	..

Out of the 439 blood meals of *Culex fatigans* tested, 258 gave a positive reaction for human blood, the anthropophilic index being 58·3 ; thereby pointing to high potentiality of active transmission of filariasis.

BREEDING HABITS.

Hatching of 3,508 culicine larvae, collected from various breeding places, was carried out, out of which 500 *Culex fatigans*. 10 *Culex vishnui*, 65 *Culex bitaeniorhynchus*, 5 *Aedes aegypti*, 2 *Aedes pallidostriatus*, 60 *Aedes albopictus*, 50 *Mansonioides annulifera*, 42 *Mansonioides uniformis* and 15 *Armigeres obturbans* hatched out.

In rural areas, *Culex fatigans* has been found breeding in almost every type of stagnant water collection contaminated with organic material, the most favourable sites being household drains, cesspools and earthen pots. Intensive breeding of *Culex fatigans* has been observed in effluent water of sugar mills, discharged in open fields. When denied dirty water, it has been observed that *Culex fatigans* breeds in clear water like pools, river-beds, etc. Breeding of *Culex fatigans* has been rarely found in tree holes, junglepools or river-bed pools far from the human habitations (Barraud, 1934).

In urban areas, most of the houses have cesspools and soakage pits which get clogged and do not function for more than six months or a year, depending on the quality and quantity of the sullage which they receive. Most of these are exposed, or loosely covered with slab or stones. Intensive breeding of *Culex fatigans* and *Armigeres obturbans* was observed in earthen-pots used for leather tanning, which had been left unused and from which water had not been changed periodically.

Mansonioides annulifera and *Mansonioides uniformis* have been found breeding in ponds containing water hyacinth and *Pistia stratiotes*. Presence of these plants and organic contamination of the water has been found essential for the prolific breeding of *Mansonioides* spp. (Iyengar, 1938).

Larval density of *Culex* spp., as well as of *Mansonioides* spp. has been noted to be reduced following heavy rainfall, which seems to be due to the lowering of the organic contamination (Iyengar, 1938).

Breeding of *Aedes aegypti*, *Aedes albopictus* and *Aedes-w-albus* has been noted in earthen pots, tree-holes, discarded tins, broken bottles, flower pots, and cut ends of bamboos. Breeding has not been found in any natural collection of water.

Armigeres obturbans breeds in enormous numbers in small pools, earthen drains grossly contaminated with urine, and in drains along which the blood from the slaughter houses flows.

Breeding of *Culex vishnui* and *Culex bitaeniorhynchus* has been noted in rice-fields, ponds and ditches.

SUMMARY.

A survey was conducted in the Gorakhpur District from January, 1958, to June, 1960, to determine the prevalence of various species of culicine mosquitoes.

The climate of the district is favourable for the breeding of culicine mosquitoes, the maximum and minimum temperature being 88°F. and 66°F., respectively, the average annual rainfall being 57·8 inches, and the relative humidity ranging from 55 to 96 per cent.

Drainage, both in rural and urban areas, is very defective, leading to the formation of cesspools, pits, ponds, tanks, stagnant pools and blocked drains, which provide ideal breeding grounds for culicine mosquitoes.

The species of culicine mosquitoes found in the Gorakhpur District are *Culex fatigans, Culex vishnui, Culex bitaeniorhynchus, Culex geliduc, Culex (Lutzia) fuscanus, Mansonioides annulifera, Mansonioides uniformis, Mansonioides indiana, Mansonia crassipes, Aedes albopictus, Aedes aegypti, Aedes (christophersomyia) thomsoni, Aedes lineatopennis, Aedes (stegomyia)-w-albus, Aedes pallidostriatus* and *Armigers obturbans.*

Amongst culicines, the most prevalent common species of the Gorakhpur District is *Culex fatigans*, its percentage being 90·44.

Precipitin tests of blood meals, from 460 mosquitoes of various species, were carried out. The anthropophilic index for *Culex fatigans* is 58·3 which points to high potentiality of active transmission of filariasis.

The resting and breeding habits of various species of culicine mosquitoes have been studied.

ACKNOWLEDGEMENT.

The authors are extremely grateful to the Insect Collectors and other staff of the National Filaria Control Unit, Gorakhpur, for their untiring help in the field.

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Indian Journal of Malariology, **15**, *4, December 1961.*

STUDIES ON CULICINE MOSQUITOES.

2. Laboratory studies on the longevity of adult *Culex fatigans* Wiedmann, 1828.

BY

B.L. WATTAL,

N.L. KALRA

AND

K.M.S. BEDI.

(*Malaria Institute of India, Delhi.*)

[June 17, 1961.]

THE question of longevity of mosquitoes, particularly the vector species, is of great interest from the point of view of epidemiology of mosquito-borne diseases. Among other factors, the efficiency of a given species, as a vector, depends on the frequency with which individual mosquitoes live long enough for the completion of the extrinsic incubation of the pathogen transmitted. The longevity of natural populations of mosquitoes has been determined, (*i*) by releasing marked specimens and subsequently recapturing them (Afridi, Majid and Shah, 1940 ; Russell and Rao, 1942) ; (*ii*) by determining the number of ovipositions completed by females in a population at the time of capture (Polovodova, 1949) ; and (*iii*) by studying the survival rates under laboratory conditions (Majid and Sinton, 1933 ; Kershaw *et al.*, 1954). The published data on the longevity of mosquitoes have been summarised, among others, by Majid and Sinton (1933), Sinton and Shute (1938), Russell and Rao (1942), Boyd (1949) and Detinova (1959). Sinton and Shute (1938 *loc. cit.*) list four factors that can conceivably influence longevity of mosquitoes in nature. These are :

1. Characteristics of the mosquito species ;
2. Activities of the individual mosquito ;
3. Climate ; and
4. Incidence of parasites and predators.

In nature, the same species will live for different periods under different ecological conditions which will vary from season to season and year to year. Therefore, longevity characteristic of a species can be understood better under laboratory conditions. The data thus collected will facilitate preparation of life tables for a species under given conditions. Information on the number of deaths for each age-interval, the number of survivors, the rate of mortality and the expectation of further life, can be forthcoming from such observations.

