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THE INDIAN COUNCIL OF MEDICAL RESEARCH

Editor :—Lieut.-Colonel JASWANT SINGH, M.B., Ch.B., D.P.H., D.T.M. & H.

Vol. IV, 1950.

PUBLISHED FOR

THE INDIAN COUNCIL OF MEDICAL RESEARCH

BY

THACKER'S PRESS & DIRECTORIES, LTD., CALCUTTA

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

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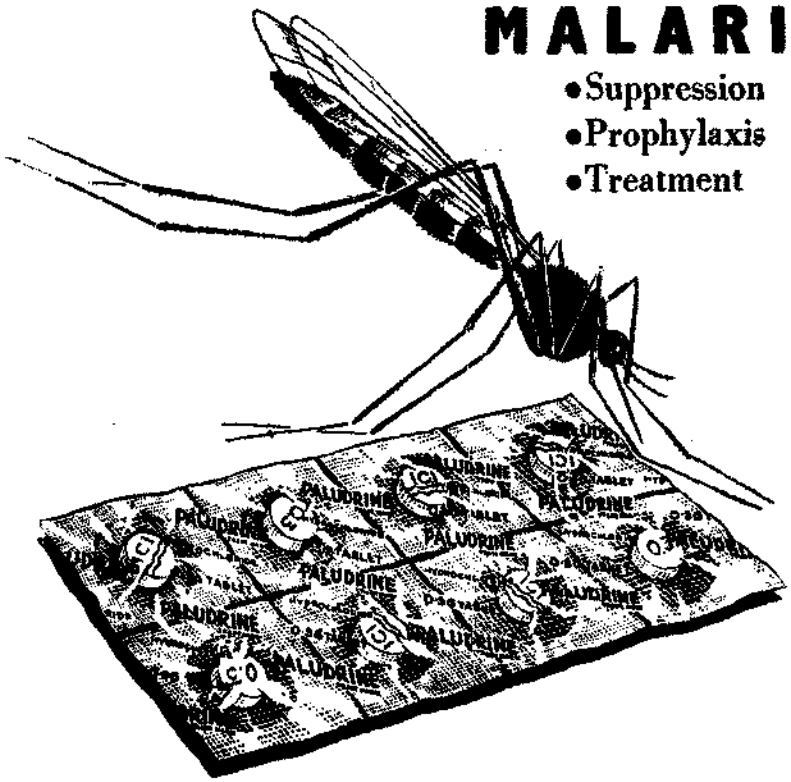

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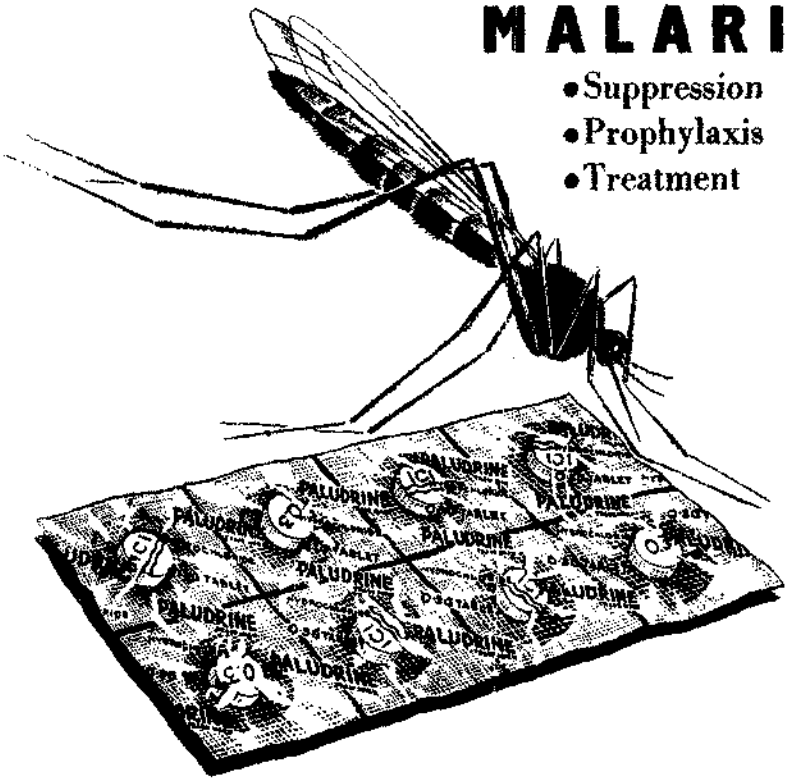

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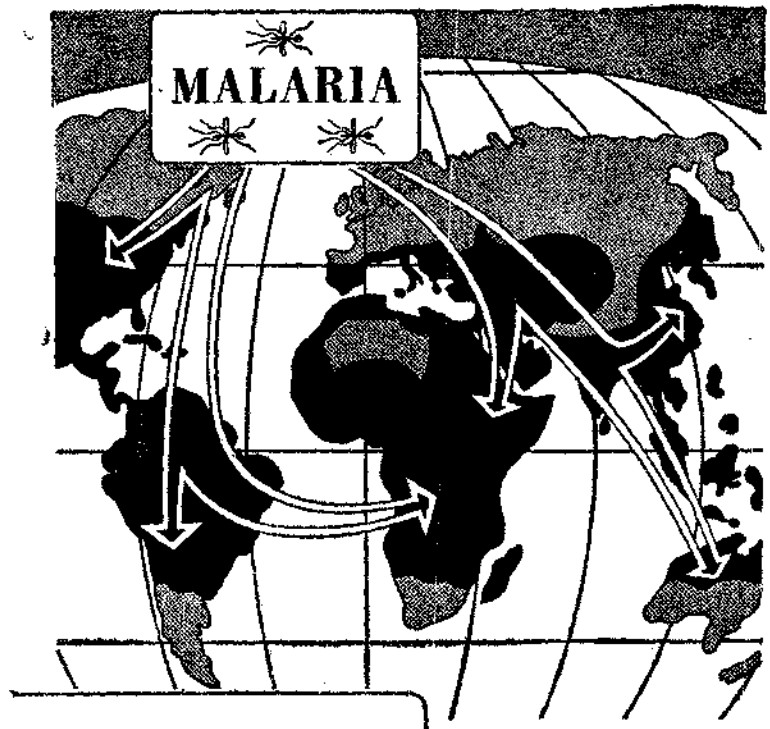

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MALARIA AND ITS VECTORS IN BORNEO.

A STUDY OF BORNEO'S GREATEST DISEASE PROBLEM, AND OF THE MOSQUITOES RESPONSIBLE FOR ITS TRANSMISSION, THROUGHOUT NORTH BORNEO AND LABUAN, SARAWAK, BRUNEI AND DUTCH BORNEO, TO THE END OF THE FIRST HALF OF THE TWENTIETH CENTURY.

BY

JOHN McARTHUR, M.R.C.S., L.R.C.P.

(*Director of Malaria Research, North Borneo, Sarawak and Brunei.*)

[February 3, 1950.]

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INTRODUCTION.

BORNEO is a beautiful, fertile, largely forest-clad and mountainous island, with a good climate and excellent prospects of agricultural and economic development.

The third largest island in the world, it is on the whole a healthy land except for malaria, which is by far its greatest disease problem and one of its greatest hindrances to progress.

In the following pages an attempt is made to summarize all the existing knowledge regarding malaria and its vectors throughout the whole of Borneo—North Borneo and Labuan, Sarawak, Brunei and Dutch Borneo—to the end of the first half of the twentieth century.

Records have been collected from all over the island, of mosquito dissections since 1914, and of mosquito identifications since 1905, while spleen rates have been collected from as far back as 1882.

These, together with information regarding the distribution and intensity of the disease, the malarial parasites, carrier species and the distribution of different species of Anopheles, have been gathered together to form so far as possible a picture of the malaria situation in Borneo as a whole.

Some of the facts recorded are the result of personal observation, but by far the greater part of the information represents the result of a study of the literature, a great deal of which was lost during the war.

Many of the following pages were recovered from the Japanese and represent much that cannot otherwise be replaced. They have been brought up to date with the inclusion of the results of more recent work.

Since much of this work represents the collection of records rather than of actual facts, it has been endeavoured to tabulate the information as nearly as possible as it was originally recorded. An attempt has been made, however, to summarize and draw conclusions in so far as this is justifiable.

It is hoped that this summary of the available knowledge will lead to a better understanding of the disease in Borneo and of the means for its eradication, and that it may contribute to the future health and prosperity of the land.

I. DISTRIBUTION AND INTENSITY OF MALARIA.

The distribution and intensity of malaria can be determined by a study of hospital records, by the examination of the population for spleen enlargement, or by blood examinations for malaria parasites.

Hospital records yield information regarding the intensity of malaria over past years. Spleen rates are an index of the intensity of malaria of recent months or perhaps a few preceding years while parasite rates are an index of the intensity of malaria at the time of examination.

Hospital records in particular, however, may be misleading, and there are few in existence in Borneo which are of value for the purpose, following their widespread destruction during the war.

Parasite examinations, although they yield much information, are troublesome and time consuming, and there are few records from Borneo which yield information of value.

By far the most valuable records for present purposes are those of spleen examinations, especially of children. In this study chief attention is therefore paid to spleen rates in children where these are obtainable. Spleen surveys have been carried out fairly extensively, especially in North Borneo, and the records of these have been largely preserved.

SOURCES OF INFORMATION.

Borneo is a relatively newly developed island, where observations on malaria have been only recent and infrequent.

The published literature and other available sources of information regarding malaria, present the records of thirty or so observers during some 65 years. These workers have in general been isolated, engaged in other occupations, and working without interchanges of knowledge, and undoubtedly much information has been lost.

The earliest reference to an assessment of malaria in Borneo appears to be that mentioned by Shircore (1937). He stated, 'It is interesting to note that Dr. Nieuwenhuis, during an investigation conducted in 1882-83, in the Sultanate of Sambas in Dutch Borneo, not far distant from the British border, obtained a spleen rate of 82·5 per cent, for the " mountain population " ', and commented that the figure certainly shows hyperendemicity to be no new thing in Borneo.

Roper (1914) recorded hyperendemic malaria round Meirbakut, on the west coast of North Borneo, in proximity to swamp, although other areas—ricefields, and rubber estates ' so long as the trees remain small ', and high land inland—were found to be healthy.

Scharff (1927), late Chief Health Officer, Singapore, found a spleen rate of 90 per cent in one place among the natives of Labuan, but only 7 per cent in the town area.

Stookes (1927) in Miri, Sarawak, stated that malaria, 'chiefly of the sub-tertian variety, was at one time very prevalent', and referred to, 'much malaria at Satang Island', off the coast of Sarawak, which was further described in Health Department Reports (Sarawak, 1930).

In 1928, Professor Walch made a tour of the east coast of Borneo, and in the following year published, with Soesilo, an account in Dutch. They recorded (Walch and Soesilo, 1929) hyperendemic malaria in Tg. Djoeata; and two areas, 500 metres apart, showing different anopheline fauna, were contrasted as having spleen rates of 8 and 49 per cent respectively.

Hutchinson (1930) found 5 enlarged spleens and 5 positive blood films out of 17 children examined at Quop, near Kuching, Sarawak; and Campbell (1931) found hyperendemic malaria in Sapong Estate, North Borneo. Campbell also visited Tambunan District, and found hyperendemic malaria throughout.

Dingle (1931), late Principal Medical Officer, North Borneo, made a tour of the interior of North Borneo and along the Kinabatangan River, finding 548 enlarged spleens among 1,235 persons examined (43·5 per cent).

Stoker (1934) recorded 100 per cent enlarged spleens in 27 children examined, and a high proportion with parasites, in Kg. Sarang Tioeng, Poeloe Laoet, an island off the south-west of Borneo; while Goelarso (1934) recorded hyperendemic malaria in the Sadjau District on the east coast.

Tregarthan (1941), in the course of four years as District Surgeon in the interior of North Borneo, confirmed Campbell's finding of hyperendemicity in the Tambunan area and elsewhere; and Clarke (1941), also District Surgeon, recorded spleen rates in 95 villages throughout a wide area. His figures suggested that malaria might be limited to altitudes of less than about 4,000 feet.

Shircore (1937) in the course of an extensive health survey of North Borneo found spleen rates well above 50 per cent in each district examined. He noted that, on the coastal plains distant from the hills, malaria was infrequent, but that in the foothills and in the interior malaria was intense, frequently with a spleen rate of 100 per cent.

In Brunei, it was found that, although malaria was practically absent from the chief centres of population, Kuala Belait and Brunei Town, it rose to high figures in the outlying districts. In 1937, for example, it was noted that there was 'comparatively little malaria on the various rubber estates, but it is very common in the rice-growing areas'.

Summarizing the observations of the Malaria Department of the Netherlands East Indies Public Health Service in Borneo, certain maps were to be found before the war in the Eykman Institute, Batavia, showing the spleen rates found in Dutch Borneo between 1930 and 1938. Out of 33 spleen rates recorded, only 5 were under 20 per cent, the majority were over 60 per cent and 13 were between 80 and 100 per cent.

In Sarawak, although few figures are given in Government reports regarding the intensity of the disease, records show that much work has been caused to the Health Department by endemic, and occasionally epidemic, malaria.

During the work of the Malaria Research Department of North Borneo before the war, Jesselton Town was found to be a healthy area, with probably no malaria transmission, but in close proximity to many areas of intense malaria. Tambunan, as elsewhere in the interior, was found to be highly malarious except in localities isolated in the middle of irrigated paddy fields or cleared areas (McArthur, 1947*a*).

During the war malaria investigation was brought virtually to a standstill, but with the reoccupation of the Island following the Japanese surrender, work was carried out by Australian Army Antimalarial Units, and in 1947 malaria research was re-established. English (1945), summarizing the findings of the army malaria units, recorded no splenic enlargements out of 325 children examined at Tarakan, and only two with parasites out of 235, but in Labuan spleen rates up to 72 per cent were recorded, in Weston 55 per cent and in Balikpapan 43 per cent out of 333 examined, although several settlements gave spleen rates of 80 to 90 per cent.

Strahan (1947) recorded a parasite rate of 60 per cent, mainly quartan, with a spleen rate of 25 per cent, at Quop, Kuching, and a spleen rate of 50 per cent at the 21st mile, in addition to the examination of schools in the Miri area.

Since the war, also, spleen surveys have been recorded from the east coast (McArthur, 1947*c*), from Labuan (Gillies, 1948*a* and *b*), from Dutch Borneo (Heilbrunn, 1948), from the Sarawak oilfields (Johnson, 1949*c* and *d*), from the Lower Rejang (Wallace, 1949), and North Borneo (Wyile, 1949; O'Neill, 1949).

Spleen and parasite surveys for the Tambunan experiment in 1949 confirmed the previous finding of hyperendemicity in the area selected for experimental control measures (McArthur, 1950).

RECORDS AND THEIR VALUE.

In Appendix I, the information from all these sources is set out to represent as nearly as possible the original records.

The value of the records is somewhat limited by the fact that it is difficult to summarize the conclusions in accurate figures. This is firstly because the results of spleen examinations may in themselves be misleading, and secondly because the different series recorded are not comparable in the degree and kind of information they present.

There are always dangers in attempting to draw conclusions from spleen rates. Schools, for example, offer a convenient means of obtaining children for examination, but tend to select the healthier children and to omit the sickly who stay at home. They also select the wealthier and may exclude certain races.

Again, there is a tendency, apart from schools, to make examinations in areas which will give an interestingly high spleen rate, and to ignore or omit to record those areas where spleen rates are likely to be nil.

In the same way, there are dangers in attempting to summarize results which may not be comparable and which may not be truly representative of an area. One examination may refer to hundreds of individuals, and another to a mere dozen. One may be a careful house-to-house examination, with a degree of accuracy, while another may be a school examination giving a totally wrong conception of the situation in the locality as a whole. Some surveys refer only to the examination

of children, and others to the adult population as well, and these things are not always specified in the records.

Finally, it is not possible to arrive at exact figures owing to the different modes of recording results, as this makes it impossible to compare one series with another; but bearing in mind the difficulties and the dangers of interpretation, the following is as nearly as possible an analysis of the study of Borneo spleen and parasite examinations.

ANALYSIS.

Since exact figures are impossible, localities have been grouped, in the summary below, according to the conventional classification of:

	Spleen rate, per cent.
Healthy areas	0–10
Areas of low endemicity	10–25
Areas of high endemicity	25–50
Hyperendemic areas	50–100

Some 30 or 40 observers have recorded more than 400 surveys carried out by themselves and others, in about 300 situations throughout Borneo.

Figures are given in Appendix I for over 32,000 individuals examined, either for spleens, or parasites, or both. About 80 of the surveys, however, do not record the number of persons examined, but give only the per cent (or in some cases only the number) positive.

On this basis, in so far as it is possible, the following is an analysis of the results of examinations in the 317 localities dealt with:

	Spleen rate, per cent.
About 30 localities were *healthy*	9
About 54 localities were *areas of low endemicity* .	11
About 82 localities were *areas of high endemicity* ...	26
About 172 localities were *hyperendemic areas* ...	54

An important point is that practically all the healthy areas are well-known places, such as Jesselton, Tawau, Labuan Town and elsewhere in North Borneo; Kuching and Miri in Sarawak; Brunei Town and Kuala Belait in the State of Brunei; and Tarakan, Sintang, and Tg. Selor in Dutch Borneo.

Twenty-three localities, or about 7 per cent, were recorded as having 100 per cent malaria.

CONCLUSIONS.

Ninety-one per cent of the areas examined throughout Borneo have been recorded as malarious.

More than half of the areas examined have been recorded as being subject to hyperendemic malaria.

Only 9 per cent of the areas examined have been recorded as healthy, and these are nearly all on the coast.

Thus, practically the whole of Borneo would appear to be malarious, most of it intensely so, and very few places indeed are healthy, most of these fortunately being the chief towns and centres of population.

MAP 1.

Distribution and Intensity of Malaria in Borneo.

I. HEALTHY AREAS.

(Spleen rate: 0 – 10 per cent.)

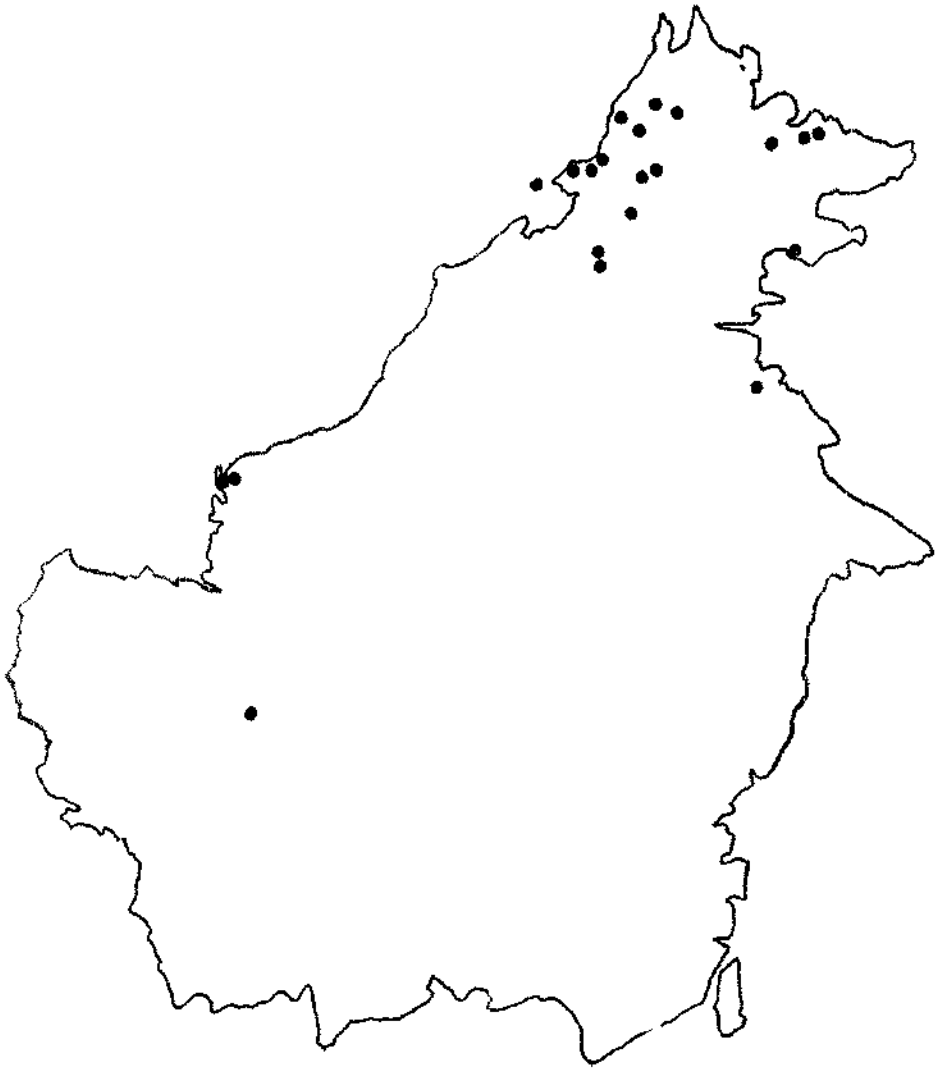
(It will be noted that some areas have yielded different results at different times, and therefore are recorded on more than one map.)

MAP 2.

Distribution and Intensity of Malaria in Borneo.

II. Areas of Low Endemicity.

(Spleen rate: 10 – 25 per cent.)



MAP 3.

Distribution and Intensity of Malaria in Borneo.

III. Areas of High Endemicity.

(Spleen rate: 25 – 50 per cent.)

MAP 4.

Distribution and Intensity of Malaria in Borneo.

IV. Areas of Hyperendemic Malaria.

(Spleen rate : 50 – 100 per cent.)

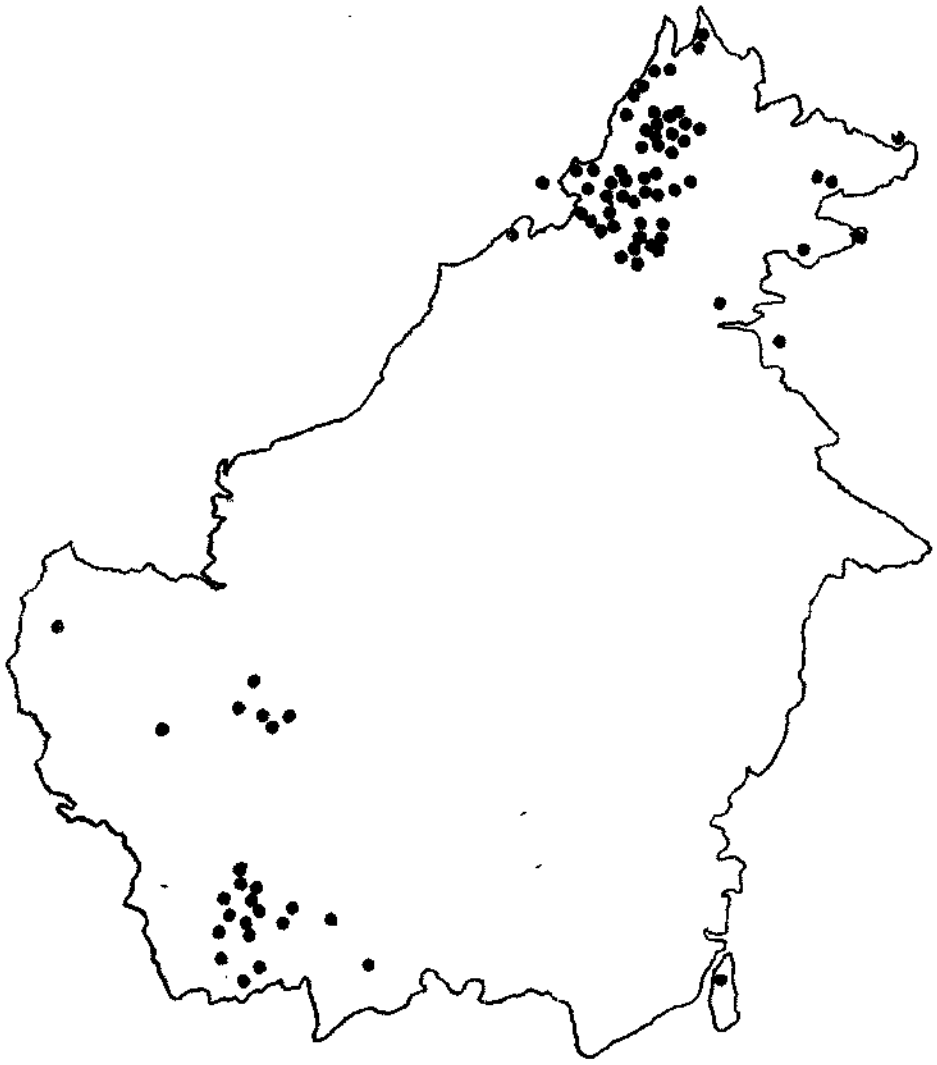
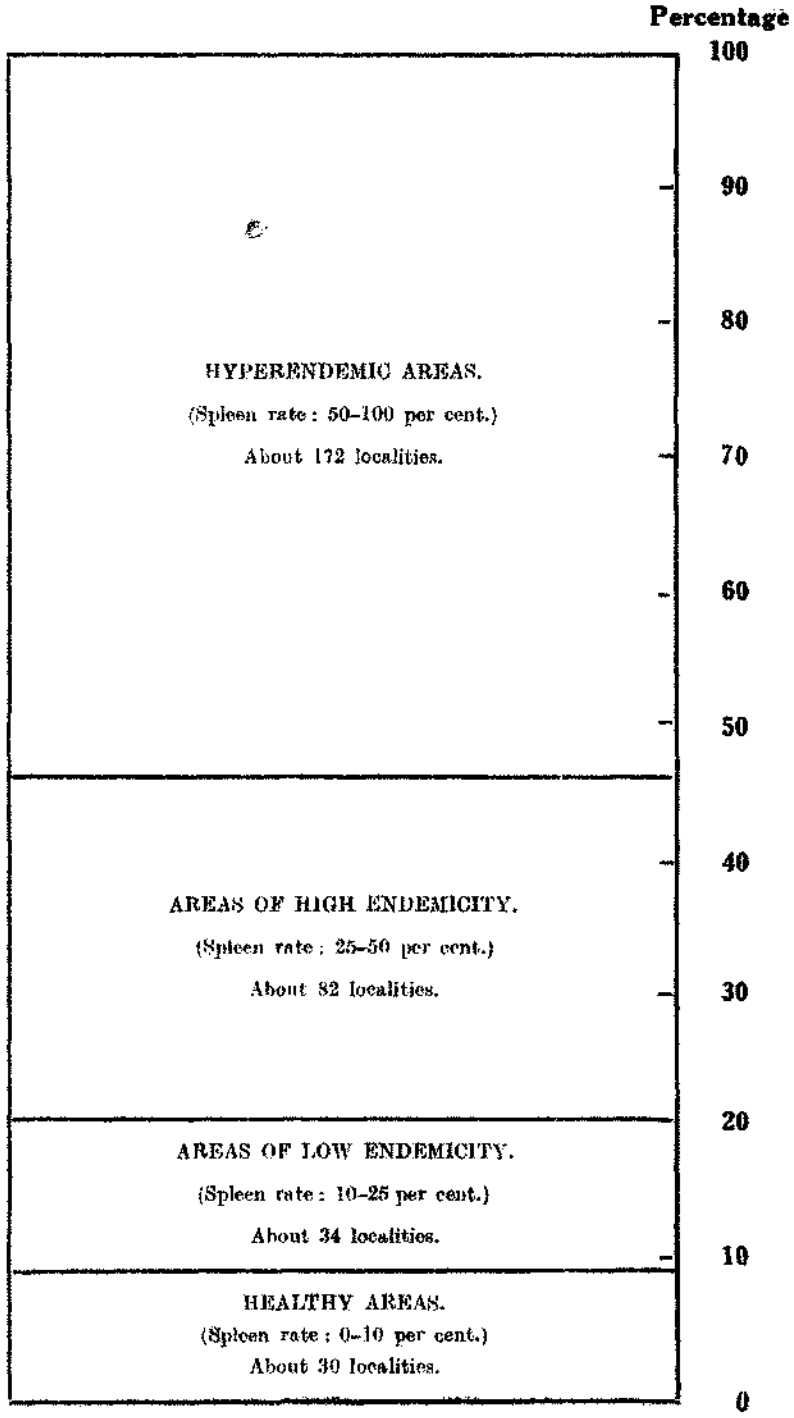


CHART 1.

INTENSITY OF MALARIA IN BORNEO.

An analysis of the 300 areas examined, according to their degree of endemicity.



II. EPIDEMIC MALARIA.

Epidemic malaria is a condition which occurs in healthy communities—that is among non-immunes—when they are exposed to unaccustomed infection.

Epidemic malaria may occur for example in a normally healthy area with some change in conditions such as the abnormal breeding of vector mosquitoes, or the introduction of large numbers of infected persons. Again epidemic malaria may occur in parties of healthy people who for some reason move to malarious situations.

Epidemic malaria does not occur among the inhabitants of intensely malarious endemic areas, except under unusual circumstances such as the introduction of some new strain of parasite, because the people have built up a degree of immunity to the local infection.

For these reasons, since healthy areas are few in Borneo, epidemic malaria is not common.

EPIDEMIC RECORDS.

Stookes (1924 ; 1927) appears to make the first record of epidemic malaria in the oilfields of Sarawak in 1915.

In about 1920, there was intense malaria, with many deaths and many cases admitted to hospital, in the newly established Northern Chinese Settlement three miles from Jesselton (McArthur, 1948). There is now no malaria there, and this epidemic was almost certainly due to the establishment of the settlement in a malarious area, from which, however, the vector has now been eradicated by jungle clearance.

Campbell (1931) records epidemic conditions following the introduction of infected Javanese workers to Sapong Estate, North Borneo, in 1925-26, a locality in which there now appears to be little or no malaria transmission.

An epidemic of malaria, so severe as to justify the abandonment of the buildings, was recorded (Sarawak, 1930) in the newly established Leper Colony on Satang Island, Sarawak, due, it appears, to the removal of a malaria-free community to a malarious situation.

Epidemic malaria appears to have been a constant problem in the Sarawak oilfields, due, partly, at least, to the constant population movement and admixture of non-immunes and infected persons, but probably also to abnormal breeding of Anopheles.

In addition to that in 1915 recorded by Stookes, there was a small outbreak in the Tg. Lobang area in 1935, and another in 1939. In Lutong it was of annual occurrence between May and July from 1924 to 1933. In Matading there was an epidemic in 1936 following the introduction of 300 coolies, and in Jerudong camp there was an outbreak in 1938-39. In Miri, it was recorded that although malaria was rife in former years, there was none since 1924 until 1941.

In 1941, there was a fairly widespread malaria epidemic throughout the oilfields area, recorded from Miri—St. John's Wood and Tg. Lobang areas—Lutong, Loak-Bakan area, and Bintulu (Webster, 1941*a*).

There are other records of epidemic malaria among healthy labour gangs going to work in malarious areas, such as that working on the Tuaran-Tenghilan Road in North Borneo in 1938.

During the war, epidemic malaria was stated by the Japanese to be in progress among the fishery staff on Banggi Island and among those employed in making the Jesselton Airfield in 1943, and it occurred among prisoners of war in both Dahan Camp, Kuching, and Labuan in 1944, all these apparently being malaria-free communities transferred to malarious areas (McArthur, 1944).

Following the Japanese surrender, a widespread epidemic of malaria occurred chiefly in coastal towns, but also in the normally malarious areas. This had probably a variety of causes, but by far the most important appeared to be the flight during the war to the malarious interior by the healthy coastal inhabitants, their infection there, and their return after the war. This resulted in epidemic conditions both in the interior during their flight, and on the coast following their return, although the epidemic on the coast may have been largely in the absence of vector mosquitoes.

This epidemic was studied by Strahan (1947), who in December 1946 recorded outbreaks along the coastline of Sarawak and Brunei from May till November, occurring at the mouth of the Kuching River, Bintulu, Miri, Lutong, Kuala Belait and Seria in Brunei. Other reports came from Medical Officers throughout the island.

CONCLUSIONS.

Thus, Borneo's malaria problem is chiefly one of intensely endemic malaria throughout the greater part of the land, which probably fluctuates little from year to year. With the existence of healthy areas in close proximity to highly malarious ones however, and with the occurrence of occasional coastal epidemics, epidemics still require to be regarded with caution in the coastal town areas and other healthy situations, and particularly among parties, for example, of healthy persons moving to the undeveloped malarious interior.

III. MALARIA PARASITES.

VALUE OF THE RECORDS.

Like other sources of information, those regarding the malaria parasite identifications of Borneo vary enormously in value. Some records were made by dressers who had received little, if any, training in microscopical diagnosis, while others were made by competent parasitologists; and between these extremes it is difficult to assess the degree of dependence that can be placed on the records.

IDENTIFICATION RECORDS.

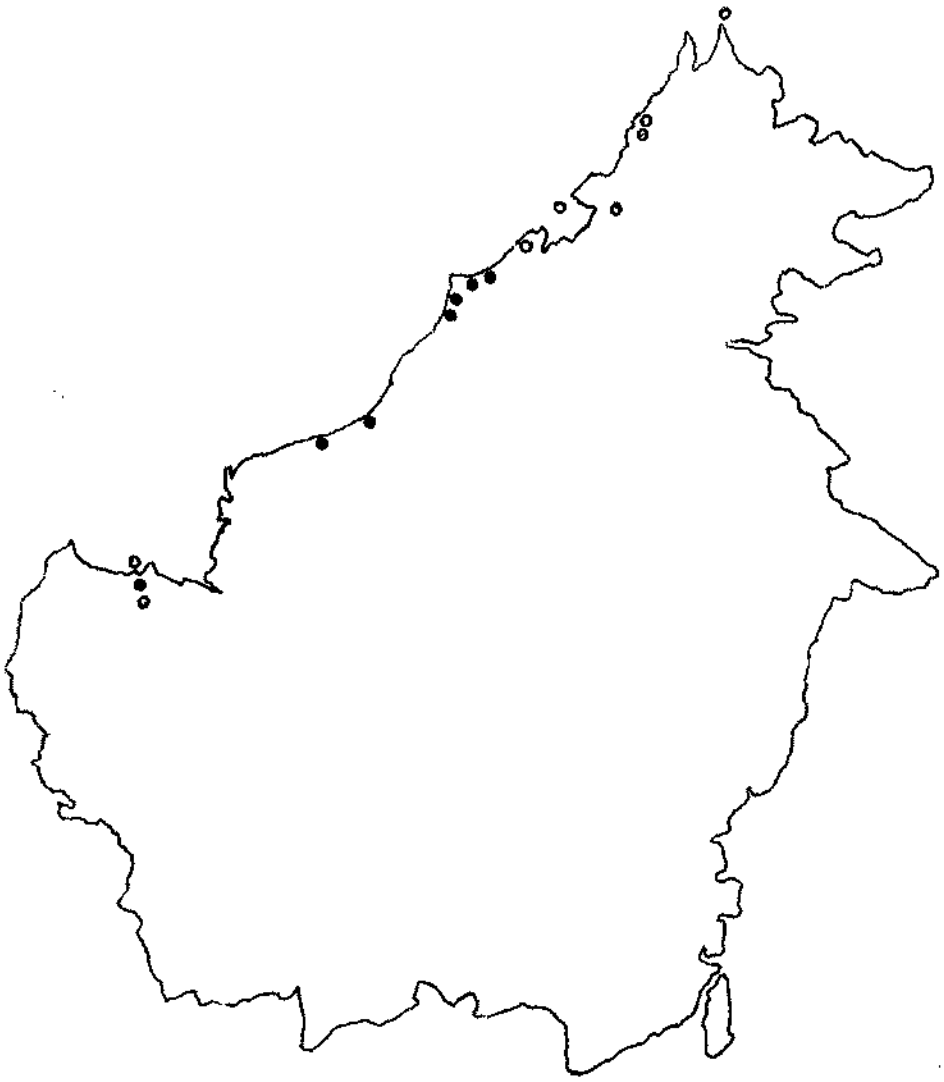
Campbell (1931) stated that from 1917 to 1925, benign tertian malaria had predominated on Sapong Estate, North Borneo, with sporadic cases of quartan and malignant tertian. Owing, however—it was claimed—to the importation in 1925–27 of infected Javanese labour, malignant tertian had become predominant.

He believed that later, as a result of treatment, the incidence of malignant tertian was reduced, benign tertian becoming predominant as a result of infection from the large Murut weeding force employed during 1928-29.

MAP 5.

The Malaria Epidemics of Borneo.

In existing healthy population — ●
In those moving to malarious areas — ○



In Jesselton Civil Hospital, during the course of 20 years, 1919–38, the species of parasites identified were recorded, and these are among the few figures which have survived the war (McArthur, 1939). It would seem that, prior to 1934, *P. falciparum* was the predominant form in the Jesselton District, replaced after that year by *P. vivax*. *P. malariæ* was recorded in the minority except in 1921. Other records of local figures preserved during the war were those of species of parasite, identified in Tambunan Dispensary in 1931–40. These figures recorded *P. falciparum* as predominant during 7 years out of 10, replaced during the other three years by *P. vivax* (McArthur, 1948).

The Sarawak Annual Medical Reports before the war, 1922–38, recorded *P. falciparum* as usually the most common species, and *P. malariæ* the least; so in Kuching, Sibu and elsewhere.

Brunei Annual Medical Reports record laboratory returns regarding malaria parasites between 1931 and 1938 for Brunei (a healthy area), Tutong (an endemic area), and Temburong (probably also an endemic area).

Shircore (1937) in North Borneo recorded a preponderance of *P. vivax* on the west coast, with *P. falciparum* even less common than *P. malariæ*; while in the interior he recorded *P. malariæ* as even more common than *P. vivax* and *P. falciparum* together. He contrasts his findings with those of Copeland, who in 1933 concluded that the predominant parasite among the Muruts was *P. falciparum*.

Ou (1941), describing the very mild malaria of Labuan at that time, recorded a considerable preponderance of *P. vivax* out of 208 classified cases of malaria.

Webster (1941*a*), describing the epidemic conditions in the Sarawak oilfields during that year, stated that the prevalent parasite was *P. falciparum*, but *P. vivax* and *P. malariæ* were also encountered.

The Malaria Research Department in Tambunan recorded 56 per cent *P. falciparum*, 26 per cent *P. vivax*, and 18 per cent *P. malariæ* out of 876 blood examinations in 1940 (McArthur, 1948). It was noted that the proportion of *P. malariæ* was higher as a rule in the more malarious villages, that there were "pockets" of high intensity *P. malariæ* in some hill ravines, and that *P. falciparum* was the parasite which was most commonly observed being introduced to the interior from estates and elsewhere.

Strahan (1947) recorded *P. falciparum* in the majority during his survey of Sarawak in the epidemic of 1946, and quoted the oil company records as showing that 90 per cent of their diagnoses were *P. falciparum*, although at Quop, near Kuching, he found an infection rate of 50 per cent, mainly quartan.

The oil company in Sarawak and Brunei quotes extensive figures for 1947, with benign tertian malaria in the majority in Miri, Kuala Belait and Seria, but malignant tertian in the majority in Lutong (Johnson, 1948).

Surveys for the Tambunan Experiment, June 1949, showed a great preponderance of *P. falciparum*, this species forming 94 per cent of infections in children aged 2 to 10 years (McArthur, 1950).

CONCLUSIONS.

It would appear therefore that the predominant parasite in Borneo tends to be generally *P. falciparum*, especially under epidemic conditions; but *P. vivax* has often been found to be in the majority. *P. malariæ* is perhaps more common than in most parts of the tropics, in some localized areas even appearing to predominate.

IV. ANOPHELES IDENTIFICATION RECORDS.

Borneo forms part—together with Sumatra, Java and Malaya—of the Malayan sub-region, and the Anopheles are closely related to those found in Malaya, although it is by no means safe to assume that they are the same in identity, habits or relation to malaria.

The sources of information regarding the identification of Anopheles in Borneo are widely distributed, and some are more reliable than others; while a good deal remains to be done regarding their precise identification and the assessment of their vectorial rôle.

IDENTIFICATION RECORDS.

Identifications and other studies have been carried out in each of the territories—North Borneo and Labuan, Brunei, Sarawak, and Dutch Borneo—and the present knowledge, incomplete though it is, is surveyed in the following pages, and summarized in Appendix IV.

The first reference to species of Anopheles in Borneo appears to be a series of seven occurring in a list of 37 mosquitoes, published by Hewitt (1905) in the Report of the Sarawak Museum.

Some years later Roper (1914), a medical practitioner on the west coast of North Borneo, published an account of some anopheline mosquitoes found in British North Borneo, with a description of a new species. His list contained 10 species.

Also in 1913 and 1914 specimens of 5 species of Anopheles were sent from Mawao Estate, North Borneo, to the collection of the Institute for Medical Research, Kuala Lumpur (Hodgkin, 1941) which were probably from the collection of Roper; and others were sent by Roper to the British Museum.

In the same year, a further list of the mosquitoes of Borneo was published by Moulton (1914), Curator of the Sarawak Museum, in collaboration with Edwards of the British Museum. This list contained the names of 92 mosquitoes, 12 of which were Anopheles. It was later supplemented by Edwards (1926) with the addition of a further Anopheles.

In 1916, Swellengrebel published in Dutch an account of Anopheles of Netherland East Indies. This was later completed and amended, and a second addition was published in 1921. This contained a list of 9 Anopheles found in Borneo, recorded by Dr. H. N. van der Heyden and Dr. Hartog Jager, in Balikpapan, Tandjong Redeb, and Moearatewe, in Dutch Borneo.

Stookes (1924), Medical Officer to the Sarawak oilfields, recorded 4 species of Anopheles in the Miri area.

Later, Rodenwaldt (1926) recorded the collection of 3 species of Anopheles in Dutch Borneo.

In the following year, two articles were published regarding Borneo Anopheles. One (Stookes, 1927) listed 10 Anopheles in the Miri area and the other (Scharff, 1927) followed a larval survey of Labuan, and listed 8 Anopheles.

The results of a larval survey in Jesselton were recorded on maps and in a report to the North Borneo Government by Dingle (1929). This report contained the names of 9 species of Anopheles; and subsequent surveys in Sandakan, Tawau and the interior of North Borneo recorded 11 species (North Borneo, 1931).

Walch and Soesilo (1929) published notes regarding tours made in Dutch Borneo and elsewhere, and recorded three species. In the same year von Kuehlewein recorded 2 species in Dutch Borneo (Goelarso, 1934); while Stoker (1931) recorded 5 species of Anopheles in Dutch Borneo and was referred to by Soesilo *et al.* (1932) as having dissected numbers of 3 others.

In North Borneo, 2 species were referred to by Campbell (1931) on Sapong Estate.

A reference was made to the Anopheles of Dutch Borneo with a list of 19 classified according to locality by Swellengrebel and Rodenwaldt (1932).

Stoker (1934), in a survey of Poeloe Laoet off South-East Borneo found 6 species of Anopheles, and was recorded by Soesilo *et al.* (1935) as having carried out large numbers of dissections on a seventh. In the same year Goelarso (1934) in Boeloengan, East Borneo, recorded 13 species of Anopheles from the coast and the interior, and Kariadi (1938), during a filariasis survey in Martapoera, South-East Borneo, also found 7 species of Anopheles.

In Brunei (1929–38) the first record, in 1929, mentioned 4 species of Anopheles as having been identified. Later, in 1932, a more careful examination was begun, and during three years 7 had been added to the list. In 1935 the enumeration of Anopheles larvæ was begun, and a systematic annual record for four years mentioned 20 species within and outside Brunei Town Sanitary Board Area, and in Kuala Belait.

In addition to the list of Anopheles published by the Sarawak Museum and by Stookes, Government Reports (Sarawak, 1930–39) record larval surveys which were carried out regularly in Kuching and in a number of outlying districts, with a total of 22 species of Anopheles identified during ten years.

In the Eykman Institute (1938), Batavia, a wall map before the war summarized the identifications made by the Malaria Division of the Netherlands Indies Public Health Service in Borneo since 1930. This in 1939 contained the names of 24 Anopheles; while a further list, summarizing the identifications of Stookes and Swellengrebel and Rodenwaldt, was published by Gater (1935).

Shircore (1937), in the course of his health survey of North Borneo, recorded 7 species; and Carlile (1941) recorded 12 species in timber camps and elsewhere on the east coast.

Webster (1941*b*), in the oilfields territory, listed 18 species, 16 in Sarawak and 11 in Brunei; and Ou (1941) referred to 11 species as having been taken, 10 in Labuan, and 1 in the neighbouring island of Pulau Da'at.

The Anopheles recorded by the Malaria Research Department in North Borneo during the three years prior to the Japanese occupation totalled 15, 13 in the interior and a further 2 at the coast (McArthur, 1948).

During the Japanese occupation of Borneo practically all investigations ceased, except for some work carried out under the extreme difficulties of internment. Eight species are recollected as having been dissected by three doctors while confined to the Kuching General Hospital (Clapham, 1948).

Three new species are stated to have been discovered by a Japanese medical officer during the war (Shinonaga, 1943), but further information regarding this has not been obtainable.