The present paper deals with the observations made during February, 1958, to June, 1959, on the survival rate and life expectancy of *Culex fatigans*, vector of *Wuchereria bancrofti*, under specified laboratory conditions.

METHODS.

All these experiments were carried out in the basement insectary of the Entomology Section of the Malaria Institute of India. The temperature in the insectary is maintained at 75° to 78°F., and the relative humidity at 78 to 82 per cent.

A laboratory colony of *Culex fatigans* (Delhi strain) has been maintained in the Entomology Section since 1948. Pupae from this colony were isolated in specimen tubes (3 inch×1 inch) provided with cotton wool plugs. Adults, emerging from these pupae during a single night, were collected for conducting these experiments.

Daily mortality rate of female mosquitoes was observed under three conditions :—

(*a*) blood-fed and fertilized ;

(*b*) blood-fed and unfertilized ;

(*c*) glucose-fed and fertilized.

In cases of '*a*', and '*c*', groups of 50 to 100 females and 30 to 75 males were kept together in regular wire-frame mosquito cages of 1 ft. cube. In case of '*b*', only the females were kept in such a cage. The daily mortality of males in cages '*a*' and '*c*' was recorded and the data were used for making life tables for males. Bulbul* was the source of blood-meal for observations under '*a*' and '*b*'. The bird was introduced in the cage every evening and was removed in the morning till the last female died. Cotton pads, moistened in 5 per cent glucose solution, provided nourishment for the males (*a*, *c*), and females in series '*c*'.

The cages were inspected everyday for the dead specimens and daily mortality was recorded. A small enamel bowl (4 inches diameter), containing water, was introduced in each cage three days after the start of the experiment to provide a site for oviposition.

The observations were repeated ten times during February, 1958, to June, 1959. The data in each experiment indicated the same trend ; therefore the data were pooled together and complete expectation of life for males and for females was worked out by standard statistical methods. Tables were constituted to give the following information :—

1. Number alive at the beginning of each day (*lx*).

* *Molpastes haemorrhous haemorrhous.*

2. Number dying in the age-interval x to $x+1$ (dx).
3. Proportion dying at the beginning of each day (qx).
4. Average number of days lived through the age-interval x to $x+1$ (Lx).
5. Total number of days lived by Lx mosquitoes (Tx).
6. Complete expectation of life at age x in average number of days lived by lx mosquitoes ($e^{\circ}x$).
7. Mortality rate per thousand population (1,000 qx).

To make the data comparable, the initial number of mosquitoes exposed to the risk of dying was standardised to 1,000. The life-tables presented here were prepared per thousand population and include the columns lx, qx and $e^{\circ}x$. Separate composite Tables were prepared to study the survivorship distribution and mortality rate per thousand population.

OBSERVATIONS.

Nine hundred females of *C. fatigans* each were studied for survivorship when blood-fed and fertilized (*a*), and when blood-fed and unfertilized (*b*). Similar observations were made on 988 females which were fed on glucose and fertilized (*c*). A total of 1,390 males were kept with females in experiments '*a*' and '*c*', and the same were observed for survivorship.

Table I indicates the number of individuals found alive at the beginning of each day till the last mosquito survived. From this and subsequent Tables, it is evident that in the experiments reported here the last male *C. fatigans* survived up to 47th day whereas the last female lived up to 52 days when blood-fed and unfertilized, and up to 53rd day when blood-fed fertilized as well as glucose-fed fertilized.

Table II and Graph 1 give the survivorship distribution of males and females per thousand population studied. In all the groups studied, there was no mortality of males or females on the first day, and all of them were alive on the second day. However, from the second day onwards, both males and females started dying. The males started dying faster than the three groups of females as shown in (Table III).

The 25 per cent mortality level for males and females was reached in about the same time (9th—11th day). After that, the males died at a much faster rate till the 75 per cent mortality level was reached. The males died at a slightly lower rate between 75 per cent and 90 per cent mortality levels. The last surviving 10 per cent males died at a very slow rate. Whereas 90 per cent mortality occurred in 24 days, the surviving 10 per cent males died within the next 23 days. The mortality in the females was gradual up to 90 per cent level, after which the mortality rate declined further (Graph I).

TABLE I.

Survivorship distribution of Culex fatigans *under different physiological conditions.*

Age (In days).	Males.	Females		
		Blood-fed fertilised.	Blood-fed unfertilised.	Glucose-fed fertilised.
0	1,390	900	900	988
1	1,390	900	900	988
2	1,390	900	900	988
3	1,332	877	875	970
4	1,290	857	846	943
5	1,242	831	819	922
6	1,204	810	797	900
7	1,154	791	770	877
8	1,112	759	742	856
9	1,044	727	706	816
10	942	696	671	753
11	868	676	654	731
12	811	665	636	699
13	762	654	624	680
14	694	621	592	649
15	616	596	572	618
16	559	572	551	588
17	483	533	518	551
18	400	493	469	510
19	341	457	428	457
20	297	427	404	412
21	247	408	377	392
22	219	372	350	360
23	190	345	323	332
24	162	312	297	303
25	135	281	270	273
26	115	267	253	257
27	102	253	236	237
28	88	222	200	203
29	81	208	186	194
30	70	188	143	172
31	73	166	112	154
32	66	143	98	132
33	60	120	90	106
34	56	101	80	80
35	43	76	74	70
36	40	58	60	61
37	35	44	48	58
38	32	40	40	56
39	30	37	30	52
40	27	37	28	43
41	19	31	22	37
42	13	26	19	32
43	12	25	16	32
44	10	25	14	31
45	9	25	14	27
46	2	15	6	20
47	1	10	2	19
48	..	10	2	14
49	..	5	2	14
50	..	5	2	14
51	..	5	1	12
52	..	3	1	6
53	..	2	..	3

TABLE II.

Survivorship distribution of Culex fatigans *under different physiological conditions per thousand population. (Standardised).*

Age (In days).	Males.	Females		
		Blood-fed fertilized.	Blood-fed unfertilized.	Glucose-fed fertilized.
0	1,000	1,000	1,000	1,000
1	1,000	1,000	1,000	1,000
2	1,000	1,000	1,000	1,000
3	958	974	972	982
4	928	952	940	955
5	894	924	910	934
6	867	900	886	912
7	831	879	856	889
8	801	844	825	868
9	752	809	785	827
10	679	774	746	763
11	626	751	727	741
12	585	740	707	709
13	550	728	694	690
14	501	692	658	659
15	445	664	636	628
16	404	638	613	598
17	349	595	576	560
18	289	550	522	518
19	246	510	476	464
20	214	476	449	418
21	178	449	419	398
22	158	415	389	366
23	137	385	359	338
24	117	348	330	308
25	98	314	300	278
26	84	298	281	262
27	75	283	262	242
28	65	248	222	207
29	60	232	206	197
30	50	210	158	173
31	55	185	124	157
32	50	159	108	124
33	46	133	99	108
34	43	112	88	82
35	33	84	81	72
36	31	64	66	63
37	27	49	53	60
38	25	45	44	58
39	23	42	33	54
40	21	42	31	45
41	15	35	24	30
42	10	29	21	34
43	9	28	18	34
44	7	28	16	33
45	6	28	16	20
46	3	17	7	21
47	1	11	2	20
48	..	11	2	15
49	..	6	2	15
50	..	6	2	15
51	..	5	1	13
52	..	3	1	7
53	..	2	..	3

GRAPH 1.

Survivorship distribution per thousand of C. fatigans under different physiological conditions.

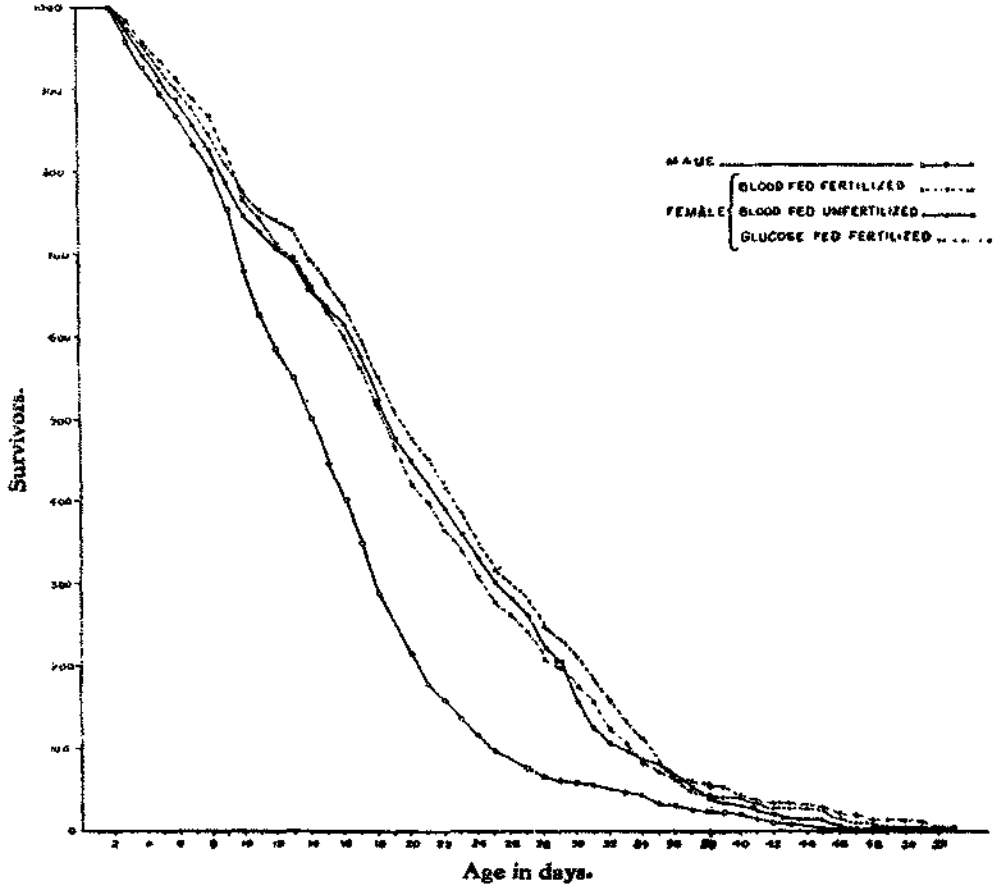


TABLE III.

Percentage of C. fatigans dying at different ages (in days).

Mortality per cent (approx.)	AGE, IN DAYS, ON WHICH THE PER CENT MORTALITY TOOK PLACE :			
	Males.	Females		
		Blood-fed fertilized.	Blood-fed unfertilized.	Glucose-fed fertilized.
25	9	11	9	10
50	13	19	18	18
75	18	27	27	26
90	24	34	32	33
95	31	36	37	39
100	47	53	52	53

To observe the differences in mortality at different ages, it was considered desirable to constitute a table (Table IV) giving mortality rate per thousand population (1,000 *qx*) at 5 days age-intervals. Proportion test of significance*, when applied to the data, revealed the following :—

Up to 5 days.—Males showed high mortality which was significantly higher than the blood-fed fertilized ($T=2{\cdot}51$) and the glucose-fed fertilized females ($T=3{\cdot}5$) but not significantly different from the blood-fed unfertilized females ($T=1{\cdot}47$). The differences in the mortality of females were found to be insignificant.

6—10 days.—The mortality among males was significantly much higher than that of females. The *T* values for the blood-fed fertilized, blood-fed unfertilized and glucose-fed females were 5·97, 4·96 and 4·84 respectively. The differences in the mortality of females were insignificant.

TABLE IV.

Mortality rate per thousand (1,000 qx.) Culex fatigans *at 5 days age-interval.*

Age-group (days).	Males.	Females		
		Blood-fed fertilized.	Blood-fed unfertilized.	Glucose-fed fertilized.
Up to 5	134	100	114	89
6 —10	279	165	179	188
11—15	356	154	157	196
16—20	558	295	316	333
21—25	634	337	320	344
26—30	365	378	557	400
31—35	452	651	536	604
36—40	525	466	633	393
41—45	895	576	727	459
46—50	1,000	333	167	400
51 and above	..	1,000	1,000	1,000

*Since the sample was large, the difference of the proportion of numbers dying, divided by the standard error of the proportion of difference, followed normal distribution. When this ratio worked out to be less than 1·96, it was considered to be statistically insignificant and when more than 1·96, it was considered statistically significant at 5 per cent level of significance. The equation used was

$$T = \frac{p_1 - p_2}{\sqrt{\frac{p_1 q_1}{n_1} + \frac{p_2 q_2}{n_2}}} \text{ Normal Distribution N (0, 1)}$$

where p_1=the proportion of n_1 dying by the age-interval.

p_2=the proportion of n_2 dying by the age-interval.