With the reoccupation of Borneo, observations were made by the Australian Army Entomological Sections. Allman (1945) listed 20 Anopheles in North Borneo, Sarawak and Brunei, and Clinton recorded 14 species in Balikpapan (English, 1945). Rutledge (1947) in Jesselton recorded dissections on 6 species.

Following work with the Australian Entomological Sections, Colless (1948*a*) published a systematic study of the Anopheles of North-West Borneo, in which he listed 21 as having been identified, and 33 as having been recorded from the area.

Strahan (1947), during his survey of the Sarawak epidemic, recorded 1 species; Heim (1948) in the Ulu Kinabatangan recorded 5: and Webster (1947) recorded 8 in the Sarawak oilfields.

Heilbrunn (1948) records 18 species as having been identified in Western Dutch Borneo during the thirteen years 1935–47. Johnson (1949*b*), in Kuala Belait, lists 18 Anopheles in the oilfield's territories, 14 in Brunei and 16 in Sarawak; Gillies (1949) has recorded 13 species from Labuan; and Wallace (1949) 6 from the Lower Rejang.

During 1949 at the Malaria Research Headquarters in Labuan, identifications have been definitely established of 29 species and sub-species in Borneo (Colless, unpublished).

CONCLUSIONS.

From these sources of information, some 58 names of Anopheles have been recorded. Of these, 29 appear to exist in Borneo beyond question, 12 are synonyms, 13 have not been confirmed, and 4 appear to be errors and almost certainly do not exist in Borneo.

Thus, some 42 species appear to have been recorded with some degree of probability, 29 of which can be accepted with fair confidence.

SUMMARY OF RECORDS.

The following list summarizes the species of Anopheles which have been collected from some 42 sources—some reliable and some otherwise—throughout the whole of Borneo between 1905 and the end of 1949 (Appendix IV).

In this list, asterisks indicate those forms whose existence in Borneo appears to be beyond question. Those in brackets are synonyms. Those queried are very doubtful and almost certainly do not occur in Borneo. Those in inverted commas have not yet been formally established.

The figures refer to the footnotes given in Appendix IV*a*.

Genus **Anopheles** Meigen
Sub-genus **Anopheles** Meigen
Group **Anopheles**

Series **Anopheles.**

* *A. aitkeni aitkeni* James (12).
* *A. aitkeni bengalensis* Puri.
* *A. aitkeni borneensis* McArthur.
(*A. aitkeni*, form II) (28).
(*A. aitkeni*, form III) (29).
* *A. aitkeni palmatus* Rodenwaldt.
* *A. brecipalpis* Roper.
A. gigas sumatrana Swellengrebel and Rodenwaldt.

Series **Myzorhynchus.**

* *A. albotaeniatus* Theobald.
* *A. bæzai* Gater.
(*A. bæzai gateri* Baisas) (23).
* *A. barbirostris* Van der Wulp.
(*A. barbirostris* var. *pallida* Swellengrebel) (18).
* *A. barbumbrosus* Strickland and Choudhury.
A. hunteri Strickland (19).
A. hyrcanus Pallas (13).
* *A. hyrcanus* var. *nigerrimus* Giles.
'*A. hyrcanus* sub. sp. nr. *sinensis*' (22).
* '*A. hyrcanus* C' (30).
* ('*A. hyrcanus* Coastal') (31).
'*A. hyrcanus* S. Venhuis (27).
* *A. letifer* Gater.
A. montanus Stanton and Hacker.
(*A. nocumbrosus* Strickland) (7).
* *A. separatus* Leicester.
A. sinensis Wiedemann (1).
* *A. umbrosus* Theobald (2).
('*A. sp. A.* nr. *umbrosus*') (24).
* '*A.* sp. *B.* nr. *umbrosus*' (25).
(*A. vanus* Theobald) (3).

Series **Lophoscelomyia.**

A. asiaticus Leicester.

Sub-genus **Myzomyia** Blanchard
Group **Neomyzomyia.**

* *A. hackeri* Edwards (14).
* *A. kochi* Dönitz.
(*A. leucosphyrus* var. *balabacensis* Baisas) (26).
* *A. leucosphyrus* Dönitz (4).
* *A. leucosphyrus* var. *pujutensis* Colless.
? *A. punctulatus* Dönitz (5).
(*A. punctulatus* var. *tessellatus* Theobald) (8).
* *A. tessellatus* Theobald.
* *A. watsoni* Leicester.
* '*A.* n. sp. nr. *watsoni*'.

Group **Myzomyia.**

A. aconitus Dönitz.
? *A. minimus* Theobald (21).
A. minimus var. *flavirostris* Ludlow.

Group **Neocellia.**

? *A. annularis* Van der Wulp (17).
A. errabundus Swellengrebel (16).
(*A. fuliginosus* Giles) (11).
* *A. karwari* James.
* *A. maculatus* Theobald.
* *A. philippinensis* Ludlow.

Group **Pyretophorus.**

? *A. litoralis* King (20).
A. ludlowi Theobald (6).
(*A. rossii* Giles) (9).
A. subpictus Grassi (15).
* *A. subpictus* var. *malayensis* Hacker.
? *A. sundaicus* Rodenwaldt.
A. vagus Dönitz (10).
* *A. vagus* var. *limosus* King.

V. MOSQUITO DISSECTIONS.

IMPORTANCE OF DISSECTIONS.

Although malaria is only carried by Anopheles mosquitoes, very few species of Anopheles—usually about 1 in 10—are actually concerned with transmission, and the rest are of little or no importance.

Unfortunately, however, species vary in their relation to malaria from place to place, and a species which is harmless in one country may be dangerous in another. It is therefore of primary importance in a malarious land such as Borneo to assess the relation to malaria of the different species that may be present.

In order to determine whether a species is a malaria vector in a given locality, considerable numbers, if possible several hundreds, must be dissected and examined for malaria parasites. If sporozoites are found in the glands of a species it is possible to know with certainty that it is a dangerous insect. If oöcysts only are found in the gut it must be regarded with suspicion. If nothing is found in adequate numbers of dissections, the supposition is that the species is of little importance or harmless.

Without dissections there are no means of knowing with certainty what mosquitoes are carrying malaria, and therefore what measures will be effective against them. Without dissections all mosquito control measures are blind, and it is impossible to know whether measures are being directed effectively or being dissipated against harmless insects. Without dissections, measures are in danger of increasing the very disease they are intended to control.

With adequate dissections, however, we are able to know with certainty what insects are dangerous and what are harmless, so that eradication work can be carried out intelligently, economically, and with confidence.

Dissections are, therefore, the most important study that can be carried out in an attempt to eradicate malaria from a land.

MOSQUITO DISSECTIONS.

The first dissections in Borneo appear to have been carried out in 1914, and since that time they have been recorded from all parts: North Borneo and Labuan, Brunei, Sarawak and Dutch Borneo.

Unfortunately, the existing dissection records are very variable in the degree of data they present, and in some cases it is impossible to express exactly, and even more difficult to summarize, all the necessary figures. It has therefore been felt best to record the information exactly as given in each reference, and to summarize where possible only the (probable) totals dissected, with if possible the number of positive gut and gland dissections, and the (probable) proportion infected. While not absolutely exact, the chances of error are not great enough to influence the final figures and the conclusions.

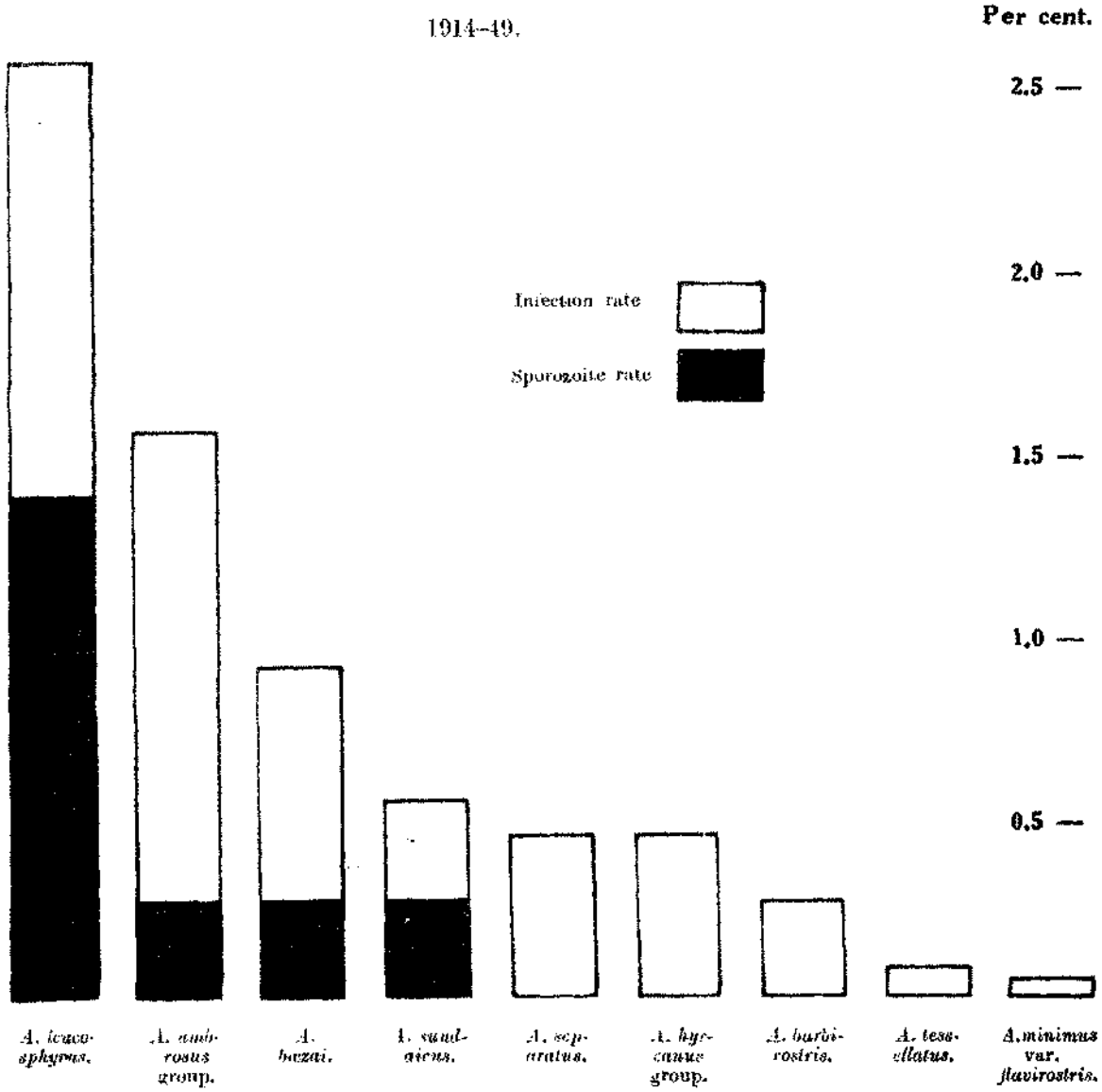
These figures are tabulated in Appendix V. They are summarized in Tables I to III and the relative proportions of the positive dissections are illustrated diagrammatically (Chart 2).

CHART 2.

THE POSITIVE MOSQUITO DISSECTIONS OF BORNEO.

From 24,000 dissections.

1914–49.



TOTAL DISSECTIONS.

Over 24,000 dissections, on 16 species or groups of Anopheles, are recorded as having been carried out throughout Borneo between 1914 and the end of 1949. Of these, 207 were positive, and 72 yielded sporozoites.

The results of these dissections probably give a fairly true picture of the relative importance of the different vectors throughout the island.

TABLE I.

Species yielding sporozoites.

Species.	Number dissected.	Number positive.	Number with sporozoites.	Percentage infected.	Sporozoite rate, per cent.
A. leucosphyrus	3,235+	90	49	2·8	1·5
A. umbrosus group ..	2,914+	50	9	1·7	0·3
A. sundaicus	4,485	29	12	0·6	0·3
A. hackai	693	7	2	1·0	0·3

TABLE II.

Species yielding oöcysts only.

Species.	Number dissected.	Number positive.	Percentage infected.
A. separatus	3,583	17	0·5
A. hyrcanus group	1,557+	8	0·5
A. barbirostris	1,002+	3	0·3
A. tessellatus	1,000	1	0·1
A. minimus var. *flavirostris* ...	2,770	2	0·07

The *A. umbrosus* group includes *A. umbrosus*, *A. letifer* and *A.* sp. *B.* (*A. novumbrosus* = true *A. umbrosus*), and before 1938 the precise identification was in doubt (Appendix IV*a*, note 2). Positives have been recorded for *A. umbrosus*, *A. letifer* and *A.* sp. *B.*

The *A. hyrcanus* group includes *A. hyrcanus* var. *sinensis*, *A. hyrcanus* var. *nigerrimus*, and the as-yet-undescribed *A. hyrcanus* Coastal, *A. hyrcanus* X and *A. hyrcanus* C (Appendix IV*a*, notes 22, 27, 30 and 31).

TABLE III.

Species negative on dissection.

Species.	Number dissected.
A. philippinensis	1,880
A. kochi ...	875
A. karwari ...	197
A. maculatus ...	186
A. albotaeniatus	25
A. brevipalpis	13
A. barbumbrosus	3+

CONCLUSIONS.

Records have been traced of over 24,000 dissections having been carried out throughout the whole of Borneo during 35 years.

From an analysis of these it is concluded that, out of 16 species or groups of Anopheles dissected, 9 have been found infected, and 4 have been found with sporozoites—and therefore proved vectors of malaria—in the following order of intensity :

Species.	Per cent infected.	Sporozoites rate, per cent.
A. leucosphyrus	2·8	1·5
A. umbrosus group ...	1·7	0·3
A. sundaicus	0·6	0·2
A. bazai	1·0	0·3
A. separatus	0·5	...
A. hyrcanus group ...	0·5	...
A. barbirostris	0·3	...
A. tessellatus	0·1	...
A. minimus var. *flavirostris* ...	0·07	...

It would appear therefore that 4 species or groups of Anopheles have actually proved on dissection to be vectors of malaria in Borneo.

Proved vectors.—*A. leucosphyrus*, although regarded as harmless in Malaya and elsewhere, has shown consistently the highest rates of infection, and appears to be most widely distributed in Borneo. It is almost certainly responsible for high hyperendemicity throughout the jungle areas of the island.

Species of the *A. umbrosus* group also are undoubtedly important, although probably not so important as *A. leucosphyrus*, and more limited in distribution.

A. sundaicus has often been claimed to be an important vector in some parts of Borneo, and as it tends to occur in coastal towns its importance may be considerable. It has only recently been found with sporozoites, however, although oöcysts have been observed on dissection for many years; and the evidence suggests that normally this insect may have a little or no relation to endemic malaria, but that periodically it may be responsible for epidemics in some localities.

A. baezai has only recently been recorded with sporozoites on dissection, and it has never been under great suspicion on the indirect evidence. While it must be regarded as a likely vector, there is as yet little evidence that it is of any great significance.

Possible vectors.—There is some evidence that *A. separatus* has a certain appetite for human blood, and the recording of a number with oöcysts adds weight to the suspicion that it may take some place in transmission in those rather limited areas in which it occurs.

Species of the *A. hyrcanus* group are also under some suspicion in view of their reputation elsewhere, and of the finding of a small proportion with oöcysts in Borneo. This group however is also limited in distribution, and does not appear to be of much importance.

A. barbirostris, with only 3 gut infections recorded out of over 1,000 dissections, and no indirect evidence of any relation to malaria, cannot be considered normally to have any importance; although the possibility of different forms, and its occasional importance in Malaya, suggests the necessity of further observation.

A. tessellatus, with a record of only one oöcyst out of 1,000 dissections, cannot be considered normally to have any importance.

A. minimus var. *flavirostris*, although found infected, and at one time claimed to be the chief vector of malaria in Dutch Borneo, is extremely limited in distribution, and has such a small index of infection that it is probably of no consequence.

Thus, in addition to the four proved vectors of Borneo malaria, five other species have been recorded as infected on dissection. None of these are under any strong suspicion on epidemiological grounds, and while further work is necessary the slender evidence available would not appear sufficient to justify any active measures against them.

Negative dissections.—Of the species found negative on dissection, only *A. philippinensis* and *A. kochi* might be considered as having been examined in adequate numbers to justify the conclusion that they are normally harmless in Borneo.

A. maculatus and *A. karwari* have scarcely been dissected yet in adequate numbers, although the evidence so far—both direct and indirect—supports the view that they take no part in Borneo malaria transmission.

This is of particular importance in the case of *A. maculatus*, for it is of major importance in Malaya, and has often been stated to be the chief vector of malaria in Borneo. The results of those dissections which have been carried out, and the study of its habits and of the epidemiological evidence, however, are in keeping with its being of little and probably no consequence in Borneo.

Thus, it appears probable that *A. leucosphyrus* is responsible for maintaining the great reservoir of malaria throughout the interior and many parts of the coast of Borneo; that members of the *A. umbrosus* group are responsible for endemic malaria to a rather more limited extent; and that *A. sundaicus*, while possibly having little relation to endemic malaria, may threaten certain coastal areas periodically with epidemics.

VI. MALARIA SITUATION.

From a summary of the facts collected from all over the island, the present conception of malaria in Borneo is of a vast reservoir of infection throughout the whole interior, with a fringe of some healthy areas around the coast.

Infection in this reservior is maintained chiefly by a shade-breeding mosquito—*A. leucosphyrus*—which is eliminated by clearing and cultivation consequent upon the extension of civilization.

Thus, in contrast to many countries, the frontiers of malaria in Borneo are pushed steadily back by the march of progress. In addition, they can probably be deliberately pushed back even more rapidly, and at a fraction of the cost of malaria eradication in other countries, by controlled agriculture and by the economic development of the island.

The facts regarding malaria, its vectors, and its control in Borneo, therefore, differ very radically from those in Malaya and other parts of the world.

This is important, because it means that a great deal of the valuable information published in Malaya and other neighbouring countries, not only does not apply to Borneo, but is the reverse of fact there.

Measures which destroy the most dangerous mosquito in Malaya, *A. maculatus*, encourage the apparently most dangerous mosquito in Borneo, *A. leucosphyrus*. Thus, measures which abolish malaria in Malaya may make it more intense in Borneo; and the very measures which must be avoided in Malaya as creating

intensely malarious conditions are those which, in Borneo, are most calculated to make a healthy land.

For this reason it is necessary completely to reverse many of our conceptions of malaria and its control in Borneo, and to forget much that has for long been taught and accepted as fact, before re-learning the facts as they have come to light during recent work.

Only by so doing, can antimalaria measures be properly directed, and an effective campaign be carried out against this greatest disease problem in the island. Antimalaria measures in Borneo which are not based upon the facts which apply locally, but are only based upon the experience of other countries, are an avoidable waste of lives, health, and economic prosperity, as well as of the time, effort, and money expended upon them.

SUMMARY AND CONCLUSIONS.

Studies have been made, by a search of the records for past years and by personal observations, regarding malaria and its vectors throughout the whole island of Borneo—North Borneo and Labuan, Sarawak, Brunei and Dutch Borneo.

These studies deal with the distribution and intensity of malaria, the occurrence of epidemic malaria, the parasites, the species of Anopheles recorded, and the results of dissections, in an endeavour to make as complete a picture as possible with the data so far available, as a foundation for future work.

A study is made of the distribution and intensity of malaria throughout the island of Borneo, as indicated by the records of about 200 situations by some 30 or 40 observers. It is concluded from this that practically the whole of Borneo is intensely malarious, with the exception mainly of the chief towns and centres of industry.

There are records of over a dozen epidemics, some of which have been due to the introduction of malaria to healthy areas, and some to the movement of healthy populations to malarious zones.

Identification records of malaria parasites have been studied from hospital, dispensary, malaria survey and other medical reports. The records are not regarded as very reliable. *P. falciparum* has generally been recorded as more predominant, but *P. vivax* often so. *P. malariæ* appears to be rather more common than in most tropical countries, having been found to predominate in some limited situations in Borneo.

Much remains to be done regarding the precise identification of Anopheles mosquitoes in Borneo. Recognizing the great possibilities of errors in identification, 42 species or sub-species have been recorded, by about 40 observers throughout the island. They are tabulated in so far as possible exactly as they were originally recorded, and an attempt has been made to assess their probable accuracy.

The importance of dissections is discussed, a survey is made of all the dissection records that can be traced, and conclusions are drawn from these regarding the relative importance of the different species of Borneo Anopheles. Records have been found, covering 35 years, of about 24,000 dissections, of which 128 were positive. Of the 42 species of Anopheles recorded in Borneo, 16 of which have been dissected, 9 have been recorded as infected with malaria, but only 4—*A. leucosphyrus*, the *A. umbrosus* group, *A. sundaicus* and *A. bazai*—have been recorded with sporozoites, and therefore possibly important vector species.

Of the positive dissections, the majority were *A. leucosphyrus*. This mosquito, apparently the most widely distributed Anopheles throughout the island, has shown the highest rate of infection, and it seems reasonable to conclude that it is the most dangerous insect in Borneo, responsible for the bulk of hyperendemic malaria throughout the interior and in many parts of the coast.

Members of the *A. umbrosus* group, of rather lesser importance than *A. leucosphyrus*, appear to be responsible for endemic malaria in those areas in which they occur.

The evidence suggests that *A. sundaicus* is responsible for periodical epidemic rather than endemic malaria, in the limited situations in which it occurs.

A. bazai must be regarded as a vector, the importance of which however requires to be assessed.

Five other Anopheles—*A. separatus*, the *A. hyrcanus* group, *A. barbirostris*, *A. tessellatus* and *A. minimus* var. *flavirostris*—must be regarded as requiring further study before any final pronouncement is made on their possibly small relation to malaria.

A. maculatus, for long claimed to be the chief vector of malaria in Borneo—as it is in the interior of Malaya—has never given any evidence of being actually concerned with transmission in Borneo, and it appears to be of little and probably no importance there.

It is concluded that Borneo is a vast reservoir of endemic, generally hyperendemic malaria, with few healthy areas; that infection is maintained chiefly by *A. leucosphyrus*, and to a lesser extent by the *A. umbrosus* group; that certain coastal areas may be threatened periodically by epidemics due to *A. sundaicus*; that *A. bazai* appears to have some place in transmission; that a number of other species are under some suspicion; and that *A. maculatus*, previously claimed to be the chief vector in Borneo, appears to have little and probably no relation to malaria transmission.

Thus, a basis is beginning to appear for rational measures directed against Borneo malaria; but the main conclusion is that further work is necessary in order to define more fully the transmission, and the means necessary for the control of this, the greatest disease problem of the land.

REFERENCES.

ALLMAN, S. L. (1945) ... Personal communication, Aug. 6, 1945, Labuan, regarding work of the *Aust. Mob. Ento. Sec.*, 1945.

BRUNEI (1929–38) ... *Govt. Annual Medical Reports.*

CAMPBELL, G. G. (1931) ... '*Med cal Reports:* Government Medical Consultant, Interior'. Singapore.

CARLILE, J. P. (1941) ... Personal communication, Oct. 31, 1941. Sandakan.

CLAPHAM, G. (1948) ... ... *Ibid.*, June 24, 1948. Kuching.

CLARKE, M. C. (1941) ... *Ibid.*, Keningau.

COLLESS, D. H. (1948*a*) ... *Proc. Linnean Soc.*, New South Wales, **73**, 3-4.

Idem (1948*b*) ... Personal communication, June 16, 1948. Labuan.

Idem (1948*c*) ... *Report of a malaria survey in the Seria and Miri Oilfields, May to June 1948.* Labuan.

Idem (1948*d*) ... *Report of a malaria survey in the Miri-Lutong Area, Sarawak, November to December 1948.* Labuan.

COPELAND (1933) ... ... See Shircore, 1937.

CRANSTON, E. (1948) ... Personal communication, April 14, 1948. Segama.

CRAWFORD (1938) ... ... *Some anopheline pupæ of Malaya.* Government Printing Office, Singapore.

DINGLE, P. A. (1929) ... *An anopheline mosquito survey of Jesselton.* Rep. to N. B. Govt., 1929. Sandakan.

Idem (1931) ... Rep. to North Borneo Govt. on a tour of the interior of N. Borneo, 1931. Sandakan.

EDWARDS, F. W. (1926) ... *Sarawak Museum J.*, December. Kuching.

ENGLISH, J. C. (1945) ... *Malaria in the Borneo Operations*, May 1 to Sept. 1. Australian Military Forces.

EYKMAN INSTITUTE (1938) ... From maps in the Institute Recording Surveys in Dutch Borneo, 1930–1938 (McArthur, 1938).

FITZGERALD, R. D. (1937 and 1939) *Reports on a Survey of Labuan.*

GATER, B. A. R. (1933) ... *Bull. Raffles Museum. Singapore*, No. 8, Dec. 1933, p. 162. Govt. Printing Office, Singapore.

Idem (1935) ... *Aids to the Identification of anopheline imagines in Malaya, Singapore.*

GILLIES, D. (1948*a*) ... ... Personal communication. Labuan.

Idem (1948*b*) ... ... *Annual Medical Report*, 1947.

Idem (1949) ... ... *Ibid.*, 1948.

Idem (1950) ... ... *Ibid.*, 1949.

GOELARSO (1934) ... ... *Geneesk. Tidjschr. Ned.-Ind.*, **74**, 21, pp. 1345–1352. Batavia.

HEILBRUNN, A. (1948) ... Personal communication. April 30, 1948, Sintang.

HEIM, F. (1948) ... ... *Report on a second visit to Ulu Kinabatangan.* Sandakan.

HEWITT, J. (1905) ... ... *Report Sarawak Museum*, pp. 7–8. Kuching.

HODGKIN, E. P. (1941) ... Personal communication, Nov. 6, 1941. Kuala Lumpur.

HOOPS, A. L. (1929) ... Report on a visit to Labuan.

HUTCHINSON, W. J. (1930) ... Sarawak Health Dept. Annual Report, Appendix XIX. Kuching.

JOHNSON, A. W. (1948) ... Personal communication, Kuala Belait.

Idem (1949*a*) ... *Ibid.*, January 25, 1949. Kuala Belait.

Idem (1949*b*) ... *Ibid.*, March 8, 1949. Kuala Belait.

Idem (1949*c*) ... *Ibid.*, July 14, 1949. Kuala Belait.

Idem (1949*d*) ... *Ibid.*, August 18, 1949. Kuala Belait.

Idem (1950) ... *Ibid.*, January 3, 1950. Kuala Belait.

KARIADI (1938) ... ... *Geneesk. Tidjschr. Ned.-Ind.*, **74**, 21.
KUHLEWEIN, VON (1929) ... *Meded. D.V.G.*, 1930, No. 1.
LABUAN (1922–49) ... ... *Annual Medical Reports.*
MAWAO ESTATE (1913-14) .. *See* Hodgkin, 1941.
McARTHUR, J. (1938) ... *A Study of Malaria in the Far East.* North Borneo Govt.

Idem (1939) .. *Report on a malaria survey of Jesselton.* Report to North Borneo Govt., Jesselton, 1939.

Idem (1940-41) . *Monthly Reports of Malaria Research Dept., Tambunan.*

Idem (1944) ... Unpublished records made during Japanese occupation. Kuching.

Idem (1945) . Malaria Transmission in Borneo. *Lancet.* July 27, 1946, p. 117. London.

Idem (1947*a*) The Transmission of Malaria in Borneo. *Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, 5, pp. 537–558.

Idem (1947*b*) *Monthly Report of Malaria Research Headquarters.* July 1947. Sandakan.

Idem (1947*c*) .. *A Malaria Survey of East Coast of North Borneo.* Sandakan. Rep. to N. B. Govt.

Idem (1948) ... *Malaria in Borneo. Report on work of the Malaria Research Dept., North Borneo, 1939 to 12.* Cyclostyled by Colonial Office, London.

Idem (1949) .. *Annual report on work of Malaria Research Dept., 1948.* Labuan.

Idem (1950) . *Annual report on work of Malaria Research Dept., 1949.* Labuan.

MOTT, G. A. (1948) ... .. Personal communication. March 2, 1948. Brunei.

MOULTON, J. C. (1914) . *13th report of the Sarawak Museum.* Govt. Printing Office.

NAIR, V. K. (1948) ... . Personal communication, April 6, 1948. Seria.
NIEUWENHUIS (1882-83) .. *See* Shircore, 1937.
NORTH BORNEO (1929) ... *Reports to N. B. Govt. on larval surveys of Sandakan, Keningau and Tambunan*, by Anti-malarial Field Assistant, Sandakan.

Idem (1931) . *Reports to N. B. Govt. on larval surveys of Tawau.*

O'NEILL, P. L. (1949) Personal communication. December 25, 1949. Jesselton.

OU, KOK BOU (1941) . *Ibid.*, April 5, 1941. Labuan.

OVERBEEK, J. G. (1941*a*) .. *Ibid.*, August 16, 1941. Batavia.

Idem (1941*b*) .. *Ibid.*, August 30, 1941. Batavia.

OVERBEEK, J. G., and STOKER, W. J. (1937). *Malaria in the N.E.I. and its control.* Batavia.
REID, J. A. (1949) ... ... *Proc. Roy. Ent. Soc. London*, Vol. 18, Parts 3-4, pp. 42–53.

RODENWALDT, E. (1926) ... *Geneesk. Tidjschr. Ned.-Ind.*, **66**, 6, pp. 787–799. Batavia.

ROPER, R. (1914) ... ... *Bull. Ent. Research*, Vol. V, Pt. 2, pp. 137–147.
RUTLEDGE, N. H. (1947) ... Personal communication. Nov. 10, 1947. Jesselton.

SARAWAK (1930–39) ... Govt. annual medical reports, Kuching.
SCHARFF, J. W. (1927) ... *Malayan Med. J.*, **2**, 3, pp. 88–93.
SHINONAGA (1943) ... .. *Nippon Times*, July 23, 1943.
SHIRCORE, J. O. (1937) ... *Report on native health, British North Borneo.* Govt. Press.

SOESILO, R. *et al.* (1932) ... *Meded. Dienst. Volksgezondh. Ned.-Ind.*, **18**, pp. 102–111. Batavia.

SOESILO, R. *et al.* (1935) ... *Afdeeling Malariabestrijding. Ned.-Ind.*, **24**, 4, pp. 386–402.

STOKER, W. J. (1931) ... *Geneesk. Tidjschr. Ned.-Ind.*, **71**, 14, pp. 1206–1209. Batavia.

Idem (1934) ... *Ibid.*, **74**, 21, pp. 1342–1344.

STOOKES, V. A. (1924) ... Notes on the control of malaria in Sarawak. *Trans. 5th Congr. F.E.A.T.M.*, pp. 38–45.

Idem (1927) ... *Trans. 7th Congr. F.E.A.T.M.*, **3**, pp. 103–115.

STRAHAN, J. H. (1947) ... *Med. J. Malaya*, **2**, 2, pp. 83–92.

SUKARMIN, bin A. NASRAN (1948) Personal communication. June 12, 1948. Labuan.

SWELLENGREBEL, N. H. (1916) ... *De Anophelinen van Nederlandsch Oost-Indie.* Amsterdam, 2nd Edn. 1921.

SWELLENGREBEL, N. H., and RODENWALDT, E. (1932). *Die Anopheln von Niederlandisch-Ostindien.* Jena.

TREGARTHAN, J. C. T. (1941) ... Personal communication. Jesselton.

WALCH, E. W., and SOESILO, R. (1929). *Meded. Dienst. Volksgezondh. Ned.-Ind. Foreign End.*, **18**, 1, pp. 199–207.

WALLACE, E. H. (1949) ... Report of a tour in the Lower Rajang. Sarawak Govt., Kuching.

WEBSTER, F. L. (1941*a*) ... *Report on an outbreak of malaria associated with* A sundaicus *showing differing breeding habits.* Personal communication. Oct. 27, 1941. Miri.

Idem (1941*b*) ... Personal communications. June 30, 1941, Aug. 12, 1941. Miri.

Idem (1947) ... *Ibid.*, Oct. 12, 1947. Seria.

WYLE, H. W. (1949) ... *Ibid.*, O'Neill, Dec. 23. 1949. Jesselton.

APPENDIX I.

Spleen and parasite surveys.

Reference and place.	Spleens.			Parasites.			Malarial involvement.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.	Number examined.	Number with malarial infection.	Percentage.
Nieuwenhuis (1882-83)									
Sambas, Dutch Borneo (Mountain population).	...	...	82·5	...	...	...	...	...	...
Roper (1914)									
Membakut, N. Borneo	92	...	54·3	...	...	...	...	...	...
Bangawan	69	...	1·42	...	...	...	...	...	...
Kimanis	52	...	11·53	...	...	...	...	...	...
Paper (Mill Hill Mission)	37	...	13·5	...	...	...	...	...	...
'Rubber Estate'—									
(A) (Prophylactic quinine) ...	...	...	21·4	...	...	...	...	...	...
(B) (No prophylactic quinine) ...	321	...	7·1	...	...	...	...	...	...
(C) (Prophylactic quinine) ...	379	...	11·87	...	...	...	...	...	...
(D) (" ") ...	322	...	25·4	...	...	...	...	...	...
Labuan (1922)									
Labuan (School children)	...	...	16	...	...	...	...	...	...
Labuan (1923)									
Labuan (School children)	...	...	11·4	...	...	...	...	...	...
Labuan (1924)									
Labuan (School children) ...	190	54	28·4	...	...	...	...	...	...
(including Bukit Kalam School) ...	61	43	70·42	...	...	...	...	...	...
Labuan (1925)									
Government English School ...	...	...	3·4	...	...	...	...	...	...

APPENDIX I—contd.

Reference and place.	Spleens.			Parasites.			Malarial involvement.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.	Number examined.	Number with malarial infection.	Percentage.
Labuan (1925)									
Malay School, Victoria Town ...	...	...	8·5	...	...	...	...	...	...
Bukit Kalam Malay School ...	...	...	71	...	...	...	...	...	...
Scharff (1927)									
Labuan, around Lyang Lyang ...	...	...	90 (nearly).	...	...	...	...	...	...
,, seashore, ditto.	30	22	...	...	...	...	...	...	...
,, inland, McArthur Road ...	18	0	...	...	...	...	...	...	...
,, town area	92	...	7	...	...	...	...	...	...
Labuan (1929)									
Bukit Kalam School	...	...	62	...	...	...	...	...	...
Hoops (1929)									
Labuan Malay School	92	5	5·44	...	...	...	...	...	...
,, Chinese School	102	4	3·92	...	...	...	...	...	...
,, Government English School ...	109	3	2·75	...	...	...	...	...	...
Walch & Soesilo (1929)									
Tg. Djocata, Dutch Borneo	42	...	59	...	...	...	...	...	...
Tg. Selor, Boeloengan, Dutch Borneo ... (*A. umbrosus* only).	...	...	8	...	...	...	...	...	...
Tg. Palas, Boeloengan, Dutch Borneo ... (*A. leucosphyrus* and *A. umbrosus*).	55	...	49	...	...	...	...	...	...
Hutchinson (1930)									
Quop, Kuching, Sarawak	17	5	...	...	5	...	...	...	...
Labuan (1930)									
Bukit Kalam School	...	...	49	...	...	...	...	...	...
Labuan (1931)									
Bukit Kalam School	...	...	91	...	...	...	...	...	...

APPENDIX I—contd.

Reference and place.	Spleens.			Parasites.			Malarial involvement.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.	Number examined.	Number with malarial infection.	Percentage.
Campbell (1931)									
Sapong Estate, N. Borneo—									
(1929 Jan.–Jun.)	...	...	...	673	535	...	...	...	...
(Jul.–Dec.)	...	...	...	...	326	...	...	...	...
(1930 Jan.–Mar.)	...	...	...	...	204	...	...	...	...
(Mar. 21, 1930—Before operations) ...	673	445	66·1	673	374	55·5	673	556	82·6
May 30, 1930 (During and after operations.)	702	230	32·7	702	42	5·9	702	272	38·7
Jul. 7, 1930 (During and after operations.)	777	261	33·5	777	31	3·9	777	276	35·5
Aug. 23, 1930 (During and after operations.)	741	155	20·9	741	14	1·8	741	163	21·9
Keningau Govt. Vernacular School ...	...	...	...	...	...	...	...	17	58
Tambunan Govt. Vernacular School ...	...	...	...	...	...	...	...	35	59
R. C. Mission	...	...	...	...	...	...	...	28	68
Keningau (Native adults)	...	...	...	...	...	...	58	...	24
Dangalad	...	...	...	...	...	...	157	...	60
Tambunan	...	...	...	...	...	...	51	...	49
Sunsurun	...	...	...	...	...	...	172	...	80
Bundu Tuhan	...	...	...	...	...	...	311	...	84
Patau	...	...	...	...	...	...	30	...	100
Keningau (Native children) ...	...	...	...	...	...	...	41	...	56
Dangalad	...	...	...	...	...	...	40	...	90
Tambunan	...	...	...	...	...	...	100	...	63
Sunsurun	...	...	...	...	...	...	168	...	84
Bundu Tuhan	...	...	...	...	...	...	208	...	69
Patau	...	...	...	...	...	...	18	...	100

APPENDIX I—contd.

Reference and place.	Spleens.			Parasites.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.
Dingle (1931)						
Tabilang, North Borneo	17	5	29·4	...	...	...
Kota Belud	45	19	42	...	...	...
Tamu Darat	53	30	56·6	...	...	...
Kaung	79	20	25·3	...	...	...
Bundu Tuhan	61	26	42·6	...	...	...
Ranau	20	5	25·0	...	...	...
Tambunan	128	57	44·5	...	...	...
Keningau	93	42	45·2	...	...	...
Kendassan	55	24	43·6	...	...	...
Tiong	50	26	52·0	...	...	...
Tulid	54	29	53·7	...	...	...
Lanas	54	20	36·7	...	...	...
Pingas	32	14	43·7	...	...	...
Mesaum	18	18	100	...	...	...
Penungah	270	168	62·2	...	...	...
Pintassan	57	17	29·3	...	...	...
Lamag	34	12	35·3	...	...	...
Bilit	52	9	17·3	...	...	...
Sukau	37	5	13·5	...	...	...
Abai	26	2	7·7	...	...	...
Brunei (1931)						
Kuala Belait	...	...	0	...	...	...
Brunei Town	...	...	3	...	...	...
Tutong Road	...	...	85	...	...	...

APPENDIX I—contd.

Reference and place.	Spleens.			Parasites.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.
Labuan (1932)						
Bukit Kalam School	67	64	96	...	...	...
Chong Hwa School (Sungei Kling) ...	16	4	25	..	...	..
Labuan (1933)						
Bukit Kalam School	67	20	29·1	...	...	...
Government English School ...	84	4	4·7	...	...	...
Chi Wen School, Victoria Town ...	61	1	1·6	...	...	...
Malay School, Victoria Town ...	63	7	11	...	...	...
Stoker (1934)						
Kota Baruh, P. Laoet, Dutch Borneo—						
0–6 years	20	20	100	20	17	70
6–15 ,, ...	7	7	100	7	3	43
Women	11	9	82	11	3	27
Men	25	14	56	25	19	60
Goelarso (1934)						
Tg. Palas, Dutch Borneo	...	...	23·4	...	...	...
Tg. Selor, ,, ,, ...	...	...	1	...	...	...
Sadjau District, Dutch Borneo ...	...	...	83·3	...	...	...
Kg. Moeara Pangean, Dutch Borneo ..	...	...	64	...	...	...
Labuan (1935)						
Bukit Kalam School	...	...	75	...	...	...
Labuan (1936)						
Bukit Kalam School	...	...	50	...	...	...
Government English School ...	71	0	0	...	...	...
Fitzgerald (1937)						
Labuan, Bukit Kalam School ...	57	33	50·77	...	...	...
Malay School	...	1	1·35	...	...	...
Government English School ...	...	0	0	...	...	...

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APPENDIX I—contd.

Reference and place.	Spleens.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.
Shircore (1937)			
Tuan	11	5	...
Malalap	6	6	...
Tenom Lama	13	12	...
Timogun Plain	34	17	...
Kemabong	22	21	...
Mailu	13	9	...
Pahl	10	10	...
Tomani	11	9	...
Lunkiting	14	14	...
Tenom District	14	12	...
Agudun	20	18	...
Apin Apin	48	44	...
Dangalud	11	10	...
Kauran	18	16	...
Keningau	26	21	...
Masak	15	13	...
Menansud	32	30	...
Keningau District	25	21	...
Nabawan	10	7	...
Trankai	12	11	...
Sipulot	20	17	...
Agis	12	12	...
Segatol	10	9	...
Sipangali	10	8	...
Pambatasan	18	18	...
Subongo	17	16	...

APPENDIX I—contd.

Reference and place.	Spleens.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.
Shircore (1937)—*contd.*			
Ponsiangan District	40	37	...
Karamatoi	4	3	...
Limbawan	7	5	...
Pau	7	6	...
Tulid	10	10	...
Tandulu	33	26	78·7
Paper and Mogong	41	28	68·3
Lintun	25	3	15·0
Pomotodon	30	21	70·0
Koranahan	20	13	65·0
Mansak	12	10	83·3
Nambaian	55	50	90·9
Dar	18	14	77·7
Bahru	7	6	85·7
Timbau	22	13	59·7
Latud and Sukong	35	35	100
Nudu	10	6	60·0
Patau	24	23	95·8
Kerokot	18	18	100
Parakagis	19	15	76·8
Rendagong	25	23	92
Sinarut	27	24	88·9
Bundu Tuhan	27	10	37·0
Tenghilan	9	6	66·6
Klawat	10	9	90·0

APPENDIX I—contd.

Reference and place.	Spleens.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.
Shircore (1937)—*concld.*			
Rangalao	16	15	93·7
Pirasan	10	9	90·0
Gunding	10	7	70·0
Tahungusi	10	3	30·0
Sumberai	10	1	10·0
Penampang	10	10	100·0
Pandassan	9	9	100·0
Mantanan	10	10	100·0
Benkungan	10	8	80·0
Senaja	15	13	86·4
Kalumpang	16	11	68·9
Simpangan	3	3	100·0
Rukan	10	9	90·0
Lakut	37	1	2·7
Surati	13	...	...
Pengalat Kechil	20	...	...
Jesselton Town	50	2	4·0
Sipitang	20	...	...
Mesapol	10	8	80
Melamam	10	10	100·0
Mansud	7	7	100
Karukan	6	4	66·6
Kota Klias	7	3	42·8
Mempakul	19	1	5·2

APPENDIX I—contd.

Reference and place.				Spleens.		
				Number examined.	Number with enlarged spleen.	Spleen rate, per cent.
Eykman Institute (1938)						
Sintang	Dutch Borneo		...			
Koemai	,,	,,	...			
Samoeda	,,	,,	...			
Kg. Pemboeang	,,	,,	...			
Tl. Bogan	,,	,,	...	...	...	0·20
Kg. Djelai	,,	,,	...			
Samarinda	,,	,,	...			
Kota Baroe	,,	,,	...			
Tg. Moelia	,,	,,	...			
Pangoalan Boen	,,	,,	...	...	...	20–40
Nanga Boelik	,,	,,	...			
Tel. Poelang	,,	,,	...			
Sepdoek	,,	,,	...			
Ng. Ketoengau	,,	,,	...			
Ng. Djetak	,,	,,	...	...	...	40–60
Ng. Maoe	,,	,,	...			
Ng. Kajang	,,	,,	...			
Ng. Pinoh	,,	,,	...			
Samblimbangan	,,	,,	...			
Bat. Pagar	,,	,,	...			
K. Waringen	,,	,,	...			
Soekamara	,,	,,	...	...	...	60–80
R. Poeloar	,,	,,	...			
Bankat	,,	,,	...			
Boenti	,,	,,	...			

APPENDIX I—contd.

Reference and place.	Spleens.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.
Eykman Institute (1938)—*concld.*			
Djamoeat Dutch Borneo ...			
Ng. Piring ,, ,, ...			
Ng. Maloe ,, ,, ...			
Bt. Toenggal ,, ,, ...			
Pedongatan ,, ,, ...			
Soengkoep ,, ,, ...			
Penaban ,, ,, ...	..	...	80–100
Kt. Moelia ,, ,, ...			
Koedangan ,, ,, ...			
S. Tocat ,, ,, ...			
Bakoengsol ,, ,, ...			
Pekalipan ,, ,, ...			
Dhibing ,, ,, ...			
Fitzgerald (1939)			
Labuan, Rancha Rancha	49	6	12·24
Kg. Lajan	17	6	35·3
Kg. Durian Tondjong ...	29	6	20·7
McArthur (1939)			
Jesselton Town	1,334	44	3·3
Northern Chinese Settlement ...	67	1	1·5
Menggatal	159	101	63·5
Jesselton (*A. maculatus* area) ...	131	3	2·3
McArthur (1940)			
Kg. Pulutan, Dusun	9	9	100
Chinese	17	1	6·0
Kg. Kamburien, Dusun	12	5	42·0
Chinese	27	2	7·0

APPENDIX I—contd.

Reference and place.	Spleens.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.
McArthur (1940)—*contd.*			
Pantai, Tanabunan (Preliminary survey)	32	6	18·7
Lumandau	18	4	22·2
Sinsurun	35	9	25·7
Gargacon	10	3	30·0
Kituntul	51	16	31·4
Kapayan	17	6	35·3
Toboh	31	11	35·5
Batung	15	6	40·0
Tibobar	17	7	41·2
Tenompok	19	8	42·1
Minadung	9	4	44·4
Ganggar	7	4	57·2
Bambungan	9	6	66·7
Mangipangi	12	8	66·7
Timbau	16	11	68·8
Tambatuan	13	9	69·2
Tantaloh	12	9	75·0
Kerumahan	22	17	77·2
Pinsan	13	12	92·3
Marakeras	17	16	94·1
Naudu	8	28	100·0
Latud	6	6	100·0
Sukong	3	3	100·0
Pagalan	15	15	100·0
Kipaku	8	8	100·0

APPENDIX I—contd.

Reference and place.	Spleens.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.
McArthur (1940)—*concld.*			
Tambunan, nearer jungle (Intensive survey).	1,682	644	38·7*
Tambunan, distant from jungle (Intensive survey).	1,202	971	80·8†
Ou Kok Bou (1941)			
Labuan (School children)	391	84	14
McArthur (1942)			
Davan	32	3	9
Monggatal	31	5	16
Dambai	17	3	18
Kaliangan	24	10	42
Lower Pulutan	19	9	47
Upper Pulutan	12	10	83
Clarke (1941)			
Apin Apin (Feb. 1940)	69	28	41
Purutan	138	58	42
Tambunan	499	209	42
Babagon	230	155	67
Kerokot	220	110	50
Mansangoh	48	34	71
Randagong	186	103	55
Ranau	292	130	45
Kundasan	67	7	10
Bundu Tuhan	339	110	33
Tgadon	10	3	30

* 2,385 blood slides examined and parasite rate found 30·5 per cent.

† Parasite rate 56·8 per cent.

APPENDIX I—contd.

Reference and place.	Spleens.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.
Clarke (1941)—*contd.*			
Tobob	113	37	33
Libang	246	142	57
Paha Paha	168	105	62
Siggitsi (Apr. 1940) ...	168	83	49
Agis	69	36	52
Sepulot	240	132	55
Pandiwan	74	36	49
Nabawan	31	14	45
Sook	57	32	56
Tetugop	58	27	47
Onop Onop (Jul. 1940)	51	36	71
Tomani	53	31	58
Kindassan (Aug. 1940) .. .	30	9	30
Pau	55	7	13
Tulid	45	14	31
Lanas	33	4	12
Masaum	101	44	44
Panangoh	223	78	35
Tonggod	135	45	33
Malagatan	96	35	36
Batang	45	17	38
Tangkulap	96	29	30
Kwamut	94	25	37
Pintassan	43	11	26
Lamag	69	8	12
Bilit	24	3	12

APPENDIX I—contd.

Reference and place.	Spleens.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.
Clarke (1941)—*contd.*			
Sukau	29	3	10
Abai	15	1	7
Kitunum (Nov. 1940)	51	31	61
Bole	43	20	47
Malaman	28	17	61
Sandumin	32	...	...
Randagong (Feb. 1941)	89	39	44
Nakapak	37	21	57
Nubatan	38	9	24
Sagindai	52	18	35
Paginatan	55	29	53
Tampias	49	24	49
Matupang	44	22	50
Muruk	60	35	58
Kuala Monsok (Jun. 1941)	17	11	65
Monsok Puntingabai	24	14	57
Ulu Monsok	117	66	56
Monsokito	19	13	68
Ulu Sungei	26	9	35
Kotonobon	21	18	86
Kaingaran	45	23	51
Pinawantai	66	43	65
Tampalang	18	10	56
Pananaboron	35	23	66
Nandai	91	56	62

APPENDIX I—contd.

Reference and place.	Spleens.			Parasites.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.
Clarke (1941)—*contd.*						
Bish (Aug. 1941)	33	23	70	...	...	...
Punti	17	11	65	...	...	...
Tatalnan	15	11	73	...	...	...
Dalit	50	28	56	...	...	...
Maliang	47	20	43	...	...	...
Mamagon	48	16	33	...	...	...
Rumidi	36	7	19	...	...	...
Pohan Batu	42	18	43	...	...	...
Pangaran	86	56	65	...	...	...
Sabingoh	65	33	51	...	...	...
Pensiangan	234	97	41	...	...	...
Sekalobot	14	8	57	...	...	...
Silom	28	12	43	...	...	...
Saliri	38	28	74	...	...	...
Katambalong	38	22	58	...	...	...
Sumintabol	37	2	5	...	...	...
Kalagunan	82	14	17	...	...	...
Batugon	44	7	16	...	...	...
Apa	52	4	8	...	...	...
Apila	74	3	4	...	...	...
Lingkawah	24	8	33	...	...	...
Toloboi	45	2	4	...	...	...

APPENDIX I—contd.

Reference and place.	Spleens.			Parasites.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.
Clarke (1941)—*concld.*						
Aluk Aluk	53	14	26	...	...	...
Pasia	166	55	33	...	...	...
Kuala Mian	75	37	49	...	...	...
Iburu	74	45	61	...	...	...
Bole	66	30	45	...	...	...
Malaman	29	14	48	...	...	...
Mansud	65	19	29	...	...	...
Tasnu Kayul	202	46	23	...	...	...
Kuala Penyu	137	40	29	...	...	...
Kota Klias	56	7	13	...	...	...
Sipitang (Adult males)	37	...	...	...	...	...
,, (Children only)	58	2	4	...	...	...
,, (,, Jan.)	37	4	7	...	...	...
English (1945)						
Tarakan, Dutch Borneo (Chinese) ...	225	*Nil*	...	225	2	1
(Indonesian) ...	100	*Nil*	...	...	...	...
Labuan (Chinese school-children) ...	102	61	60	...	...	...
(Javanese ,, ,,) ...	670	511	76	...	...	...
(Civilian School)	...	...	...	191	(61)	32
Weston, North Borneo (Malay) ...	...	...	55	...	...	...
(Chinese) ...	...	...	54	...	...	...
Balikpapan	333	(141)	43	...	...	...

APPENDIX I—contd.

Reference and place.	Spleens.			Parasites.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.
English (1945)—*concld.*						
Riko Villages	...	...	80-90	...	...	...
Balikpapan	...	...	...	277	41	15
Brunei, Kampong (1939)	561	...	1	...	...	...
Kuala Belait (1939)	275	...	1	...	...	..
Strahan (1947)						
Miri (Chinese School)	120	(4)	3	48	(22)	40
Kg. Krokop (Malay School) ...	75	24	33	...	...	...
Lutong-Pujut (Chinese School) ...	137	15	11	...	...	...
Kg. Pujut	...	...	...	26	(23)	80
Kg. Kedayan	...	...	...	12	3	25
Quap	...	...	...	...	...	50
21st Mile	...	...	...	...	...	50
Mott (1948)						
Kuala Belait (R. C. School) ...	78	1	1·2	78	...	...
(Chung Hua School) ...	448	3	0·7	448	...	...
(Government School) ...	85	2	2·3	85	...	...
McArthur (1947)						
Tambisan	30	30	100	...	...	...
Lahad Datu	21	20	95	...	...	...
,, ,, (inland village) ...	7	4	57	...	...	...
Segama	...	...	55	...	...	...
Semporna	38	20	53	...	...	...

APPENDIX I—contd.

Reference and place.	Spleens.			Parasites.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.
McArthur (1947)—*concld.*						
Tawan	18	1	6	...	...	...
,, Table Estate 1	10	10	100	...	...	...
,, ,, ,, 2	8	8	100	...	...	...
,, ,, ,, 3	10	5	50	...	...	...
,, ,, ,, 4	6	1	18	...	...	...
Gillies (1948*a*)						
Baboiboe Laut (age 20 years 3 months)	25	10	40	25	5	20
Bulukut	8	4	50	8	3	38
Kg. Java and Tuan Ku	11	7	64	11	4	36
Patau Patau No. 1	26	7	27	26	9	35
,, ,, No. 2	16	6	38	16	6	38
Sungei Kling	14	1	7	14	1	7
Tandjong Aru	21	12	57	21	13	62
Rancha Rancha	16	10	63	16	5	31
Sungei Bedann	16	4	25	16	4	25
Sagocking and Geresok	49	10	20	49	11	22
Liang Lianganan (left of Anchor Inn) ...	19	6	32	19	1	5
,, ,, (right ,, ,,) ...	13	10	77	13	5	38
Batu Manikan	18	17	94	18	7	39
Sungei Labu	10	0	0	7	0	0
Sungei Labu Darat	9	5	55	9	3	33
Sungei Lada	16	15	94	16	9	56

APPENDIX I—contd.

Reference and place.	Spleens.			Parasites.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.
Gillies (1948*a*)—*concld.*						
Sungei Miri and Paga	5	5	100	5	2	40
Lajan	16	16	100	16	12	75
Seguman	10	9	90	10	4	40
Labok Temiang	20	9	45	20	6	30
Rural Board Area	74	15	20	74	19	26
Kg. Nagalang	21	11	52	21	5	23
Gangarak	7	4	57	7	5	71
Kg. Pantai	17	5	29	17	1	6
Town	14	9	14	64	11	17
Kg. Dawar	17	10	59	18	6	35
Bukit Kuda	25	16	64	25	9	36
Bukit Kalam	16	12	75	16	13	10
Heilbrunn (1948)						
Sintang, Dutch Borneo (Chinese Schools)	159	1	0.6	159	19	12
(Indonesian Schools)	497	76	15.6	497	145	27
Ng. Pinch (Chinese Schools)	319	1	0.3	319	21	6.6
(Indonesian Schools)	255	16	6	255	35	14
Heim (1948)						
Kg. Abai	...	...	...	40	4	...
Kg. Minurulu	...	...	...	46	8	...

APPENDIX I—contd.

Reference and place.	Spleens.			Parasites.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.
Heim (1948)—*concld.*						
Kg. Pintassan	...	...	...	40	3	...
Ulu Kinabatangan	264	217	82.1	...	...	...
Nair (1948)						
Bukit Sawat Camp, Brunei (1938) ...	9	4	...	...	...	...
Kg. Penkalan Siong (1938)	19	15	...	...	...	...
McArthur (1948*b*)						
Mempakul	8	7	87.5	...	...	...
Menumbok	20	9	45.0	...	...	...
Colless (1948*c*)						
Kuala Belait—Seria Road	19	12	37	...	...	...
Colless (1948*d*)						
Pangkalan Gdns., Lutong	47	13	28	...	...	...
Kg. Loak	21	8	38	...	...	...
Cranston (1948)						
Lahad Datu, Segama Estate ...	197	...	26.4	...	...	...
Johnson (1949*c*)						
Luak Bay, Miri (Under 10)	35	...	37	...	...	4·5
Sungei Krokop (February 1947) ...	...	...	51	...	...	...
(July 1949)	112	...	6·25	232	...	1·7

APPENDIX I—contd.

Reference and place.	Spleens.			Parasites.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.
Johnson (1949*d*)						
Liang Saw Mill (Under 8)	...	...	...	36	...	...
Kg. Gana (,,)	11	6	54·5	17	5	29·4
Kg. Lumut (,,)	10	8	80	30	9	30
Tungu Liang (,,)	...	...	...	2	1	50
Kg. Kalmyo (,,)	...	...	...	12	...	0
Kuala Lumut (,,)	18	14	77·7	119	18	27·7
McArthur (1950)						
Kg. Segu (Age 2–10)	55	5	9	...	...	...
Wallace (1949)						
Matu Village, Lower Rajang (Under 10)	23	4	13	23	1	4
Kuala Matu ,, ,, ...	11	2	18	11	3	27
Sungei Sok ,, ,, ...	17	2	11	17	6	35
Kampong Tien ,, ,, ...	33	...	...	33	1	3·3
Kampong Sai ,, ,, ...	16	4	25	16	2	12·5
Dyaklong House ,, ,, ...	19	1	5	19	...	0
Wyile (1949)						
Tuaran (Bajau children)	45	20	...	...	...	...
(Other children)	17	2	...	...	...	...
Jesselton (Chinese children) ...	30	8	...	...	...	...
(Brunei children)	12	7	...	...	...	...
(Other children)	57	26	...	...	...	...

APPENDIX I—concld.

Reference and place.	Spleens.			Parasites.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.
O'Neill (1949)						
Tanjong Aru, Jesselton (Bajau Kampong)	38	18	...	...	...	...
Railway Workshop, Jesselton ...	35	12	...	...	...	...
Tanjong Aru, Jesselton (Asian population)	25	7	...	...	2	...
Kampong Likas, Jesselton (Bajau) ...	23	21	...	...	...	...
Mile 4, Tuaran Road	15	8	...	...	...	...
Penampang (Dusan) (Jan.)	36	7	...	...	...	...
(Apr.)	51	14	...	...	...	...
Kampong Madziang	8	8	...	...	...	...
Sunsurun (Tambunan)	25	19	...	...	...	...
Maatshapi Estate Village, Labuad Datu ...	25	15	...	...	...	...
McArthur (1950)						
Tambunan Experiment—						
Kg. Sukong	49	39	80	25	25	48
Kg. Latud	34	20	59	37	14	38
Kg. Piasau	37	30	81	41	19	46
Kg. Timbau	85	54	64	90	24	27
Kg. Batung	56	30	54	59	12	20

APPENDIX Ia.

Health of localities in Borneo.

Areas recorded as healthy (Spleen rates 0-10 per cent.)

Bangawan, N. Borneo	Abai, N. Borneo	Sungei Labu Dorat (Labuan)
Jesselton Town ,,	Sundumin ,,	Miri, Sarawak
Menpakul ,,	Sumintobol ,,	Kg. Segu ,,
N. Chinese Settlt. ,,	Apa ,,	Kg. Tantien ,,
Lakut (Papar) ,,	Apila ,,	Brunei Town, Brunei
Surati ,, ,,	Tolobol ,,	Kuala Belait ,,
Pengalat Kechil ,,	Tawau ,,	Tg. Selor, Dutch Borneo.
Pulutan (Menggatal) ,,	Victoria Town (Labuan)	Tarakan ,,
Kambirien ,, ,,	Sungei Kling ,,	Sintang ,,
Darau ,, ,,	Sungei Labu ,,	Ng. Pinoh ,,

Areas recorded as of low endemicity (Spleen rates 10-25 per cent.)

Kimanis, N. Borneo	Bilit, N. Borneo	Sungei Lada (Labuan)
Papar ,,	Nubutan ,,	Labuan Rural Board Area
Sukau ,,	Rumidi ,,	Kg. Matu, Sarawak
Lintuo ,,	Kalangunan ,,	Kuala Matu ,,
Pantai ,,	Batugon ,,	Sungei Sok ,,
Lumanau ,,	Tamu Kayui ,,	Kg. Sai ,,
Menggatal ,,	Kota Klias ,,	Tg. Palas, Dutch Borneo
Dambai ,,	Rancha Rancha (Labuan)	Sintang ,,
Kundassan ,,	Kg. Durian Tanjong ,,	Lutong-Pujut, Sarawak
Pau ,,	Patau Patau ,,	Tuaran, N. Borneo
Lanas ,,	Sungei Bedaun ,,	Penampang ,,
Lamag ,,	Sagoeking ,,	

Areas recorded as of high endemicity (Spleen rates 25-50 per cent.)

Tabilang, N. Borneo	Tagagon, N. Borneo	Linkawah, N. Borneo
Kota Belud ,,	Toboh ,,	Aluk Aluk ,,
Kaung ,,	Siggatal ,,	Pasia ,,
Bunau Tuhan ,,	Pandiwan ,,	Kuala Mian ,,

APPENDIX Ia—contd.

Areas recorded as of high endemicity (Spleen rates 25-50 per cent.)—*concld.*

Ranau,	N. Borneo	Nabawan,	N. Borneo	Mansud	N. Borneo
Keningan	,,	Tetugop	,,	Kuala Penyu	,,
Kandassan	,,	Kindassan	,,	Table Estate	,,
Lanas	,,	Tulid	,,	Menumbok	,,
Pingas	,,	Massaum	,,	Segama Estate	,,
Lamag	,,	Panangoh	,,	Penampang	,,
Bilit	,,	Tonggod	,,	Tg. Aru	,,
Tahungusi	,,	Malagatan	,,	Kg. Lajan (Labuan)	
Kota Klias	,,	Batang	,,	Lyang Lyang	,,
Sunsurun	,,	Tangkulop	,,	Buboeloe Laut	,,
Gargaron	,,	Kwamut	,,	Bulukut	,,
Kituntul	,,	Pintassan	,,	Lubok Temiang	,,
Kapayan	,,	Bole	,,	Kg. Pantai	,,
Toboh	,,	Rendagong	,,	Tg. Palas, Dutch Borneo	
Batung	,,	Lubatan	,,	Balikpapan	,,
Tibobar	,,	Segindau	,,	Kg. Krokop, Sarawak	
Tenompok	,,	Tampias	,,	Quop	,,
Minadung	,,	Ulu Sungei	,,	Lutong	,,
Kaliangan	,,	Maliang	,,	Kg. Lasok	,,
Lower Pulutan	,,	Mantagon	,,	Pankalan Gdns., Lutong	
Apin Apin	,,	Pohon Batu	,,	Luak Bay	,,
Purutan	,,	Pensiangan	,,	Bukit Sawat Camp, Brunei	
Silon	,,	Malaman	,,	Kuala Belait-Seria Rd.	

Areas recorded as hyperendemic (Spleen rates over 50 per cent.)

Membakut,	N. Borneo	Tenghilan,	N. Borneo	Dalit,	N. Borneo
Sapong	,,	Klawat	,,	Pangaran	,,
Keningau	,,	Rangalan	,,	Sabingoh	,,
Tambunan	,,	Pirasan	,,	Sekalobot	,,
Dangalad	,,	Gunding	,,	Saliri	,,

APPENDIX Ia—contd.

Areas recorded as hyperendemic (Spleen rates over 50 per cent.)—*contd.*

Sunsuran,	N. Borneo	Penampang,	N. Borneo	Kalambalong,	N. Borneo
Bundu Tuhan	,,	Pandassan	,,	Weston	,,
Patau	,,	Mantanan	,,	Kg. Likas	,,
Tanun Darat	,,	Benkungan	,,	Kg. Madziang	,,
Tiong	,,	Senaja	,,	Maatohapi Estate	,,
Tulid	,,	Kalumpang	,,	Ulu Kinabatangan	,,
Mesanin	,,	Simpingan	,,	Liang Liang (Labuan)	
Penungoh	,,	Rukam	,,	Kg. Java	,,
Melalap	,,	Mesapol	,,	Tg. Aru	,,
Tenom Lama	,,	Melamam	,,	Ramka Ramka	,,
Timogun Plain	,,	Mansud	,,	Batu Mankian	,,
Kamabong	,,	Karukan	,,	Sungi Miri	,,
Mailu	,,	Menggatal	,,	Lajac	,,
Pahi	,,	Ganggar	,,	Segunan	,,
Tomani	,,	Bambangan	,,	Kg. Nagalang	,,
Lunkiting	,,	Mangipangi	,,	Gangarah	,,
Tenom District	,,	Tambatuan	,,	Kg. Dawar	,,
Agudun	,,	Tantaloh	,,	Bukit Kuda	,,
Apin Apin	,,	Piasau	,,	Bukit Kalan	,,
Kaaran	,,	Marakaras	,,	Quop,	Sarawak
Masak	,,	Pagalan	,,	Sungei Krokop	,,
Menansud	,,	Kipaku	,,	Kg. Lunut	,,
Nabawan	,,	Upper Pulutan	,,	Kuala Lunut	,,
Trankai	,,	Iburu	,,	Sambas,	Dutch Borneo
Sepulot	,,	Babagon	,,	Tg. Djoeata	,,
Agis	,,	Mensangoh	,,	Kota Baruh	,,
Segatol	,,	Randagong	,,	Sadjau District	,,
Sepangali	,,	Libang	,,	Kg. Moera, Pangean,	,,
Pambalasan	,,	Pahu Pahu	,,	Rikko Villages	,,
Subongo	,,	Sook	,,	Bat. Pagar	,,

APPENDIX 1a—concld.

Areas recorded as hyperendemic (Spleen rates over 50 per cent.)—*concld.*

Pensiangan, N. Borneo		Onop Onop, N. Borneo		K. Waringen, Dutch Borneo	
Karamatoi	,,	Kitunum	,,	Soekamara	,,
Limbawan	,,	Nalapak	,,	R. Poeloar	,,
Pan	,,	Paginatan	,,	Bangkat	,,
Tandulu	,,	Matupang	,,	Boenti	,,
Papar	,,	Muruk	,,	Djemoeat	,,
Mogong	,,	Kuala Monsok	,,	Ng. Piring	,,
Pomotodon	,,	Monsok Pintingabai	,,	Ng. Maloe	,,
Keranhan	,,	Ulu Monsok	,,	Bt. Toenggal	,,
Mansok	,,	Monsokito	,,	Pedongatan	,,
Nambaian	,,	Kotonobon	,,	Soengkoep	,,
Dar	,,	Kaingaran	,,	Penahan	,,
Bahru	,,	Pinawantai	,,	Kt. Moelia	,,
Timbau	,,	Tampalang	,,	Koedangan	,,
Latud	,,	Pananaboron	,,	S. Toeat	,,
Sukong	,,	Nandai	,,	Bakoengsol	,,
Naudu	,,	Biah	,,	Pekalipan	,,
Patau	,,	Punti	,,	Dhibing	,,
Kerokot	,,	Tambisan	,,	Tutong Road, Brunei	
Parakagis	,,	Lahad Datu	,,	Kg. Penkalan Siong	,,
Rendagong	,,	Segama	,,	Kg. Gana, Sarawak	
Smarut	,,	Mempakul	,,		
		Tataluan	,,		

(It will be noted that some areas have yielded different results at different times, and therefore fall into more than one group.)

APPENDIX II.

Recorded epidemics of malaria in Borneo.

Year.	Place.	Recorded by	Year.	Due to local conditions.	Due to population movement.
1915	Sarawak Oilfields ...	Stookes	1927	?	...
1920	North Chinese Settlement ...	McArthur	1948	...	+
1925–27	Sapong Estate	Campbell	1931	...	+
1930	Satang Island, Sarawak ...	Sarawak	1930	...	+
1935	Tg. Lobang, Miri ...	Webster	1941*a*	+	...
1939	Do.	,,	1941*a*	+	...
1924–33	Lutong (Annually May–July)	,,	1941*a*	+	...
1936	Matading	,,	1941*a*	+	+
1938-39	Jerudong, Brunei ...	,,	1941*a*	...	+
1941	Miri	,,	1941*a*	+	...
1941	Lutong	,,	1941*a*	+	...
1941	Loak-Bakan Area ...	,,	1941*a*	+	...
1941	Bintulu	,,	1941*a*	+	...
1938	Tuaran-Tenghilan Rd. ...	McArthur	...	...	+
1943	Banggi Island	,,	1944	...	?
1944	Dahan Camp	,,	1944	...	+
1944	Labuan	,,	1944	...	+
1946	Kuching River	Strahan	1947	+	...
	Bintulu	,,	1947	+	...
	Miri	,,	1947	+	...
	Lutong	,,	1947	+	...
	Kuala Belait	,,	1947	+	...
	Seria	,,	1947	+	...

+ = Yes.

APPENDIX III.

Malaria parasites of Borneo.

Year.	Recorded by	Place.		Numbers with malaria parasites.			Percentage.		
				M.T.	B.T.	Q.	M.T.	B.T.	Q.
1922–38	Sarawak ...	Kuching Laboratory	1922 ...	13	34	14	...	...	...
			1923 ...	28	10	2	...	...	...
			1924 ...	35	24	14	...	...	...
			1925 ...	87	16	9	...	...	...
			1926 ...	120	16	9	...	...	...
			1927 ...	77	46	14	...	...	...
			1929 ...	136	65	14	...	...	...
			1930 ...	91	143	18	...	...	...
			1931 ...	138	131	16	...	...	...
			1932 ...	89	103	13	...	...	...
			1933 ...	145	138	14	...	...	...
			1936 ...	38	80	7	...	...	...
			1937 ...	138	33	4	...	...	...
			1938 ...	153	51	33	...	...	...
		Sibu	1925 ...	9	1	6	...	...	...
			1926 ...	70	19	4	...	...	...
			1927 ...	26	9	1	...	...	...
			1930 ...	85	5	2	...	...	...
			1931 ...	28	7	4	...	...	...
			1932 ...	31	8	8	...	...	...
			1933 ...	45	12	7	...	...	...
			1936 ...	35	17	7	...	...	...
			1937 ...	84	27	16	...	...	...
1929	Campbell ...	Sapong Estate	1927 ...	...	...	...	81	19	...
			1928 ...	...	...	...	18	82	...

M.T. = malignant tertian. B.T. = benign tertian. Q. = quartan.

APPENDIX III—contd.

Year.	Recorded by	Place.		Numbers with malaria parasites.			Percentage.		
				M.T.	B.T.	Q.	M.T.	B.T.	Q.
1929	Campbell ...	Sapong Estate	1929 ...	...	...	...	20	80	...
			1930 ...	...	...	...	25	75	...
1931–38	Brunei ...	Brunei	1931 ...	4	14	5	...	...	...
			1932 ...	3	13	10	...	...	...
			1933 ...	14	45	11	...	...	...
			1934 ...	17	14	6	...	...	...
			1935 ...	33	55	17	...	...	...
			1936 ...	109	66	30	...	...	...
			1937 ...	68	32	33	...	...	...
			1938 ...	101	78	25	...	...	...
		Temburong	1934 ...	1	...	...	...	...	...
			1935 ...	1	10	2	...	...	...
			1936 ...	...	6	1	...	...	...
			1937 ...	1	2	...	...	...	...
			1938 ...	1	1	...	...	...	...
		Tutong	1936 ...	1	7	...	...	...	...
			1937 ...	1	4	1	...	...	...
			1938 ...	5	14	1	...	...	...
		Brunei Total	...	349	317	137	...	...	...
		Temburong Total	...	4	19	3	...	...	...
		Totong Total	...	7	25	2	...	...	...
1933	Copeland ...	Tenom & Interior	...	...	...	...	62	37	1
1934	Stoker ...	Poeloe Laoet, age 0–6	...	9	5	0	...	...	...
		,, 6–15	...	2	1	0	...	...	...
		Women	...	3	0	0	...	...	...
		Men	...	11	3	1	...	...	...

M.T.=malignant tertian. B.T.=benign tertian. Q.=quartan.

APPENDIX III—contd.

Year.	Recorded by	Place.	Numbers with malaria parasites.			Percentage.		
			M.T.	B.T.	Q.	M.T.	B.T.	Q.
1937	Shircore ...	North Borneo Interior ...	...	...	...	29·8	15·8	54·4
		,, ,, West Coast	...	...	...	17·6	49·1	33·3
1939	McArthur ...	Jesselton 1919 ...	37	14	8	63	23	13
		1920 ...	60	46	16	49	38	13
		1921 ...	21	7	8	58	19	22
		1922 ...	13	4	2	68	21	10
		1923 ...	20	16	18	37	29	33
		1924 ...	14	14	9	38	38	24
		1925 ...	11	10	3	46	41	12
		1926 ...	4	5	4	31	38	31
		1927 ...	16	9	4	55	31	14
		1928 ...	20	10	4	59	29	11
		1929 ...	33	25	3	54	41	5
		1930 ...	36	16	4	64	29	7
		1931 ...	8	5	2	53	33	13
		1932 ...	4	4	2	40	40	20
		1933 ...	33	29	6	48	42	9
		1934 ...	39	88	5	29	67	3
		1935 ...	27	73	25	21	58	20
		1936 ..	9	47	4	15	78	6
		1937 ...	5	49	5	8	83	8
		1938 ...	10	73	13	10	76	13
1940	McArthur ...	Tambunan	188	230	158	56	26	18
1940	Ou ...	Labuan	77	117	14	...	...	...
1941	Webster ...	Matading, Sarawak ...	72	28	3	..	...	...
		Jerudong, Brunei ...	35	55	4	...	...	...

M.T.=malignant tertian. B.T.=benign tertian. Q.=quartan.

APPENDIX III—contd.

Year.	Recorded by	Place.	Numbers with malaria parasites.			Percentage.		
			M.T.	B.T.	Q.	M.T.	B.T.	Q.
1946	English ...	Tarakan	9	21	1	...	...	...
		Labuan, in-patients ...	32	18	...	...	...	...
		out-patients, children	...	4	...	...	...	...
		out-patients, adults	7	8	—	...	...	...
		Civilian School ...	32	27	...	...	...	...
		Javanese Compound.	44	98	...	...	...	...
		Balikpapan ...	24	17	...	...	...	...
		Balikpapan, in-patients.	14	68	...	...	...	...
1947	Strahan ...	Miri School (Chinese) ...	13	8	...	...	...	...
		Kg. Pujut (Malay) ...	15	7	...	...	...	...
		Kg. Kedayan (Kedayans) ...	0	2	...	...	...	...
		Quop	...	...	+	...	...	...
		Sarawak 1946 Jan.	13	2	...	...	...	...
		Feb.	17	7	2	...	...	...
		Mar.	51	39	2	...	...	...
		Apr.	78	28	...	...	...	...
		May	222	38	4	...	...	...
		Jun.	244	40	1	...	...	...
		Jul.	470	72	...	...	...	...
		Aug.	446	21	...	...	...	...
1948	McArthur ...	Tambunan Dispensary 1931	3	—	1	75	25	...
		1932	5	4	—	56	44	—
		1933	2	3	1	33	55	17
		1934	6	3	...	67	33	—
		1935	12	2	—	86	14	—

M.T. = malignant tertian. B.T. = benign tertian. Q. = quartan.

APPENDIX III—concld.

Year.	Recorded by	Place.	Numbers with malaria parasites.			Percentage.		
			M.T.	B.T.	Q.	M.T.	B.T.	Q.
1948	McArthur ...	Tambunan Dispensary 1936	14	5	1	70	25	5
		1937	25	21	11	44	37	19
		1938	8	23	13	18	52	30
		1939	3	13	2	17	72	11
		1940	17	11	3	54	35	9
		Total	95	85	32	45	40	15
1948	Johnson ...	Miri 1947	284	361	6	...	...	...
		Lutong 1947	352	349	4	...	...	...
		Kuala Belait & Seria 1947	192	481	3	...	...	...
1948	Johnson ...	Sintang Indonesian Schools	60	97	—	...	...	...
	Heilbrunn ...	Sintang Chinese Schools ...	13	6	—	...	...	...
		Ng. Pinoh Indonesian Schools.	14	22	...	...	...	...
		Ng. Pinoh Chinese Schools	7	14	—	...	...	...
1948	Gillies ...	Labuan 1947	399	145	1	75	24	—
1948	Heim ...	Kg. Abai	3	1	...	...	...	...
		Minurulu	4	4	...	...	...	...
		Pitassan	2	1	...	...	...	...

M.T. = malignant tertian. B.T. = benign tertian. Q. = quartan.

APPENDIX IV.

Recorded Anopheles identifications of Borneo.

Species.	Footnote numbers.	Hewitt, 1905.	Roper, 1914.	Mawao Estate, 1914 (Hodgkin, 1941).	Moulton, 1916.	Edwards, 1926.	Swellengrebel, 1921.	Stookes, 1924.	Rodenwaldt, 1926.	Scharff, 1927.	Stookes, 1927.	Dingle, 1929.	North Borneo, 1929–31.	Walch and Soesilo, 1929.	von Kuehlewein, 1929.
A. sinensis	1	+	—	—	+	—	+	+	+	+	+	+	+	—	—
A. barbirostris ...	...	+	+	+	+	—	+	+	—	+	+	+	+	—	—
A. umbrosus ...	2	+	+	—	+	—	+	+	+	+	+	+	+	+	+
A. vanus ...	3	+	—	—	—	—	—	—	—	—	—	—	—	—	—
A. leucosphyrus ...	4	+	+	+	+	—	+	+	—	—	+	+	+	+	+
A. kochi ...	...	+	+	—	+	—	+	—	—	+	+	+	+	—	—
A. maculatus ...	...	+	+	—	+	—	—	—	—	—	—	+	+	—	—
A. brevipalpis ...	...	—	+	+	+	—	—	—	—	—	+	—	—	—	—
A. punctulatus ...	5	—	+	—	—	—	+	—	—	—	—	—	+	—	—
A. ludlowi ...	6	—	+	—	+	—	—	—	—	+	+	+	+	+	—
A. separatus ...	...	—	+	—	+	—	—	—	—	+	+	—	—	—	—
A. albotæniatus ...	...	—	+	—	+	—	—	—	—	—	+	—	—	—	—
A. tessellatus ...	...	—	—	+	—	—	—	+	—	—	—	+	+	—	—
A. novumbrosus ...	7	—	—	+	—	—	—	—	—	—	—	—	—	—	—
A. punctulatus var. *tessellatus.*	8	—	—	—	+	—	—	—	—	—	—	—	—	—	—
A. karwari ...	...	—	—	—	+	—	—	—	—	+	—	—	—	—	—
A. watsoni ...	...	—	—	—	—	+	—	—	—	—	—	—	—	—	—
A. rossii ...	9	—	—	—	—	—	+	—	—	—	—	+	—	—	—
A. vagus ...	10	—	—	—	—	—	+	—	—	+	—	—	+	—	—
A. aconitus ...	...	—	—	—	—	—	+	—	—	—	—	—	—	—	—
A. fuliginosus ...	11	—	—	—	—	—	—	—	—	—	—	—	—	+	—
A. montanus ...	...	—	—	—	—	—	—	—	—	—	—	—	—	—	—

+ = recorded. — = not recorded.

APPENDIX IV—contd.

Species.	Footnote numbers.	Campbell, 1931.	Stoker, 1931.	Soesilo *et al.*, 1932.	Swellengrebel and Bodenwaldt, 1932.	Stoker, 1934.	Goetsch, 1934.	Brunei, 1929–35.	Sarawak, 1930–39.	Gater, 1935.	Webster, 1941b.	Ou, 1941.	Shircore, 1937.	Carlile, 1941.	McArthur, 1948.
A. sinensis ...	1	—	—	—	—	+	—	+	+	—	+	—	—	—	—
A. barbirostris ...	...	—	—	—	+	—	+	+	+	+	+	+	+	+	+
A. umbrosus ...	2	—	+	+	+	—	+	+	+	+	+	+	+	+	—
A. vanus	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. leucosphyrus ...	4	—	+	+	+	+	+	+	+	+	+	+	+	+	+
A. kochi	...	+	—	—	+	—	+	+	+	+	+	+	+	+	+
A. maculatus ...	...	—	—	—	+	+	+	+	+	+	+	+	+	+	+
A. brevipalpis ...	...	+	—	—	—	—	—	+	—	+	+	—	—	—	—
A. punctulatus ...	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. ludlowi ...	6	—	—	—	+	—	—	+	+	?	—	—	—	—	+
A. separatus ...	...	—	—	—	+	—	—	+	+	+	+	+	—	+	—
A. albotæniatus ...	...	—	—	—	+	—	+	+	+	+	+	—	—	—	—
A. tessellatus ...	...	—	—	+	—	—	+	+	+	+	+	+	+	+	+
A. novumbrosus ...	7	—	+	—	—	—	+	+	+	—	+	—	—	+	—
A. punctulatus var. *tessellatus.*	8	—	—	—	+	—	+	—	—	—	—	—	—	—	—
A. karwari ...	...	—	—	—	+	—	—	+	+	+	+	+	—	—	+
A. watsoni ...	...	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. rossii	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. vagus	10	—	—	—	+	—	+	+	+	+	+	—	—	—	—
A. aconitus ...	...	—	—	—	+	—	—	+	—	+	—	—	—	—	—
A. fuliginosus ...	11	—	—	—	+	—	—	—	—	—	—	—	—	—	—
A. montanus ...	...	—	+	—	—	—	+	—	+	+	—	—	—	+	—
A. aitkeni ...	12	—	+	—	+	+	+	+	+	+	+	—	+	—	+

+ = recorded. — = not recorded.

APPENDIX IV—contd.

Species.	Footnote numbers.	Campbell, 1931.	Stoker, 1931.	Soesilo *et al.*, 1932.	Swellengrebel and Rodenwaldt, 1932.	Stoker, 1934.	Goelaeo, 1934.	Brunei, 1929–35.	Sarawak, 1930–39.	Gater, 1935.	Webster, 1941b.	Ou, 1941.	Shircore, 1937.	Carlile, 1941.	McArthur, 1948.
A. hyrcanus ...	13	—	—	—	+	+	—	+	—	+	—	—	—	—	—
A. aitkeni palmatus ...	...	—	—	—	+		—	—	—	+	—	—	—	+	+
A. leuca var. *hackeri* ...	14	—	—	—	+	—	+	+	—	+	—	—	—	—	—
A. subpictus ...	15	—	—	—	—	—	—	—	—	+	—	—	—	—	—
A. errabundus ...	16	—	—	—	+	—	—	—	—	+	—	—	—	—	—
A. barbumbrosus ...	...	—	—	—		+	—	+	+	—	+	—	—	+	+
A. minimus flavirostris	...	—	—	—		+	—	—	—	—	—	—	—	—	—
A. hæzai ...	...	—	—	—	—	—	—	+	—	—	+	+	—	—	+
A. annularis ...	17	—	—	—	—	—	—	+	—	+	—	—	—	—	—
A. barbirostris var. *pallidus.*	18	—	—	—		—	+	—	—	—	—	—	—	—	—
A. philippinensis ...	...	—	—	—		—	—	+	—	—	—	+	—	—	+
A. aitkeni bengalensis	...	—	—	—		—	—	—	—	—	—	—	—	+	+
A. asiaticus ...	...	—	—	—	—	—	—	—	+	—	—	—	—	—	—
A. subpictus malayensis	...	—	—	—	—	—	—	—	+	—	—	—	—	—	—
A. hyrcanus nigerrimus	...	—	—	—	—	—	—	—	+	—	—	—	—	—	—
A. sundaicus ...	...	—	—	—	—	—	—	—	—	+	+	+	—	+	—
A. gigas sumatrana ...	...	—	—	—		—	—	—	+	—	—	—	—	—	—
A. hunteri ...	19	—	—	—		—	—	—	—	—	—	—	—	—	—
A. litoralis ...	20	—	—	—		—	—		—	—	—	—	—	—	+
A. aitkeni borneensis ...	...	—	—	—	—	—	—	—	—	—	—	—	—	—	+

+ = recorded. — = not recorded.

APPENDIX IV—contd.

Species.	Footnote numbers.	Eckman Institute, 1939.	Clapham, 1948.	Allman, 1945.	English, 1945.	Rutledge, 1947.	Colless, 1948a.	Heilbronn, 1948.	Heim, 1948.	Webster, 1947.	Strahan, 1947.	Johnson, 1949b.	Gillies, 1949.	Wallace, 1949.	Colless, 1949 (unpublished).
A. sinensis ...	1	—	+	+	—	—	—	+	—	—	—	+	—	—	?
A. barbirostris ...	...	+	+	+	+	+	+	+	+	+	—	+	+	—	+
A. umbrosus ...	2	+	—	+	—	+	+	+	—	+	—	+	—	+	+
A. vagus ...	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. leucosphyrus ...	4	+	+	+	+	+	?	+	+	—	—	+	+	—	+
A. kochi ...	...	+	+	+	+	—	+	+	+	—	—	+	+	—	+
A. maculatus ...	...	+	—	+	—	—	+	+	—	—	—	—	+	—	+
A. brevipalpis ...	...	+	—	+	—	—	+	+	—	+	—	+	—	—	+
A. punctulatus ...	5	—	—	—	—	—	—	+	—	—	—	—	—	—	—
A. ludlowi ...	6	—	—	—	—	—	?	—	—	—	—	—	—	—	?
A. separatus ...	...	+	—	+	+	+	—	+	—	+	—	+	+	+	+
A. albotaeniatus ...	...	+	—	+	+	—	+	+	—	—	—	+	—	+	+
A. tessellatus ...	...	—	+	+	+	—	+	—	—	+	—	+	+	—	+
A. novimbrosus ...	7	+	—	+	+	—	—	+	—	—	—	—	—	—	—
A. punctulatus var. *tessellatus*.	8	+	—	—	—	—	—	+	—	—	—	—	—	—	—
A. karwari ...	...	+	—	+	—	—	+	+	—	—	—	—	+	—	+
A. watsoni ...	...	—	—	—	—	—	—	—	—	—	—	+	—	—	+
A. ossii ...	9	—	—	—	—	—	—	—	—	—	—	—	—	—	
A. vagus ...	10	+	—	—	+	—	?	+	—	—	—	—	—	—	?
A. aconitus ...	...	+	—	—	—	—	?	—	—	—	—	—	—	—	?
A. fuliginosus ...	11	+	—	—	—	—	—	—	—	—	—	—	—	—	—
A. montanus ...	...	+	—	—	?	—	?	—	—	—	—	—	—	—	?
A. aitkeni ...	12	+	—	+	—	—	+	—	—	—	—	+	—	—	+

+ = recorded. — = not recorded.

APPENDIX IV—contd.

Species.	Footnote numbers.	Eykman Institute, 1939.	Clapham, 1948.	Allman, 1945.	English, 1945.	Rutledge, 1947.	Colless, 1948a.	Heilbrunn, 1948.	Heim, 1945.	Webster, 1947.	Strahan, 1947.	Johnson, 1949b.	Gillies, 1949.	Wallace, 1949.	Colless, 1949 (unpublished).
A. hyrcanus ...	13	—	—	+	—	—	—	—	—	+	—	—	+	+	—
A. aitkeni palmatus ...	...	—	—	—	—	—	?	—	—	—	—	—	—	—	+
A. leucosphyrus var. *hackeri*	14	+	—	—	+	—	?	+	—	—	—	+	—	—	+
A. subpictus ...	15	+	—	—	—	—	?	—	—	—	—	—	+	—	?
A. sundaicus ...	16	+	—	—	—	—	?	—	—	—	—	—	—	—	?*
A. barbumbrosus ...	...	+	+	—	—	—	+	+	—	—	—	+	—	—	+
A. minimus flavirostris	...	—	—	—	—	—	—	—	—	—	—	—	—	—	?
A. hazei ...	...	—	+	+	+	—	—	—	—	+	—	+	+	+	+
A. annularis ..	17	—	—	+	—	—	?	—	—	—	—	—	—	—	?*
A. barbirostris var. *pallidus.*	18	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. philippinensis ...	...	—	—	+	—	+	+	—	+	—	—	—	+	—	+
A. aitkeni bengalensis	...	—	—	—	—	—	—	—	—	—	—	—	—	—	+
A. asiaticus ...	...	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. subpictus malayensis.	...	—	—	—	—	—	—	—	—	—	—	—	—	—	+
A. hyrcanus nigerrimus.	...	—	+	+	+	—	+	—	—	—	—	+	—	—	+
A. sundaicus ...	...	+	—	+	+	+	+	+	+	+	+	+	+	—	+
A. gigas sumatranus	...		—	—	—	—	—	—	—	—	—	—	—	—	—
A. hunteri ..	19	—	—	—	+	—	—	—	—	—	—	—	—	—	—
A. litoralis ...	20	—	—	—	—	—	?	—	—	—	—	—	—	—	?*
A. aitkeni borneensis	...	—	—	—	—	—	—	—	—	—	—	—	—	—	+
A. minimus ..	21	+	—	—	—	—	—	—	—	—	—	—	—	—	—
A. vagus limosus ...	...	—	—	+	—	—	+	—	—	—	—	—	—	—	+

+ = recorded. — = not recorded.

* = Forms whose existence in Borneo appears to be beyond question.

APPENDIX IV—concld.

Species.	Footnote numbers.	Eykman Institute, 1939.	Clapham, 1948.	Allman, 1947.	English, 1943.	Rutledge, 1947.	Colless, 1948a.	Heilbronn, 1948.	Heim, 1948.	Webster, 1947.	Strohar, 1947.	Johnson, 1949b.	Gillies, 1949.	Wallace, 1949.	Colless, 1949 (unpublished).
'*A. hyrcanus* subsp. nr. *sinensis*'.	22	—	—	—	—	—	+	—	—	—	—	—	—	—	—
A. hazari gateri ...	23		—	—	—	—	+	—	—	—	—	—	—	—	—
'*A.* sp. 4 nr. *umbrosus*'.	24	—	—	—	—	—	+	—	—	—	—	—	—	—	—
'*A.* sp. *B* nr. *umbrosus*'.	25	—	—	—	—	—	+	—	—	—	—	+	—	—	+
A. leuco. balabacensis	26		—	—	—		+	—	—	—	—	—	—	—	—
A. leuco. pujudensis ...		—	—	—	—	—	+	—	—	—	—	—	+	—	+
'*A. hyrcanus*, X.' ...	27		—	—	—	—	—	+	—	—	—	—	—	—	?
A. aitkeni, Form II	28		—	—	—	—	—	+	—	—	—	—	—	—	—
A. aitkeni, Form III	29	—	—	—	—	—	—	+	—	—	—	—	—	—	—
A. letifer ...	...	—	—	—	—	—	—	—	—	—	—	+	—	—	+
'*A. hyrcanus*, C.' ...	31	—	—	—		—	—	—	—	—	—	—	—	—	+
'*A. hyrcanus*, Coastal'.	31		—	—	—	—	—	—	—	—	—	—	—	—	+
A. n. sp. nr. *Watsoni*'.	...	—	—	—	—	—	—	—	—	—	—	—	—	—	+

+ = recorded. — = not recorded.