$q_1 = 1 - p_1$.

$q_2 = 1 - p_2$.

$n1/n2$=the respective number of mosquitoes alive at the beginning of each age-interval.

When the sample size was small, the following equation was used :

$$T = \frac{p_1 - p_2}{\sqrt{\frac{p_1 q_1}{n_1 - 1} + \frac{p_2 q_2}{n_2 - 1}}} \text{ is distributed as t with } n_1 + n_2 - 2 \text{ degrees of freedom.}$$

Therefore, 't' table was referred to when the size of the surviving population was small.

11—15 days.—The mortality among males continued to be significantly much higher than that of females and definitely higher than in the '6-10 days' age-interval. The *T* values for the three groups of females mentioned earlier were 9·45, 9·21 and 7·3 respectively. The mortality in the blood-fed fertilized and blood-fed unfertilized, and blood-fed unfertilized and glucose-fed fertilized, was not significantly different. However, the mortality in the glucose-fed fertilized was found to be significantly higher than the blood-fed fertilized females.

16—20 days.—The mortality among males continued to be significantly much higher than that of females. The *T* values for the three groups of females were 9·27, 8·94 and 7·86. The differences in the mortality among females were insignificant.

21—25 days.—The mortality pattern was the same as in the '6—10 days' age-interval. *T* values for the three groups of females were 4·95, 4·74 and 4·78 respectively.

26—30 days.—The mortality in males was not significantly different from the mortality in the blood-fed fertilized and glucose-fed fertilized females. The mortality in the blood-fed unfertilized females was significantly higher than the mortality in males ($T=3·41$), blood-fed fertilized females ($T=4·27$) and glucose-fed fertilized females ($T=3·59$).

31—35 days.—The mortality in males was significantly less than the blood-fed fertilized ($T=2·86$), and glucose-fed fertilized females ($T=2·16$). The differences in the mortality among females were insignificant.

36—40 days.—The mortality in males and the different sets of females was not significantly different. The blood-fed unfertilized females showed highest mortality, which was significantly higher than the glucose-fed fertilized females ($T=2·74$).

41—45 days.—The mortality in males was significantly higher than the blood-fed fertilized ($T=3·3$) and glucose-fed fertilized females ($T=4·01$). The blood-fed unfertilized females had significantly higher mortality than the glucose-fed fertilized females.

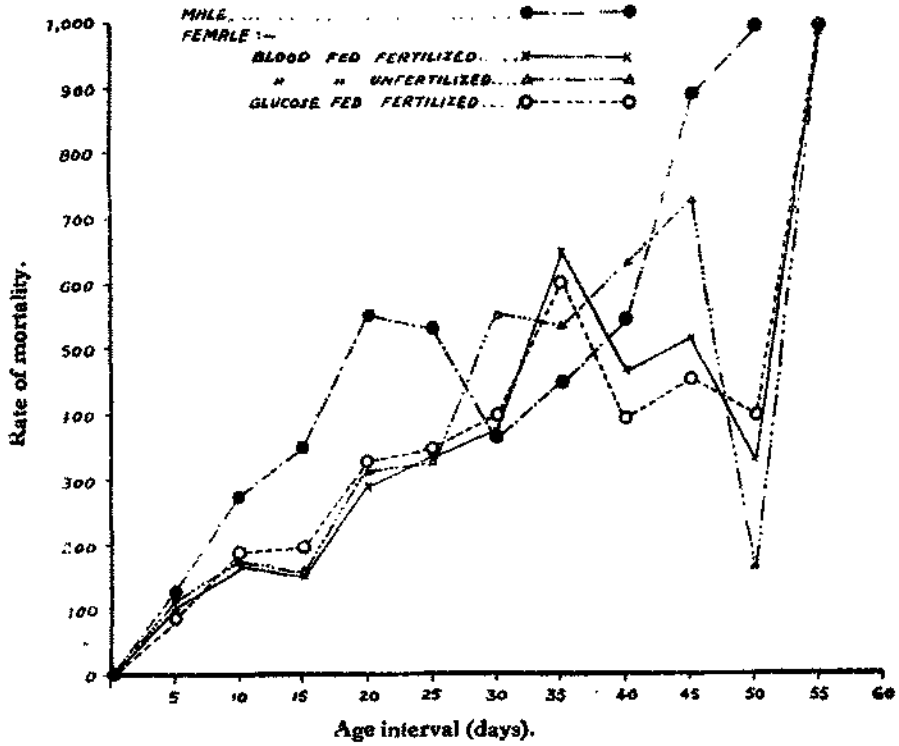
46—50 days.—The last male died on the 47th day. Therefore, only the mortality differences among females were tested and found to be insignificant.

Table IV and Graph 2 show that the rate of mortality in males is higher up to the 25th day stage, by which time nearly 90 per cent of the males die. Then there is a temporary decline of the death rate till the last few surviving mosquitoes die at which time the death rate again is higher than the females. The mortality-rate curves for the blood-fed fertilized and the glucose-fed fertilized females seem to run the same course, *i.e.*, a steep mortality rate up to 10 days, then a slight decrease, followed by another steep rise (15—20 days interval), then gradual increase up to the 30th day and another steep rise up to the 35th day. By this time, the 90 per cent mortality level is reached and the remaining 10 per cent behave like

males. The blood-fed unfertilized females deviate from the curves already mentioned. They show a steep rise in mortality during the 25-30 days age-interval, then a slight decline on the 35th day, again a steady and sharp increase up to the 45th day, followed by a sudden dip before the final total mortality.

GRAPH 2.

Mortality rate per thousand C. fatigans *at 5 days age-interval.*



By observing the actual number dying every day (*dx* table, not included in this paper) and the rate of mortality (Table IV, Graph 2), it was evident that the death rate was not constant. The rates of mortality showed definite increase with age till the 90 per cent mortality level was reached. After this level, the mortality fluctuated till there was a sharp increase in mortality when the last surviving individuals died.