Forms in inverted commas have not yet been formally established.

APPENDIX IVa.

Footnotes to table of Borneo Anopheles identifications.

[The preceding table of Borneo Anopheles identifications is indebted to Mr. D. H. Colless for the following footnotes.]

1. *A. sinensis.*—Many records certainly refer to the unnamed '*A. hyrcanus* subsp. nr. *sinensis*' of Colless (1948), now referred to as the '*A. hyrcanus*, Coastal form.' in accordance with Malayan practice. Some may refer to the other *sinensis*-like forms which have been recently recognized in Malaya (Crawford, 1938). There is no definite evidence yet of the occurrence in Borneo of the true *A. sinensis*.

2. *A. umbrosus.*—Numerous possibilities of confusion exists here. Adult records will for the most part refer indiscriminately to the true *A. umbrosus*, *A. letifer*, and the *A.* sp. *B* nr. *umbrosus* of

APPENDIX IVa—concld.

Colless (1948), and the earlier records will also include *A. bæzai*. Larval records may include any of these, with the remarkable exception of the true *A. umbrosus*, which would be recorded as *A. novumbrosus*. The larvæ of *A. breripalpis* and *A. separatus*, particularly the former, may also have been occasionally recorded as '*umbrosus*'.

3. *A. annus.*—Probably refers to *A. hyrcanus nigerrimus*.

4. *A. leucosphyrus.*—Almost certainly includes records of *A. pujutensis*, which is indistinguishable from *A. leucosphyrus*, in the larval stage.

5. *A. punctulatus.*—Almost certainly to *A. tessellatus*.

6. *A. ludlowi.*—Apart from McArthur (1948), all refer to *A. sundaicus*.

7. *A. novumbrosus.*—*A. umbrosus* [syn. by Colless (1948)].

8. *A. punctulatus* var. *tessellatus.*—*A. tessellatus*.

9. *A. rossii.*—*A. subpictus* (including *A. subpictus malayensis* in many records from Borneo).

10. *A. sagus.*—Probably in many cases refers to *A. sagus limosus*. The type form appears to be absent from North Borneo at least.

11. *A. fuliginosus.*—*A. annularis*.

12. *A. aitkeni.*—Probably most records refer to the 'Malayan type' of Gater (1933).

13. *A. hyrcanus.*—Impossible to relate to any definite form of this group. At least 4 occur in Borneo.

14. *A. leucosphyrus* var. *hackeri.*—Probably some records (if not most) refer to *A. pujutensis*.

15. *A. subpictus.*—Probably includes *A. subpictus malayensis*.

16. *A. errabundus.*—Probably *A. philippinensis*.

17. *A. annularis.*—Almost certainly all refer to *A. philippinensis*.

18. *A. barbirostris* var. *pallida.*—*A. barbumbrosus*.

19. *A. hunteri.*—Possible, though very likely refers to *A. separatus*.

20. *A. litoralis.*—This record refers almost certainly to the Borneo form of *A. sundaicus*.

21. *A. minimus.*—This, if present, is extremely rare. The single record seems highly doubtful.

22. *A. hyrcanus* subsp. nr. *sinensis.*—This form is as yet unnamed. It occurs in Malaya where it is referred to as '*A. hyrcanus*, Coastal form', a practice which is now being followed in Borneo Malaria Research.

23. *A. bæzai gateri.*—Probably *A. bæzai*.

24. '*A.* sp. *A* nr. *umbrosus*'.—*A. lelifer*.

25. '*A.* sp. *B* nr. *umbrosus*.—This species has not yet a published name.

26. *A. leucosphyrus* var. *balabacensis.*—*A. leucosphyrus leucosphyrus* (syn. by Reid, 1949).

27. '*A. hyrcanus*, X'.—An unnamed *nigerrimus*-like form of the *hyrcanus* group.

28. '*A. aitkeni*, form II'.—*A. aitkeni* 'Malayan type' of Gater (1933).

29. '*A. aitkeni*, form III'.—*A. aitkeni* var. *bengalensis*.

30. '*A. hyrcanus*, C'.—An unnamed *nigerrimus*-like species of the *hyrcanus* group.

31. '*A. hyrcanus*, Coastal form'.—'*A.* subsp. nr. *sinensis*' of Colless, 1948 (*see* note 22).

APPENDIX V

Mosquito dissections of Borneo

Year, by whom dissected, reference and place.	Species.	Dissections.			Number positive.			Positive percentage.		
		G.	Gl.	Total.	G.	Gl.	Total.	G.	Gl.	Total.
Roper (Roper, 1914)										
Membakut, North Borneo ...	*A. kochi*	...	...	8	...	...	-	...	...	-
"	*A. leucosphyrus* ...	1	...	17	...	...	-	...	...	-
"	*A. barbirostris* ...	...	...	3	...	...	-	...	...	..
"	*A. umbrosus* ...	...	...	130	...	1	...	...	...	..
"	*A. brevipalpis* ...	...	...	5	...	...	...	...	...	
1924, Stookes (Stookes, 1927)										
Miri, Sarawak	*A. barbirostris* ...	...	...	3	...	-	...	...	...	...
"	*A. ludlowi*	...	...	22	3	...	3	13·6	...	...
"	*A. kochi*	...	...	+	...	...	-	...	...	-
1927, Scharff (Scharff, 1927)										
Labuan	*A. ludlowi*	...	...	1	...	...		...	...	-
1931, Health Department (Sarawak, 1932)										
Bau, Sarawak	*A. leucosphyrus* ...	...	...	+	...	...	...	...	...	
"	*A. barbirostris* ...	...	...	+	...	...	-	...	...	-
"	*A. umbrosus*	...	...	+	...	...	-	...	...	

1931, Stoker (Soesilo *et al.*, 1932)											
Sanggau, Dutch Borneo ...	*A. umbrosus*	...	..	689	...	...	3	..	...	..
.. ..	*A. tessellatus* ...	...	...	10	...	...	..	...	...	..
.. . ...	*A. leucosphyrus* ...	...	...	1	...	...	-	...	...	..
1934, Stoker (Stoker, 1934)											
Kg. Surang Tiong	*A. maculatus*	...	...	11	...	...	...	...	...	—
Pontos Lanoi	*A. hyrcanus* var. *sinensis*	...	...	32	...	...	...	...	...	...
Dutch Borneo	*A. leucosphyrus* ...	...	...	57	6	...	6	...	...	..
Kg. Pedumbaan	*A. leucosphyrus* ...	...	...	53	1	...	1	...	...	0·4
1933-34, Goelarso (Goelarso, 1934)											
Tg. Selor, Dutch Borneo ...	*A. kochi*	...	...	13	...	...		...	...	..
Tg. Palas	*A. kochi*	...	...	8	...	...	.	...	...	..
Sadjau	*A. leucosphyrus* ...	5	...	5	...	...	..	...	...	...
Binai River	*A. leucosphyrus* ...	1	...	1	...	...	..	...	...	..
Kg. Moeara Pangean	*A. leucosphyrus* ...	...	...	13	...	...	1	...	...	..
1933, MacMahon (Sukarmin, 1948)											
Labuan	*A. sundaicus*	...	...	23	1	.	...	...	...	...
.. ..	*A. leucosphyrus* ...	...	...	6		—	...	...	...	...
.. ..	*A. kochi*	...	...	20	..		...	...	...	.
.. ..	*A. tessellatus*	...	...	10	—		...	...	...	..
.. ..	*A. barbirostris* ...	...	...	15	—	—	...	...	...	...

+ = recorded. — = not recorded. G. = gut. Gl. = gland.

APPENDIX V—contd.

Year, by whom dissected, reference and place.	Species.	Dissections.			Number positive.			Positive percentage.		
		G.	Gl.	Total.	G.	Gl.	Total.	G.	Gl.	Total.
1933, MacMahon (Sukarmin, 1948)—*concld.*										
Labuan	*A. bazai*	...	...	9			...	...	...	...
.. ..	*A. separatus*	...	...	7			...	...	...	...
1934, ? (Soesilo *et. al.*, 1935)										
Kg. Semblinbingan, P. Laoet ...	*A. minimus* var. *flavirostris*	...	...	2,137	...	...	1	...	...	0·05
Overbeek, 1941*b*										
Kg. Tengah	*A. minimus* var. *flavirostris*	...	...	401	...	...	1	...	...	0·25
Kg. Semboeloeoe ...	*A. minimus* var. *flavirostris*	...	...	252	...	...	—	...	...	—
1935, Soesilo (Overbeek and Stoker, 1937)										
Soengei Kakap, Pontianak ...	*A. umbrosus*	...	...	59	...	...	...	...	...	15·2
1936, Stoker (Overbeek, 1941*a*)										
Tanah Grogot, Dutch Borneo ...	*A. hyrcanus*	...	...	186	...	...	4	...	...	2·15
1938, Sarawak Oilfields (Webster, 1941)										
Bukit Sawat & Brunei	*A. umbrosus*	...	...	75	3	2	3	..	...	4·9
Medical Staff (Webster, 1941)										
Bukit Sawat & Brunei	*A. nonumbrosus* ...	...	...	7	...	...	—	...	...	...
.. ..	*A. brevipalpis* ...	...	...	1	...	...	—	...	...	...

.. ..	*A. separatus*	...	...	5	...	...	...	...	...	...
. ...	*A. barbirostris* ...	...	...	1	...	...	—	...	...	...
.. ..	*A. tessellatus*	...	...	1	...	...	.	...	...	...
.. ..	*A. albotaeniatus* ...	...	...	1	...	...		...	...	..
Jerudong, Brunei	*A. umbrosus*	...	...	13	...	...	—	...	...	...
.. ..	*A. barbirostris* ...	...	...	36	...	...	—	...	...	...
.. ..	*A. kochi*	...	...	10	...	...	—	...	...	...
.. ..	*A. leucosphyrus* ...	...	...	28	...	...	—	...	...	...
Matading, Sarawak	*A. sundaicus*	...	...	261	...	...	—	...	...	...
.. ..	*A. hyrcanus* var. *sinensis*	...	...	4	...	...	.	...	...	...
.. ..	*A. bazai*	...	...	3	...	...	—	...	...	...
.. ..	*A. umbrosus*	...	...	1	...	...	.	...	...	...
Miri, Sarawak	*A. sundaicus*	...	...	52	...	...	—	...	...	...
1940, McArthur (McArthur, 1947)										
Tambunan, North Borneo ...	*A. kochi*	...	...	237	—	...	..	...	...	—
.. ..	*A. philippinensis* ...	...	...	231	..	—	—	...	...	
. ...	*A. leucosphyrus* ...	...	...	41	...	—	..	...	...	
.. ..	*A. karwari*	...	...	32	...	..	—	...	...	.
.. ..	*A. maculatus*	...	...	28	—	—	—	...	...	—
.. ..	*A. barbirostris* ...	...	...	26	—	—	—	....	...	—
Purutan, North Borneo ...	*A. leucosphyrus* ...	...	...	6	—	—	—	...	...	—
"	*A. maculatus*	...	...	1	—	—	—	...	...	—

+ = recorded. — = not recorded. G. = gut. Gl. = gland.

APPENDIX V—contd.

Year, by whom dissected, reference and place.	Species.	Dissections			Number positive.			Positive percentage.		
		G.	Gl.	Total.	G.	Gl.	Total.	G.	Gl.	Total.
1940, McArthur (McArthur, 1947) —*concld.*										
Apin Apin, North Borneo ...	*A. leucosphyrus* ...	...	...	34	—	—	—	...	...	—
"	*A. barbirostris* ...	...	...	6	—	—	—	...	...	—
"	*A. kochi*	...	...	6	—	—	—	...	...	—
"	*A. maculatus*	...	...	2	—	—	—	...	...	—
"	*A. philippinensis* ...	...	...	2		—	—	...	...	—
Jesselton, North Borneo ...	*A. litoralis*	...	...	30	—	—	—	...	...	—
"	*A. leucosphyrus* ...	...	...	2	—	—	—	...	...	—
"	*A. philippinensis* ...	...	...	1	—	—		...	...	—
"	*A. maculatus*	...	...	1	—	—	—	...	...	—
"	*A. barbirostris* ...	...	...	1	—	—	—	...	...	—
Menggatak, North Borneo ...	*A. philippinensis* ...	...	...	58	—	—	—	...	...	—
"	*A. kochi*	...	...	8	—	—	—	...	...	—
"	*A. barbirostris* ...	...	...	2	—	—	—	...	...	—
"	*A. litoralis*	...	...	1	—	—	—	...	...	—
1941, McArthur (McArthur, 1947)										
Tambunan, North Borneo ...	*A. leucosphyrus* ...	—	—	639	7	12	19	...	...	3·0
"	*A. barbirostris* ...	...	...	482	—	—	—	...	...	—

...	*A. kochi*	...	...	290	—	+		...	...	—
...	*A. philippinensis*	...	...	274	—	—	—	...	...	—
...	*A. maculatus*	...	...	138	—	—	—	...	...	
...	*A. karwari*	...	...	70	—	+	+	...	...	—
...	*A. tessellatus*	...	...	12	—	—	—	...	...	—
...	*A. barbumbrosus*	...	...	2	—	—	—	...	...	—
Apin Apin ...	*A. leucosphyrus*	...	...	11	0	2	2	...	...	18·2
...	*A. maculatus*	...	...	1	—	+	—	...	...	—
Puntan ...	*A. leucosphyrus*	...	...	13	—	1	1	...	...	7·7
...	*A. maculatus*	...	...	3	—	—	—	...	...	—
Apin Apin ...	*A. leucosphyrus*	...	...	15	1	2	3	...	...	20·0
...	*A. barbirostris*	...	...	3	—	—	—	...	...	—
...	*A. philippinensis*	...	...	1	—	—	—	...	...	—
1942-43, O'Connor, Clapham and Allen (Clapham, 1948)										
Kuching, Sarawak ...	*A. leucosphyrus*	...	...	100+	1	...	...	...	...	...
...	*A. hyrcanus* var. *sinensis* / *A. hyrcanus* var. *nigerrimus*	...	...	100+	...	...	—	...	...	...
...	*A. barbirostris*	...	...	50+	...	...	—	...	...	...
...	*A. tessellatus*	...	...	+	...	...	—	...	...	...
...	*A. barbumbrosus*	...	...	+	...	...	—	...	...	...
...	*A. kochi*	...	...	—	...	...	—	...	...	...
...	*A. baezai*	...	...	—	...	...	—	...	...	...

+ = recorded. — = not recorded. G. = gut. Gl. = gland.

APPENDIX V—*contd*

Year, by whom dissected, reference and place.	Species.	Dissections.			Number positive.		
		G.	Gl.	Total.	G.	Gl.	Total.
1945, Allman (Allman, 1945)							
Labuan	*A. philippinensis*	...	...	11	...	...	...
"	*A. hyrcanus* var. *nigerrimus*	...	...	5	...	...	...
"	*A. barbirostris*	...	...	4	...	...	...
"	*A. kochi*	...	...	2	...	...	...
"	*A. tessellatus*	...	...	1	...	...	...
1945-46, Rutledge (Rutledge, 1947)							
Jesselton	*A. leucosphyrus*	...	...	178	8	1	...
"	*A. ambrosus*	...	...	56	4		...
"	*A. sundaicus*	...	...	50	...		...
"	*A. barbirostris*	...	...	80	1	...	...
"	*A. separatus*	...	...	50	...	—	...
"	*A. philippinensis*	...	...	25			...
1947, McArthur (McArthur, 1947*b*)							
Sandakan	*A. leucosphyrus*	...	12	12	1		1
1947, B.M.P. and S.O.L. Medical Staffs (Webster, 1947)							
Serin, Brunei	*A. sundaicus*	...	5	5	1		...
"	*A. lazai*	...	237	237	237		...

...	*A. ambrosus*	205	205	205		
...	*A. barbirostris*	7	7	7		
...	*A. brevipalpis*	1	1	1		
...	*A. scutratus*	8	8	8		
...	*A. hyrcanus*	8	8	8		
...	*A. tessellatus*	2	2	2		
Kuala Belait, Brunei ...	*A. sundaicus*	31	31	31	3	
...	*A. bezzai*	139	139	139		
...	*A. barbirostris*	3	3	3		
...	*A. hyrcanus*	61	61	61		
...	*A. separatus*	16	16	16		
...	*A. tessellatus*	9	9	9		
...	*A. ambrosus*	8	8	8		
Lutong, Sarawak ...	*A. sundaicus*	25	25	25	2	
...	*A. tessellatus*	1	1	1		
...	*A. bezzai*	19	19	19		
...	*A. ambrosus*	16	16	16		
...	*A. brevipalpis*	1	1	1		
...	*A. hyrcanus*	11	11	11		
...	*A. barbirostris*	1	1	1		
...	*A. separatus*	4	4	4		
Miri, Sarawak ...	*A. hyrcanus*	1	1	1		

+ = recorded. — = not recorded. G. = gut. Gl. = gland.

APPENDIX V—contd

Year, by whom dissected, reference and place.	Species.	Dissections.			Number positive.		
		G.	Gl.	Total.	G.	Gl.	Total.
1945, Allman and Colless (Colless, 1948b)							
Brooketon, Sarawak	*A. separatus*	?	102	102	...	...	
Brunei	*A. leucosphyrus*	1	1	1	...	...	
Kuala Belait	*A. separatus*	116	123	124	...	...	
"	*A. sundaicus*	21	29	29	...	...	
"	*A. umbrosus*	3	3	3	...	...	
"	*A. novumbrosus* ...	3	5	5	...	...	
"	*A. albotaeniatus*	1	1	1	...	...	
"	*A. tessellatus*	7	9	10	...	...	
"	*A. hyrcanus*	9	8	9	...	...	
Labuan	*A. leucosphyrus*	3	6	6	...	...	
"	*A. hyrcanus* var. *nigerrimus*	60	68	68	...	...	
"	*A. philippinensis* ...	27	35	35	...	...	
"	*A. barbirostris*	4	4	4	...	...	
"	*A. kochi*	2	2	2	...	...	
1945, Colless (Colless, 1948b)							
Jesselton	*A. separatus*	0	24	24	...	...	
"	*A. barbirostris*	1	4	4	1		
"	*A. sundaicus*	0	1	1	...	...	

1947, Gillies (Gillies, 1948)

Labuan	...	..	..	..	*A. sundaicus*	...	...	...	144	2	...	...
..	..	...	..	...	*A. hyrcanus*	...	...	...	66	2	..	..
..	...	...	...	...	*A. tessellatus*	...	...	...	20	...	..	—
..	...	..	...	...	*A. barbirostris* ...	...	...	...	11	...	...	—
..	...	...	...	...	*A. separatus* ...	...	...	...	7	...	...	—
..	...	...	...	...	*A. leucosphyrus* ...	...	...	...	6	...	...	—
..	...	...	...	...	*A. baezai* ...	...	...	...	3	...	...	

+ = recorded. — = not recorded. G. = gut. GL = gland.

APPENDIX V—contd.

Year, by whom dissected, reference and place.	Species.	Dissections.			Number positive.			
		G.	Gl.	Total.	G.	Gl.	G.+Gl.	Total.
1948, Gillies (Gillies, 1949)								
Labuan	*A. sundaicus*	382	402	410	—	—	...	...
,, ...	*A. tessellatus*	7	8	8	—	—	...	...
,, ...	*A. philippinensis* ...	23	33	33	—	—	...	...
,, ...	*A. karwari*	—	1	1	—	—	...	...
,, ...	*A. leucosphyrus*	89	129	133	1	—	...	...
,, ...	*A. hyrcanus* var. *sinensis* ...	4	4	4	—		...	...
,, ...	*A. hyrcanus* var. *nigerrimus*	1	1	1		—	...	...
,, ...	*A. kochi*	17	20	22	—	—	...	...
,, ...	*A. barbirostris*	13	14	14	—	—	...	...
,, ...	*A. baezai*	3	3	3	—	—	...	...
1948, B.M.P. and S.O.L. Medical Staffs (Johnson, 1949)								
Kuala Belait & Seria, Brunei ...	*A. albotaeniatus* ...	...	...	15	...	...	...	...
,, ...	*A. baezai*	...	...	17	...	...	...	...
,, ...	*A. hyrcanus*	...	...	144	...	...	...	...
,, ...	*A. separatus*	...	...	125	...	...	...	...
,, ...	*A. tessellatus*	...	...	41	...	...	...	...
,, ...	*A. umbrosus*	...	...	209	9	3	...	...

,,	...	...	...	*A. letifer*	...	...	27	..	1	..	...
,,	...	...	...	*A. spec.* 'B'	...	...	11	...	...	...	..
Miri & Lutong, Sarawak		...	...	*A. sundaicus*	...	...	5	...	...	...	...
,,	...	...	...	*A. albotaeniatus* ...	...	...	1	...	...	...	...
,,	...	...	...	*A. bazai*	...	...	1	...	...	...	...
,,	...	...	...	*A. hyrcanus*	...	...	69	...	...	...	...
,,	...	...	...	*A. separatus*	...	...	29	...	...	...	...
,,	...	...	...	*A. tessellatus*	...	...	7	...	...	...	...
,,	...	...	...	*A. umbrosus*	...	...	5	...	...	...	...
,,	...	...	...	*A. sundaicus*	...	...	235	...	...	...	...
,,	...	...	...	*A. barbirostris*	...	...	19	...	...	...	...
,,	...	...	...	*A. barbumbrosus* ...	...	...	1	...	...	...	...
,,	...	...	...	*A. brevipalpis*	...	...	3	...	...	...	...
1948, Malaria Research Department Staffs (McArthur, 1949)											
Labuan	...	...	...	*A. leucosphyrus*	641	715	727	9	16	5	30
,,	...	...	...	*A. philippinensis* ...	704	770	784	...			
,,	...	...	...	*A. sundaicus*	328	401	414	2		..	2
,,	...	...	...	*A. tessellatus*	94	115	119	–	...	..	...
,,	...	...	...	*A. kochi*	157	178	184	–	...	–	...
,,	...	...	...	*A. barbirostris*	27	32	33	–	..	..	...
,,	...	...	...	*A. hyrcanus* var. *nigerrimus*	13	23	26	–	..	–	...

+ = recorded. – = not recorded. G. = gut. Gl. = gland

APPENDIX V—contd.

Year, by whom dissected, reference and place.	Species.	Dissections.			Number positive.			
		G.	Gl.	Total.	G.	Gl.	G.+Gl.	Total.
1948, Malaria Research Department Staffs (McArthur, 1949)—*concld.*								
Labuan	*A. separatus*	7	8	8	—	—	—	...
"	*A. karwari*	3	3	3	—	—	—	...
1948, Colless (Colless, 1948*c*)								
Seria-Kuala Belait Road (mile 5½ to 8½)	*A. umbrosus*	91	131	131	6	1	1	8
"	*A. sp. B. nr. umbrosus* ...	3	3	4	1	—	—	1
"	*A. separatus*	5	9	9	—	—	—	...
"	*A. letifer*	6	7	7	—	—	—	...
"	*A. tessellatus*	1	2	2	—	—	—	...
Seria	*A. albotæniatus* ...	3	4	4	—	—	—	...
1948, Colless (Colless, 1948*d*)								
Seria	*A. umbrosus*	3	11	11	—	—	—	...
Kg. Loak	*A. hyrcanus*, Coastal ...	0	2	2	—	—	—	...
"	*A. umbrosus*	8	8	8	...	...	...	...
"	*A. hyrcanus*, Coastal ...	1	1	1	...	...	...	...
Kg. Senadin	*A. sundaicus*	1	1	1	...	...	...	...
"	*A. hyrcanus*, Coastal ...	29	36	36	...	...	...	...

"	...	...	...	*A. separatus*	12	15	15	...	..	...	...
"	...	...	...	*A. tessellatus*	9	14	14	...	...	...	...
Pangkalan g'dus., Latong		...	...	*A. barbirostris*	4	4	4	...	...	...	...
"	...	...	...	*A. bucai*	41	48	48	...	...	...	...
"	...	...	...	*A. hyrcanus*, Coastal ...	3	4	4	...	...	...	...
"	...	..	...	*A. separatus*	16	22	22	...	...	...	..
"	...	...	...	*A. tessellatus*	4	5	5	...	...	...	...
"	...	...	...	*A. umbrosus*	2	2	2	..	...	...	...

+ = recorded. — = not recorded. G. = gut. Gl. = gland.

APPENDIX V—contd.

Year, by whom dissected, reference and place.	Species.	Dissections.			Number positive.				Positive percentage.		
		G.	Gl.	Total.	G.	Gl.	G.+Gl.	Total.	G.	Gl.	Total.
1949, Wallace (Wallace, 1949)											
Binatang, Lower Rejang ...	*A. separatus*	...	...	1	—	—	—	—	...	...	—
Kuala Matu	*A. hyrcanus* var. *sinensis*	...	...	1	—	—	—	—	...	...	—
,, ...	*A. hyrcanus* var. *nigerrimus*	...	...	18	—	—	—	—	...	...	—
,, ...	*A. umbrosus*	...	...	7	...	...	...	...	...	...	...
,, ...	*A. separatus*	...	...	23	...	...	...	...	...	...	...
Sungei Sok	*A. hyrcanus* var. *nigerrimus*	...	...	4	...	...	...		...	...	...
Kg. Tien	*A. separatus*	...	...	4	...	...	...	...	...	...	...
1949, Gillies (Gillies, 1950)											
Labuan	*A. philippinensis* ...	42	64	64	...	...	...	...	...	...	...
,, ...	*A. karwari*	27	25	28	...	...	...	...	...	...	...
,, ...	*A. baezai*	23	23	23	...	...	...	...	...	...	...
,, ...	*A. leucosphyrus* ...	9	21	21	...	...	...	...	...	...	...
,, ...	*A. hyrcanus* var. *nigerrimus*	14	18	18	...	...	...	...	...	...	...
,, ...	*A. separatus*	8	8	8	...	...	...	...	...	...	...
,, ...	*A. sundaicus*	4	6	6	...	...	...	...	...	...	...
,, ...	*A. baezai*	1	1	1	...	...	...	...	...	...	...
,, ...	*A. maculatus*	1	1	1	...	...	...	...	...	...	...

1948, B.M.P. & S.O.L. Medical Staffs (Johnson, 1950)											
Miri, Lutong & outstations ...	*A. sundaicus* ...	1,497	1,506	1,507	2	3	...	5	...	...	...
"	*A. baezai*	129	130	130	5	1	...	6	...	...	...
"	*A. umbrosus* ...	165	166	167	5	—	...	5	...	...	...
"	*A. separatus* ...	2,673	2,693	2,704	16	—	...	16	...	...	...
"	*A. barbirostris* ...	155	157	157	1	—	...	1	...	...	...
"	*A. tessellatus* ...	437	447	450	1	—	...	1	...	...	...
"	*A. hyrcanus* var. *sinensis*...	233	233	233	...	...	...	...	...	...	...
"	*A. hyrcanus* var. *nigerrimus*	1	1	1	...	...	...	...	...	...	...
"	*A. albotæniatus* ...	3	3	3	...	...	...	...	...	...	...
"	*A. letifer*	4	4	4	...	...	...	...	...	...	...
"	*A. kochi*	11	11	11	...	...	...	...	...	...	...
"	*A. brevipalpis* ...	1	1	1	...	...	...	...	...	...	...
Kuala Belait, Seria & outstations (Brunei).	*A. sundaicus*	...	...	520	1	7(13?)	...	8(14?)	...	...	...
"	*A. baezai*	...	...	47	-	1	...	1	...	...	..
"	*A. sp. B. umbrosus* ...	...	...	26	2	--	...	2	...	...	..
"	*A. letifer*	...	...	538	2	--	...	2	...	...	...
"	*A. separatus* ...	...	...	279	1	-	...	1	...	...	...
"	*A. hyrcanus* var. *sinensis*	...	...	344	2	—	...	2	...	...	...
"	*A. umbrosus* ...	...	...	445	—	—	...	—	...	...	...
"	*A. tessellatus*	...	...	87	—	—	...	—	...	...	...
"	*A. brevipalpis* ...	...	...	1	—	...	...	...	...	...	...

+ = recorded. — = not recorded. G. = gut. Gl. = gland.

APPENDIX V—concld.

Year, by whom dissected, reference and place.	Species.	Dissections.			Number positive.				Positive percentage.		
		G.	Gl.	Total.	G.	Gl.	G.+Gl.	Total.	G.	Gl.	Total.
1949, Malaria Research Dept. Staff (McArthur, 1950)											
Labuan	*A. leucosphyrus* ...	582	1,096	1,109	6	8	...	15	1·2	0·8	1·4
,, ...	*A. sundaicus* ...	588	705	712	...	2	...	2	0·3	—	0·3
,, ...	*A. tessellatus* ...	159	185	191	...	...	...	...	...	...	...
,, ...	*A. philippinensis* ...	328	356	361	...	...	...	...	...	...	...
,, ...	*A. hyrcanus* var. *nigerrimus*.	6	8	100	...	...	...	...	...	...	...
,, ...	*A. barbirostris* ...	28	30	30	...	...	...	...	...	...	...
,, ...	*A. kochi*	48	53	54	...	...	...	...	...	...	...
,, ...	*A. separatus* ...	9	9	9	...	...	...	...	...	...	...
,, ...	*A. baezai*	13	14	14	...	...	...	...	...	...	...
,, ...	*A. karwari*	55	65	63	...	...	...	...	...	...	...
,, ...	*A. hyrcanus* C ...	1	1	1	...	...	...	...	...	...	...

+ = recorded. — = not recorded. G. = gut. Gl. = gland.

APPENDIX Va.

Positive series of Borneo dissections, 1914–1949.

Reference and place.	Species.	Dissections.			Number Positive.			
		G.	Gl.	Total.	G.	Gl.	G.+Gl.	Total.
Roper, 1914								
Membakut, North Borneo	*A. umbrosus*	—	—	130	—	1	—	1
Stookes, 1927								
Miri, Sarawak	*A. ludlowi*	—	—	22	3	—	—	3
Sosilo *et al.*, 1932								
Sanggau, Dutch Borneo	*A. umbrosus*	—	—	680	...	...	...	3
Stoker, 1934								
Kg. Sarang Tioong, Poeloe Laoet ...	*A. leucosphyrus* ...	...	...	57	6	—	—	6
Kg. Pedambaan, Dutch Borneo ...	*A. leucosphyrus* ...	...	...	53	1	—	—	1
Gestarso, 1934								
Kg. Moeara Pangean, Dutch Borneo ...	*A. leucosphyrus* ...	...	...	13	...	...	...	1
Sukarmin, 1948								
Labuan	*A. sundaicus*	...	...	23	1	—	—	1
Sosilo *et al.*, 1935								
Kg. Semblimbingan, P. Laoet ...	*A. minimus* var. *flavirostris*	...	...	2,137	...	...	...	1

+ = recorded. — = not recorded. G. = gut. Gl. = gland.

APPENDIX Va—contd.

Reference and place	Species.	Dissections.			Number positive.			
		G.	Gl.	Total.	G.	Gl.	G.+Gl.	Total.
Overbeek, 1941*b*								
Kg. Tengah, P. Laoet	*A. minimus* var. *flavirostris*...	...	...	401	...	...	...	1
Overbeek and Stoker, 1937								
Soengei Kakap, Pontian	*A. umbrosus*	...	...	59	...	...	...	9
Overbeek, 1941*a*								
Tanah Grogot, Dutch Borneo ...	*A. hyrcanus*	...	...	186	...	...	...	4
Webster, 1941								
Bukit Sawat and Brunei	*A. umbrosus*	...	...	75	3	2	—	3
McArthur, 1947								
Tambunan, North Borneo	*A. leucosphyrus*	—	—	639	7	13	—	19
Apin Apin	*A. leucosphyrus*	...	...	11	0	2	—	2
Purutan	*A. leucosphyrus*	...	...	13	—	1	—	1
Apin Apin	*A. leucosphyrus*	...	...	15	1	2	—	3
Clapham, 1948								
Kuching, Sarawak	*A. leucosphyrus*	...	...	100	1	—	—	1
Rutledge, 1947								
Jesselton	*A. leucosphyrus*	...	...	178	8	1	—	9
,, ...	*A. umbrosus*	...	...	56	4	—	—	4
,, ...	*A. barbirostris*	...	...	80	1	—	—	1

McArthur, 1947b									
Sandakan	*A. leucosphyrus*	12	12	12	1	—	—	1
Webster, 1947									
Seria, Brunei	*A. sundaicus*	5	5	5	1	—	—	1
Kuala Belait, Brunei	*A. sundaicus*	31	31	31	3	—	—	3
Lutong, Sarawak	*A. sundaicus*	25	25	25	2	—	—	2
Colless, 1948b									
Jesselton	*A. barbirostris*	1	4	4	1	—	—	1
Gillies, 1948									
Labuan	*A. sundaicus*	...	...	141	2		—	2
,, ...	*A. hyrcanus*	...	...	66	2		—	2
Gillies, 1949									
Labuan	*A. leucosphyrus*	89	129	133	1		—	1
Johnson, 1949a									
Kuala Belait & Seria, Brunei ...	*A. ambrosus*	...	...	209	8	3		11
,, ...	*A. latifa*	...	...	27		1		1
Malaria Research Department, 1949									
Labuan	*A. leucosphyrus*	641	715	727	9	16	5	30
,, ...	*A. sundaicus*	328	401	414	2	—	—	2
Colless, 1948c									
Seria-Kuala Belait Road (mile 5¼ to 8½)	*A. ambrosus*	91	131	131	6	1	1	8
,, ...	*A.* sp. *B.* nr. *ambrosus* ...	3	3	4	1	—	—	1

+ = recorded. — = not recorded. G. = gut. Gl. = gland.

APPENDIX Va—concld.

Reference and place.	Species.	Dissections.			Number Positive.			
		G.	Gl.	Total.	G.	Gl.	G.+Gl.	Total.
Johnson, 1950								
Miri, Lutong & outstations ...	*A. sundaicus*	1,497	1,506	1,507	2	3	—	5
,, ...	*A. baezai*	129	130	130	5	1	—	6
,, ...	*A. umbrosus*	165	166	167	5	—	—	5
,, ...	*A. separatus*	2,673	2,693	2,704	16	—	—	16
,, ...	*A. barbirostris*	155	157	157	1	—	—	1
,, ...	*A. tessellatus*	437	447	450	1	—	—	1
,, ...	*A. sundaicus*	—	...	520	1	13	—	14
Kuala Belait, Seria, Brunei & outstations	*A. baezai*	...	...	47	—	1	—	1
,, ...	*A.* sp. *B.* nr. *umbrosus* ...	...	...	26	2	—	—	2
,, ...	*A. letifer*	...	...	538	2	—	—	2
,, ...	*A. separatus*	...	...	279	1	—	.	1
,, ...	*A. hyrcanus* var. *sinensis* ...	...	...	344	2	—	—	2
McArthur, 1950								
Labuan	*A. leucosphyrus*	582	1,096	1,109	6	8	1	15
,, ...	*A. sundaicus*	588	705	712	—	2	—	2

+ = recorded. — = not recorded. G. = gut. Gl. = gland.

Indian Journal of Malariology, **4**, 1, March 1950.

MALARIA TRANSMISSION IN FORMER UDAIPUR STATE, CENTRAL PROVINCES.

BY

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[January 16, 1950.]

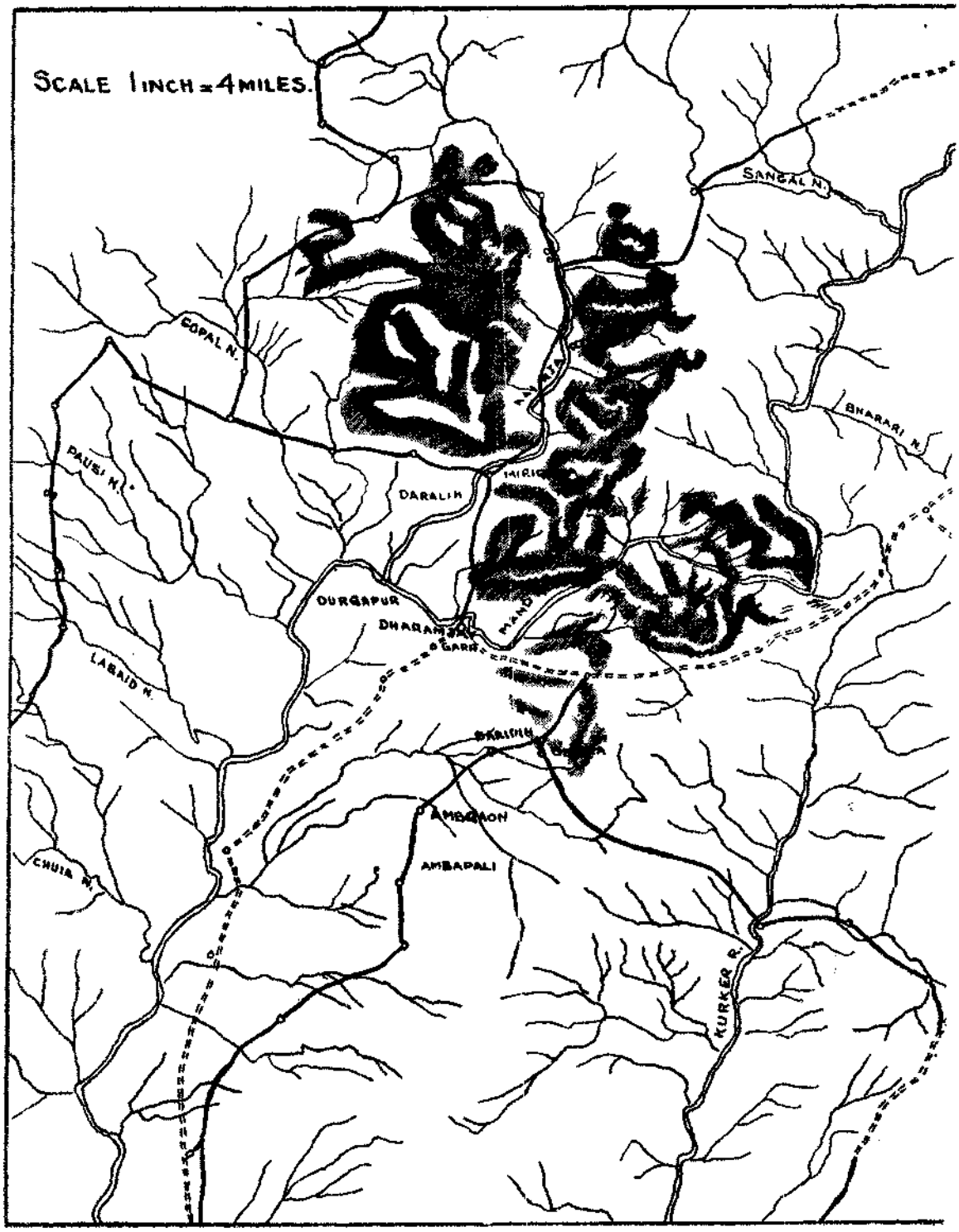
INTRODUCTION.

ROY AND BISWAS (1942) investigated malaria at Dharamjaigarh, the capital of former Udaipur State. They recorded 10 species of Anopheles and out of 2,053 specimens dissected between August and November 1941, found a sporozoite rate of 0·5 per cent in 969 *A. culicifacies* and of 0·7 per cent in 854 *A. pallidus*. They dissected only 38 specimens of *A. fluviatilis*, all negative.

Senior White (1943) has made the following remarks : 'The nearest collections to Udaipur State made by the present author are those from the three Eastern States Agency stations, about 50 miles south-east of Udaipur. In this locality, 486 dissections of *A. pallidus* were made without a single infection being found. The 939 negative dissections at Jharsuguda, some 70 miles from Udaipur, may also be considered.' Senior White goes on to say, 'Roy and Biswas show *A. culicifacies* rising to an October peak, whereas in the length Jharsuguda to Jamga, numbers fall from August to October. For *A. pallidus*, they show a steady incidence level until October, followed by a fall almost to nil in November, which in my length was the month of peak incidence.' He concludes by saying, 'Thus the unusual mensual incidence of *A. culicifacies* and *A. pallidus* as well as the high infection rates found in Udaipur State as compared with widely distributed contrary findings in both respects in the same part of India seem to call for independent investigations in confirmation.'

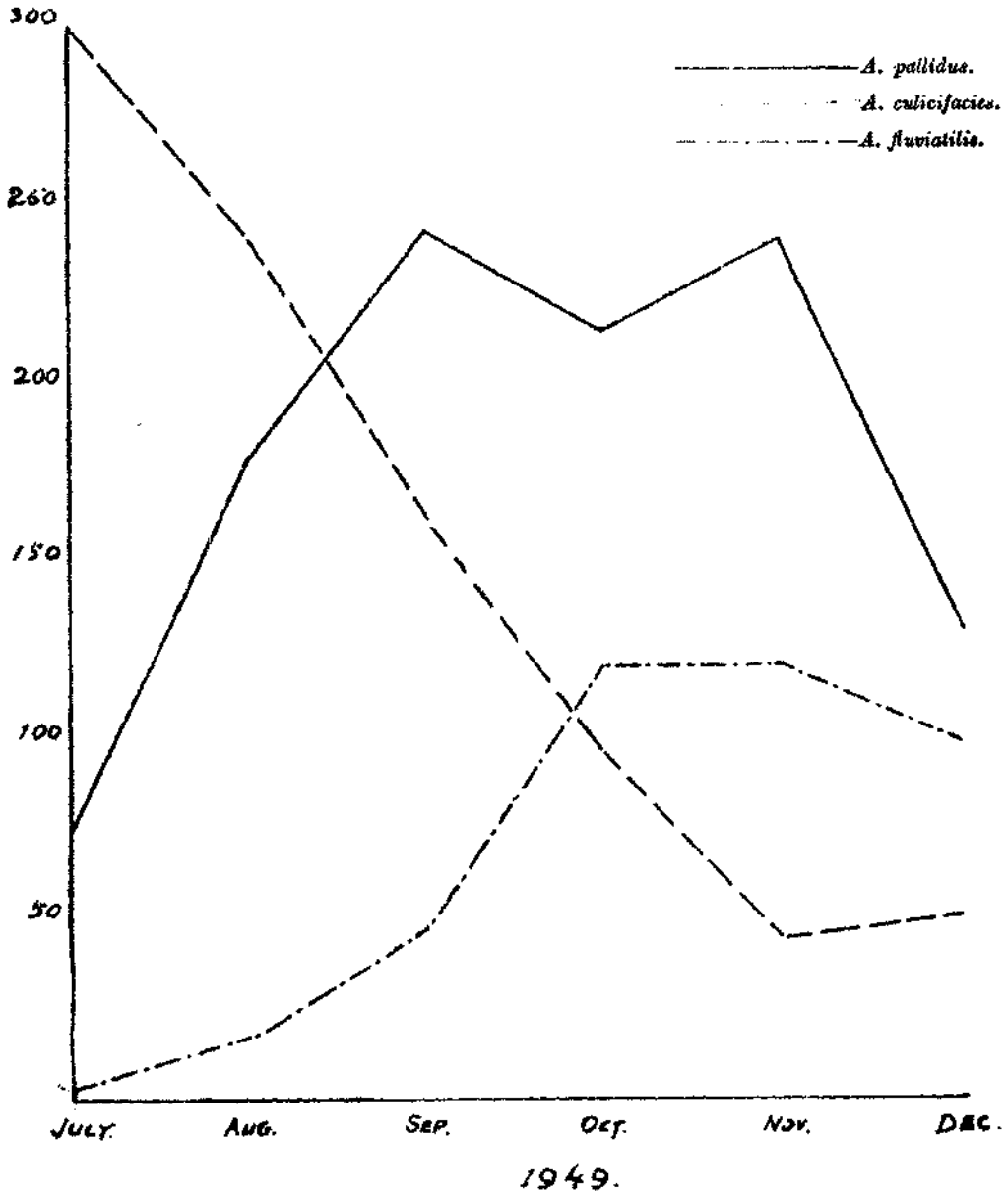
In 1948, Udaipur State along with other Chhattisgarh States was merged with Central Provinces and Berar. Early in 1949, malaria control operations were extended to these States and this opportunity was taken to investigate the vectors of malaria in and around Dharamjaigarh, the capital of former Udaipur State.

MAP.


SCALE 1 INCH = 4 MILES.
SANGAL N.
GOPAL N.
BHARARI N.
PAUSI N.
DARALIH
DURGAPUR
DHARAMGARH
LABAID N.
AMBGAON
AMBAPALI
CHUIA N.
KURKER R.

Four villages Amapalli, Daredih, Durgapur and Miriguda within a radius of 6 miles from Dharamjaigarh were selected for this investigation (Map). Mosquitoes were regularly collected from these villages, brought to Dharamjaigarh and

GRAPH 1.



dissected. Dissections were limited to the finding of gland infections only. The investigations commenced on June 1, 1949, and are continuing. Since observations so far recorded are extremely illuminating, the authors felt that these should be published at this stage.