Life tables of *C. fatigans* males, and the blood-fed fertilized, blood-fed unfertilized and glucose-fed females are presented in Tables VI, VII, VIII and IX respectively. Table V and Graph 3 give the life expectancy of *C. fatigans* males and females, as studied in the present experiments, at 5 days age-interval.

TABLE V.

Life expectancy ($e^{o}x$) of Culex fatigans *at 5 days age-interval.*

Sex.	Age-interval (days).											
	0	5	10	15	20	25	30	35	40	45	50	55
Male :	14·80	11·51	8·76	7·04	7·06	7·87	7·23	5·91	3·00	1·00	0·0	..
Female :												
Blood-fertilized.	20·16	16·55	14·22	11·38	9·54	8·14	5·86	5·80	5·57	2·72	2·60	0·0
Blood-fed unfertilized.	19·26	15·82	13·70	10·61	8·94	7·14	5·33	4·66	4·14	1·71	2·00	0·0
Glucose-fed fertilized.	19·42	16·55	13·36	10·70	9·69	8·29	6·69	8·11	6·60	4·33	2·07	0·0

GRAPH 3.

Life expectancy ($e^{o}x$) of C. fatigans *at 5 days age-interval.*

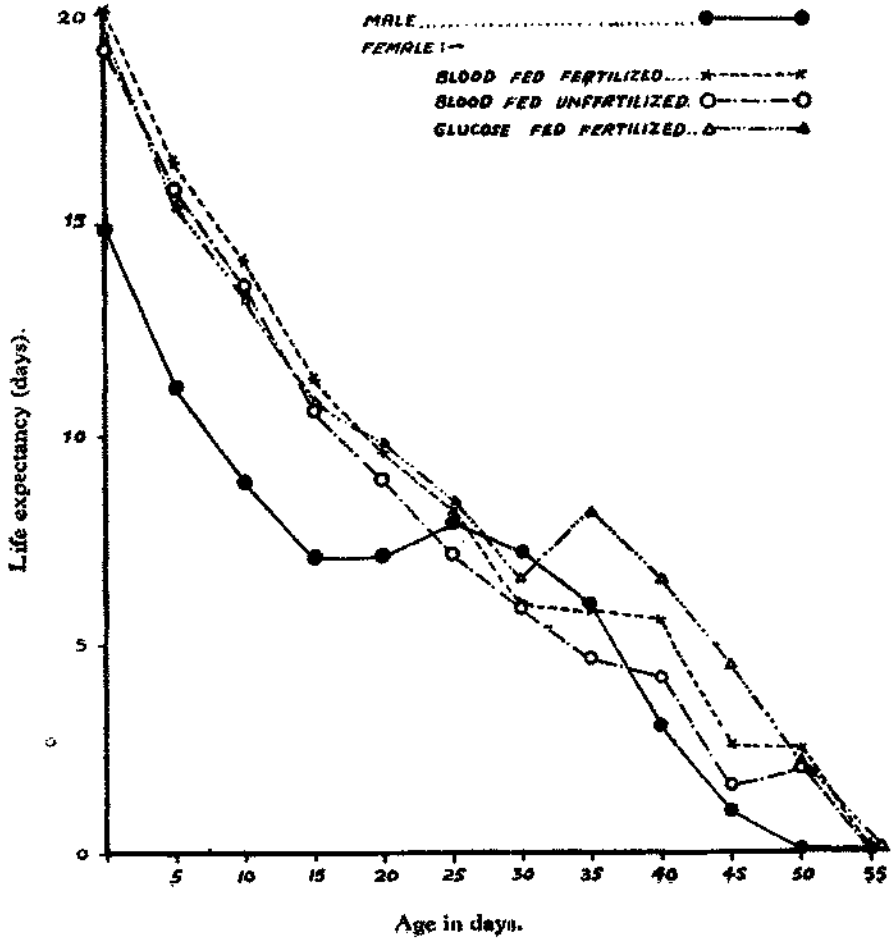


TABLE VI.

Life table for Culex fatigans *males.*

Age (In days).	*lx.*	*qx.*	*e°x.*	Age (In days).	*lx.*	*qx.*	*e°x.*
0	1,000	0·00	14·80	24	117	0·17	7·48
1	1,000	0·00	13·80	25	98	0·15	7·87
2	1,000	0·04	12·80	26	84	0·11	8·16
3	958	0·03	12·34	27	75	0·14	8·14
4	928	0·04	11·72	28	65	0·08	8·35
5	894	0·03	11·15	29	60	0·02	8·04
6	867	0·04	10·49	30	59	0·08	7·23
7	831	0·04	9·92	31	55	0·10	6·78
8	801	0·06	9·28	32	50	0·09	6·44
9	752	0·10	8·85	33	46	0·07	6·03
10	679	0·08	8·76	34	43	0·23	5·43
11	626	0·07	8·46	35	33	0·07	5·91
12	585	0·06	8·02	36	31	0·13	5·30
13	550	0·09	7·50	37	27	0·09	4·97
14	501	0·11	7·19	38	25	0·06	4·38
15	445	0·09	7·04	39	23	0·10	3·63
16	404	0·14	6·71	40	21	0·30	3·00
17	349	0·17	6·68	41	15	0·32	3·00
18	289	0·15	6·96	42	10	0·08	3·23
19	246	0·13	7·08	43	9	0·17	2·50
20	214	0·17	7·06	44	7	0·10	1·80
21	178	0·11	7·38	45	6	0·78	1·00
22	158	0·13	7·26	46	2	0·50	1·50
23	137	0·15	7·30	47	1	1·00	1·00

Legend : *lx*=number alive at the beginning of each day.
qx=proportion dying at the beginning of each day.
e°x=complete expectation of life at age *x* in average number of days lived by *lx* mosquitoes.

The life expectancy of males at birth was observed to be 14·8 days, whereas the life expectancy of females was observed to be 20·16, 19·26 and 19·42 days for the blood-fed fertilized, blood-fed unfertilized and glucose-fed fertilized respectively. The life expectancy of males at birth was significantly less than that of females. The different physiological conditions, under which the females were kept, did not seem to affect their life expectancy at birth.