The mosquitoes which were collected belonged to the following species :— *A. culicifacies, A. fluviatilis, A. minimus, A. jeyporiensis, A. stephensi, A. pallidus, A. splendidus, A. subpictus, A. barbirostris, A. karwari, A. vagus, A. hyrcanus, A. annularis, A. tessellatus, A. jamesi, A. theobaldi, A. maculatus.*

Graph 1 shows the curves for *A. culicifacies, A. pallidus* and *A. fluviatilis.*

The number of *A. culicifacies* shows a steady fall from July to November. In the case of *A. pallidus* there is a steady increase in number from July to November ; similarly in the case of *A. fluviatilis* though not to the same extent as in the case of *A. pallidus*. The authors' findings in respect of *A. pallidus* and *A. culicifacies* seem to generally agree with the findings of Senior White (1943).

NATURAL INFECTION IN ANOPHELES.

The results of anopheline dissections are given in Table I.

TABLE I.

Results of dissections.

Species.	Number dissected.	Number showing sporozoites in salivary gland.
A. pallidus ...	7,121	0
A. culicifacies ...	2,277	2
A. fluviatilis ...	1,335	9
A. subpictus ...	502	0
A. hyrcanus ...	286	0
A. splendidus ...	234	0
A. annularis ...	106	0
A. vagus ...	54	0
A. barbirostris ...	42	0
A. minimus ...	11	0
A. karwari ...	2	0
A. theobaldi ...	1	0
Total ...	11,971	...

SEASONAL INCIDENCE OF INFECTION IN ANOPHELES.

In order to ascertain the seasonal incidence of infection in the vectors, the findings relating to the two species, namely *A. culicifacies* and *A. fluviatilis*, have been analysed to bring out the monthly incidence of infection.

The authors' observations are given in Table II.

TABLE II.

Results of monthly dissections of A. culicifacies *and* A. fluviatilis.

Month.	*A. culicifacies.*			*A. fluviatilis.*		
	Number dissected.	Number with sporozoites in gland.	Sporozoite rate, per cent.	Number dissected.	Number with sporozoites in gland.	Sporozoite rate, per cent.
1949.						
Jun. ...	660	2	0·30	1	0	...
Jul. ...	402	0	0	0	0	...
Aug. ...	443	0	0	16	0	...
Sep. ...	183	0	0	56	1	1·7
Oct. ...	376	0	0	280	5	1·8
Nov. ...	75	0	0	419	2	0·5
Dec. ...	138	0	0	563	1	0·2
TOTAL ...	2,277	2	0·08	1,335	9	0·7

The infection rate of 0·08 per cent in *A. culicifacies* suggests that in this area *A. culicifacies* plays almost very small part in malaria causation.

Dissections of 7,121 *A. pallidus* without coming across a single infected specimen and the finding of gland infections in *A. fluviatilis* during September, October, November and December is in general conformity with Senior White's observations that the principal malaria vector in the Eastern States Agency and in the Hazaribagh Range is *A. fluviatilis* and that *A. culicifacies* transmits to some extent.

SUMMARY.

The density of *A. culicifacies* shows a steady fall from July to November, whereas *A. pallidus* density steadily increases in number from July to November, so also with *A. fluviatilis* though not to the same extent. Contrary to the findings of Roy and Biswas (1942), no natural infection in *A. pallidus* was encountered by the authors during the period June 1949 to December 1949 in and around Dharamjaigarh, capital of former Udaipur State.

A. culicifacies and *A. fluviatilis* were found naturally infected, sporozoite rate in them being 0·08 and 0·7 per cent respectively.

CONCLUSION.

The principal vector, in the former Udaipur State, is *A. fluviatilis* which commences its transmission in September. *A. culicifacies* plays at the most a very small part in malaria causation.

ACKNOWLEDGMENT.

The authors wish to express their indebtedness to Shri D. B. S. P. Patnaik, Antimalaria Supervisor, and Shri Krishna Bahadur Singh, Insect Collector, for helping them in the laboratory.

REFERENCES.

ROY, D. N., and BISWAS, T. C. (1942). On the importance of *A. pallidus* as a carrier of malaria in Udaipur State, C. P. *J. Mal. Inst. Ind.*, **4**, 3, pp. 417–420.

SENIOR WHITE, R. (1943) ... On malaria transmission in the Hazaribagh Ranges. *Ibid.*, **5**, 2, pp. 207–231.

Indian Journal of Malariology, 4, 1, March 1950.

SUPPRESSIVE ACTION OF MEPACRINE, APHACRINE, CHLOROQUIN, CAMOQUIN AND PROGUANIL AGAINST *P. KNOWLESI* IN *S. RHESUS* MONKEYS.

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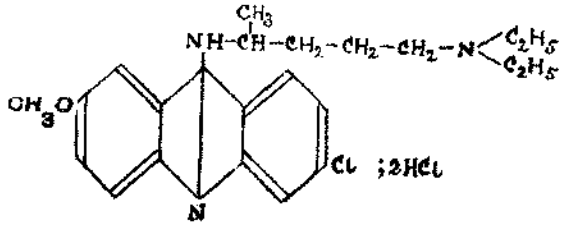
[March 31, 1950.]

RESULTS of therapeutic trials with the above antimalarials on *rhesus* monkeys experimentally infected with *P. knowlesi* have recently been published (Jaswant Singh *et al.*, 1949). Concurrently, efficacy of these antimalarials as suppressants was also being carried out against *P. knowlesi* and investigations have covered a period of three years. Based partly on some of these observations, large-scale field trials on human malarias had been undertaken in various parts of India in selected hyperendemic areas and results with proguanil (paludrine) (Afridi, 1947 ; Ray, 1948) and chloroquin (Goldsmith, 1946 ; Ray, *loc. cit.*) have already appeared in literature.

The chemical structure and formulæ of mepacrine, chloroquin, camoquin and proguanil (paludrine) have been indicated in the previous paper (Jaswant Singh *et al.*, *loc. cit.*), and details of aphacrine (Ladco) which were not included therein, are given below :—



Chemical name :—2 methoxy-6 chloro-9-(4′-diethylamino-1′-methyl-butyl)-amino-acridine dihydrochloride.

METHODS AND MATERIALS.

The trials were conducted against blood-induced *P. knowlesi* infection. One hundred and thirty-eight northern Indian monkeys (*S. rhesus*) were used for these investigations and each one received an inoculation of 1 c.c. of blood from donor monkeys showing on an average one parasite per 5 to 10 microscopic fields of a thin film. Blood smears, both thick and thin, stained with J.S.B. stain (Jaswant Singh and Bhattacharji, 1944) were examined daily in all cases throughout the period of investigations.

The drugs were tested in varying dosages from 10 to 50 mg. in the majority of the cases, and up to 100 mg. in respect of a few antimalarials like mepacrine and proguanil when biweekly and weekly schedules were instituted. Administration of drug was restricted to some cases only before the infective inoculation, while in others the animals received the drug either after the inoculation or both before and after the inoculation of parasites. The under-mentioned treatment schedules were followed :—

1. Daily drug administration for 7 days prior to inoculation.
2. Daily drug administration for 7 days subsequent to inoculation.
3. Daily drug administration for 7 days prior and for 7 days subsequent to inoculation.
4. Daily drug administration for 7 days prior and for 7 days subsequent to inoculation, and thereafter for 21 days at biweekly interval.
5. Biweekly drug administration for a week prior and for 4 weeks subsequent to inoculation.
6. Biweekly drug administration for 4 weeks prior and for 10 weeks subsequent to inoculation.
7. Weekly drug administration for 2 weeks prior and 4 weeks subsequent to inoculation.
*8. Weekly drug administration for 4 weeks prior and for 10 weeks subsequent to inoculation.

In all cases the drug was administered orally under the personal supervision of one of the authors.

The various treatment schedules, dosage of the antimalarials and the number of monkeys involved in the investigation under each regime, have been grouped in Tables I to VIII.

In majority of the cases, two animals were placed under each dosage regime. In certain cases, however, larger series were utilized.

The follow-up of the cases which did not develop infection on completion of the treatment regimes lasted usually from 2 to 5 months. In a few cases, observations were recorded only up to about one month while in others up to a period of 9 months. Complete sterilization of infection, latent infection and susceptibility were assessed by :—

(*a*) Sub-inoculation to healthy animals.
(*b*) Adrenalin injection (1 c.c.).
(*c*) Super-inoculation with the homologous strain (susceptibility tests).
(*d*) Splenectomy in a few cases.

---

* Under Schedule 8, only chloroquin was used.

TABLE I.

Schedule 1.

Daily drug administration for 7 days prior to infective inoculation.

Drug.	10 mg.				20 mg.				50 mg.			
	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)
Mepacrine ...	2	*Nil*	*Nil*	2 (3rd and 6th)	2	*Nil*	*Nil*	2 (3rd both)	2	*Nil*	*Nil*	2 (7th and 10th)
Proguanil ...	7	*Nil*	*Nil*	7 (3rd to 6th)	8	*Nil*	*Nil*	8 (4th to 7th)	3	*Nil*	*Nil*	3 (3rd to 7th)
Aphacrine ...	2	*Nil*	*Nil*	2 (6th to 7th)	2	*Nil*	*Nil*	2 (4th and 5th)	2	*Nil*	*Nil*	2 (5th and 8th)
Chloroquin ...	2	*Nil*	1 (37th)	1 (9th)	2	1	*Nil*	1 (5th)	2	1	1 (11th)	*Nil*
Camoquin ...	...	...	...	...	2	*Nil*	*Nil*	2 (10th both)	...	...	...	

RESULTS.

The success or otherwise under each drug regime is represented in the tables as (*a*) 'completely suppressed', meaning that no parasites were detected during the period of observation; (*b*) 'partially suppressed', to indicate delay of over 10 days in the appearance of parasites from the date of inoculation; (*c*) 'failure', meaning the drug having no effect on that particular schedule and regime.

Under this schedule it will be observed that except chloroquin, antimalarials failed to suppress the infection. In 50 mg. dosage under chloroquin, there was no failure and out of 2 animals, infection in one was completely suppressed while in the other was partially so; with 20 mg. dosage, infection was suppressed completely in one while failed in the other. With 10 mg., there was only partial suppression in one case and failure in the other.

TABLE II.

Schedule 2.

Daily drug administration for 7 days subsequent to infective inoculation

Drug.	10 mg.				20 mg.			
	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)
Mepacrine ...	2	*Nil*	1 (14th)	1 (7th)	2	*Nil*	1 (13th)	1 (10th)
Proguanil ...	6	*Nil*	*Nil*	6 (4th and 9th)	6	2	2 (12th)	2 (8th)
Aphacrine ...	2	*Nil*	*Nil*	2 (4th both)	...	...	...	...
Chloroquin ...	4	2	1 (10th)	1 (5th)	2	2	*Nil*	*Nil*
Camoquin ...	2	*Nil*	2 (18th and 46th)	*Nil*	2	2	*Nil*	*Nil*

It is interesting to note that in 20 mg. dosage, both chloroquin and camoquin completely suppressed the infection, though with 10 mg., the results are not so striking. Both proguanil and aphacrine were proved ineffective in 10 mg. dosage though proguanil was partially successful in 20 mg. dosage.

TABLE III.

Schedule 3.

Daily drug administration for 7 days prior and 7 days subsequent to infective inoculation.

Drug.	10 mg.				20 mg.			
	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)
Chloroquin ...	2	2	*Nil*	*Nil*	...	...	...	...
Camoquin ...	2	*Nil*	1 (23rd)	1 (8th)	2	2	*Nil*	*Nil*

The schedule was followed up with chloroquin and camoquin only. While the former proved successful in 10 mg. dosage, the latter required double the dose.

The outstanding feature of this schedule is that while chloroquin proved effective in 10 mg. dosage, double the dose was required for camoquin, and five times for mepacrine, proguanil and aphacrine for similar effects.

Where chloroquin proved effective in 10 mg. dosage, camoquin, mepacrine and proguanil required 20 mg. to produce similar effect and aphacrine 50 mg. dosage. When camoquin was tried biweekly on 50 mg. dosage, complete suppression was obtained. This regime was not tried with the other drugs.

Chloroquin proved successful in biweekly schedule even in 10 mg. dosage, while mepacrine showed similar results with 100 mg. Proguanil failed to suppress the infections in 50 or even with 100 mg. dosage.

The main features under this schedule are that on a weekly regime, proguanil even on 100 mg. dosage proved to be a failure. Mepacrine was partially successful whereas chloroquin was completely so in 50 mg. dosage. Camoquin was tried only in 20 mg. dosage but failed to suppress infection as compared to chloroquin where partial suppression was effected in the same dosage. From our previous observations on 10 and 20 mg. doses, trials with mepacrine, proguanil and aphacrine on a weekly schedule were not considered necessary.

TABLE IV.

Schedule 4.

Daily drug administration for 7 days prior and subsequent to infective inoculation and thereafter biweekly for 3 weeks.

Drug.	10 mg.				20 mg.				50 mg.			
	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.	Failure. (Day of appearance of parasites.)
Mepacrine ...	...	...	...	...	1	*Nil*	1 (12th)	*Nil*	1	1	*Nil*	*Nil*
Proguanil ...	1	*Nil*	*Nil*	1 (7th)	1	*Nil*	1 (12th)	*Nil*	2	2	*Nil*	*Nil*
Aphacrine ...	...	...	...	...	...	...	...	...	1	1	*Nil*	*Nil*
Chloroquin ...	1	1	*Nil*	*Nil*	1	1	*Nil*	*Nil*	1	1	*Nil*	*Nil*
Camoquin ...	2	*Nil*	*Nil*	2 (8th)	2	2	*Nil*	*Nil*	...	...	...	...

TABLE V.

Schedule 5.

Daily drug administration for 7 days prior and 4 weeks (28 days) subsequent to infective inoculation. Last item, viz. camoquin, administered biweekly throughout.

Drug.	10 mg.				20 mg.				50 mg.			
	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)
Mepacrine (daily) ...	2	*Nil*	*Nil*	2 (5th and 6th)	1	1	*Nil*	*Nil*	1	1	*Nil*	*Nil*
Proguanil (daily) ...	1	*Nil*	1 (11th)	*Nil*	1	1	*Nil*	*Nil*	1	1	*Nil*	*Nil*
Aphacrine (daily) ...	...	...	...	...	2	1	*Nil*	1 (5th)	1	1	*Nil*	*Nil*
Chloroquin (daily) ...	1	1	*Nil*	*Nil*	1	1	*Nil*	*Nil*	1	1	*Nil*	*Nil*
Camoquin (daily) ...	...	...	...	...	2	2	*Nil*	*Nil*	...	...	...	...
Camoquin (biweekly)	...	...	...	...	...	...	...	...	2	2	*Nil*	*Nil*

TABLE VI.

Schedule 6.

Biweekly drug administration for 4 weeks prior and 10 weeks subsequent to infective inoculation.

Drug.	10 mg.				20 mg.				50 mg.				100 mg.			
	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)
Mepacrine ..	..	..	..	..	..	..	..	..	2	1	*Nil*	1 (4th)	2	2	*Nil*	*Nil*
Proguanil ..	..	..	..	..	..	..	..	..	2	*Nil*	*Nil*	2 (4th and 8th)	2	*Nil*	*Nil*	2 (3rd and 5th)
Chloroquin ..	2	2	*Nil*	*Nil*	2	2	*Nil*	*Nil*	1	1	*Nil*	*Nil*	..	..	..	..
Camoquin ..	2	*Nil*	*Nil*	2 (6th and 9th)	..	..	..	..	..	..	..	..	..	..	..	..

TABLE VII.

Schedule 7.

Weekly drug administration for 2 weeks prior and 4 weeks subsequent to infective inoculation.

Drug.	10 mg.				20 mg.				50 mg.				100 mg.			
	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)
Mepacrine ..	..	..	..	..	..	..	..	..	2	1	..	1 (6th)	2	*Nil*	1 (37th)	1 (4th)
Proguanil ..	..	..	..	..	..	..	..	..	2	*Nil*	*Nil*	2 (5th and 8th)	2	*Nil*	*Nil*	2 (7th and 8th)
Chloroquin ..	2	1	1	1 (4th)	2	1	..	1 (6th)	2	2	*Nil*	*Nil*	..	..	..	..
Camoquin ..	..	..	..	..	2	*Nil*	*Nil*	2 (8th and 9th)	..	..	..	..	..	..	..	..

TABLE VIII.

Schedule 8.

Weekly drug administration for 4 weeks prior and 10 weeks subsequent to infective inoculation.

Drug.	50 mg.			
	Number of animals used.	Completely suppressed.	Partially suppressed. (Day of appearance of parasites.)	Failure. (Day of appearance of parasites.)
Chloroquin ...	2	2	*Nil*	*Nil*

Chloroquin proved highly effective.

DISCUSSION.

From the foregoing results it is noted that out of 33 monkeys receiving varying doses of chloroquin in the different treatment schedules, the number of break-through cases were only 5; three out of 25 under Schedules 1 to 6, where drug was administered daily or biweekly; and two out of 6 under Schedules 7 and 8, where drug administration was resorted to at weekly intervals. The break-throughs occurred only when the dosages were between 10 and 20 mg. In 7 out of 8 cases, where a dose of 50 mg. chloroquin was administered daily, biweekly or weekly, the suppression was complete and no parasitaemia was detected throughout the period of observation. Tests for latent infections were negative indicating thereby that complete sterilization had occurred. Similar investigation with respect to other drugs were also conducted in those cases which did not break through. It is interesting to note that in most cases a dose of 10 mg. had been effective, particularly noticeable in Tables II to VII. On the other hand, all the other drugs in Schedule 1 had completely failed to suppress infection in all cases irrespective of the doses used.

When daily drug administration was instituted subsequent to inoculation, and continued for 7 to 28 days, mepacrine and proguanil were successful in 50 mg. under all schedules and partially so in 20 mg. dose. Under biweekly schedule, proguanil proved ineffective even in 100 mg. doses whereas mepacrine proved effective, partially in 50 mg. and completely so in 100 mg. doses. Similarly, under weekly schedule, proguanil gave poorer results while mepacrine proved only partially successful. Results obtained with aphacrine were similar to those obtained with mepacrine or proguanil. Camoquin when administered daily was 100 per cent successful (Schedules 2 to 5) in 20 mg. dose and partially so in 10 mg. dosage.

Although the number of test series under each schedule is not large but when all the series under the eight tables are compared, it will be seen that chloroquin

even in a very small dose is effective, partially in cases where daily drug administration was instituted only before the infective inoculation and no suppressive treatment given afterwards, and fully so, in most of the cases when the regime was continued for varying periods after the inoculation. Chloroquin also proved effective when drug administration was instituted biweekly or weekly.

Camoquin, though not tested as extensively as chloroquin, showed effective suppressive activities though somewhat of a lower order than chloroquin, in that it proved effective only when drug was administered subsequent to infective inoculation and in those schedules where the dose was more than 10 mg.

While the relative merits of mepacrine and proguanil may appear to be the same as observed from the results shown in Tables I to V, in weekly and biweekly schedules, mepacrine showed marked superiority as a suppressant over proguanil, even though statistically the number of animals utilized was not large. These studies are well comparable to the previous observations made by workers in this country and abroad. Jaswant Singh *et al.* (*loc. cit.*) had previously reported that in therapeutic dosage, where much larger doses were administered than in the present series, the action of chloroquin and camoquin on parasites was found to be prompt, and much quicker than mepacrine or proguanil; mepacrine being slightly superior to proguanil; and again both these were found to be quicker than aphacrine and metachloridine. In weekly schedules, chloroquin proved superior to all others and confirm the effective suppressive action in human malaria observed by Goldsmith (*loc. cit.*), Packer (1947), Elmendorf (1948), Ray (*loc. cit.*), Smith *et al.* (1948), Coatney *et al.* (1949), Butt (1950) and others.

Contrary to the observations made against sporozoite-induced infections where proguanil was found to be effective in biweekly regimes (Ray, *loc. cit.*; De Rook, 1949), etc., analysis of Table VI shows that in blood-passaged infection of *P. knowlesi*, biweekly dosage of mepacrine gave somewhat better results than proguanil; complete suppression being effective in 100 mg. and partially so in 50 mg. dosage. Against sporozoite-induced infection in human malaria, mepacrine was found ineffective in biweekly or weekly regimes (Fairley, 1945).

The better results obtained with proguanil against sporozoite-induced infection than mepacrine can easily be explained as proguanil is supposed to act directly against the pre-erythrocytic stages of *P. falciparum* and has deleterious inhibitory action against the tissue phase of *P. vivax* (Fairley, 1946) appearing in peripheral blood as erythrocytic forms. Action of mepacrine is restricted to only against erythrocytic phase and that is why more frequent drug administration is necessary to keep the erythrocytic forms suppressed or destroyed.

In case of blood-induced infection, however, the position is different. The action against erythrocytic parasites will depend on the (*a*) presence or absence of the drug in the system depending on the absorption, excretion, degradation, etc., on the one hand and (*b*) the inherent molecular activity of the chemicals on the other. It is generally agreed that compounds which are rapidly absorbed from the gastro-intestinal tract and show considerable localization in tissues are more effective if loading or priming doses are given at the start of the therapy. In those where the rate of elimination is slow, the compounds will be retained in the body longer and permit wider spacing of doses (Cooper, 1949). While the above-

mentioned drugs are quickly absorbed and markedly localized in the leucocytes, tissues like kidney, spleen, etc., proguanil is more rapidly disposed off when compared to atabrin, chloroquin and camoquin (Cooper, *loc. cit.*; Jaswant Singh, 1950). This may perhaps explain the failure of proguanil in Schedule 6 even in doses of 100 mg.

With regard to mepacrine, chloroquin and camoquin, while the absorption, localization and the general pattern of distribution are very similar with a slow rate of excretion and degradation, the drop in plasma concentration after cessation of drug administration is slightly more rapid with chloroquin and camoquin (60 per cent per week) as compared to atabrin (50 per cent per week) (Wiselogle, 1945; Loeb *et al.*, 1946; Cooper, *loc. cit.*; Coatney, 1949). Although the better localization and slow elimination may be the factors for explaining better suppressive action of these drugs when compared to quinine or proguanil, the wide and marked variations in the results obtained in the different schedules between mepacrine on the one hand and camoquin and chloroquin on the other, and between chloroquin and camoquin, may be difficult to explain. Nor can these explain the wide range of 'quinine equivalents' observed in respect of these drugs (atabrin 2 to 6, chloroquin 15, and camoquin 8 to 30 against *P. gallinaceum*—Wiselogle, *loc. cit.*). From these observations, the relative effectiveness of chloroquin is in conformity with the quinine equivalents of the different drugs. Whether this difference in the biological activity in compounds of more or less similar group of chemical composition depends on the nature of molecular structure of the drugs or their metabolites, is a matter for consideration and this may explain the differences observed in their activities. As suggested by Work and Work (1948), study of the relationship between structure and activity of various chemicals may indicate a profitable line of investigation.

SUMMARY.

Suppressive action of antimalarial drugs—mepacrine, chloroquin, camoquin, aphacrine, proguanil, in doses lower than previously used for therapeutic purposes—were tried under eight different schedules. Chloroquin proved to outrank all others though camoquin showed considerable activities.

REFERENCES.

AFRIDI, M. K. (1947) .. *Ind. J. Mal.*, **1**, pp. 347–360.
BUTT, D. C. A. (1950) .. *J. Nat. Mal. Soc.*, **9**, pp. 44–49.
COATNEY, G. R. (1949) ... *Bull. Pan Amer. San. Bureau*, **28**, p. 401.
COATNEY, G. R., RUBE, D. S., COOPER, W. C., JOSEPHSON, E. S., and YOUNG, M. D. (1949). *Amer. J. Hyg.*, **49**, pp. 49–59.
COOPER, W. C. (1949) ... *Pub. Hlth. Rep.*, **64**, pp. 717–732. U.S. Public Health Service, Federal Security Agency.
DE ROOK, H. (1949) ... *Trop. Dis. Bull.*, **47**, p. 206.
ELMENDORF, J. E. (1948) ... *Amer. J. Trop. Med.*, **28**, pp. 425–436.
FAIRLEY, N. H. (1945) ... *Trans. Roy. Soc. Trop. Med. Parasit.*, **38**, pp. 311–366.
Idem (1946) ... *Ibid.*, **40**, pp. 229–273.
GOLDSMITH, K. (1946) ... *J. Mal. Inst. Ind.*, **6**, pp. 311–316.
JASWANT SINGH (1950) ... Antimalarial Drugs. (In press.)
JASWANT SINGH and BHATTACHARJI, L. M. (1944). *Ind. Med. Gaz.*, **79**, pp. 102–104.

JASWANT SINGH, RAY, A. P., and NAIR, C. P. (1949). *Ind. J. Mal.*, **3**, pp. 387–403.
LOEB, R. F., *et al.* (1946) ... *J. Amer. Med. Assoc.*, **130**, pp. 1069-1070.
PACKER, H. (1947) ... ... *J. Nat. Mal. Soc.*, **6**, pp. 147–154.
RAY, A. P. (1948) ... ... *Ind. J. Mal.*, **2**, pp. 35–66
SMITH, H. F., DY, F. J., and CABRERA, D. J. (1948). *Acta Med. Philippina*, **5**, pp. 1–16.
WISELOGLE, F. Y. (1945) ... *Survey of Antimalarial Drugs*, 1941–1945. Edwards Bros., Ann Arbor, Michigan, U.S.A.
WORK, T. S., and WORK, E. (1948). *The Basis of Chemotherapy*, pp. 304–361. Interscience Publishers, Inc., N.Y.

Indian Journal of Malariology, **4**, 2, *June 1950*.

A FATAL CASE OF *P. FALCIPARUM* MALARIA.

BY

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[February 7, 1950.]

An old man of 75 was admitted into the hospital with a history of remittent fever and diarrhœa of 8 days' duration. The patient came to the hospital walking and was weak, emaciated and anæmic with icteric tinge of the conjunctivæ. The temperature on admission was 98°F., hands and feet were cold, pulse feeble, 100 per minute and blood pressure 80/55. Heart sounds were weak and lungs clear. The tongue was coated and rather dry; liver and spleen were not palpable.

The blood smear showed a heavy *falciparum* infection. A few crescents and schizonts were also seen. Number of parasites—1,093,440 per c.mm.; 35·6 per cent of red cells were infected, some of them containing 2, 3 or 4 rings in each; total plasma protein 4·5 per cent; hæmoglobin 8·8 gm. per cent (64 per cent Hellige scale), red cells 2·72 millions per c.mm., white cells 16,000 per c.mm., polymorphs 78 per cent, lymphocytes 14 per cent and monocytes 8 per cent. Most of the polymorphs and monocytes had pigment within their cytoplasm.

Stool examination showed nothing abnormal. Urine could not be collected for examination.

TREATMENT.

Paludrine lactate 0·2 gm. was given intravenously and 0·2 gm. intramuscularly on admission at 3·00 p.m. This was followed by 3 more intravenous injections, 0·1 gm. each, first two at an interval of 4 hours and the third of 6 hours, i.e. till 5·00 a.m. the following morning. Normal saline with 5 per cent glucose (10 ounces) was also administered intravenously by drip method on admission and alkaline mixture was given every 6 hours.

On the second day the patient was drowsy but responded to questions. He had one liquid motion and had passed some urine in linen. Temperature 98°F.,

pulse feeble, rate 124 per minute, extremities cold, B.P. 88/60, crepitations were heard on both lung bases.

Blood examination on the second day revealed—*falciparum* rings a few, crescents +, hæmoglobin 6·05 gm. per cent (44 per cent Hellige scale), red cells 2·0 millions per c.mm., white cells 39,000 per c.mm., polymorphs 82 per cent, lymphocytes 12 per cent and monocytes 6 per cent.

He was given another intramuscular injection of paludrine lactate 0·3 gm. at 11·00 a.m. and was put on 0·2 gm. t.d.s. by mouth. Besides, as a supportive measure, he was given a plasma transfusion and put on atropine with coramine and suprarenal cortical hormone injections every 4 hours alternately. In view of the lung signs and marked leucocytosis, he was also given penicillin 50,000 units followed by 25,000 units every 3 hours. He had oxygen inhalation and frequent liquid feeds in small amounts.

Towards the evening the condition further deteriorated, hiccough started; the patient became comatose and died at night, 34 hours after admission.

POSTMORTEM REPORT.

Postmortem was very much delayed (34 hours after death) and the body was partially decomposed.

Rigor mortis—present. Cornea—hazy.

Site of intravenous injection.—The medial cubital vein on the right side in which he was given the first 3 paludrine injections was thrombosed. At the sites of the intramuscular injections no necrotic area was visible to the naked eye.

Lungs.—Right 540 gm. Left 520 gm. Adhesions present with a few emphysematous bullæ. They were congested, œdematous and crepitant. All the cut pieces floated in water.

Microscopically—Congested vessels, exudates in alveoli and bronchioles.

Heart.—Small and flabby, postmortem clot present, coronary arteries patent, wall of right ventricle 3 mm., left 12 mm.

Microscopically—Dilated capillaries in between muscle fibres containing malaria parasites (mostly grown-up asexual forms and schizonts). The capillaries packed with infected red cells.

Heart blood.—Showed plenty of crescents and a few asexual parasites. There were also a few clumps of nucleated cells which were apparently myeloblasts.

Spleen.—400 gm., soft and flabby, slate-coloured, decomposed.

Microscopically—Impression smear showed malaria parasites and pigments. Decomposed.

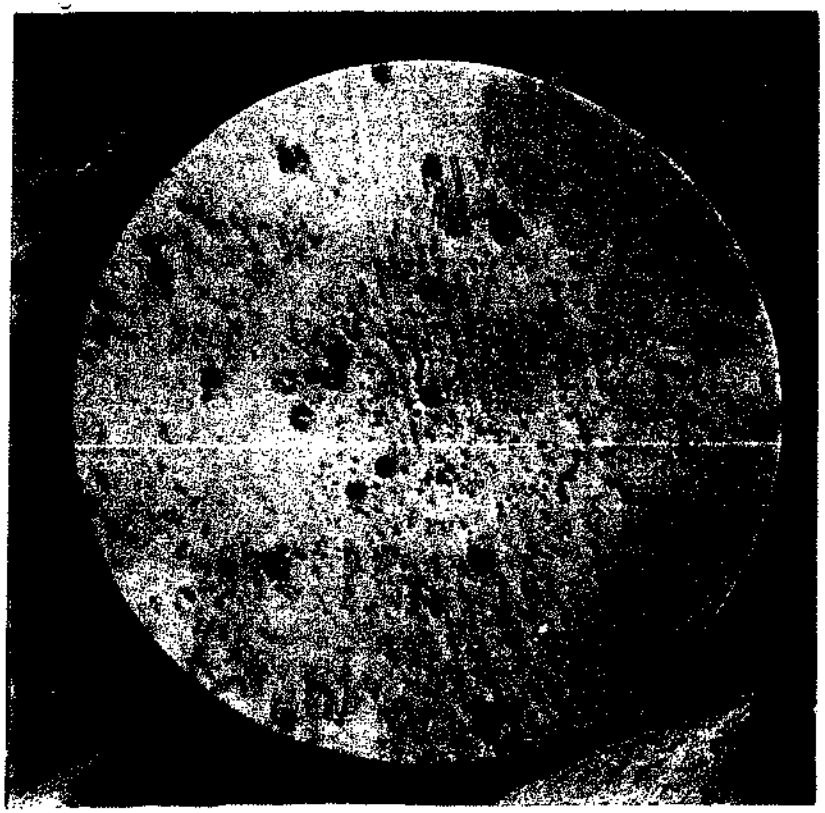
Liver.—900 gm., dark and congested. Impression smear showed parasites and pigments.

Stomach and intestines.—Congested areas seen.

Microscopically—Section of small intestines showed capillaries of villi packed with infected red cells.

Suprarenal glands.—Decomposed.

PLATE I.

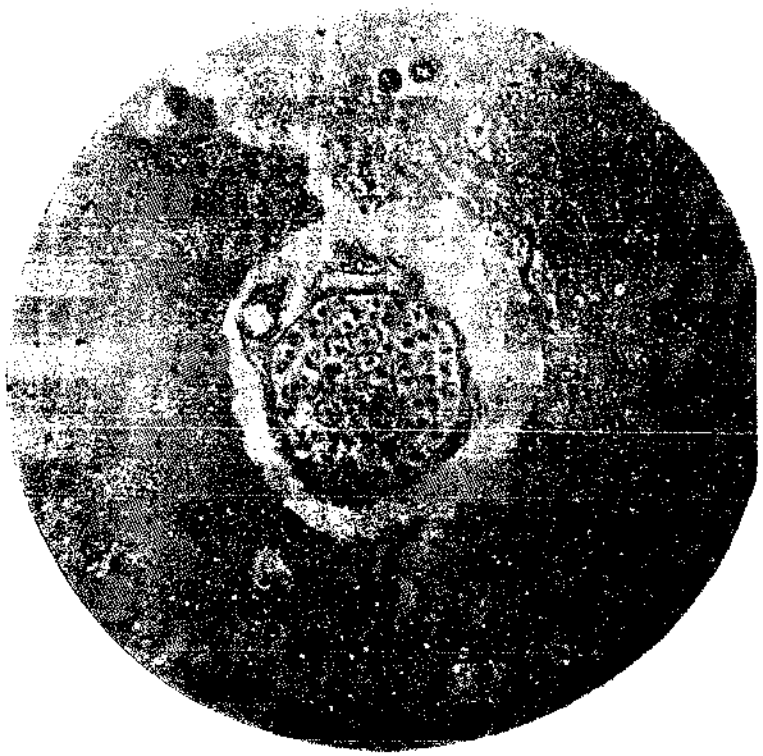


Thick smear of blood (J.S.K. stain).

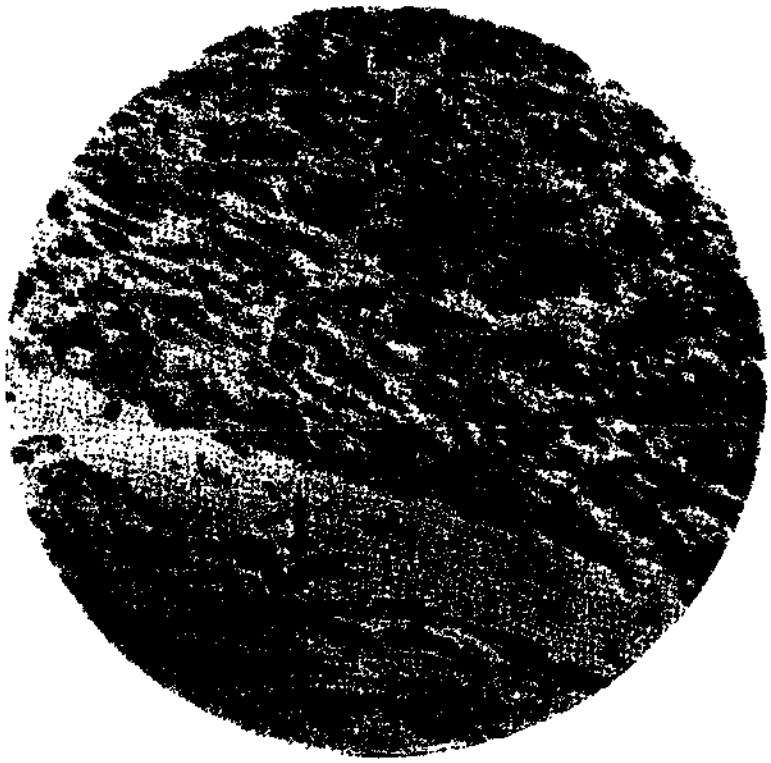
Thin smear of blood 1 to 4 rings in the R.B.C.

PLATE II.

Section of brain: the capillaries are packed with malaria parasites and pigments.



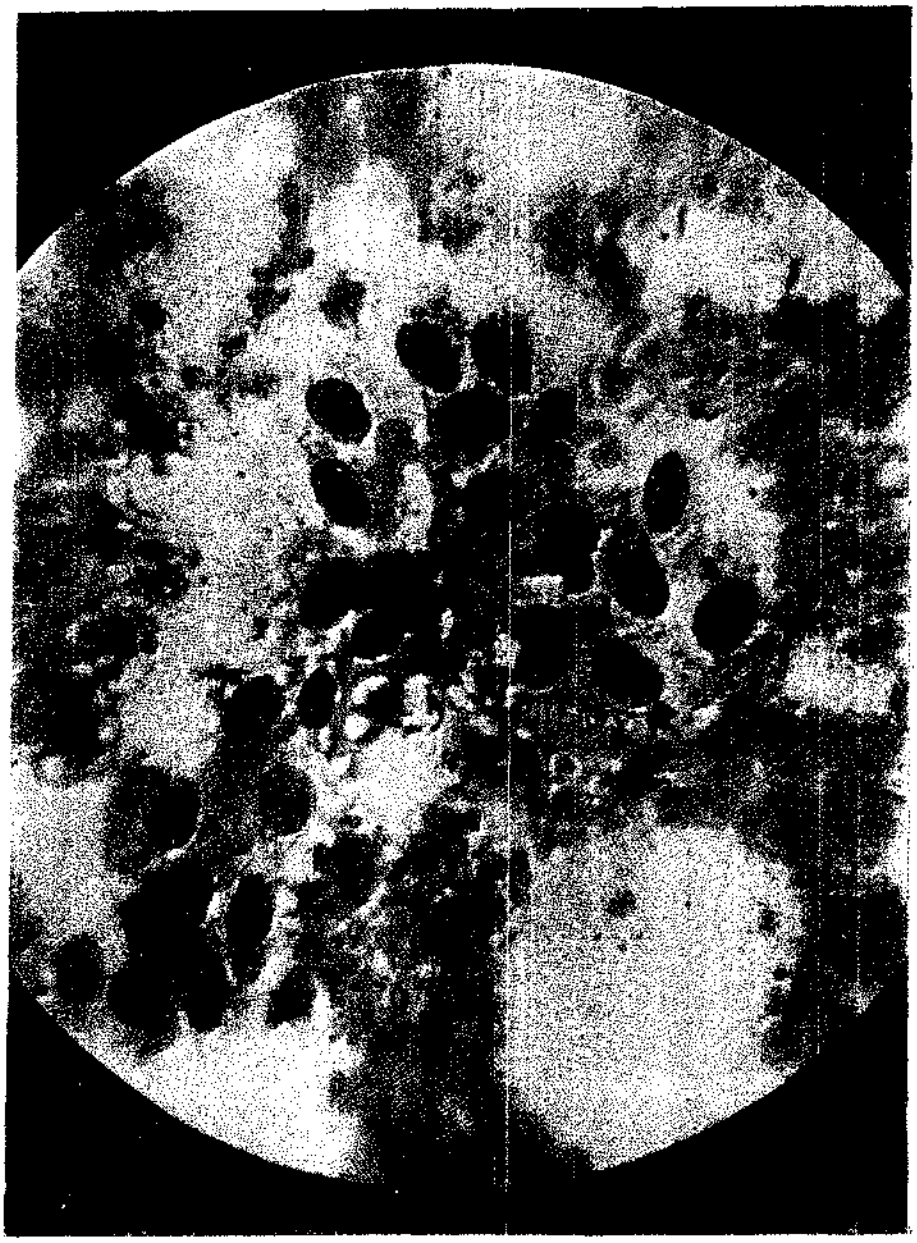
Section of brain showing one blood vessel filled with parasitized red cells.



Section of heart: the capillaries between the muscle fibres are packed with infected red cells.

Section of intestinal villi; the engorged capillaries are filled with parasites and pigments.

PLATE IV.



Smear of heart's blood showing myeloblasts.

Sternum.- Slate-coloured bone marrow.

Kidneys.—Right 115 gm. Left 100 gm. Haemorrhagic spots on surface.

Microscopically—A few parasitized cells in glomerulus. Degeneration of tubular epithelium. Medulla showed numerous capillaries filled with parasitized red cells.

Brain. 1,220 gm., meninges congested, brain lead-coloured, congested and oedematous.

Microscopically—Brain capillaries filled with parasitized red cells.

DISCUSSION.

The case presents some notable features:

The patient, an old man of 75, had a very heavy *falciparum* infection which was untreated for 8 days when the fever subsided, probably from failure of body reaction. He was, however, able to walk to the hospital though in a very weak condition. The spleen was not palpable. He was given an intensive paludrine therapy, 1·4 gm., in little over 24 hours along with supportive treatment, yet he could not be saved. Paludrine caused a great reduction in the number of parasites in the peripheral blood but had little or no effect on their proliferation in the internal organs. Diarrhœa, cardiac weakness and drowsiness were apparently due to blocking of capillaries with parasitized red cells seen in the intestinal wall, heart and brain.

A rapid change in the blood picture was noticed within 24 hours.

(*a*) Fall of hæmoglobin by 20 per cent (Hellige).

(*b*) Fall of red cell count by $\frac{3}{4}$ million.

(*c*) A rise of white cell count from 16,000 to 39,000 per c.mm. (80 per cent polymorphs).

(*d*) Increase in the number of crescents with reduction of rings.

(*a*) and (*b*) can be explained by destruction of parasitized red cells. An increase of crescents may be a part of natural malarial process, possibly activated by paludrine administration. Some leucocytosis is expected due to hæmo-concentration, but the count of white blood cells of 39,000 per c.mm. appeared abnormally high. The fall of hæmoglobin and R.B.C. was apparently not due to hæmo-dilution only as the W.B.C. count increased. Besides, the patient had only 10 oz. of saline and 10 oz. of plasma by intravenous drip.

A number of cells, apparently myeloblasts, were found postmortem in the heart's blood. This may be an indication of abnormal leucopoiesis which can explain the marked leucocytosis. The cause of this could be either overwhelming infection or the effect of heavy dose of paludrine or both. Reference to the literature shows that malaria itself may cause slight increase of myelocytes and simultaneous administration of paludrine may increase their number still further. Myelocytes have also been seen to appear in the blood of volunteers receiving 1·0 gm. daily.

Another question arises: could the patient be saved if he was given quinine or mepacrine instead of paludrine or both? It is difficult to say definitely, because deaths had been recorded even after adequate quinine therapy in similar cases.

In severe *falciparum* malaria, general anoxaemia and tissue anoxia may arise. In the earlier stage, with treatment the change is reversible, but when late, it is irreversible and any antimalarial may fail. Intravenous paludrine has been reported to be effective (Chaudhuri and Chakravarti, 1949). But considering the failures of the drug in *falciparum* malaria, it would be best to treat serious cases with quinine which has stood the test of time. Covell *et al.* (1949) reported failure to effect a radical cure of Lagos strain of *falciparum* malaria with paludrine alone. He also considers that this drug is not sufficiently rapid in action to warrant its use in extreme urgency (Covell, 1949).

It is noted that intravenous paludrine caused thrombosis of the vein injected while intramuscular injection was painful; there was however no visible necrosis.

Lastly, it cannot be ignored that the patient was an old undernourished man of 75. His system was flooded with malaria parasites especially affecting all the vital organs, i.e. brain and heart, and his body reactions were failing when admitted although he could walk to the hospital.

REFERENCES.

CHAUDHURI, R. N., and CHAKRAVARTI, H. (1949). Intravenous 'Paludrine' (Proguanil). *Brit. Med. J.*, Jan. 15, pp. 91–93.

COVELL, G. (1949) ... .. The treatment of malignant tertian malaria. *Ibid.*, Oct. 8, pp. 775–777.

COVELL, G., NICOL, W. D., and SHUTE, P. G. (1949). 'Paludrine' (Proguanil) in prophylaxis and treatment of malaria infections caused by a West African strain of *P. falciparum*. *Ibid.*, Jan. 15, pp. 88–91.

Indian Journal of Malariology, **4**, 3, *June 1950*.

SUPPRESSIVE TREATMENT OF MALARIA.*

BY

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WITH STATISTICAL ANALYSIS.

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[March 21, 1950.]

INTRODUCTION.

THIS report relates to an enquiry on the prophylactic value of paludrine, chloroquine and camoquin in malaria which was carried out in a rural area. A preliminary trial was first made in 1947 with paludrine alone in a village, Katalgarh, a brief description of which is given below, so that a proper perspective may be obtained of the conditions in which the experiment was undertaken. The following year the enquiry was extended to include some neighbouring villages, with more or less similar features using all the three drugs in selected groups.

Katalgarh is a small village in the Hooghly District of West Bengal, some 40 miles from Calcutta with a population of about 300, mostly poor and dependent on cultivation. Its two striking features are numerous bamboo groves and water tanks, large and small, some of which hardly serve any other purpose than breeding mosquitoes. There are extensive paddy fields all round, which separate this village from the others, which are equally malarious. Malaria has long been prevalent in this area breaking out during the rains and attaining its peak in October and November. Kala-azar was once common, and the village was visited by the great pandemic of influenza in 1918 which greatly reduced the population. Although it has since remained free from epidemics, malaria still flourishes causing much morbidity and mortality. Many among the well-to-do people have migrated to towns owing to the perennial fear of malaria. To alleviate the intense sufferings, a band of young men got together about thirty years ago and formed an antimalaria

* The investigations were financed by the Indian Research Fund Association (now Indian Council of Medical Research).

society which was affiliated to the Central Antimalaria Society, Calcutta, an all-Bengal organization. Later they formed a local centre for giving free treatment to kala-azar patients. This centre became very popular and was well attended even by patients from neighbouring villages and no doubt helped to stamp out the disease, though to the author's surprise, he discovered in the course of the present investigation two cases of kala-azar which readily responded to antimony treatment. The efforts of the young men in the eradication of malaria were however far from successful, for although they cleared the jungles which abounded, and distributed quinine free, they were handicapped by limited funds and lack of scientific guidance. During and since the war, their work practically came to a standstill.