The life expectancy decreased progressively with the increase in age. In the case of males there was progressive decrease in the life expectancy till the 15th day age-interval. From the 15th to 30th day, the mortality was less and there was the same or greater expectancy of life. After the 30th day, the life expectancy decreased progressively till the last surviving male died.

Although the life expectancy of males was generally lower than that of females, in the age-interval of '25th to 35th day' it was found to be higher than that of females.

The life expectancy of females under different physiological conditions was found to be similar from 0 to 15th day, although the blood-fed fertilized females showed slightly higher life expectancy than the other two groups. From the

TABLE VII.

Life table for Culex fatigans *female — blood-fed and fertilized.*

Age (In days).	*lx.*	*qx.*	*e°x*	Age (In days).	*lx.*	*qx.*	*e°x.*
0	1,000	0·0	20·16	27	283	0·12	6·93
I	1,000	0·0	19·16	28	248	0·06	6·82
2	1,000	0·03	18·16	29	232	0·10	6·25
3	974	0·02	17·63	30	210	0·12	5·86
4	952	0.03	17·02	31	185	0·14	5·57
5	924	0·03	16·55	32	159	0·16	5·39
6	900	0·02	15·96	33	133	0·16	5·32
7	879	0·04	15·34	34	112	0·25	5·24
8	844	0·04	14·96	35	84	0·24	5·80
9	809	0·04	14·60	36	64	0·24	6·45
10	774	0·03	14·22	37	49	0·09	7·34
11	751	0·01	13·65	38	45	0·08	7·02
12	740	0·02	12·85	39	42	..	6·57
13	728	0·05	12·06	40	42	0·16	5·57
14	692	0·04	11·67	41	35	0·16	5·55
15	664	0·04	11·38	42	29	0·04	5·54
16	638	0·07	10·58	43	28	..	4·72
17	595	0·08	10·32	44	28	..	3·72
18	550	0·07	10·12	45	28	0·40	2·72
19	510	0·07	9·88	46	17	0·33	3·20
20	476	0·07	9·54	47	11	..	3·60
21	449	0·08	9·07	48	11	0·50	2·60
22	415	0·07	8·79	49	6	..	3·60
23	385	0·10	8·44	50	6	..	2·60
24	348	0·10	8·28	51	6	0·40	1·60
25	314	0·05	8·14	52	3	0·33	1·33
26	298	0·05	7·54	53	2	1·00	1·00

Legend : *lx* = number alive at the beginning of each day.
qx = proportion dying at the beginning of each day.
e°x = complete expectation of life at age *x* in average number of days lived by *lx* mosquitoes.

15th day age-interval onwards till the 45th day, the life expectancy of the glucose-fed fertilized females was high, followed by the blood-fed fertilized and blood-fed unfertilized groups. These differences were found to be significant between the "35th—45th day" age-intervals. Between the "15th—50th day" age-interval, life expectancy of the blood-fed unfertilized females was found to be generally less than the females in the other two groups.

DISCUSSION.

Life tables have been extensively used to study the mortality relation within human populations at specific age-intervals. The data thus collected have been used to determine the expectation of life of individuals in different countries, under varying socio-economic and environmental conditions. Pearl and his associates (*see* Pearl, 1940 ; Allee *et al.*, 1950) studied with great precision the life-tables for lower organisms, particularly that of the fruit fly, *Drosophila melanogaster* and the flour beetle, *Tribolium confusum*. But so far, no such tables are available for mosquitoes. This is so largely, as Kershaw *et al.* (1954) mention, because of the limited numbers

TABLE VIII.

Life table for Culex fatigans *females — blood-fed and unfertilized.*

Age (In days).	*lx.*	*qx.*	$e^{o}x$.	Age (In days).	*lx.*	*qx.*	$e^{o}x$.
0	1,000	0·00	19·26	27	262	0·15	6·01
1	1,000	0·00	18·26	28	222	0·07	6·00
2	1,000	0·00	17·26	29	206	0·23	5·42
3	972	0·03	16·74	30	158	0·22	5·83
4	940	0·03	16·30	31	124	0·13	6·30
5	910	0·03	15·82	32	108	0·08	6·13
6	886	0·03	15·24	33	99	0·11	5·63
7	856	0·04	14·76	34	88	0·08	5·28
8	825	0·05	14·29	35	81	0·19	4·66
9	785	0·05	14·00	36	66	0·20	4·63
10	746	0·03	13·70	37	53	0·17	4·67
11	727	0·03	13·04	38	44	0·25	4·50
12	707	0·02	12·40	39	33	0·07	4·83
13	694	0·05	11·63	40	31	0·21	4·14
14	658	0·03	11·23	41	24	0·14	4·14
15	636	0·04	10·61	42	21	0·16	3·74
16	613	0·06	9·99	43	18	0·12	3·81
17	576	0·09	9·60	44	16	0·00	2·71
18	522	0·09	9·54	45	16	0·57	1·71
19	476	0·06	9·41	46	7	0·67	2·33
20	449	0·07	8·94	47	2	0·00	5·00
21	419	0·07	8·55	48	2	0·00	4·00
22	389	0·08	8·17	49	2	0·00	3·00
23	359	0·08	7·81	50	2	0·50	2·00
24	330	0·09	7·45	51	1	0·00	2·00
25	300	0·06	7·14	52	1	1·00	1·00
26	281	0·07	6·57				

Legend: *lx*=number alive at the beginning of each day.
qx=proportion dying at the beginning of each day.
$e^{o}x$=complete expectation of life at age *x* in average number of days lived by *lx* mosquitoes.

in the population groups for which survival/mortality rates were studied. The above mentioned authors used graphical method to study the survival of *Anopheles quadrimaculatus*, *A. hyrcanus* var. *sinensis*, *A. gambiae*, *A. funestus*, *A. maculipennis* var. *atroparvus* and *Aedes aegypti*. They came to the conclusion that "the general pattern of survival for both sexes is the same, but the survival of the males is less than that of the females". The life-tables worked for insects other than mosquitoes (Allee *et al.*, 1950 *loc. cit.*), also show that generally females live longer than the males. The differences in the longevity of two sexes have been reported to be significant. Russell and Rao (1942) reported the same for *A. culicifacies*. In the experiments presented here it was observed that female *Culex fatigans*, when blood-fed fertilized, blood-fed unfertilized and glucose-fed fertilized, lived longer (52 to 53 days) than the males (47 days). The life expectancy of females (Table V) under all conditions was found to be significantly higher than males (20·16, 19·26 and 19·42 days respectively as against 14·80 days). There is no comparable data available in literature for *C. fatigans*. However, Gill (1921) succeeded in keeping 100 females alive for more than 10 days on raisins at a temperature of 27°C. and a relative

TABLE IX.