The inhabitants of the village are composed of landowners and labouring classes who may be subdivided into permanent settlers and Santhals, some of whom have settled there for a good many years while others had only recently come from a neighbouring village. The Santhals are the poorest of the lot. The attitude of the people towards malaria is one of resignation and their mental and physical qualities are reflected in lack of initiative and poor out-turn in the fields. The village, therefore, seemed suitable for testing antimalarial drugs. When preliminary arrangements were being made to start the work, it was reported that one of the new-comer Santhals lost his little child from an attack of 'pernicious malaria', a name which has become familiar even among the illiterates. Poverty did not permit calling any of the two local doctors, and the child died within 24 hours. Alarmed at the rapidity with which death took place, the Santhals seriously considered leaving the village to which they had only lately come, but being very poor, gave up the idea. This event however speeded up the efforts and it was decided to start prophylactic work with paludrine without further delay.

1. PRELIMINARY ENQUIRY.

Malaria was already occurring when on September 11, 1947, a preliminary survey of the village was carried out. Of the 83 children examined, 47 had enlarged spleen (57 per cent) and 24 had parasites in the blood (29 per cent). The splenic enlargement was mostly within 3 fingers, and only four of them showed enlargement up to 4 fingers. Nineteen (79 per cent) out of the 24 positive cases had malignant tertian infection, justifying the common belief in the prevalence of this type of malaria.

SELECTION OF MATERIAL.

For reasons given below, prophylactic treatment had to be confined to a small section of the population. Those of the landlord class who stayed in the village, though really not in prosperous condition, could afford to purchase antimalarial drugs and indeed some of them were in the habit of taking such medicines during the malaria season, though not systematically. As mentioned before, the Santhals were the poorest, ill-clothed and ill-fed and seldom bothered about medical aid even when they were dangerously ill. The others were economically not so worse off.

It was therefore decided to put the Santhals under the scope of the prophylactic treatment and with them were included a few families of non-Santhal labourers who lived nearby and whose children frequently suffered from fever. The total number of men, women and children who were thus selected were 60 and the

remaining population was regarded as the comparison group. The spleen and parasite indices of children in the two groups, viz. paludrine and comparison, are given in Table I.

TABLE I.

Spleen and parasite rates in children selected for paludrine and the comparison groups.

Group.	Total number of children.	Number with enlarged spleen.	Spleen rate, per cent.	Number with malaria parasites.	Parasite rate, per cent.
Paludrine ...	32	23	72	16	50
Comparison ...	51	24	47	8	16

Plan of work.—The original idea of dividing the whole village into two equal groups—one for paludrine and the other for comparison—had to be given up, firstly because there was not enough paludrine to go round, and secondly, for the more important reason, no reliable person was at first available who would take the trouble to conscientiously distribute the medicine in the desired way. Past experience had shown that if any reliance was placed on the villagers to turn up at a selected centre at a certain hour on appointed days, two or three times a week, there would be no prospect of completing the experiment. Some would no doubt come, and that too at their own convenience, but the majority would fail to appear for treatment. The only way of making it a success was to take the medicine to their doors and make each individual swallow it on the spot. This became possible through the generosity of a local gentleman who voluntarily agreed to go from house to house twice a week (Sundays and Wednesdays) and see that each person swallowed the dose in his presence with a draught of water, and it was mainly for his convenience that the number was kept at a low level. Actually paludrine was started on the very day the preliminary survey was carried out (September 11) and this was continued regularly till the end of December except for a break of ten days in October when three treatment days were missed owing to the illness of the distributor. The usual dose was one tablet (0·1 gm.) but to children under 4 years, half a tablet was given. Contrary to the author's fears, there was unusual keenness in taking the medicine but the enthusiasm began to wane in December when there was a natural decline in the incidence of malaria. No other prophylactic measure was adopted during the course of paludrine treatment.

RESULTS.

After the treatment was completed, the children were re-examined for spleen and parasites. The results are given in Table II.

(*a*) *Parasites in the blood.*—Five out of 29 children in the paludrine group were found with malaria parasites, i.e. 17 per cent against the original 50 per cent, while 11 out of 50 children in the comparison group had parasites, i.e. 22 per cent against the original 16 per cent.

TABLE II.

Spleen and parasite rates after the experiment.

Group.	Total number of children.	Number with enlarged spleen.	Spleen rate, per cent.	Number with malaria parasites.	Parasite rate, per cent.
Paludrine ...	29*	13	45	5	17
Comparison ...	50†	23	46	11	22

* Excluding 3 children, one of whom died of dysentery, one left the village and one was diagnosed to be suffering from kala-azar.

† Excluding 1, who had left the village.

(*b*) *Splenic enlargement.*—Thirteen out of 29 children had still enlarged spleen in the paludrine group, i.e. 45 per cent, against 72 per cent at the preliminary examination. There was reduction in the size of the spleen in the majority of cases except in one whose spleen instead of diminishing actually increased from 2 to 4 fingers. This was later proved to be due to kala-azar. In the comparison group, 23 children or 46 per cent had enlarged spleen against the original 47 per cent.

(*c*) *Incidence of fever.*—When paludrine was started, fever was common in the village and in the selected group as many as 16 out of 32 children were found with malaria parasites in the blood. With the drug given twice a week, the fever began to decline but owing to ten days' interruption in treatment in October when malaria was at its worst, there was a break through and a good many went down with fever again. No special treatment was given for this and on resumption of paludrine, the fever quickly came under control though not quite completely, for five children had one or more short attacks of malaria in November, and one of these had another attack in December as well. After the first week of December, no one had any fever until the end of the month when paludrine was discontinued and the period of observation ceased. In contrast, the non-paludrine group suffered much from malaria during the same period.

CONCLUSION.

Thus it is seen that paludrine was able to reduce the splenic rate from 72 to 45 per cent and the parasite rate from 50 to 17 per cent in a hyperendemic rural area. In those who did not have this suppressive therapy, the spleen rate was practically unaffected and the parasite rate had actually increased compared with the original finding. Though paludrine took some time to control the fever in a few cases, there was no doubt that the incidence of fever was much less than in previous years. The experiment, however, suffered from a few defects—the number of subjects was small, the prophylaxis was started rather late, i.e. about 2 months after the onset of malaria transmission season, and there was a ten days' break in the treatment during the peak of the malaria season. Attempts were made to remedy these defects in the enquiry that followed.

II. MAIN ENQUIRY.

The results of the first enquiry were so encouraging that in the year 1948 the scope was widened with a grant from the Indian Research Fund Association (now

known as the Indian Council of Medical Research). Some additional villages were included and paludrine as well as chloroquine and camoquin were used for determination of the relative efficacy of these drugs and as to how far it was practicable to introduce them in mass prophylaxis of malaria. Though the work still continues, an account of the author's experience gained in 1948-49 with the above mentioned drugs is given.

PRELIMINARY DETAILS.

For the purpose of this experiment, the population of 8 villages, each separated from the others by a distance of about a mile, in the selected area, was divided into five groups each comprising about 250 persons; four groups for chemoprophylactic investigations and one for comparison. There were four groups in the former category because it was decided to try besides chloroquine and camoquin, paludrine in two different dosages.

A survey carried out in May 1948 of 1,249 men, women and children, showed spleen and parasite rates as 62·8 and 10·7 per cent respectively. Various nutritional deficiencies were evident in 44·4 per cent of the population examined. The hæmoglobin value, estimated in 1,097 individuals by the Adams-Tallqvist scale, was found to be below 50 per cent (7·8 gm.) in 13 per cent, between 50 and 70 per cent (7·8 gm. to 10·8 gm.) in 70 per cent, and over 70 per cent (10·9 gm.) in 17 per cent of the cases. The total serum protein determined in 61 individuals by the copper sulphate method, however, showed almost normal values.

The transmission period in these villages starts, as already stated, with the onset of rains, i.e. about July; October and November months being the period when malaria incidence is usually at its highest.

The parasite rates were determined at successive stages of the experiment, at intervals of 2 months during the active malaria season and thereafter at intervals of 3 months. The number examined at each stage did not remain the same as some of the subjects did not submit to blood examination or were absent, but it included the majority of the subjects. Spleen rates were examined at the beginning of the experiment in May 1948 and again at its termination, in February 1949.

DOSAGE.

The prophylactic doses administered were according to the schedule given in Table III.

TABLE III.

Schedule of prophylactic dosage.

Treatment groups.	Drug and frequency of administration.	For subjects.		
		Above 10 years.	4–10 years.	Below 4 years.
I	Paludrine twice weekly ...	0·1 gm.	0·05 gm.	0·03 gm.
II	Paludrine once weekly ...	0·3 gm.	0·2 gm.	0·1 gm.
III	Camoquin once weekly ...	0·2 gm.	0·1 gm.	0·05 gm.
IV	Chloroquine once weekly ...	0·25 gm.	0·125 gm.	0·062 gm.

The number of subjects allotted to each treatment are shown below :—

Treatment.	Number of persons.
I	239
II	276
III	246
IV	241
V (Comparison) ...	266
TOTAL ...	1,268

The regular administration of the drug to the first four groups was begun from the first week of July 1948 and the comparison group received quinine sulphate only in case of fever. The blood of every case reported to be ill was examined, and if parasites were detected, a single therapeutic dose of the drug prescribed for prophylactic purpose in that group was administered. No other prophylactic measures were adopted. To facilitate the work, one Field Assistant was appointed to administer the medicine individually. The work was continued till the end of December.

Certain difficulties were at first encountered, leading to some irregularity in the administration during the earlier period. Although the Field Assistant went from house to house with the tablets, some persons would not swallow them immediately but would put it off on some pretext or the other. There were some who found it difficult to swallow three tablets of paludrine at a time (Group II) and actually vomited them out. This in turn discouraged others from taking the treatment. There was also counter-propaganda from interested persons, alleging various ill-effects of the medicines. These difficulties were however eventually overcome.

RESULTS.

Parasite rates. The infection rates shown in Table IV for the different treatments in the months of September 1948, November 1948, February 1949 and May 1949 reveal certain prominent features. It will be observed that in May 1949, i.e. shortly before the malaria season began, these rates in the villages varied from 7 to 11 per cent, the average being 10 per cent.

TABLE IV.

Infection rates per 100 at different stages of the experiment.

Group.	May 1948.	September 1948.	November 1948.	February 1949.	May 1949.
I	11·20 (235)	3·15 (222)	4·48 (223)	1·36 (221)	4·10 (167)
II	9·42 (276)	9·13 (219)	5·73 (227)	3·09 (259)	9·56 (241)
III	10·57 (246)	8·02 (212)	1·89 (212)	0·63 (159)	5·95 (84)
IV	7·00 (227)	5·00 (101)	2·60 (193)	1·60 (261)	1·87 (190)
V	11·32 (265)	15·03 (173)	36·87 (179)	14·57 (199)	17·40 (224)

The figures within brackets indicate the number of subjects whose blood was examined.

While the rates in the comparison group rose from 11 to 15 in September and reached the peak figure of 37 in November, the rates arising from the treatment, on the other hand, went down and were very much lower. This was the case with all the treatment regimes throughout. The decrease in November when the potentiality for malarial incidence appeared greatest, was striking. In February, the parasite infection was at a minimum in all groups, due no doubt to the cessation of the transmission period and to the cold weather when the general health of the people is at its best. The rise in May was probably due to relapses which usually occur in the spring months and which could no doubt have been prevented, had the drugs been continued instead of being stopped in December, and even then, the rates were much lower than that in the comparison group. The parasite rates for each treatment and comparison group during the different stages of the experiment are shown diagrammatically in Fig. 1.

Taking into consideration the different treatments separately, it is to be noted that treatment Group II showed a comparatively higher rate of infection. This treatment consisted of 3 tablets of paludrine (0·1 gm. each) once a week—at that time 0·3 gm. tablets were not available and it was too much for some persons to swallow the dose without vomiting and this caused some panic, and attempts were made to evade it. This may have been the cause of the unsatisfactory response.

As regards treatment Groups I, III and IV, they were more or less equally effective, no significant difference between them being noticeable.

Analysis of variance (Appendix, Table A) brings out the comparison between the four treatment groups taking account of their performances for the four periods of the experiments collectively. The graduated infection rates per 100 examined for the four treatments is shown in Appendix (Table C) and in Fig. 2. Statistical analysis showed that the significant differences observed between the treatments is due to the presence of treatment Group II in the collection, which consistently showed higher infection rate whereas the others, viz. treatment Groups I, III and IV, did not show significant differences between themselves. To establish the superiority of the treatments over the comparison, it would suffice if it can be shown that the worst amongst them, viz. treatment Group II, showed significantly lower infection rate than the comparison. The table of mean differences and the values of 't' shown in Appendix (Table B) clearly showed that, in the months November 1948, February 1949 and May 1949, treatment Group II showed significantly lower infection rate than comparison group; the difference being most striking, as mentioned before, in November 1948.

The comparison between treatment Group II and the comparison group is diagrammatically shown in Fig. 3.

Another feature revealed by the analysis is that a significant number of subjects belonging to the comparison group who showed positive results in the blood test in September 1948, continued to show the same up to the month of February 1949. This is indicated by the correlations between the rates observed during September 1948, November 1948 and February 1949, the correlation coefficients between rates of September and November, September and February, and November and February being 0·28, 0·27 and 0·19 respectively. This is a characteristic only of the comparison group. For the treatment groups similar correlation coefficients are almost zero.

FIG. 1.

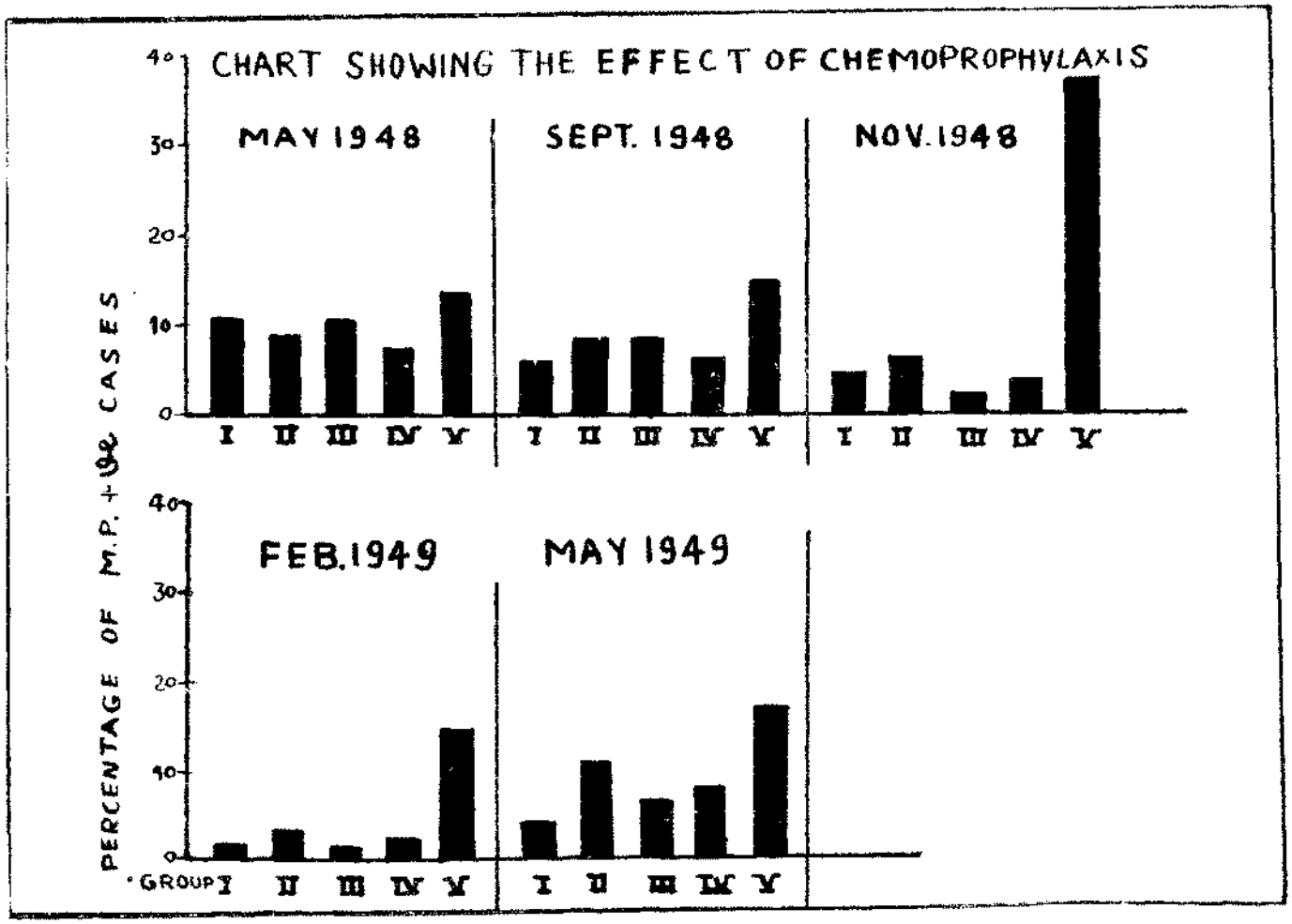


CHART SHOWING THE EFFECT OF CHEMOPROPHYLAXIS
MAY 1948
SEPT. 1948
NOV. 1948
FEB. 1949
MAY 1949
PERCENTAGE OF M.P. + Ve CASES
40
30
20
10
0
GROUP I II III IV V

Fig. 2.

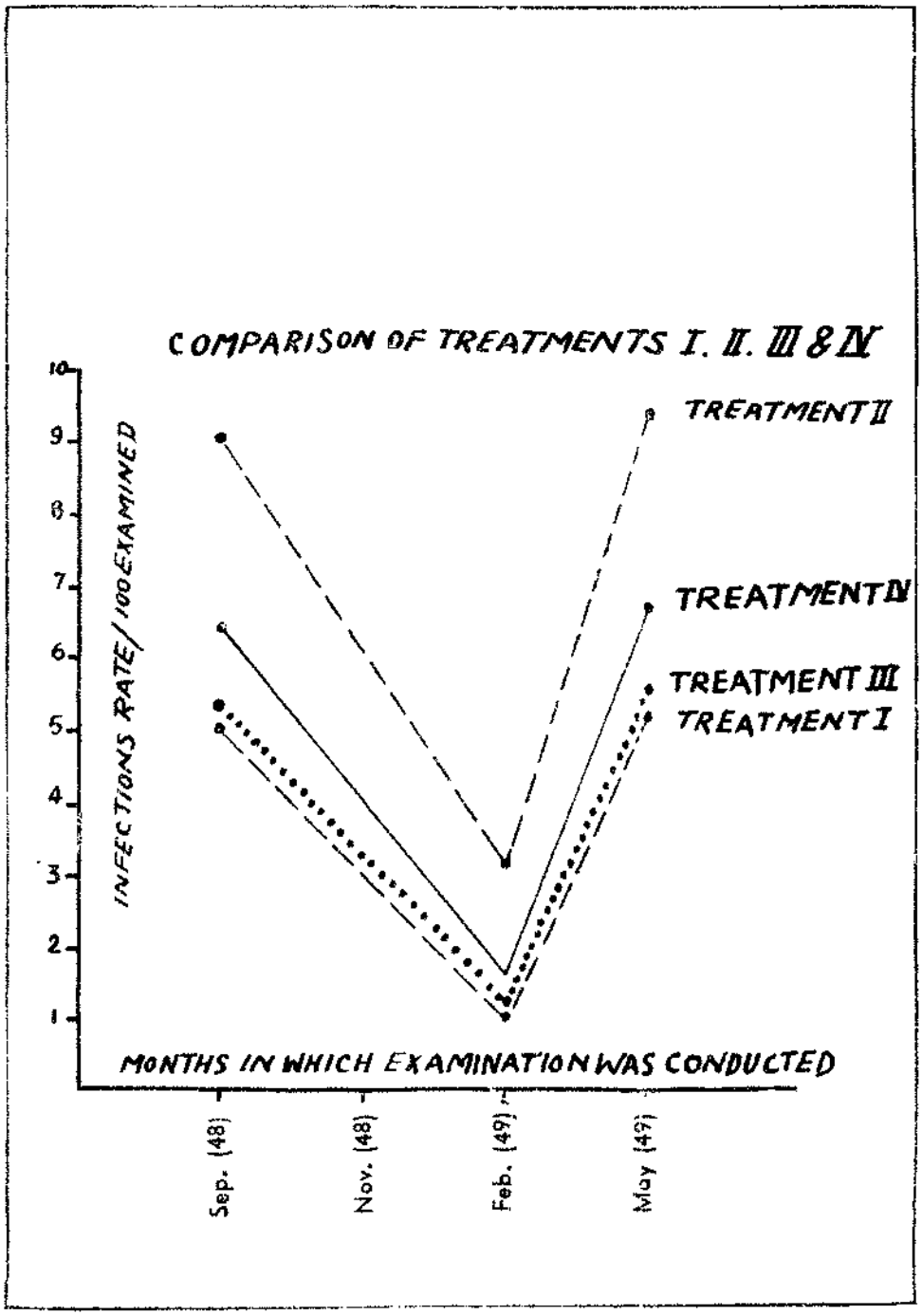

COMPARISON OF TREATMENTS I. II. III & IV
INFECTIONS RATE / 100 EXAMINED
10
9
8
7
6
5
4
3
2
1
TREATMENT II
TREATMENT IV
TREATMENT III
TREATMENT I
MONTHS IN WHICH EXAMINATION WAS CONDUCTED
Sep. (48)
Nov. (48)
Feb. (49)
May (49)

Fig. 3.

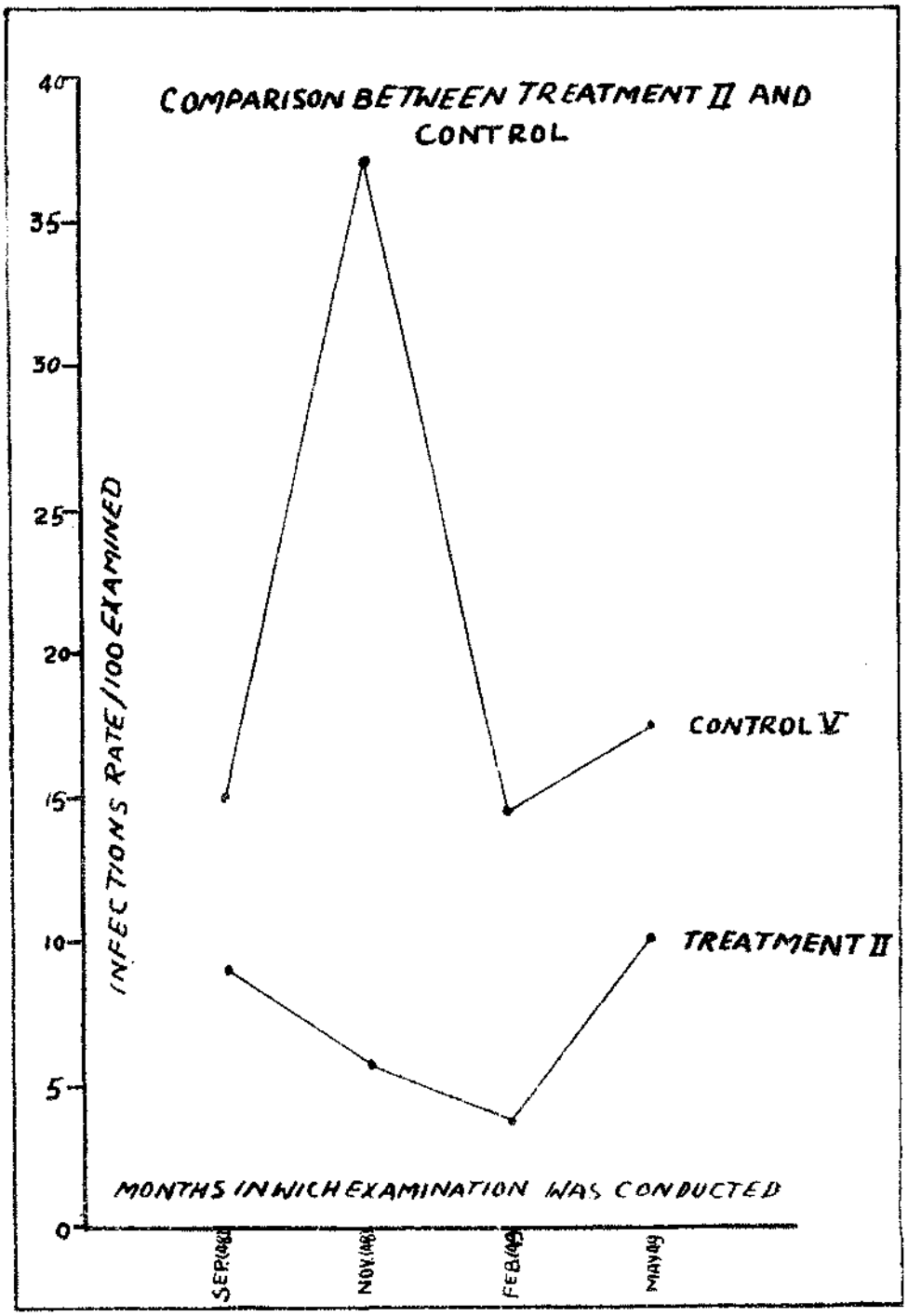
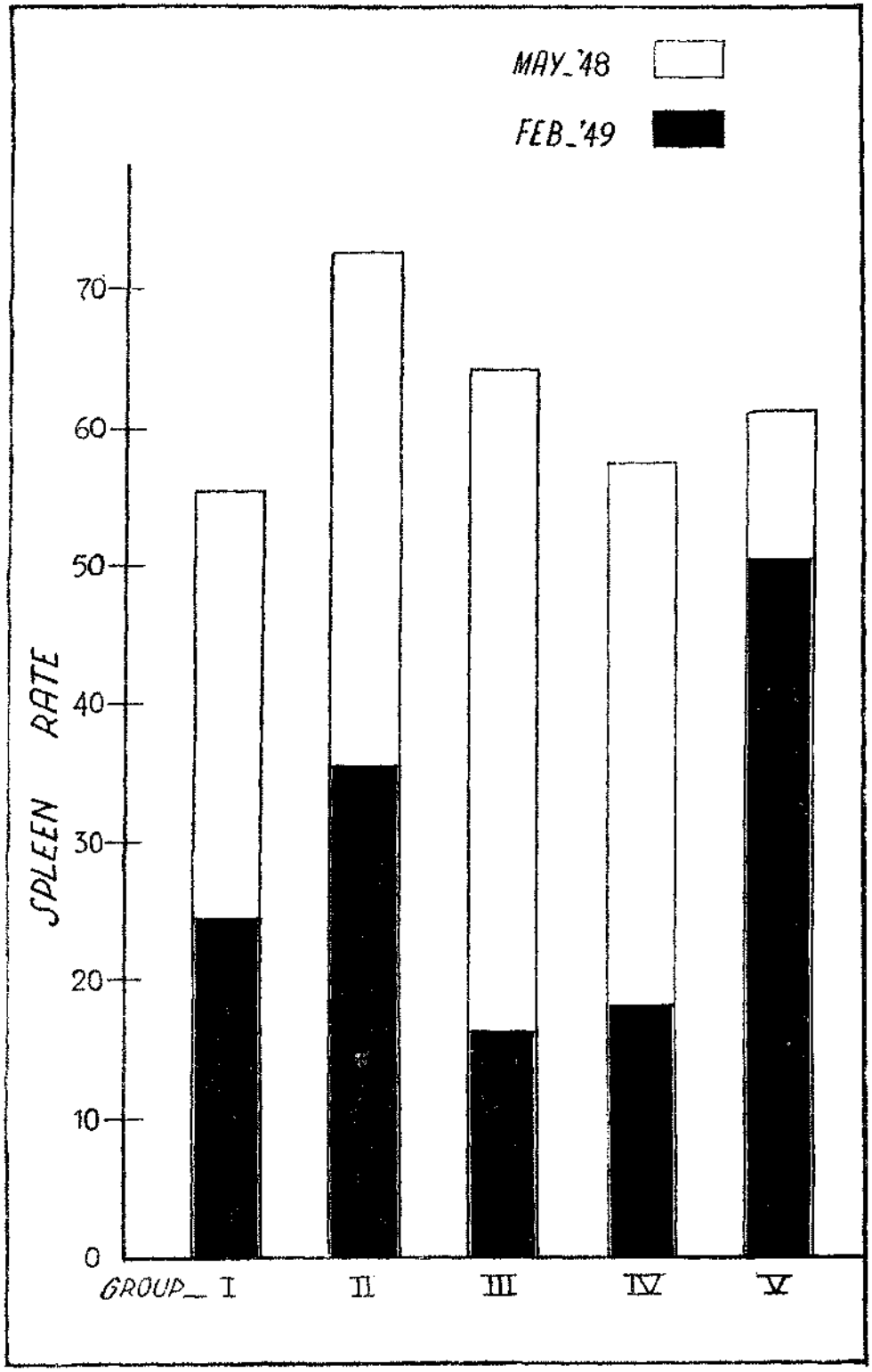

COMPARISON BETWEEN TREATMENT II AND CONTROL
INFECTIONS RATE/100 EXAMINED
40
35
30
25
20
15
10
5
0
CONTROL V
TREATMENT II
MONTHS IN WICH EXAMINATION WAS CONDUCTED
SEP(46)
NOV(46)
FEB(49)
MAY(49)

Fig. 4.



Spleen rates.—After the preliminary investigation in May 1948, the spleen rate was again determined in February 1949, after the treatment was completed. This showed a marked reduction in the treatment groups as will be seen from Table V and Fig. 4. This reduction was more noticeable in the camoquin and chloroquine groups (Tables V and VI). The higher rate under Group II may be due to reasons already given.

TABLE V.

Reduction in spleen rate per cent.

Group.	I.	II.	III.	IV.	V.
May 1948 ...	55·4	72·8	64·2	57·7	61·2
Feb. 1949 ...	24·4	35·6	16·4	18·2	50·6

For a proper evaluation of the drugs, so far as the spleen rates were concerned, a statistical analysis was made of the subjects who had enlarged spleen at the commencement of the experiment and those who had no such enlargement. Table VI shows the effect of the drugs on those who had enlarged spleen at the initial stage of the experiment.

TABLE VI.

Effects on enlarged spleen.

Group.	Number of cases with enlarged spleen.		Reduction per cent.
	Initial stage.	Final stage.	
I ...	96	26	27
II ...	116	50	43
III ...	85	10	12
IV ...	66	12	18
V ...	80	51	64

It is evident that the reduction of the spleen was most marked in Group III and nearly to the same extent in Group IV as compared with Groups II and V (comparison), the effect being intermediate in Group I.

Statistical tests bringing out the comparison between the treatments are shown in the Appendix (Table D).

Table VII shows the effect of the drugs in the prevention of spleen enlargement.

TABLE VII.

Effects on prevention of spleen enlargement.

Groups.	Cases without spleen enlargement at initial stage.	Cases with enlarged spleen at final stage.	Per cent.
I ...	73	11	15
II .	32	4	12
III .	47	0	0
IV ..	31	0	0
V	56	15	27

The striking feature is that the spleen was not palpably enlarged at the end of the experiment in any of those belonging to Groups III and IV who had not shown it in the beginning.

The statistical analysis for the comparison of the treatment effects are shown in Appendix (Table E).

The analysis thus tends to show that chloroquine and camoquin were much more effective than paludrine not only in reducing the size of enlarged spleen but also in preventing its enlargement. No toxic symptoms were encountered.

As regards fever, there was clear indication supported by voluntary statements of the people themselves, that those who took the drugs regularly suffered very little from malaria while many in the comparison group suffered from it badly. Among the three, chloroquine and camoquin were found to be definitely superior to paludrine.

DISCUSSION.

In the absence of a causal prophylactic drug, the value of malaria control by drug is limited except under unstable or emergent situation. Previous work on drug control with quinine 5 grains daily or mepacrine 0·4 to 0·6 gm. in the course of a week showed that the incidence of malarial attacks could be considerably minimized, but it was held that this method should not be relied upon as the sole measure for prevention of malaria. During World War II, the success attained with drugs suppressing clinical attacks was remarkable. Various workers (Findlay and Stevenson, 1944; Fairley *et al.*, 1945) observed the superiority of mepacrine to quinine for suppression of malaria. The Board for the Co-ordination of Malarial Studies (1946) stated that chloroquine was even more active than mepacrine, being effective in a single weekly dose of 0·3 gm. Berberian and Dennis (1949) noted that weekly doses, 0·5 gm., of chloroquine diphosphate without any recourse to anti-mosquito measures, not only effectively controlled malaria but also reduced the splenic index from 59 to 6 per cent within an average treatment period of 26 weeks. When the effect of malaria control with residual D.D.T. spraying was compared, the splenic index decreased only from 70 to 50 per cent

within that period. Maier (1948) is of opinion that one tablet (0·3 gm. base) weekly is as effective in suppressing relapses of *vivax* malaria as a daily 0·1 gm. dose of mepacrine in the Philippines. Berberian (1948) while undertaking experiments in 2 hyperendemic villages in Lebanon with chloroquine, 0·5 gm. once a week in one village and 1 gm. (4 tablets) once a fortnight in the second village, also recorded that no relapses occurred while under suppressive medication. Thereafter the villages were thoroughly sprayed with D.D.T. when the rate of relapses observed within the following 9 months was 2·6 per cent.

Enough field trials with camoquin have not yet been carried out. Deane (1948) used it in a small Amazonian village in weekly doses (for children 1–4 years, 0·1 gm.; 4–14 years, 0·2 gm.; 15 years and up, 0·3 gm.) and obtained good results.

Paludrine has been widely used in various countries by various workers in varying doses (Fairley *et al.*, 1946; Coatney, 1948; Van Riel, 1947; Robertson, 1948; Blanc, 1949). The use of 0·3 gm. once a week has now been recommended as a suppressive by most workers and various organizations. The dose recommended by the Colonial Medical Research Committee (1949) for prophylaxis in endemic areas is 0·1 gm. daily. (For children 5 years, 0·025 gm.; 6–12 years, 0·05 gm. daily). When daily dose is not practicable, one dose of 0·3 gm. should be taken every week. In the case of persons, other than the indigenous inhabitants resident in the endemic areas for sometime or who have been otherwise already exposed to malaria infection, a full therapeutic course should be taken before entering on the suppression regime. Maegraith (1948) also states that a dosage of 0·1 gm. twice or thrice weekly appears to be more effective but even this dose may in some cases be insufficient. The most satisfactory dose is probably 0·1 gm. daily and no toxic symptoms have been encountered from such dosage. Where daily dosage is not practicable he advocated the use of 0·3 gm. weekly and in case of individuals already heavily infected with *falciparum* malaria, a full therapeutic dosage should be preceded before prophylaxis is begun. Beltran and Nieto (1948), after experiments for 30 weeks in Mexico while using 0·3 gm. weekly, 0·1 gm. weekly and 0·1 gm. bi-weekly, concluded that 0·1 gm. twice weekly gave the best results.

Covell *et al.* (1949*a*), while investigating on the action of paludrine on a West-African strain of *P. falciparum* which was relatively insensitive to the drug, found that even with this strain complete protection was achieved by using 0·1 gm. daily, 0·1 gm. twice weekly and 0·3 gm. weekly. With the non-immunes they advocated generally the daily dosage of 0·1 gm.

In India, Viswanathan and Bailey (1947) observed that 0·1 gm. of paludrine weekly is not absolutely effective and recommended 0·1 gm. twice a week. It has also been suggested by Afridi (1947) and Lomax (1947) that paludrine 0·1 gm. twice a week would be satisfactory for mass administration. Ray (1948) working in a tea estate in North Bengal reported on its use, 0·3 gm. weekly and 0·1 gm. bi-weekly and chloroquine 0·25 gm. weekly, and noted in each case a remarkable decline in the incidence of fever during the period of drug administration. Goldsmith (1946) observed successful suppression of malaria among the labourers in Assam with one tablet of chloroquine given weekly. Some paludrine refractory strains of parasites have occasionally been reported in India, Africa and elsewhere and they are best

treated with another antimalarial drug of a different group (Chaudhuri, 1948; Chaudhuri and Chakravarty, 1950; Covell *et al.*, 1949*b*; Edison and Field, 1950). Fairley (1949) points out that in Australia where many thousands of patients with relapsing *vivax* malaria were under suppressive medication with paludrine 0·1 gm. bi-weekly, no increased resistance to the drugs was observed. Recently Edison and Field (*loc. cit.*) pointed out that paludrine resistance is not yet a serious problem in Malaya but it may arise from casual and irregular suppression in an hyperendemic area.

Further, controlled field experiments are necessary to demonstrate the development of paludrine-resistant strains during mass administration and time alone will prove if they have any significant value in mass prophylaxis.

The present study shows the comparative effectiveness of paludrine, chloroquine and camoquin in mass chemoprophylaxis of malaria carried out for 26 weeks in a hyperendemic rural area of West Bengal. Compared with the comparison group, the suppressive value is remarkably significant, the parasite, spleen and fever rates being markedly reduced. The drugs are well tolerated even by children and pregnant women. Chloroquine and camoquin are more effective than paludrine, the latter appearing to reduce the parasite rate more with the bi-weekly dose (0·1 gm.) than with the weekly dose (0·3 gm.).

By suppression therapy only, it was possible for the Army during the last war to operate in hyperendemic areas of the Far East. Drug prophylaxis should be of equal or even greater importance to indigenous populations continuously exposed to malarious infections. The subject has been well discussed by Boyd (1949). There is the prospect of benefit to be derived by decreasing clinical attacks as well as reducing the human infectivity although there are considerable practical difficulties. The Malaria Commission of the League of Nations (1937) points out that it is not possible to reach all inhabitants of an area, it is expensive and it is only partially protective and suffices only to diminish morbidity. In spite of the various difficulties, it is apparent from the wartime research and developments that by regular administration of drugs, much can be done to protect the chronically infected villagers who are frequently exposed to repeated infections. Suppression therapy should be fully utilized, especially where anopheline control is yet not possible for various reasons, viz. practical difficulties, housing conditions, habit of sleeping outside uncovered, etc. Best results would possibly be obtained by the adoption of antimosquito measures in combination with drug prophylaxis.

SUMMARY.

1. Suppressive treatment of malaria in a rural area during the period July to December is described.

2. In 1947 a preliminary trial with paludrine in a small village gave very encouraging results.

3. In 1948 the experiment was repeated on a larger scale, including a group of villages. The population was divided into five groups, one of which served as comparison and the other four were treated with :—

I. Paludrine 0·1 gm. twice weekly.

II. Paludrine 0·3 gm. once weekly.

III. Camoquin 0·2 gm. once weekly.

IV. Chloroquine 0·25 gm. once weekly.

Each group consisted of about 250 men, women and children.

4. Blood examinations were carried out periodically from May 1948 to May 1949 and the spleen was examined before the investigation was begun and again at its termination.

5. The reduction in parasite rates due to the four treatments was significant in all periods, especially in the month of November when the potentiality for transmission was highest. Treatment II (paludrine 0·3 gm. once a week) proved to be inferior to the three treatments, even though it showed considerable difference with the comparison group.

6. So far as reduction in spleen is concerned there was marked difference between the treatment groups and the comparison group. Treatment Groups III and IV (chloroquine and camoquin) proved to be very effective not only in reducing the spleen but also in the prevention of spleen enlargement. Here also Treatment II showed itself as inferior to the other treatments, Treatment I occupying an intermediate position between Treatments III and IV on one side and Treatment II on the other.

7. Malarial fever was scarce among those who took the drugs regularly while many in the comparison group suffered from it repeatedly.

8. Certain difficulties encountered in this experiment are mentioned.

9. The value of malaria control by drugs is discussed.

10. Suppression therapy should be fully utilized especially where anopheline control is not possible.

ACKNOWLEDGMENT.

The author's grateful thanks are due to Dr. N. K. Chakravarty, Mr. C. L. Mitter and Mr. S. Mitter for valuable assistance in the field investigations. Thanks are also due to Messrs. Imperial Chemical Industries (India) Ltd., and to Messrs. Parke, Davis & Co. for free supply of paludrine and camoquin in liberal quantities. The author is also indebted to the Malaria Department of National Institute of Health, U.S.A., from whom they obtained chloroquine through the courtesy of Lieut.-Colonel Jaswant Singh of the Malaria Institute of India, Delhi, and Professor K. V. Krishnan of the All-India Institute of Hygiene and Public Health.

REFERENCES.

AFRIDI, M. K. (1947) ... *Ind. J. Mal.*, **1**, p. 347.

BELTRAN, E., and NIETO, C. (1948). *Rev. Inst. Salubridad y Enfermedades Trop.*, Mexico, **9**, p. 123.

BERBERIAN, D. A. (1948) ... *Proc. Fourth Int. Cong. Trop. Med. Mal.*, Washington, **1**, p. 766.

BERBERIAN, D. A., and DENNIS, E. W. (1949). *Amer. J. Trop. Med.*, **29**, p. 463.

BLANC, F. (1949) ... ... *Med. Trop.*, **9**, p. 143.

BOARD FOR THE CO-ORDINATION OF MALARIAL STUDIES (1946). *J. Amer. Med. Assoc.*, **131**, p. 963.

BOYD, M. F. (1949) ... ... *Malariology*, W. B. Saunders Co., Philadelphia.

CHAUDHURI, R. N. (1948) ... *Ind. Med. Gaz.*, **83**, p. 225.

CHAUDHURI, R. N., and CHAKRAVARTY, N. K. (1950).	*Ind. J. Mal.*, **4**, p. 111.
COATNEY, G. R. (1948) ...	*Proc. Fourth Int. Cong. Trop. Med. Mal.*, Washington, **1**, p. 768.
COLONIAL MEDICAL RESEARCH COMMITTEE (1949).	*Lancet*, **1**, p. 578. *Brit. Med. J.*, **1**, p. 585.
COVELL, G., NICOL, W. D., and SHUTE, P. G. (1949*a*).	*Brit. Med. J.*, **1**, p. 88.
COVELL, G., NICOL, W. D., SHUTE, P. G., and MARYAN, M. (1949*b*).	*Trans. Roy. Soc. Trop. Med. Hyg.*, **42**, p. 343.
DEANE, L. M. (1948) ...	*Proc. Fourth Int. Cong. Trop. Med. Mal.*, Washington **1**, p. 768.
EDISON, J. F. B., and FIELD, J. W. (1950).	*Brit. Med. J.*, **1**, p. 147.
FAIRLEY, N. H. (1949) ...	*Ibid.*, **2**, p. 891.
FAIRLEY, N. H., *et al.* (1945) ...	*Trans. Roy. Soc. Trop. Med. Hyg.*, **38**, p. 311.
Idem (1946) ...	*Ibid.*, **40**, p. 105.
FINDLAY, G. M., and STEVENSON, A. C. (1944).	*Ann. Trop. Med. Parasit.*, **38**, p. 168.
GOLDSMITH, K. (1946) ...	*J. Mal. Inst. Ind.*, **6**, p. 311.
LEAGUE OF NATIONS (1937) ...	*Fourth general report on the treatment of malaria*, Geneva.
LOMAX, P. W. (1947) ...	*Ind. J. Mal.*, **1**, p. 389.
MACGRATH, B. G. (1948) ...	*Proc. Fourth Int. Cong. Trop. Med. Mal.*, Washington, **1**, p. 742.
MAIER, J. (1948)	*Amer. J. Trop. Med.*, **28**, p. 407.
RAY, A. P. (1948)	*Ind. J. Mal.*, **2**, p. 35.
ROBERTSON, J. D. (1948) ...	*Zanzibar Med. San. Rc.*, 1947, *Appendix* II, p. 24.
VAN RIEL, J. (1948) ...	*Ann. Soc. Belge Med. Trop.*, **28**, p. 85.
VISWANATHAN, D. K., and BAILEY, J. D. (1947).	*Ind. J. Mal.*, **1**, p. 307.

APPENDIX

Note.—The treatment rates for any one month are uncorrelated with the corresponding rates for any other month. Therefore, the four rates observed for each treatment can be considered as four independent experiments with each treatment, the difference in the duration of the treatment and differences due to seasons being solely responsible for the differences between these four rates. An ordinary analysis variance with these rates would have sufficed if the mutual differences were not considerable. However, the seasonal plus duration differences (month effect) are considerable and, therefore, the rates were transformed to a new set of variable given by y- Sin $^{-1}/\sqrt{p}$ (p being the infection rate). The analysis of variance table as well as the mean difference table given in Table A are based on these new variables y.

TABLE A.

Analysis of variance.

(Treatments I, II, III and IV.)

Source of variation.	D.F.	Sum of squares.	Mean squares.	F.	5 per cent.	Significance.
(1) Due to treatments ...	3	11,587	3,862	4·70	2·60	Significant.
(2) Due to seasons and duration of treatment.	3	31,288	10,429	12·70	2·60	Do.
(3) Interaction between (1) and (2)	9	7,468	830	1·01	1·89	Not significant.
(4) Error	...	...	821	...	...	...

TABLE B.

Table of mean differences.