Life table for Culex fatigans *females—glucose-fed and fertilized.*

Age (In days).	*lx.*	qx.	*e°x.*	Age (In days).	*lx.*	qx.	*e°x.*
0	1,000	0·00	19·42	27	242	0·14	7·39
1	1,000	0·00	18·42	28	207	0·04	7·55
2	1,000	0·02	17·42	29	197	0·11	6·88
3	982	0·03	16·73	30	175	0·10	6·69
4	955	0·02	16·22	31	157	0·21	6·42
5	934	0·02	15·55	32	124	0·13	6·97
6	912	0·03	14·92	33	108	0·24	6·94
7	889	0·02	14·30	34	82	0·13	8·04
8	868	0·05	13·64	35	72	0·13	8·11
9	827	0·08	13·29	36	63	0·05	8·24
10	763	0·03	13·36	37	60	0·03	7·64
11	741	0·04	12·74	38	58	0·07	6·89
12	709	0·03	12·31	39	54	0·17	6·38
13	690	0·05	11·64	40	45	0·14	6·60
14	659	0·05	10·17	41	39	0·14	6·59
15	628	0·05	10·70	42	34	0·00	6·56
16	598	0·06	10·22	43	34	0·03	5·56
17	560	0·07	10·78	44	33	0·13	4·71
18	518	0·10	9·63	45	29	0·26	4·33
19	464	0·10	9·69	46	21	0·05	4·65
20	418	0·05	9·69	47	20	0·26	3·84
21	398	0·08	8·16	48	15	0·00	4·07
22	366	0·08	3·93	49	15	0·00	3·07
23	338	0·09	8·64	50	15	0·14	2·07
24	308	0·10	8·42	51	13	0·50	1·33
25	278	0·06	8·29	52	7	0·50	1·17
26	262	0·08	7·78	53	3	1·00	1·00

Legend: *lx*=number alive at the beginning of each day.
qx=proportion dying at the beginning of each day.
e°x=complete expectation of life at age *x* in average number of days lived by *lx* mosquitoes.

humidity of 65 to 80 per cent. And Majid and Sinton (1933) succeeded in keeping 40 females alive for 105 days and 5 for 189 days when given one blood-meal, followed by sugar solution. The observations of Majid and Sinton (1933 *loc. cit.*) were made during cold months when the average temperature was 16·9°C., and therefore the mosquitoes could be expected to live longer than at higher temperature (25°C.) and humidity (80 per cent), *i.e*, the conditions under which the present observations were made.

From the life-tables (Tables VI, VII, VIII, IX) presented here, the longevity characteristic of *C. fatigans*, particularly those of males, seems to be much higher than normally realised. Of course, in these experiments optimum conditions for survival were provided. It is unlikely that natural population of this mosquito will behave similarly. However, considering the fact that *C. fatigans* is largely a domestic species, breeding in peri-domestic waters, it is reasonable to expect a large proportion of the adult females to survive under optimum temperature and humidity. These optimum climatic conditions are also favourable for the development of the stages of *Wuchereria bancrofti* in the mosquito. Therefore,

under optimum climatic conditions the mosquitoes could be expected to survive in large numbers to keep on transmission of bancroftian filariasis.

On the work of Russell and Rao (1942), Macdonald (1952) came to the conclusion that under natural conditions mosquito mortality remained unchanged with age. and that this was supported by available literature concerning the longevity of mosquitoes kept under laboratory conditions. However, Kershaw *et al.* (1954 *loc. cit.*), from their experiments with *Aedes aegypti*, *Chrysops* spp. and *Simulium damnosum*, and by scanning the literature on survival of mosquitoes, concluded that the rate of mortality of mosquitoes, maintained under laboratory conditions, increases with age. The present experiments revealed that the mortality in either sex of *C. fatigans* was not constant (Table IV, Graph 2). There was no mortality from "0 to 1st day". Mortality in males as well as females started on the 2nd day (Graph 1) and the death rate increased with the increase in age till 90 to 98 per cent mortality (males, blood-fed and glucose-fed fertilized females, and blood-fed unfertilized females respectively) amongst the original populations occurred (Table IV, Graph 2). The last 2 to 10 per cent survivors, made up of individuals who have the ability to outlive considerably their companions, showed fluctuations in the rate of mortality.

Although the pattern of survival (Graph 1) for males and females was similar, males died at a significantly higher rate than the females till 90 per cent males died (25th day). Between 26 to 30 days and 36 to 40 days, mortality rate in males was not significantly different from the rate of mortality in females. The rate of mortality in males was significantly less than the females between 31 to 35 days period. From the 41st day onwards, the last few surviving males died at a significantly higher rate till the 47th day when the last male died.

Christophers (1960), while dealing with the normal duration of life of *Aedes aegypti*, mentions that "if given a blood-meal within the first 7 days there is usually little mortality until after oviposition, when it may be considerable as at subsequent ovipositions". He further mentions that "Bonne-Wepster and Brug (1932) state that life is longer where the insects are given sugar and water as food instead of blood. In this case there is no mortality following oviposition. Connor (1924) states that 70 per cent of females die after the first batch of eggs has been laid". In the present experiments, the life expectancy of the ovipositing *C. fatigans* females was found to be similar to the non-ovipositing females. The $e^{o}x$ (Table V, Graph 3) for the former worked out to be 20·16 days while for the non-ovipositing females it was 19·26 days (blood-fed unfertilized) and 19·42 days (glucose-fed fertilized). From these results, it was also evident that the blood feed or glucose feed did not alter the chances of survival (Tables I, II). It was observed that the mortality followed the same course and the last surviving female died on the 53rd day in both blood-fed fertilized and glucose-fed fertilized females. Glucose-fed females survived better than the blood-fed batches only in the "25th to 45th day" age-interval (Table V) when 90 to 95 per cent of the females had died.

SUMMARY.

Laboratory experiments were carried out during the period February, 1958, to June, 1959, to study the longevity of *Culex fatigans* males maintained on 5 per cent glucose solution and females when blood-fed fertilized, blood-fed unfertilized and glucose-fed (5 per cent solution) fertilized. From the data thus obtained, survival rates, death rates and life tables for either sex were worked out.

The observations revealed that *C. fatigans* females lived longer than males. The life expectancy of males at birth (14·80 days) was found to be much higher than normally realised, but significantly less than that of females (19·26 to 20·16 days). The females under the different physiological conditions did not show any significant difference in their life expectancy. The last surviving male died on the 47th day whereas the females survived up to 52nd to 53rd days.

Contrary to the general belief, there was no mortality on the first day and the rate of mortality was not constant. Mortality started from the 2nd day and the rate of mortality increased with increase in age till 90 to 98 per cent of the initial populations died. Although, the pattern of survival in case of males and females was similar, the males died at a significantly faster rate than the females.

Food and mating status of females, as conditioned in the present experiments, did not seem to affect their survival.

It was concluded that *C. fatigans*, with a high longevity characteristic and being predominantly a domestic mosquito, could be expected to survive in large numbers under favourable ecological conditions.

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Indian Journal of Malariology, **15**, *1, December 1961.*

GYNANDROMORPHISM IN *CULEX FATIGANS* WIED.

BY

B. S. KRISHNAMURTHY

(Malaria Institute of India, Delhi).

[June 22, 1961.]

GYNANDROMORPHISM in mosquitoes, though rare, has been reported from time to time. Over 40 cases of gynandromorphs have been recorded so far among *Culex*, *Aedes* and *Mansonia* spp. (Lum, 1960). It has also been observed in *Haemagogus* spp. (Bates, 1949). Mostly this has been observed in the laboratory colonies of mosquitoes. Blazquez (1958), while attempting to obtain a strain of *Culex fatigans* resistant to DDT, obtained as many as 50 gynandromorphs between F6 and F10 generations. In India, Ausat and Koshi (1955) reported a gynandromorph of *C. fatigans* from a colony maintained for ten years. The specimen described by them had antennae and palpi of male, while the abdomen and genitalia were those of female.

A gynandromorph was found among individuals of a laboratory strain (DE/Ha)* of *C. fatigans* maintained at the Malaria Institute of India, Delhi. The mosquito hatched on March 20, 1961. The specimen was alive for 19 days and was later mounted in canada balsam for detailed examination. A description of the specimen is given in this note :

The left antenna and palp (Plate I, Fig. 1) are typically that of a normal female and the right antenna and palp of a normal male. The last segment of the left front leg is that of a normal female while the right front tarsus is that of a male. The external genitalia are of a normal male but the last segment, including the terminalia, has not undergone the usual torsion, resulting in an inverted condition of the genitalia (Plate I, Fig. 2).

Efforts were made to determine the behaviour of the gynander by offering a blood-feed and also by allowing it to mate with normal females. The specimen made feeble efforts to bite, but was not successful in piercing the skin. The egg rafts, obtained from the normal females which had been given an opportunity to mate with the gynander, were unfertilized and did not hatch. The spermathecae, when dissected, had no sperms in them.

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*A *C. fatigans* strain obtained by crossing a female of Delhi with the males of Hamburg strain.

Thanks are due to Shri Bhagat Ram and Shri Sohan Singh for their help in raising the laboratory cultures.

PLATE 1.

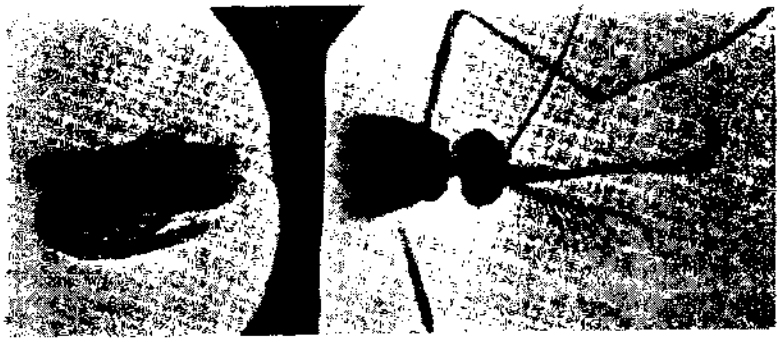

FIG. 1. Close-up view of the unrotated terminalia and head of gynandromorphism of *Culex fatigans*.

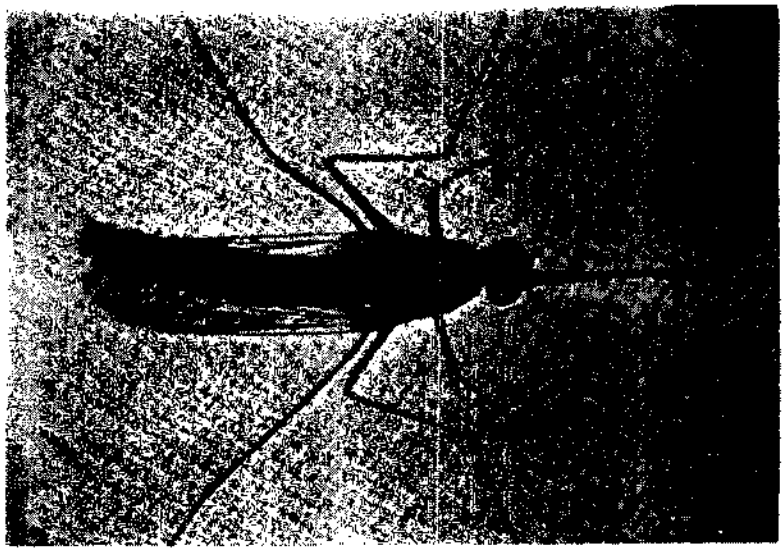

FIG. 2. Dorsal view of gynandromorph of *Culex fatigans* showing both the male and female head appendages and male genitalia.

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