Month.	Mean difference.	Value to 't'.	5 per cent 't'.	Significance.
Sept. 1948	5·23	1·80	1·96	Not significant.
Nov. 1948	23·53	8·22	...	Significant.
Feb. 1949	12·13	4·49	...	Do.
May 1949	6·27	2·36	...	Do.

TABLE C.

Graduated infection rates per 100 examined for the four treatments I, II, III and IV.

Treatments.	September 1948.	November 1948.	February 1949.	May 1949.
I ...	4·92	2·81	0·93	5·20
II ...	0·02	6·23	3·15	9·39
III ..	5·25	3·15	1·09	5·56
IV ...	6·36	4·01	1·62	6·67

ANALYSIS OF SPLEEN INDEX.

The subjects belonging to each treatment and comparison group were divided into two classes 'A' and 'B', 'A' being those who showed enlarged spleen at the beginning of the experiment and 'B' being those who did not show enlarged spleen then. For each of these classes of subjects, separate comparisons of the treatments by a X^2 test is shown in Tables D and E.

TABLE D.

(Subjects classified as 'A'.)

Test between treatments.	Degree of freedom.	Value of X^2 observed.	5 per cent margin for X^2.	Significance.
(1) I, II, III, IV and comparison	4	64·29	9·49	Significant.
(2) I, II and comparison ...	2	23·87	5·99	Significant.
(3) I and II	1	5·87	3·84	Significant.

TABLE E.

(Subjects classified as 'B'.)

Test between treatments.	Degree of freedom.	Value of X^2 observed.	5 per cent margin for X^2.	Significance.
(1) I, II, III, IV and comparison	4	21·95	9·49	Significant.
(2) I, II and comparison ...	2	3·85	5·99	Not significant.

Indian Journal of Malariology, **4**, 2, June 1950.

COMPARISON OF THE ACTION OF PALUDRINE (PROGUANIL), CHLOROQUINE AND CAMOQUIN IN SINGLE DOSES.

BY

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[June 16, 1950.]

THIS investigation was undertaken with a view to devising a simple form of treatment for malaria to suit the masses in rural areas who are for various reasons unable to take a full therapeutic course. The object was to find a single non-toxic dose from the newer antimalarial drugs, paludrine, chloroquine and camoquin, which would be able to effect a clinical cure, after which the patient can himself continue to take the drug once or twice a week during the malarial season or longer. Comparative therapeutic trials of these three drugs in single doses (0·5 gm. each) were carried out in a series of 75 patients admitted into the hospital. They included 31 *falciparum*, 40 *vivax*, 2 mixed (*falciparum* and *vivax*) and 2 *malariae* infections and were divided into three groups 25 in each; Group 1 having paludrine hydrochloride, 5 tablets 0·1 gm. each; Group 2 having chloroquine diphosphate, 2 tablets 0·25 gm. base each; and Group 3 having camoquin, 10 tablets 0·05 gm. base each. The tablets were administered in the presence of one of the authors. Two patients of Group 1 and one of Group 3 vomited soon after administration and they are not included in this series. The immediate effects of the three drugs on the temperature and the parasites are shown in Table I.

TABLE I.

Effect of paludrine, chloroquine and camoquin on the temperature and parasites.

Serial number.	Infection.	Duration (in days) of fever.	Duration (in days) of asexual parasites.	Observation period (days).	Relapses after completion of treatment (days).
		(A) Paludrine (0·5 gm. single dose).			
1	F	Normal same day	1	13	17
2	F	2	3	15	23
3	F	2	2	13	21
4	F	1	1	21	...
5	V	2	2	18	120
6	V	Normal same day	1	14	...
7	F	3	2	20	49
8	V	3	3	36	...
9	V	4	2	60	14
10	V	2	1	10	...
11	FV	3	3	52	13
12	V	3	2	46	14
13	V	2	4	21	...
14	V	3	2	10	...
15	V	4	1	31	...
16	F	3	1	18	...
17	V	1	1	17	...
18	F	3	1	15	...
19	F	3	1	13	...
20	F	4	5	24	...
21	V	3	1	14	...
22	F	4	5	12	13
23	F	1	1	10	...

F = *falciparum*. V = *vivax*. M = *malariæ*.

The above results are summarized in Table II.

TABLE I—*contd.*

Serial number.	Infection.	Duration (in days) of fever.	Duration (in days) of asexual parasites.	Observation period (days).	Relapses after completion of treatment (days).
		(A) Paludrine (0·5 gm. single dose)—*concld.*			
24	V	1	1	35	...
25	F	3	4	28	...
		(B) Chloroquine (0·5 gm. single dose).			
1	F	1	2	11	...
2	V	Normal same day	1	24	...
3	F	3	1	31	...
4	F	2	2	27	53
5	F	1	2	16	...
6	F	3	1	14	...
7	F	3	2	38	...
8	V	2	1	24	...
9	V	1	1	30	...
10	F	2	2	25	...
11	V	3	2	28	...
12	V	1	1	30	...
13	V	1	1	70	...

F = *falciparum.* V = *vivax.* M = *malariæ.*

The above results are summarized in Table II.

TABLE I—*contd.*

Serial number.	Infection.	Duration (in days) of fever.	Duration (in days) of asexual parasites.	Observation period (days).	Relapses after completion of treatment (days).
		(B) Chloroquine (0·5 gm. single dose)—*contd.*			
14	V	1	1	15	...
15	V	2	2	45	...
16	V	1	1	60	...
17	F	1	1	21	...
18	V	1	2	13	...
19	V	1	1	90	...
20	V	1	1	65	49
21	V	1	1	12	...
22	F	1	1	30	...
23	F	2	1	13	...
24	V	1	1	15	...
25	F	2	2	20	...
		(C) Camoquin (0·5 gm. single dose).			
1	V	3	2	20	...
2	F	2	1	23	28
3	V	3	2	12	...

F = *falciparum.* V = *vivax.* M = *malariæ.*

The above results are summarized in Table II.

TABLE I—*concld.*

Serial number.	Infection.	Duration (in days) of fever.	Duration (in days) of asexual parasites.	Observation period (days).	Relapses after completion of treatment (days).
		(C) Camoquin (0·5 gm. single dose)—*concld.*			
4	F	3	1	30	...
5	F	1	1	60	20
6	F	Normal same day	3	45	13
7	V	2	1	28	..
8	FV	3	3	25	27
9	V	2	1	30	...
10	V	3	3	30	...
11	V	1	1	30	...
12	V	1	1	14	...
13	V	2	1	28	...
14	F	1	2	15	..
15	F	2	2	28	.
16	V	2	3	56	...
17	M	1	3	34	...
18	V	3	2	16	..
19	V	1	2	20	.
20	F	2	3	18	...
21	V	1	2	25	...
22	M	2	2	35	...
23	F	Normal same day	2	12	...
24	F	Do.	2	16	...
25	V	2	2	28	75

F = *falciparum*. V = *vivax*. M = *malariæ*.

The above results are summarized in Table II.

TABLE II.

Summary of Table I.

Days of treatment.	ASEXUAL PARASITES.						TEMPERATURE.					
	POSITIVE.				NEGATIVE.		RAISED.				NORMAL.	
	B.T.	M.T.	Q.	Mixed M.T. and B.T.	Number.	Percentage.	B.T.	M.T.	Q.	Mixed M.T. and B.T.	Number.	Percentage.
Paludrine												
(0·5 gm.) ...	12	12	0	1	0	0	11	11	0	1	2	8
After 1st day ...	6	6	0	1	12	48	9	9	0	1	4	16
,, 2nd ,, ...	2	4	0	1	6	24	6	7	0	1	5	20
,, 3rd ,, ...	1	3	0	0	3	12	2	2	0	0	10	40
,, 4th ,, ...	0	2	0	0	2	8	0	0	0	0	4	16
,, 5th ,, ...	0	0	0	0	2	8	...	...	...	...	...	...
Chloroquine												
(0·5 gm.) ...	15	10	0	0	0	0	14	10	0	0	1	4
After 1st day ...	5	4	0	0	16	64	4	6	0	0	16	56
,, 2nd ,, ...	0	0	0	0	9	36	1	3	0	0	6	24
,, 3rd ,, ...	0	0	0	0	0	...	0	0	0	0	4	16
Camoquin												
(0·5 gm.) ...	13	9	2	1	0	0	13	6	2	1	3	12
After 1st day ...	8	6	2	1	8	32	9	4	1	1	7	28
,, 2nd ,, ...	2	2	0	1	12	48	4	1	0	1	9	36
,, 3rd ,, ...	0	0	0	0	5	20	0	0	0	0	6	24

M.T. = malignant tertian. B.T. = benign tertian. Q. = quartan.

Clinical cure.—It will be seen that the asexual parasites disappeared from the peripheral blood within 3 days after the treatment was given in 84 per cent of cases treated with paludrine and 100 per cent cases with chloroquine and camoquin.

The corresponding figures as regards fever were also the same. Comparison between the latter two drugs showed that all chloroquine cases and 80 per cent camoquin cases became parasite-free by the end of the second day after treatment was started, the corresponding figures as regards temperature being 84 and 76 per cent. Thus all the three drugs seem effective to the extent of achieving clinical cure—chloroquine being quick, paludrine slow and camoquin intermediate in action. The results are also illustrated in the temperature charts (Figs. 1, 2, 3, 4, 5 and 6).

Relapses.—The patients were under observation for 1 to 4 months. None of them had any treatment after the single dose they had received on admission. During this period, 9 out of the 25 patients treated with paludrine had recurrence of fever (Table III). Three of them who had *vivax* infection on admission relapsed with homologous parasites in the blood within a fortnight after treatment while still in the hospital; the remaining six had, after discharge, clinical malaria (blood was not examined) which responded well to antimalarial drugs.

TABLE III.

Relapses in patients treated with paludrine.

Serial number.	Infection.	Interval between 1st and 2nd attack, days.	Type of relapse.
1	F	17	Clinical.
2	F	23	,,
3	F	49	,,
4	V	14	V
5	F and V	13	V
6	V	14	V
7	V	120	Clinical.
8	F	21	,,
9	F	13	,,

F = *falciparum*. V = *vivax*.

Two patients with *vivax* infection in 25 cases of chloroquine series relapsed (homologous parasites found) after seven weeks.

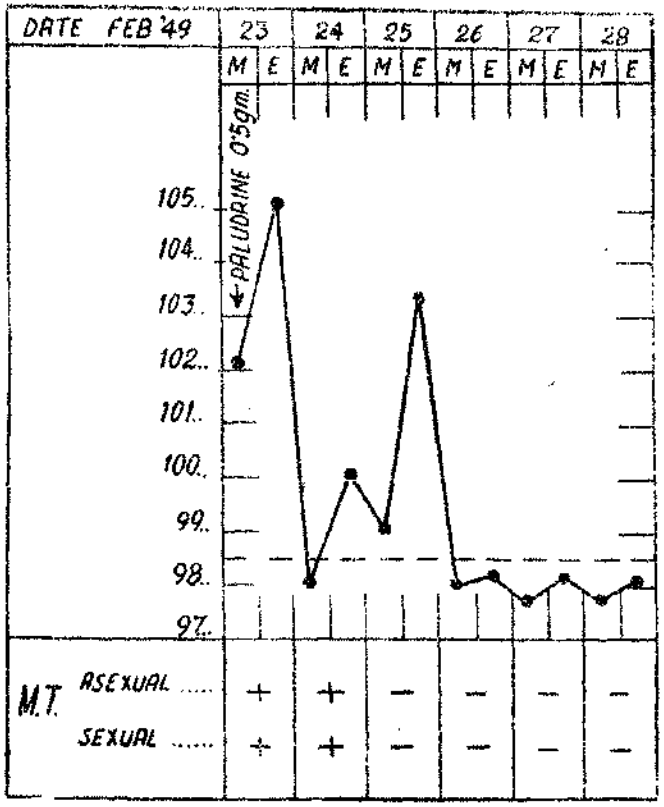

Fig. 1.—*Falciparum* malaria treated with single dose of 0·5 gm. of paludrine.

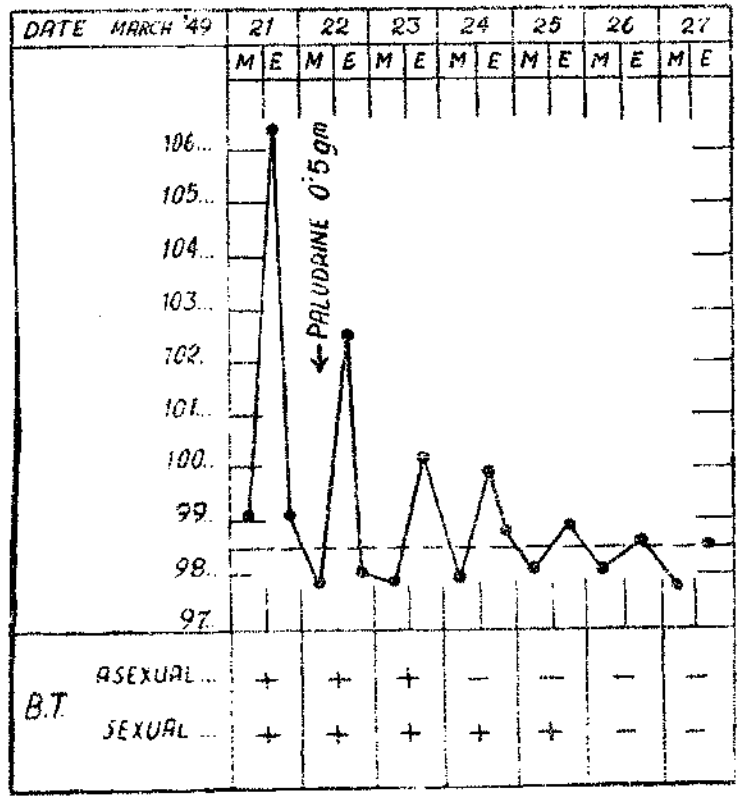

Fig. 2.—*Vivax* malaria treated with single dose of 0·5 gm. of paludrine

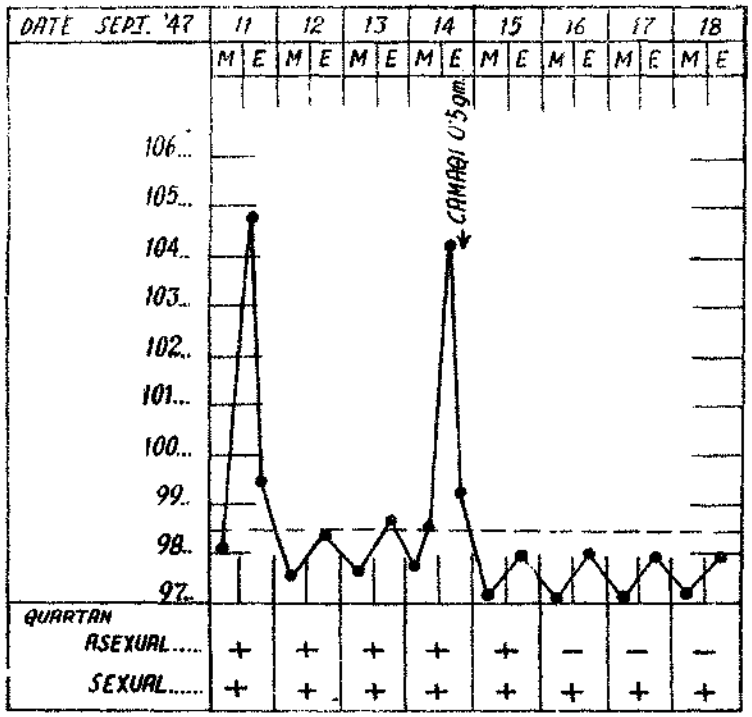

FIG. 3.—*Quartan* malaria treated with single dose of 0·5 gm. of camoquin.

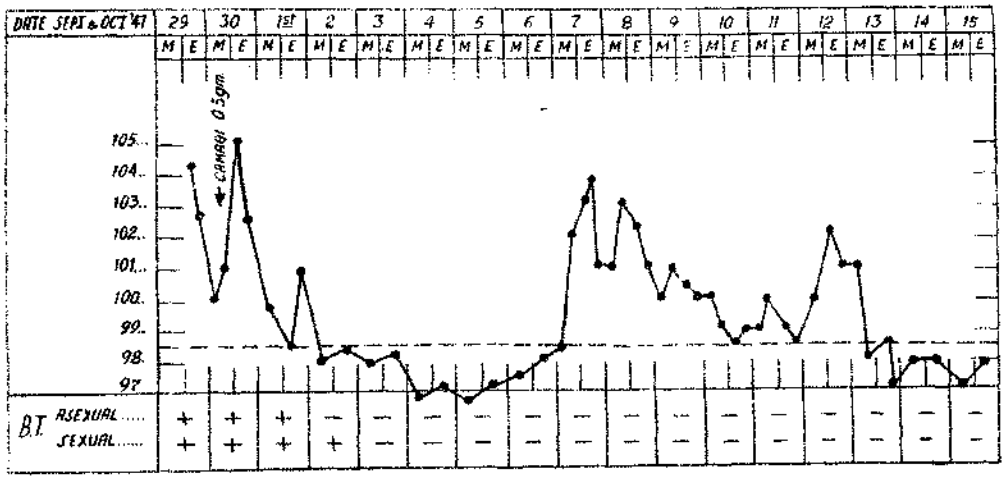

FIG. 4.—*Vivax* malaria treated with single dose of 0·5 gm. of camoquin. The patient had an attack of dengue during convalescence.

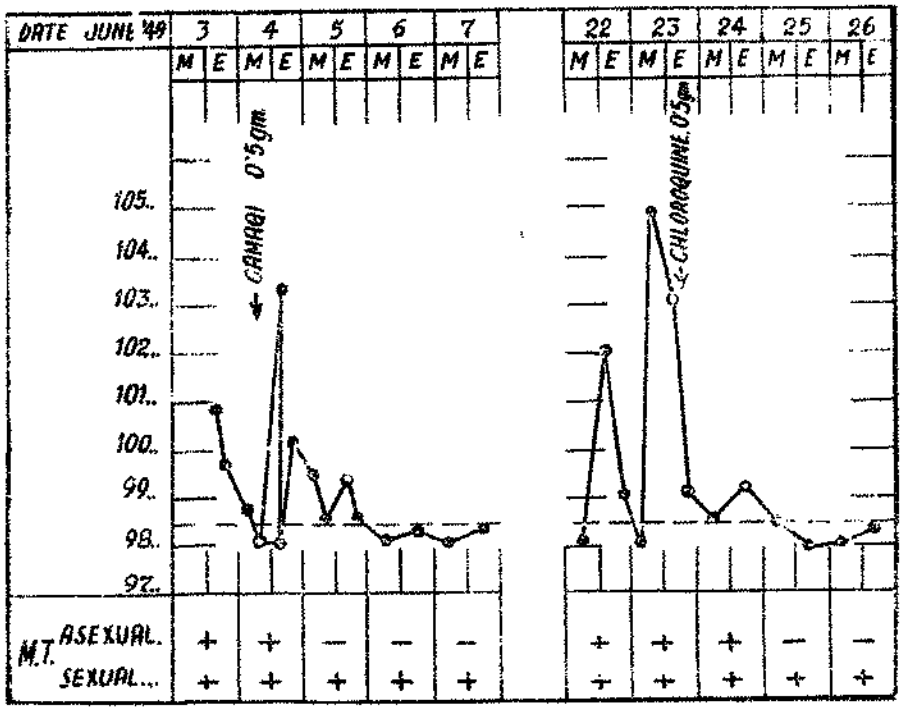

FIG. 5.—*Falciparum* malaria treated with single dose of 0·5 gm. of camoquin. Relapse after 16 days—treated with chloroquine, 0·5 gm. in single dose.

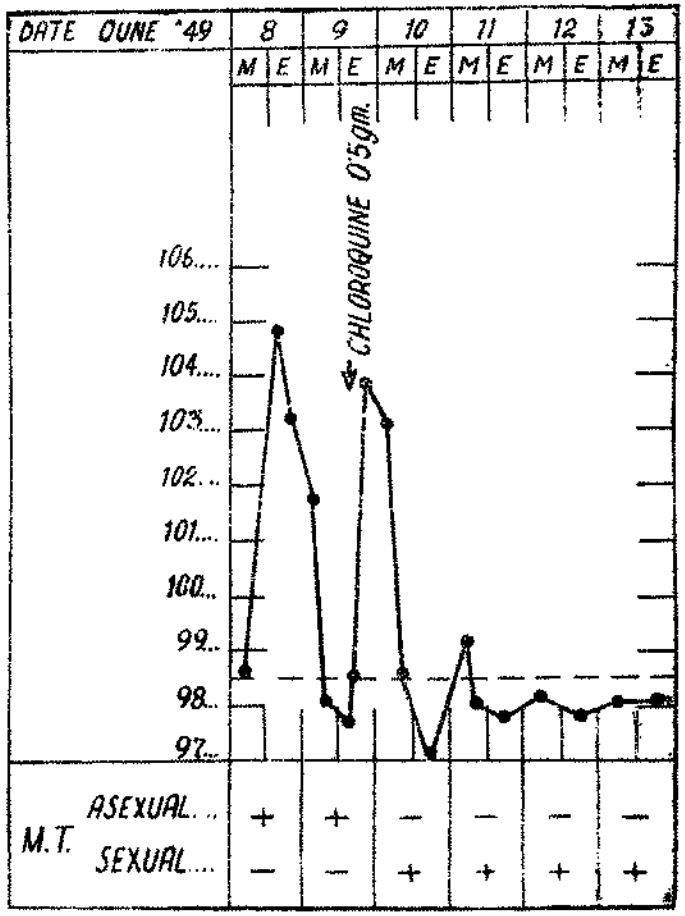

FIG. 6.—*Falciparum* malaria treated with single dose of chloroquine, 0·5 gm.

Five amongst the camoquin series of 25 cases had recurrence of fever after the treatment was given as shown below. Of these, three patients, two due to *falciparum* and one due to *vivax* infection, relapsed (homologous parasites being found) after 20, 13 and 27 days while still in the hospital. The second case had no fever but only parasitæmia for 4 days. The two other cases had recurrence of fever after one and 2½ months (the blood was not examined).

TABLE IV.

Relapses in patients treated with camoquin.

Serial number.	Infection.	Interval, days.	Type of relapse.
1	F	28	Clinical.
2	F	20	F
3	F	13	F
4	F and V	27	V
5	V	75	Clinical.

F = *falciparum*. V = *vivax*.

Absorption and excretion of paludrine.—Of the three compounds under discussion, the excretion and absorption of paludrine in human beings using single oral dose of 0·5 gm. of paludrine hydrochloride were studied. The method of Spinks and Tottey (1946) based on the hydrolysis of paludrine or its metabolic degradation products to p-chloroaniline and development of azo colour by coupling the diazotized p-chloroaniline with sulphatoethyl m-toluidine was employed. The blood was withdrawn four hours after the administration of the drug and examined and this was repeated at the same hour on the following days till it was negative. The results of whole blood concentration of paludrine in ten patients are represented in Table V.

TABLE V.

Whole blood concentration of M-4888 (paludrine) concentration (micrograms per litre) days of treatment.

Patient.		1 (4 hours).	2	3	4
500 mg. paludrine hydrochloride					
G	...	720	380	220	60
SM	...	600	320	180	...
AK	...	680	300	120	50
H	...	780	400	140	...
SB	...	640	360	260	70
M	...	800	380	210	70
LR	...	750	340	200	50
SR	...	590	280	180	...
PS	...	620	300	150	...
LC	...	700	290	120	...

There was a rapid rise in concentration of the drug in the blood after four hours, it was reduced to nearly half on the second day and to negligible figure on the fourth day. The concentrations reached on the last day are near the limits of the analytical technique and therefore their measurements are not very accurate. The data in Table V shows that the drug disappears from the blood in about four days. The results of whole blood concentration and urinary output of three typical cases are illustrated in Figs. 7, 8 and 9.

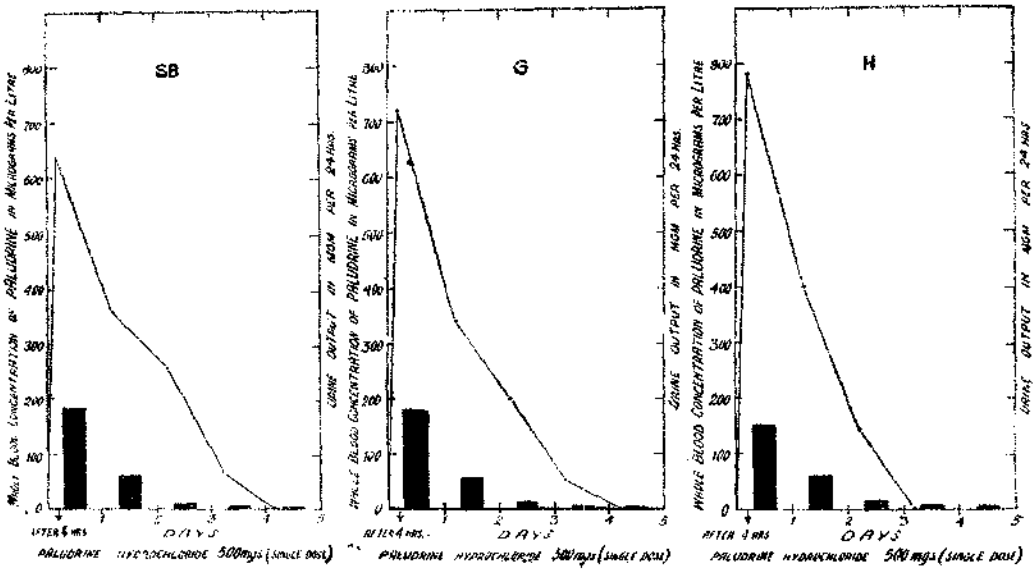

FIG. 7. FIG. 8. FIG. 9.

FIGS. 7, 8 and 9.—Represent the whole blood concentration and urinary excretion of paludrine in three patients SB, G and H.

Urinary excretion after a single oral dose of 0·5 gm. of paludrine hydrochloride has also been studied in most of these subjects. It has been observed that the drug disappears from the urine generally in four to five days. The results of three cases are shown in Table VI. Most of the drug was excreted during the first and second twenty-four hours and the amount excreted from the third day onwards fell to negligible figures. The total excretion of the drug amounted to about 50 per cent of the administered drug.

TABLE VI.

Urinary output (500 mg. paludrine hydrochloride) in mg. calculated as paludrine hydrochloride.

Time.	Patient.		
	SB	G	H
1st 24 hours	188·2	178·2	150·5
2nd 24 hours	65·5	58·3	60·3
3rd 24 hours	9·9	12·6	13·5
4th 24 hours	6·5	5·4	7·1
5th 24 hours	2·1	1·9	2·8
TOTAL ...	272·2	256·4	234·2
Percentage of intake ...	54·4	51·3	46·8

DISCUSSION.

The present study indicates that the clearance of asexual parasites and control of fever can be effected, though at varying rates, by a single dose of any one of the three drugs, chloroquine being quick, paludrine slow and camoquin intermediate in action. This is also confirmed by the authors' experience with prophylactic treatment now being carried out in a rural area of West Bengal, which shows that chloroquine and camoquin are superior to paludrine, acting more quickly and reducing the relapse rate to a greater extent.

It has been observed that with single doses varying from 0·3 to 0·6 gm. of paludrine hydrochloride, the concentration of the drug in the blood reaches a maximum after 4 hours, drops to about 50 per cent after 24 hours and it becomes negligible after 3 days due to rapid excretion in the urine. This is in marked contrast to the fate of chloroquine and camoquin which, like mepacrine, become localized in the tissues and are only slowly eliminated in the urine. It has been reported that chloroquine (Berliner *et al.*, 1948) and camoquin (Wiselogle, 1946) persist in the body for a fairly long time even after the end of treatment and that the plasma concentrations drop only by about 60 per cent per week. Quinine, on the other hand, is only slightly localized, disappearing from the plasma by about 90 per cent within 24 hours after the administration of the drug is discontinued. These facts go to a certain extent in explaining the relative values of these drugs in the control of malaria.

The question arises whether quinine and mepacrine can be similarly used. The authors have not sufficient experience in this respect, but recently six cases of acute malaria were treated with single dose of quinine sulphate (20 grains in

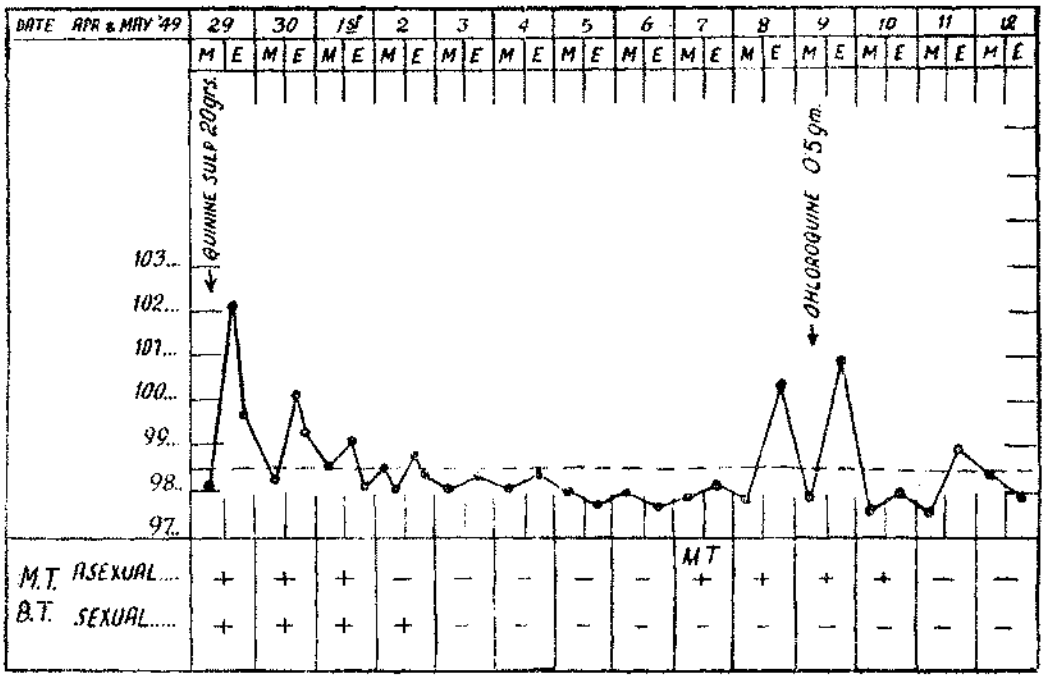

FIG. 10.—Mixed *vivax* and *falciparum* malaria treated with single dose of quinine gr. xx. Recrudescence of *falciparum* infection.

1½ oz. of water). The immediate clinical response was good in five cases, but there was recrudescence or relapse within 5 to 7 days (Fig. 10). The remaining case was uninfluenced with regard to temperature as well as parasites in the blood.

Simmeons and Chhatre (1947) and Chaudhuri and Chakravarty (1948) had reported the satisfactory response to a single dose of camoquin. It was pointed out that it may prove a suitable remedy for mass treatment of malaria in rural areas. The present study also bears it out. Relapses and reinfection can be prevented by subsequent weekly doses. In the field experiments during 1948 and 1949, the results of weekly doses of chloroquine 0·25 gm. and camoquin 0·2 gm. for about 6 months were very good as compared with the comparison and also paludrine and quinine groups. No toxic sign or symptom was encountered. Unfortunately neither of them is cheap nor available for general use.

Paludrine even in a single dose of 0·3 gm. has already been reported in India by various workers (Afridi, 1947; Chaudhuri and Rai Chaudhuri, 1947; Viswanathan and Bailey, 1947; Viswanathan, 1948) to effect a clinical cure in an average case of malaria among semi-immune population; so it is no wonder that 0·5 gm. should be equally effective. Whilst 0·3 gm. dose had occasionally been a failure, there was none at least in the present series although relapses were common. This would indicate that 0·5 gm. or preferably 0·6 gm. (i.e. 2×0·3 gm.

tablets) would be better than 0·3 gm. (one tablet) for the single dose treatment among the mass population. This simple non-toxic therapy may even be placed in the hands of lay persons; indeed it is necessary if such a preventive programme is to be carried out. But confidence in paludrine has lately been shaken in view of its certain limitations which the earlier investigations failed to reveal. It would be desirable especially for individual cases to give a dose daily for the following 3 or 4 days or until the fever subsides. Paludrine is now issued in 0·3 gm. tablets. The patients should be made to swallow 2 of these tablets (0·6 gm. or 0·52 gm. of the base) immediately and given 3 or 4 tablets with instruction to take one a day. Such treatment should be particularly suitable in dispensary practice in rural areas where for various reasons the poorer classes are usually unable to undergo the full course of treatment. Alternatively, quinine sulphate or cinchona febrifuge may be used in daily divided doses of 15 grs. for 5 days, or mepacrine in more or less same doses as paludrine. Further success, however, depends on continuing, after the control of fever, paludrine in 0·3 gm. doses, once a week especially during the malaria season, for, practically speaking, it is in this way only that relapses and incidentally reinfection can be considerably suppressed. The results have also been borne out by field studies.

Paludrine refractory strains of parasites have occasionally been reported in India (Chaudhuri, 1948; Chaudhuri and Rai Chaudhuri, 1947); these are best treated with quinine or mepacrine. Paludrine has however no place even with larger doses in the treatment of seriously ill patients for whom quinine is the drug of choice (Covell, 1949; Chaudhuri and Chakravarty, 1950).

ACKNOWLEDGMENT.

The authors wish to record their thanks to Sri C. L. Mitter and Sri S. Mitter for valuable assistance and to the Medical Services Department of Messrs. Imperial Chemical Industries (India) Ltd., Calcutta, and Messrs. Parke, Davis & Co., for the supply of paludrine and camoquin respectively.

REFERENCES.

AKRIDI, M. K. (1947) ... *Ind. J. Mal.*, **1**, p. 317.
BERLINER, R. W., *et al.* (1948) ... *J. Clin. Invest.*, **27** (No. 3, Part 2), p. 98.
CHAUDHURI, R. N. (1948) ... *Ind. Med. Gaz.*, **83**, p. 225.
CHAUDHURI, R. N., and CHAKRAVARTY, N. K. (1948). ... *Ind. J. Mal.*, **2**, p. 115.
Idem (1950) ... *Ibid.*, **4**, p. 111.
CHAUDHURI, R. N., and RAI CHAUDHURI, M. N. (1947). ... *Ind. Med. Gaz.*, **82**, p. 217.
Idem (1949) ... *Ind. J. Mal.*, **3**, p. 305.
COVELL, G. (1949) ... ... *Brit. Med. J.*, **ii**, p. 773.
SIMMONS, A. T. W., and CHHATRE, K. D. (1947). ... *Ind. Med. Gaz.*, **82**, p. 255.
STANKS, A., and TOPPEY, M. M. (1946). ... *Ann. Trop. Med. & Parasit.*, **40**, p. 101.
VISWANATHAN, D. K. (1948) ... *Proc. Fourth Int. Cong. Trop. Med. & Mal.*, Washington, **1**, p. 878.
VISWANATHAN, D. K., and BAILEY, J. D. (1947). ... *Ind. J. Mal.*, **1**, p. 307.
WISELOGLE, F. Y. (1946) ... *A Survey of Antimalarial Drugs*. J. W. Edwards, Michigan.

Indian Journal of Malariology, **4**, 2, June 1950.

MALARIA CONTROL MEASURES IN THE TARAI AREA UNDER THE TARAI COLONIZATION SCHEME, KICHHA, DISTRICT NAINI TAL: SEPTEMBER 1947 TO DECEMBER 1948.

FIRST REPORT.

BY

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[February 25, 1950.]

INTRODUCTION.

THE past story of the development of the Tarai on the whole is rather depressing. Attempts made so far by individuals and agencies to develop the Tarai and bring it under cultivation and inhabitation as a permanent measure of settlement have invariably ended in failures. Such a gloomy past was a great hindrance in future progress and all efforts to develop the Tarai were more or less given up. The question of development of Tarai was again seriously taken up at the end of the World War II in 1946 when Government was faced with the immediate problem of settling the returning ex-servicemen on demobilization and also with the acute problem of food shortage in the country. The partition of the country in 1947 complicated the problem and necessitated a real serious effort at the development of the Tarai, chiefly for the rehabilitation of displaced persons from Pakistan.

The hub of the problem in the Tarai is malaria. No efforts at development of this area could be successful without adequate malaria control. To achieve this, one antimalaria unit was established in this area with effect from September 1, 1947.

GENERAL DESCRIPTION OF THE AREA.

The Tarai in Naini Tal District is that strip of land which extends at the foot of the Himalayas between the waterless Bhabar to the north and the cultivated

plain of Rohilkhand to the south. It stretches from the Sarda River on the east to Kashipur Pargana on the west and lies between 28° 53′ and 29° 26′ north latitude and between 78° 53′ and 80° east longitude with an average breadth of 11 miles from north to south. Its general appearance is that of a plain sloping gently towards the south-east, the average fall being 12 feet in the mile.

The country in general is a tract of forests and swamps with scattered patches of cultivated land. The northern half of the tract chiefly consists of jungle or savannahs of grass and reeds and a number of springs. These springs lie in a series of morasses that are formed along the southern edge of the Bhabar by syphonic influence on the subterranean drainage and are strongest and most numerous where the breadth of the Bhabar above them is greatest. From this line of springs flow a number of sluggish streams which are constantly doubling back their courses and the channel is seldom well defined. The land in between is slightly undulating, rising as it leaves one channel and falling as it approaches the next. The undulations to the north are small and decided, while to the south, as the distance between the rivers increases, the country is more level.

SOIL.

The soil of the Tarai is a moist alluvial deposit without any sign of rock formation of the Bhabar. It is naturally fertile and owing to its inherent capability, the outturn is so satisfactory that manure is seldom thought of or required. Consequently good crops can be raised with little effort and this fact combined with low rents is the chief attraction to an immigrant.

DRAINAGE.

The drainage system of the Tarai, as a whole, resembles the reticulations of a leaf. The streamlets on the edge of the moist country unite to form large channels which again feed the arterial lines of drainage and all eventually join the great mid-rib stream. Most of the rivers are subject to heavy floods during rains. They are nearly all used for irrigation. The Tarai streams are often blocked in their courses with fallen trees and overhanging branches to an extent almost inconceivable to one not acquainted with them, and in high floods the velocity is greatly diminished by their presence.

CLIMATE.

The climate of the Tarai differs from that of the adjoining plains country in great variation of the day and night temperatures. This is due to the moist nature of the soil. The excessive moisture combined with great heat of the summer renders the climate distinctly enervating. These conditions of temperature and humidity combined with the general water-logged condition of the Tarai are suitable both to the mosquito and the malarial parasite, and it is therefore not surprising that malaria is prevalent in hyperendemic form in this part of the country.

RAINFALL.

The average rainfall, although not so heavy as in the Bhabar tract, is considerably greater than that in the neighbouring plains districts of Rohilkhand. It is comparatively heavier in the eastern portions of the Tarai than in the western. Thus Kilpuri in the east has an average of 64·88 inches while Rudrapur in the centre of Tarai shows 57·97 inches and Kashipur in the west only 46·4 inches. The average rainfall recorded at Kichha is about 50 inches. July and August are the wettest months and November and December the driest

SPRING WATER LEVEL.

This varies in different parts of the area from 1 to 21 feet.

CROPS.

Crops resemble somewhat those of the plains districts. Due to the natural fertility of the soil good crops can be raised with little effort. Rice is the main staple crop of the Tarai particularly of the Tharus and Bhuksas who form the bulk of the non-indigenous population of the area. Besides rice, other kharif crops are maize, jowar and sugarcane. Rabi crops are wheat, gram, barley and lahi.

IRRIGATION.

In the Tarai, the streams have been used from the earliest time for irrigation. Construction of simple dams across the streams has been in vogue. This system has ruined both the land and the climate, and has formed the arable land into immense swamps. The soil became water-logged and gave rise to severe form of malaria which carried away a great many of the inhabitants. Endeavours were then made to remedy this evil by introducing the system of canals and reclamation of the swamps. This somewhat improved the condition but later, due to lack of constant care and attention on the part of those in charge of these works, the improvement effected in the beginning did not last long and rapid deterioration took place making the condition even worse than what it was in the beginning.

FOOD OF THE PEOPLE.

Rice is generally the staple diet specially with the Tharus and Bhuksas who are also great fish-eaters. Wheat is only in partial consumption chiefly on occasions of entertainment at marriages and the like.

COMMUNICATIONS.

In the Tarai, metalled roads are very scarce. Low-lying portions of the country are difficult of access for a large portion of the year. Frequently the thoroughfares in common use are no better than mere tracks cut through the forest, difficult for even country carts to negotiate.

Three railway lines pass through the tract. The Bareilly-Kathgodam line runs north and south about the middle with railway stations at Gokulnagar and Kichha.

Pilibhit-Tanakpur railway line across the extreme east of the tract also runs north and south with stations at Khatima, Chakarpur and Banbassa.

A branch line connects Lalkua on the Bareilly-Kathgodam line to Kashipur with railway stations at Gularbhoj in Bhabar and Bazpur in Tarai.

The provincial metalled road connecting Bareilly and Kathgodam runs parallel to the railway line and passes through Kichha, Gokulnagar and Haldwani. There is also a pucca road between Kichha and Rudrapur. Rest of the roads in the area are kutcha. A new metalled road which is under construction will on completion connect Rudrapur to the Bareilly-Kathgodam provincial road at a short distance above Gokulnagar.

PEOPLE.

Earlier records show that the Tarai was a famous forest and grazing land. It was more densely populated in the latter part of the 18th century than at any previous or subsequent period. This density of population was attributed to thousands of families leaving Rajputana for fear of Maratha invaders and settling in the Tarai. Subsequently the population began to decline steadily and is still on the decline. The population has thus been mainly of a somewhat shifting nature particularly in the southern parts of the Tarai where immigration from the adjoining districts of Rohilkhand was a constant feature.

The population mainly consists of two classes of people—the indigenous or Desi and non-indigenous Tharus and Bhuksas. Both the latter are closely allied races and thrive well in the unhealthy Tarai. The indigenous, on the other hand, suffer very badly from the distressing climate and malaria. In almost all villages inhabited by the indigenous, the majority, both amongst adults and children, consists of anæmic debilitated individuals with protuberant abdomen more than half filled with spleens.

SOCIO-ECONOMIC CONDITIONS.

The people of the Tarai are essentially agriculturists. Rents and taxes are low and considerable facilities are given to the residents by Government to encourage cultivation of land. Paddy is the chief crop. There are large areas of grazing land and every village has its own herd of cattle. Kharif crop is usually harvested in October and Rabi sown in November. These are, therefore, the months of the greatest stress and strain to the villagers. Night watching of the fields, poor clothing and hard labour contribute to a large extent to lowering of natural resistance against disease. This is comparatively more marked amongst the indigenous population. The non-indigenous Bhuksas and Tharus who usually enjoy good physique are not so much affected. They are found cheerful and full of energy and appear to enjoy life. Their children are sturdy little fellows who behave and amuse themselves like those met with in other healthy parts of the country.

The houses of the indigenous are clumsy structures made of thick mud walls with no adequate provision for lighting and ventilation. Cattlesheds are usually inside houses or close by. Usually the surroundings are dirty and there is invariably overcrowding in the houses. The houses of Bhuksas and Tharus are built according to a definite plan and are neat and clean with comparatively better arrangement for lighting and ventilation. Cattlesheds are usually built at some distances from their houses.

ANTIMALARIAL ACTIVITIES OF KICHHA COLONIZATION SCHEME.

Area under antimalaria unit scheme.

The area taken up constituted a triangular block of 18,000 acres of fallow land in the Naini Tal Tarai, with Kichha, Lalkua and Rudrapur at its three corners and another wedge-shaped piece of land measuring 12,000 acres south of the road to Rampur from Kichha extending up to the northern boundary of the Baheri Tehsil of District Bareilly. Kichha and Lalkua are situated on roadsides and are also connected by a metalled road from Bareilly to Naini Tal and Almorah. Rudrapur is not connected by any railway line but is situated on the metalled road from Kichha to Rampur and Moradabad. Most of this area has been lying uncultivated and undeveloped since ages and is being maintained as reserve forest by the Tarai and Bhabar Government estates. The high malariogenic conditions prevailing in the area had stood in the way of its development so far.

Topographical and hydrographical features.

The characteristic topographical feature of the region comprises of vast stretch of dense jungle of huge bushy trees and tall elephant grass.

There are a number of small parallel streams in the area. These streams are densely covered with vegetation all over. The subsoil water level is high being 3 to 3½ feet below the surface resulting in extreme dampness of the soil and a large number of surface water collections forming fruitful sources of mosquito breeding. Artesian wells are a well-marked hydrographical feature of the region and form the only source of supply of drinking water.

Population.

Out of a total number of 103 villages in the whole of this tract, 40 have been taken up for development as a preliminary measure. Of these 40 villages, 23 are inhabited with an approximate population of 2,556 persons and the rest are uninhabited (Table I). People, as in other parts of this tract, are migratory in habit. They live in kutcha huts with thatched roofing unprotected from the bites of mosquitoes.

TABLE I.

Names of villages taken up for development as a preliminary measure with their population.

Name of village.	Population.	Name of village.	Population.
1. Kichha	1,029	21. Gidhpura	Uninhabited.
2. Bundia	76	22. Chhinki	..
3. Sesai	17	23. Naugna	..
4. Soneri	54	24. Malpura	..
5. Sirouli	346	25. Lalpur	175
6. Dupaharya	102	26. Pachpera	14
7. Pateri	121	27. Chukti	10
8. Pantpura	83	28. Mehoryan	34
9. Aujania	22	29. Jehanabad	14
10. Nandarni	141	30. Khamaria	53
11. Jagroonagar ...	23	31. Keoria	Uninhabited.
12. Richoli	Uninhabited.	32. Rudrapur	6
13. Darau	..	33. Rudrapur Majhera ..	41
14. Sahjana	..	34. Rampura	91
15. Rajpura	..	35. Bhadaipura ..	43
16. Deora Piper ...	..	36. Dila Majhra ..	15
17. Kurria	..	37. Bagwara	46
18. Ramnagar	..	38. Gauntia	Uninhabited.
19. Billoongar	..	39. Pratabpur	..
20. Racanpura	..	40. Rameswarpur ..	..

METEOROLOGICAL CONDITIONS.

The mean annual rainfall of the area is 50 inches. The maximum and minimum temperatures recorded in this area are 108·4°F. and 36·7°F. in June and December respectively. The relative humidity varies from 40 to 75 per cent.

EXTENT OF MALARIAL PREVALENCE.

(*a*) *Spleen and parasitic surveys.*—Examinations of children between 2 and 10 years of age and of adults for enlargement of spleen were carried out in pre-monsoon and post-monsoon periods and results recorded (Table II). The spleen rates varied generally between 50 and 100 per cent, both amongst children and adults, showing prevalence of hyperendemic malaria in the tract.

TABLE II.

Child spleen index.

Month of survey.	Village.	Population.	Number of children examined.	Number with enlarged spleen.	Spleen rate, per cent.
Dec. 1947 ...	Kichha ...	1,029	20	14	70·0
May 1948 ..	Do. ...	1,029	78	51	65·4
Dec. 1947 ...	Bundia ..	76	4	3	75·0
Jun. 1948 ...	Do. ...	76	15	8	53·3
Dec. 1947 ...	Sesai ...	17	3	3	100·0
Dec. 1947 ...	Soneri ...	54	...	...	...
Jun. 1948 ..	Do. ..	54	2	Nil	0
Jan. 1948 ..	Pachpera ...	14	3	1	33·3
May 1948 ...	Do. ...	14	3	1	33·3
Jan. 1948 ...	Chukti ...	10	1	1	100·0
Jun. 1948 .	Do. ...	10	2	2	100·0
Jan. 1948 .	Meheryan ..	34	4	4	100·0
Jun. 1948 ...	Do. ...	31	4	4	100·0
Jan. 1948 ...	Jehanabad ...	14	2	2	100·0
Jan. 1948 ...	Khamaria ...	53	6	5	83·3
Jun. 1948 ...	Do. ...	53	8	8	100·0
Apr. 1948 ...	Siroali ...	316	16	13	81·3
May 1948 ..	Pantpura ...	83	11	7	63·3
Jun. 1948 ...	Poteri ...	121	12	10	83·3
Jun. 1948 ..	Rampura ...	91	9	8	87·5
Jun. 1948 ..	Rudrapur Majhera.	11	10	10	100·0
Jun. 1948 .	Rudrapur ...	6	...	...	...
Jun. 1948 ..	Anjania ...	22	4	3	75·0
Jun. 1948 ...	Naudarni ...	141	19	11	57·8
Mar. 1948 ...	Lalpur ...	175	29	29	100·0
May 1948 ...	Dupaharya	102	9	8	87·5

TABLE II—*contd.*

Adult spleen index.

Month of survey.	Village.	Spleen rate, per cent.
Dec. 1947	Kiehha ...	40·0
May 1948	Do. ...	78·2
Dec. 1947	Bundia ...	100·0
Jun. 1948	Do. ...	58·8
Dec. 1947	Sesai ...	100·0
Dec. 1947	Soneri ...	56·6
Jun. 1948	Do. ...	80·0
Jan. 1948	Pachpera ...	20·0
May 1948	Do. ...	33·0
Jan. 1948	Chukti ...	100·0
Jun. 1948	Do. ...	60·0
Jan. 1948	Meheryan ...	66·6
Jun. 1948	Do. ...	75·0
Jan. 1948	Jehanabad ...	...
Jan. 1948	Khamaria ...	80·0
Jun. 1948	Do. ...	78·6
Apr. 1948	Sironli ...	90·9
May 1948	Pampura ...	69·2
Jun. 1948	Pateri ...	54·5
Jun. 1948	Rampura ...	86·9
Jun. 1948	Rudrapur Majhera	66·7
Jun. 1948	Rudrapur ...	50·0
Jun. 1948	Anjania ...	71·4
Jun. 1948	Naudarai ...	57·8
Mar. 1948	Lalpur ...	82·6
May 1948	Dupaharya ...	93·1

The records of blood examinations indicate preponderance of benign tertian infection (Table III).

TABLE III.

Child parasitic index.

Month of survey.	Village.	Number of children examined.	Species of Malaria parasites found.			Malaria parasites not found.	Parasitic rate, per cent.
			Vivax.	*Falciparum.*	*Malariae.*		
Dec. 1947 ...	Kichha ..	20	...	..	..	20	0
May 1948 .	Do. ..	78	2	...	..	76	2·6
Dec. 1947 .	Bundia ..	4		...	..	4	0
Jun. 1948 ..	Do. ..	15	..	..	.	15	0
Dec. 1947 ..	Sesai ..	3	..	...	..	3	0
Dec. 1947	Soneri ...	...	..	...	..	...	...
1948.							
Jun. . .	Soneri ..	2	...	...	...	2	0
May . .	Pachpera ...	3	.	..	..	3	0
Jun.	Chukti ..	2	..	..	..	2	0
Jun. .	Meheryan ..	4	...	...	..	4	0
Jun.	Khamaria ..	6	...	...	...	6	0
Jun. .. .	Sirouli ...	16	3	...	..	13	17·6
May	Pantpura ...	11	...	...	..	11	0
Jun.	Pateri ..	12	1	...	..	11	8·3
Jun.	Ranipura ..	9	...	...	..	9	0
Jun.	Rudrapur Majhera	10	...	...	..	10	0
Jun.	Rudrapur ...	..	...	...	..	...	...
Jun.	Anjania ..	7	...	...	...	7	0
Jun.	Naudarni ..	19	1	...	..	18	5·2
Mar. .. .	Lalpur ..	29	28	...	...	1	96·5
May	Dupaharya ...	9	...	...	...	9	0

Adult parasitic index.

Month of survey.	Village.	Number of children examined.	Species of malaria parasites found.			Malaria parasites not found.	Parasite rate per cent.
			Vivax.	*Falciparum.*	*Malaria.*		
Dec. 1947 ...	Kichha ...	10	...	...	...	10	0
May 1948 ...	Do. ...	54	2	...	...	52	3·6
Dec. 1947 ...	Bundia ...	5	...	...	...	5	0
Jun. 1948 ..	Do. ..	17	...	...	...	17	0
Dec. 1948 ...	Sesai ...	1	...	...	...	1	0
Dec. 1947 ...	Someri ...	6	...	...	...	6	0
1948.							
Jun.	Someri ..	15	...	...	...	15	0
May	Pachpera ...	3	...	...	...	3	0
Jun. .. .	Chukti ..	5	...	...	...	5	0
Jun.	Meheryan ...	12	...	...	...	12	0
Jun.	Khamaria ...	14	...	...	...	14	0
Apr.	Sirouli ..	34	10	...	...	24	30·3
May . ..	Pantpura ...	42	9	...	...	33	21·4
Jun.	Pateri ...	49	1	...	...	48	2·04
Jun.	Rampura ...	22	...	...	...	22	0
Jun. ...	Rudrapur Majhera	15	...	...	...	15	0
Jun.	Rudrapur ...	4	...	...	...	4	0
Jun.	Aujana ...	7	...	...	...	7	0
Jun. .. .	Naudarni ...	25	1	...	...	24	4
Mar.	Lalpur ...	46	40	...	...	6	87
Mar.	Dugaharya ...	42	5	...	...	37	12

INCIDENCE OF MALARIA.

A study of the percentage of attendance of malaria cases to total cases in Kichha Dispensary (Table IV) gives an idea of the malarial incidence of this area.

TABLE IV.

Percentage of malaria cases to total cases who attended Kichha Dispensary, Naini Tal District, during 1942–1948.

Months.	PERCENTAGE OF MALARIA CASES TO TOTAL CASES.		
	Average of 5 years (1942–1946).	1947.	1948.
Jan.	26	28	25
Feb.	25	25	16
Mar.	22	20	19
Apr.	23	24	23
May	30	28	18
Jun.	23	25	19
Jul.	24	27	17
Aug.	31	40	25
Sep.	46	55	26
Oct.	50	62	29
Nov.	52	54	28
Dec.	49	34	16

From this Table it will appear that September to December are the months of highest malarial incidence and after the establishment of the antimalaria unit

in Kichha in September 1947 and the institution of malaria control measures, there has been a noticeable decline in the percentage of malaria cases to total cases in the year 1948.

(*b*) *Entomological observations.*—These observations were regularly made. Several mosquito catching stations were established in operational villages as well as in comparison villages to assess the adult densities particularly that of the known vector species of the Tarai (*A. minimus*). There was preponderance of *A. culicifacies* and *A. fluviatilis*, while *A. minimus* was found in very small numbers (Table V). In the pre-monsoon period, mosquito breeding was restricted chiefly to the bed of the Kichha River where *A. culicifacies*, *A. fluviatilis* and *A. splendidus* were found breeding in large numbers. In the post-monsoon period, there was enormous breeding almost all over in the large number of water collections and marshes. In the winter months of November, December and January, breeding, practically of all the species, ceased more or less completely.

TABLE V.

Species of anopheles found in the area.

1.	*A. culicifacies*	56·2
2.	*A. annularis*	15·8
3.	*A. fluviatilis*	14·5
4.	*A. splendidus*	7·4
5.	*A. subpictus*	4·9
6.	*A. minimus*	0·5
7.	*A. pallidus*	0·7
8.	*A. pigus*	
9.	*A. vagus*	
10.	*A. maculatus*	
11.	*A. stephensi*	
12.	*A. barbirostris*	
13.	*A. hyrcanus*	
14.	*A. theobaldi*	

Number of mosquitoes available for dissection was small: *A. culicifacies* 91, *A. minimus* 12 and *A. stephensi* 1 were dissected to detect gland infection but without positive findings.

MALARIA CONTROL MEASURES.

Anti-adult measures: (1) *Antimalaria staff.*—A list of antimalaria staff sanctioned for the unit is given (Table VI). In planning the control measures, the main objective in view was to keep the entire population including the staff employed on various developmental activities fit and free from malarial attacks. With this end in view both D.D.T. residual spraying and paludrine prophylaxis and treatment were employed.

TABLE VI.

Staff sanctioned for the unit.

Name of post.	Number.
Antimalaria Officer ..	1
Malaria Supervisors ...	5
Accounts Clerk ...	1
Senior Malaria Field Workers ...	5
Malaria Field Workers ...	25
Mistri	1
Laboratory Assistants ...	2
Insect Collectors	2
Motor Driver	1
Peon	1
Orderlies	2
Motor Cleaner	1
Sweepers	2

(2) *Spraying material used.*—Applications of D.D.T. were made at intervals of 6 weeks in all village huts, bungalows and other structures excepting temporary camps and thatched gang huts which were sprayed at intervals of three weeks. During the first round, 2·5 per cent D.D.T. suspension prepared with gum acacia and gelatine according to the formula recommended by the Malaria Institute of India was used giving a dose of 50 mg. of D.D.T. per sq. ft. Supervision in the preparation of suspension was exercised by the Antimalaria Officer or in his absence by one of the Malaria Supervisors. In subsequent rounds 1·25 per cent D.D.T. suspension was used.

(3) *Equipment.*—Stirrup pumps were mostly used for spraying. These were fitted with a nozzle having a diameter of 1/32 inch. Only indoor residual spraying was undertaken in village huts including cowsheds. Catching stations were not sprayed. Tents and thatched gang huts were sprayed both inside and outside. A uniform dose of 50 mg. of D.D.T. per sq. ft. was used in all spraying operations.

It is, however, not claimed that every section of the sprayed buildings received a uniform dosage of D.D.T. as it could never be possible to obtain such an exactitude in a large-scale field operation. Superior staff kept watch over the work of the spraying gangs so as to ensure efficient spraying as far as possible. Before spraying operation was actually undertaken in any village or camp, the surface area to be sprayed was recorded beforehand and the quantity of paste required was then issued to the spraying gang for spraying. The paste was diluted with water to make the watery suspension of the required strength at the site of operation.

Paludrine prophylaxis and treatment.—Paludrine hydrochloride tablets, 100 mg. each, were administered to the local residents, the Government personnel and labourers working under the contractors. The regime followed was 3 tablets per adult in a single dose once every week and proportionately lower dosage to persons of lower age groups. Such prophylactic courses continued throughout the entire period except in the months of December and January.

Each malaria supervisor maintained a register in which he showed the names of permanent as well as of migratory population. While distributing weekly paludrine prophylaxis, he used to verify attendance with the help of his register. This was helpful in so far that very few people could escape the weekly prophylactic dose of paludrine.

A statement showing the number of persons administered paludrine prophylaxis and the number of paludrine tablets used is given in Table VII.

TABLE VII.

Prophylactic distribution of paludrine hydrochloride.

Month, 1948.	Total number of persons to whom paludrine given.	Total number of tablets given.
Jan. ..	640	6,154
Feb. .	5,082	13,557
Mar. ..	8,649	29,375
Apr. ...	10,626	30,903
May .	12,383	34,874
Jun. .	16,365	46,283
Jul. ..	15,451	45,245
Aug. ...	16,246	49,556
Sep. ...	14,763	45,562
Oct. ..	16,801	46,925
Nov. ..	19,558	58,018
Dec. ...	14,451	44,898

For curative purposes, paludrine was administered to microscopically positive malaria cases. The regime followed was 3 tablets per day for 7 to 10 days followed by 3 tablets once a week for 2 to 3 weeks and then the usual prophylactic dose of 3 tablets once weekly for adults. Proportionately smaller doses were given to persons of lower ages.

In a few cases, paludrine was found to create some gastric disturbance, such as vomiting. No other ill-effects of the drug were noticed in any case.

Result of control measures.

The control measures as described above have proved satisfactory in the sense that the development schemes that were introduced in the area continued to operate without any hindrance on account of malaria and the new settlers who were afraid of malaria on arrival remained free from malaria.

D.D.T. spraying was effective in reducing mosquito nuisance with consequent fall in the number of fever and malaria cases amongst the residents.

SUMMARY.

1. The hydrographical, topographical and meteorological features of the region and their effects on malarial conditions prevailing in the area have been discussed.

2. That the low economic status of people exerts a favourable influence on the transmission of malaria has been emphasized.

3. Malaria as judged from spleen rate is hyperendemic in the area.

4. *A. minimus* which was previously incriminated as the chief vector of Tarai in Bazpur area was found in very small numbers in this area.

5. The staff employed in the unit and the technique of D.D.T. spraying and paludrine prophylaxis and treatment have been described.

6. Control measures have proved satisfactory in the sense that the mosquito nuisance and malarial attendance have been considerably reduced and the population passed the period with much less incidence of malaria as judged from the Kichha Dispensary attendance figures.

7. Expenditure on the scheme has been rather high due to the inclusion of non-recurring items in the first year of the scheme and also due to piling up of the stores that became available from medical and scientific stores for use in the succeeding year.

ACKNOWLEDGMENT.

The author is indebted to the Antimalaria Officer of this unit, Dr. A. K. Chakrabarti, under whose supervision the malaria control work was carried out in this area and who supplied the data for incorporation in this paper. The author also wishes to record his thanks to the Assistant Malaria Officer, Dr. Diwan Chand, for his valuable assistance in compiling this report.

Indian Journal of Malariology, **4**, 2, June 1950.

CERTAIN UNCOMMON HABITS OF *CULEX BITÆNIORHYNCHUS* (TYPE FORM).

BY

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[April 17, 1950.]

COLONIZING.

THE colony of *C. bitaeniorhynchus* was started with egg-rafts in preference to the larvæ and pupæ which were both of the type form and var. *ambiguus* and between which the question of sexual barriers had to be considered.

The egg-rafts, collected from the natural breeding habitats, were reared individually in separate basins. The larvæ thrived well only in the presence of green algæ regardless of the supply of other food materials. Green algæ after washing in a few changes of water were frequently replaced in the rearing basins. Larvæ, if not properly reared, would produce undersized adults which would but exhibit poor performance.

Pupæ of the same progeny were kept in separate cages for emergence. Adults of only the type form were selected for colonization and introduced into the colony cage measuring approximately 2 × 2 × 2 feet. The colony cage, a wooden frame-work, supported on legs about 2 inches high, is fitted on three sides with half an inch thick fibre-board, rough surface inward, the bottom being of thick galvanized iron sheet coated with white paint. The top of the frame-work is covered with a 3 ply-wood board having a central aperture of 2 inches square, secured firmly with fine aluminium wire gauze. The front side of the cage is covered, the upper half with glass to provide good visibility while the lower half is screened with fine aluminium wire gauze having a central cloth sleeve entrance (10 × 10 inches).

The males were fed on 10 per cent glucose solution on cotton-wool kept in tubes which fitted into the sockets of a special wooden rack. The rack is kept in an inclined position. This serves the nutrient to the mosquitoes at all levels inside the cage without their having to make a special effort if food were served at any one spot. Females were given the opportunity to imbibe a blood meal on rabbit which was properly caged after being shaved on the back.

* Financed by the Indian Council of Medical Research.

In the beginning, only a small proportion of the females was found to have become fertilized as revealed by sample dissections. The percentage of the inseminated females increased with the passage of time. Most of the fertilized specimens were found amongst the gravid females. The gravid females persistently refused to deposit eggs although one or two bowls containing ordinary plain water were kept inside the colony cage. The reluctance on the part of the females to lay eggs in captivity was overcome by introducing green algal growth into the bowls. Females were thus induced to oviposit fairly readily. The eggs being viable were reared into adults which further reinforced the stock in the colony, making it self-perpetuating and in-bred.

OBSERVATIONS.

Eggs.—Unlike other mosquitoes, the eggs of *C. bitæniorhynchus*, when freshly deposited, show light green coloration. Because of a cumulative effect, the egg-raft itself shows much deeper shade than the individual eggs forming the same raft. If the supply of chlorophyll is made deficient, say, to the half-grown larvæ, by not replacing the decaying algæ, the resulting females (undersized) will lay eggs of faint cream colour. Variations in the shade of the colour of freshly laid egg-rafts are presumably related to the amount of chlorophyll ingested by the mosquito in the larval stage. The question of coloration of the subsequent rafts by the same females has not been investigated.

In nature, egg-rafts of this species have been found to be as long as half an inch or slightly more in arc length. The rafts are usually dark brown in colour. The larvæ hatch out by cutting a circular cap at the anterior end of the eggs. After the escape of the larvæ, the caps remain attached to the eggs, often lying fairly close to the cut end. Empty egg-rafts do not disintegrate over considerable periods, thus giving an exaggerated impression of the intact rafts that may actually be present. Empty rafts, which lie flat and which on being lifted with a strip of paper will readily bend on account of the loss of stiffness, can be easily distinguished from the unhatched rafts which, when handled, retain their boat-shaped appearance.

Larvæ.—Barraud (1934) records that the larva of *C. bitæniorhynchus* 'usually feeds amongst green algæ which it resembles in colour'. When paddy fields are flooded with water after transplantation of rice, green algæ appear over extensive areas. In such situations, larvæ and pupæ and also egg-rafts are found abundantly.

The larvæ are found breeding in streams, irrigation drains and pools but only in intimate association with algal growths. Breeding begins to decline with the aging of green algæ. When they become yellowish brown, that is, rotten, larvæ disappear completely.

In the paddy fields, a very high percentage of the larvæ is found infected with round worms. A thickened body particularly in grown-up larvæ is a sure sign of worm infection.

Attempts to rear larvæ without the supply of green algæ were uniformly unsuccessful. The technique of rearing mosquito larvæ as described by Russell and Mohan (1939*b*) for *A. stephensi* (type) totally failed in the case of *C. bitæniorhynchus* unless supplemented with fresh green algæ. Curd prepared from a skimmed milk, which has been successfully used for rearing the larvæ of *A. fluviatilis* (Mohan,

PLATE V.

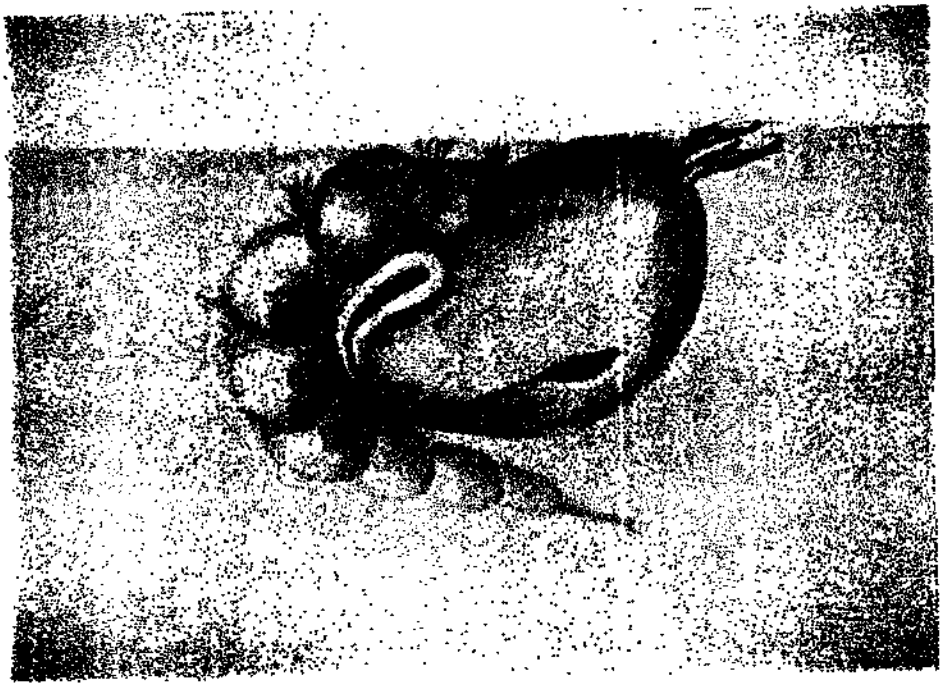

FIG. 1.—*C. bitaniorhynchus* pupa.

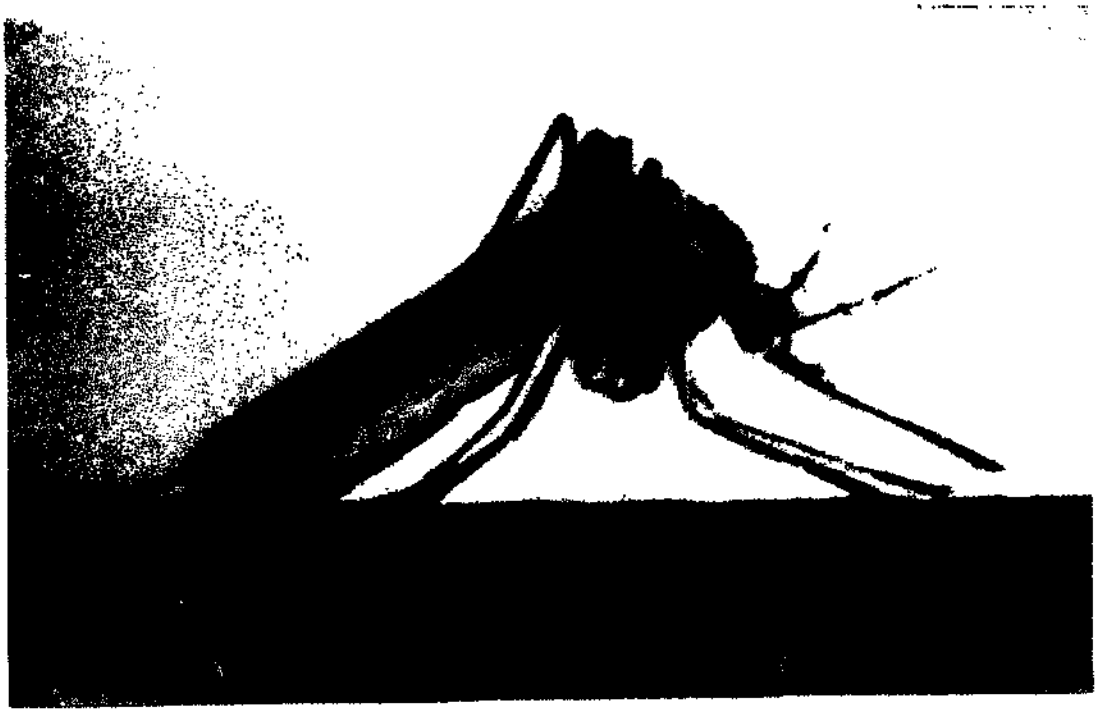

FIG. 2.—*C. bitaniorhynchus* female.

1945), and later adopted for the larvæ of colonies of *A. stephensi* (type) and *Aedes aegypti*, was likewise found to be absolutely unserviceable without the provision of green algæ.

In curd water, the first stage larvæ of *C. bitæniorhynchus* died within two or three days while those of the intermediate stages took a little longer. Active healthy larvæ, on being placed in curd water, were found to become gradually sluggish with the progressive depletion of the particulate greenish matter previously ingested from the rearing basins. The habit of 'shamming dead' soon disappeared completely. The larvæ remained for longer intervals at the bottom of the water. They became enfeebled, so much so that they could be easily lifted up with the tip of a needle. In the same water containing the same food, while the larvæ of *C. bitæniorhynchus* showed cent per cent mortality, those of other species in comparable stages thrived well.

With the supply of fresh green algæ which also promoted the growth of plankton, it was hardly necessary to supplement the larva food with yeast or any other material. Larvæ were invariably found concentrated among green algæ. When disturbed, they would almost immediately return to the algal matting. When green algæ were kept in a fresh condition, mortality among the larvæ was remarkably low and the adults produced were robust. But when green algæ were allowed to decay or rot, the growth of the larvæ was adversely affected, particularly in the younger stages. Attempts to rear larvæ on the plankton associated with green algæ were all abortive. Plankton was obtained by centrifuging several samples of water in contact with fresh and decaying algæ and served in fresh tap water.

The above findings clearly indicate that green algæ are of fundamental importance to the larvæ of *C. bitæniorhynchus* not only for their growth but also for their survival.

Pupa.—The breathing trumpets of the pupa of *C. bitæniorhynchus* are a unique feature and readily distinguish this species from all other known mosquitoes. Quite unlike the known pupæ of other mosquitoes, the breathing trumpets of the pupa of *C. bitæniorhynchus* extend anteriorly well beyond the extreme anterior outline of cephalothorax; they may converge apically and touch at the extreme ends or lie parallel; very rarely, if at all, do they diverge wide apart (Plate V, fig. 1). These variations in the placement of the breathing trumpets were found in the pupæ of the same progeny.

It is rather curious that the breathing trumpets of *C. bitæniorhynchus*, by which this species can at once be recognized, should have remained unnoticed in India. Ingram and Macfie (1919) in their description of the pupa of *Culex ager* var. *æthiopicus*, Edward (synonym), from West Africa note that the trumpets 'are directed anteriorly in the living pupa'.

Adult: (a) Resting posture.—*C. bitæniorhynchus* adults exhibit two different resting postures, one during day time and the other at night. When a mosquito is not flying, it may be 'sitting' or 'standing', so to speak. In the 'sitting' posture (Plate V, fig. 2), the body of the adult is brought close to the resting surface, so that the hind end of abdomen, depending on its contents, touches or nearly touches it. The femur of the front leg on either side, appressed to the pleural surface, is raised upwards almost vertically, projecting slightly above the level of dorsum,

and tibia is directed downwards in the same plane, forming a very narrow inverted V, with femur and tibia appearing as a single segment in side view; tarsus from the distal end of tibia, which lies close to the resting surface, slopes down gently towards head-end with the last two segments usually touching the resting surface.

The inverted V, formed by femur and tibia of the middle leg, lies very close, and external to that formed by the hind leg, the ends of the two inverted V's (tibio-femoral joints), appearing as a single protuberance a little above the level of scutellum when seen from behind. Tarsus of the middle leg on each side is reclined to the resting surface with 3 to 4 of its terminal segments touching it. Tarsi of the hind legs usually remain in the air.

When a mosquito is in the 'standing' posture, tibio-femoral joints widen out and the first two tarsal segments or so of the fore and middle legs become less reclined, so that the body of the mosquito is raised more or less in a horizontal plane. The height of the mosquito from the resting surface would depend on the degree of the angle between tibio-femoral joints and the extent to which the tarsal segments become less reclined or straighten out. Thus, a mosquito may be in partial or more fully 'standing' posture. The 'standing' posture of *C. bitæniorhynchus* adults is met with mostly at night when the brisk alternate movements of the hind legs become very noticeable.

(*b*) *General behaviour.*—*C. bitæniorhynchus* has been occasionally captured from the cattlesheds in routine collections. In a few searches, the adults, both males and females, were found outdoors, resting chiefly among stunted vegetation along the margins of irrigation drains in arecanut gardens in Kallar. The females were in all stages of gonotrophic cycle.

It has already been stated that under natural conditions eggs of *C. bitæniorhynchus* are found on algal growths. The females of this species can exercise discrimination in the selection of algal sites for the purposes of oviposition. Small pits were dug near the algal growths in a stream in which larvæ of *C. bitæniorhynchus* were breeding. Being close to the bank of the stream, the pits became filled with stream water through percolation. Green algæ from the stream after being thoroughly washed with water were floated on sticks in the four pits on one side of the bank while similar four pits on the opposite side were left for comparison.

The following morning, 17 rafts (4, 7, 3 and 3) were recovered from the pits with algæ but none from any of the four comparison pits. With flower pots, which were partially sunk into the bed of stream, similar results were obtained.

During day time, practically all the males and females of *C. bitæniorhynchus* in the colony cage were seen resting in the 'sitting' posture. Generally speaking, they did not take alarm at the approach of a moving object towards them. Their volition appeared to be in abeyance so much so that they would hardly move unless bodily disturbed. This sedentary habit is in the constitutional make-up of this species, as no departure from this behaviour has been observed at any part of the year.

As soon as it darkened in the insectory room, which was about half an hour or so earlier than the time of sunset, mosquitoes were seen taking up 'standing'

posture and becoming active. Swarming time ranged for about an hour during which some pairing couples could be seen.

If *dim* light from a torch were made to fall inside the colony cage in every *nook and corner* for about *half* an hour, mosquitoes were seen to become activated in relatively larger numbers. The adults, on being disturbed, took more of 'exercise' which presumably resulted in the sharpening of their instincts. Dim light was helpful only if *used at dusk*; *late in the night* it had *practically* little or no effect. Most of the feeding and ovipositing were completed during the early part of the night.

Females imbibed blood meal usually on the second night after emergence. Rabbit was the only animal used as a source of blood meal for the females. Dark rabbit seemed to be more attractive to the females than a white one. This observation, which is only impressionistic, requires further investigation. If fed to repletion, a single blood meal was found sufficient for the full maturation of ova.

Feeding activity exhibited by the females was of a low grade. In the presence of both glucose solution (10 per cent) and rabbit (caged and shaved on back), a higher proportion of the females was found to feed on glucose water rather than imbibe a blood meal on rabbit. Removal of glucose from the colony cage before evening resulted in a relatively higher proportion of the females partaking of the blood meal.

In general, under cage conditions, nocturnal activities of *C. bitaeniorhynchus* mosquitoes such as swarming, feeding and oviposition were hardly seen to reach the high mark as has been observed by the writer in other species of mosquitoes, both anophelines and culicines.

(*c*) ***Relation to disease.***—*C. bitaeniorhynchus* came into limelight when Williamson and Mohomed Zain (1937) reported human malarial infections in this species under experimental conditions. Russell and Mohan (1939*a*), however, found the strain of this species collected from Cooum River, Guindy, Madras, to be extremely resistant to human malaria. While they detected no infection in *C. bitaeniorhynchus*, *A. stephensi* fed on the same doners at the same time were highly infected, in one lot to the extent of 100 per cent. Russell and Mohan (1942) succeeded in infecting this species with *P. praecox* in Coonoor sparrows up to the sporozoite stage.

DISCUSSION.

From a consideration of the foregoing account, it is evident that *C. bitaeniorhynchus* exhibits one or more uncommon characteristics in every stage of its life. The light green coloration of eggs, when freshly deposited, has not so far been recorded for any known mosquito. The colour, which is derived from green algæ, varies in shade with the amount of chlorophyll carried over by the mosquito through its larval stage.

Certain species of mosquitoes on account of their specialized breeding habits have been referred to as puddle-breeders, stream-breeders, etc. On the same analogy and perhaps with greater justification, *C. bitaeniorhynchus* may well be called 'green algæ breeder' or simply 'algæ breeder' for the sake of euphony. In *C. bitaeniorhynchus*, the habit of selective breeding is remarkably restricted to sites of algal growths whether they be in ricefields, streams, pools or irrigation

drains. Barber, Komp and Hayne (1927) state that 'the character of the water seemed to be a more important consideration than the size or the contour of the body in which it is contained'. Perhaps this statement is in no other mosquito species so strikingly applicable as to the larva of *C. bitæniorhynchus*. The presence of green algæ has been shown to be of vital importance not only for the growth but also for the survival of the larvæ of *C. bitæniorhynchus*. To say 'no green algæ, no *C. bitæniorhynchus*' may be as true as 'no Anopheles, no malaria'.

The larva and adult of this species can be easily distinguished from other species by the diagnostic characters given by Barraud (*loc. cit.*). As shown earlier, the pupa of *C. bitæniorhynchus* can be readily recognized at first glance from all other known pupæ of mosquitoes by the configuration of its breathing trumpets which are oriented anteriorly and project well beyond the front extremity of cephalothorax in a convergent or parallel position.

The adult insect also exhibits some very uncommon habits. The sedentary habits of the adults coupled with their relatively low level of activity under laboratory conditions, their 'sitting' posture during day in contrast to the 'standing' posture at night, and their vital association with green algæ for oviposition and breeding requirements—these salient points mark this species out without a parallel among the known Indian mosquitoes.

One may hazard the suggestion that *C. bitæniorhynchus* might provide for its utilization as test insect of value in some other fields of investigation. To a nutritionist it may prove an asset for evaluating the nutritive value and metabolism of green algæ without which the larvæ cannot reach maturity. Experts in malaria control have advocated 'dishing' experiments as one of the means to determine the presence or absence of the residual toxicity of insecticide sprays. Preliminary field trials tend to support the impression that *C. bitæniorhynchus* can be employed as D.D.T.-detector with advantage. It may be very useful to equate *C. bitæniorhynchus* with the vector species as regards its susceptibility to residual spray in terms of dosage-time-mortality.

SUMMARY AND CONCLUSIONS.

1. The procedure adopted for the laboratory colonization of *C. bitæniorhynchus* (type form) has been described. Oviposition by the gravid females is induced by the presence of green algæ which otherwise is usually postponed.

2. Green algæ are indispensable for the growth of *C. bitæniorhynchus* larvæ without which it is impossible to rear them to maturity.

3. The pupa can be macroscopically recognized by the configuration of its breathing trumpets.

4. Under laboratory conditions, the adults are sedentary in habit, showing no marked avidity for any tropisms except the tropism of resting. The resting posture exhibited by the adults during day is different from that typified mostly at night.

5. A plea has been put forward for the utilization of this insect in nutrition problems by a nutritionist and as D.D.T.-detector by a malariologist.

REFERENCES.

BARBER, M. A., KOMP, W. H. W., and HAYNE, T. B. (1927).	*U.S.A. Pub. Hlth. Repts.*, **42**, 41, pp. 2487–2502.
BARRAUD, P. J. (1934) ...	*The Fauna of British India. Diptera. Vol. V. Family Culicidæ. Tribes Megarhinini and Culicini.* Taylor and Francis, London, p. 463.
INGRAM, A., and MACFIE, J. W. S. (1919).	*Bull. Ent. Res.*, **10**, pp. 59-69.
MOHAN, B. N. (1945) ...	*Annual Report of the Malaria Institute of India*, pp. 10-14.
RUSSELL, P. F., and MOHAN, B. N. (1939*a*).	*J. Mal. Inst. Ind.*, **2**, 4, pp. 425-431.
Idem (1939*b*) ...	*Ibid.*, **2**, 4, pp. 433-437.
Idem (1942) ...	*J. Parasit.*, **28**, 2, pp. 126-129.
WILLIAMSON, K. B., and MOHAMED ZAIN (1937).	*Trans. Roy. Soc. Trop. Med. & Hyg.*, **31**, pp. 111-114. (Also *Nature*, **139**, p. 714.)

Indian Journal of Malariology, **4**, 2, *June 1950*.

ON THE CONTROL OF PHLEBOTOMUS (SANDFLIES) WITH D.D.T. AND B.H.C. (GAMMEXANE).

BY

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[June 1, 1950.]

SANDFLIES are responsible for the spread of three important diseases in India, e.g. kala-azar, oriental sore and sandfly fever. Since a long time attention has been directed towards its control and though many suggestions have been made from time to time, yet the elimination of the fly has been found to be difficult.

With the discovery of D.D.T. as a powerful insecticide which is effective against most of the insect pests, certain experiments have been carried out to determine its residual action on the adult sandfly. B.H.C. has also been tried for this purpose.

Hertig and Fisher (1945) made extensive tests in Palestine where the pucca built living quarters were sprayed with 5 per cent D.D.T. solution by weight in kerosene at the rate of 1 to 1·5 U.S. gallons per 1,000 square feet (approximately 200 to 300 mg. per square foot). The interior walls of the houses and the edges of the ceilings were sprayed side by side with comparison buildings where spraying was done on the outside walls from ground to eaves including doors, windows, screens and outside openings with little of the surrounding walls. Both these reduced sandfly population to 25 per cent and proportionately fewer bites were reported. The effectiveness was noticed to last for at least 24 days, no observation being made thereafter. Similar experiments, but on a wider scale, were carried out by Jacusiel (1947), also in Palestine. The internal surfaces of walls and ceilings of the pucca built houses were sprayed with 5 per cent D.D.T. solution in kerosene with a dosage of 100 mg. per square foot area. A high degree of protection was obtained for 52 to 58 days when the sandfly season came to an end. It was assumed, however, that the residual effect was not lost and would continue to be active for months more. Hertig and Fairchild (1948) came to a more definite observation and found that the residual effect of D.D.T. lasted for 12 to 19 months. Their experiments were carried out in Peru for 2 years and the observations were made in Surco, a place highly infested with sandflies. Here also the spraying was done in pucca built houses with 5 per cent D.D.T. at the rate of 1·5 gallons per 1,000 square feet

(approximately 300 mg. per square foot) in such a way, that the surfaces became wet without an excess running down. Treatment of boundary stone walls (the principal outdoor shelters of adult sandflies) produced marked reduction in population and combined treatment of stone walls and house spraying (inner and outer walls only) reduced the sandflies to an extremely low level, as also protecting persons indoors from bites.

In none of the above experiments huts with mud walls have been included. In this case the recommended dose of 1 to 2 gallons per 1,000 square feet (approximately 200 to 400 mg. per square foot) would be very inadequate, as the mud walls would absorb a good deal of the solution quickly and make it innocuous to the sandflies. The present investigation, therefore, was carried out to control *P. argentipes* and *P. papatassi*, the two most prevailing species of sandflies in Bengal and to assess the effectiveness of the residual action of D.D.T. and B.H.C. spray in different strengths on the mud walls, the treatment being done up to a certain height on the walls on their interior surfaces only. The observations were made for two years for which the financial assistance was given by the Indian Research Fund Association (now known as Indian Council of Medical Research).

CHOICE AND DESCRIPTION OF THE SITE OF WORK.

The experiments were carried out in Kaorapooker and adjoining villages about 11 miles south of Calcutta. These villages were highly infested with sandflies and endemic in kala-azar. Altogether five such villages were selected and except Kaorapooker others contained only huts, built of mud having thatched or tinned roof. In Kaorapooker, there was a Mission School with attached hostels built of brick with tinned or tiled roofs. The living quarters and the cowsheds were close to each other in all the villages, often they were attached. The average size of a living room was $12 \times 8 \times 9$ feet and that of a cowshed $10 \times 6 \times 9$ feet. Every cowshed was provided with an earthenware trough for feeding the cows and was usually placed on a mud platform having a depression on its centre. This depression always remained wet and made it an admirable place for the breeding of sandflies. In almost every cowshed there could be found a large number of rat holes which were sometimes also present around the living quarters.

Each living quarter and cowshed in the different villages was numbered and labelled. Daily catches were made from each house in all the different villages before spraying but after spraying inspections were made on alternate days. Collections used to start at 6 a.m. in the morning and continued till 2 p.m. in the afternoon, spending at least 10 minutes in each room. The prevalence of sandflies was carefully studied from day to day before actual spraying operations were undertaken. It was observed that the population started rising from early June and reached its peak during the later part of August. After this the density started falling, reaching minimum in the middle of January. The villages, however, never remained absolutely sandfly-free. Amongst the prevailing species, *P. argentipes* was most abundant. *P. papatassi* came next to it and *P. minutus* was the least.

The breeding places were searched for as a routine procedure and the loose sodden soils from the suspected breeding places were collected and larva recovered by flotation method of Young, Richmond and Brendish (1926). The recovered larva were then reared in the laboratory by Smith's (1925) method whereby adults

bred out within 19 to 20 days. In spite of heavy larval mortality, large numbers of *P. argentipes* and *P. papatassi* bred out. No *P. minutus*, however, could be bred this way. Thus, it was discovered that the natural breeding places of *P. argentipes* were under the earthenware feeding troughs and in the moist dark corners of the cowsheds and to a certain extent in the moist rat holes in and around them. *P. papatassi* bred under the same conditions as also under the stumps of the dead banana trees where the soil was wet. Breeding places of *P. minutus* could not be discovered. The temperature and humidity of the place during the peak incidence varied from 82 to 90°F. and 75 to 85 per cent respectively.

SPRAYING AND TECHNIQUE.

Spraying was done with either 5 or 2·5 per cent D.D.T. solution in kerosene. For this a stock solution of 5 per cent D.D.T. was prepared with 8 ounces of pure D.D.T. powder dissolved in one gallon of grade III kerosene oil. The powder was first mixed with a small quantity of kerosene in a pestle and mortar which was thereafter mixed with the rest of kerosene, constantly stirring while the kerosene was added. The mixture was then kept exposed to sun for at least 24 hours and was frequently agitated till no undissolved particle of D.D.T. was seen at the bottom. This 5 per cent stock solution was diluted with an equal amount of kerosene whenever 2·5 per cent solution was needed. The spraying operation was performed by means of a hand-operated Jim Dandy type of sprayer with 2 gallons capacity. The adjustable nozzle of the sprayer was so adjusted that it gave out a fine spray and no large droplet of the solution leaked out during spraying.

Spraying was done only to the inner surfaces of the inner walls of the living quarters and the cowsheds up to a height of 3 feet. The dry mud walls quickly absorbed a large quantity of the solution, therefore, the spraying was continued till the surface became thoroughly wet without an excess running down. Besides the walls of the cowsheds, the feeding trough with its platform and the dark corners were given particular attention and sprayed. An average dose of 6 to 7 gallons of the prepared solution was required for 1,000 square feet surface (approximately 1,500 mg. per square foot).

The whole area under operation was divided into five groups for comparative studies. Each group was treated individually in a different manner, keeping one group amongst them untreated for comparison.

GROUP A. *Kuorapooker Village.*—This was the largest of all, having an area of about 3 square miles. Except the London Mission with its attached school and hostel which were brick built, all others were huts built of mud. Altogether 26 living rooms and 16 cowsheds were treated amongst which 8 living rooms were pucca. The daily catch of sandflies before spray was 5 to 20 in bed rooms and 20 to 40 in cowsheds. The prevailing species were *P. argentipes* and *P. papatassi* and only a few *P. minutus* were caught during the whole season. The breeding places were discovered to be in the cowsheds, in the rat holes in and around them and under the stumps of the banana trees. Spraying was done with 5 per cent solution during the early part of June. Two living huts were re-painted with mud one day after the spray in spite of best efforts by the spraying team to prevent it. The residual effect was lost and sandflies appeared inside within 2 days.

GROUP B. *Midday Chak.*—This was a small village with an area of about a square mile, consisting of mud built huts only. The total number of cowsheds were only four in number and were very close to the living rooms. Daily sandfly catch before spray ranged between 3 to 10 in bed rooms and 12 to 18 in cowsheds. Breeding places were found to be in the cowsheds only; none was discovered outside. The species of sandflies caught were identified as *P. argentipes* and *P. papatassi*. No *P. minutus* was caught during the season. Only the cowsheds were sprayed with 5 per cent D.D.T. solution during early part of July, particular attention having been given to the feeding troughs and the dark corners.

GROUP C. *Thakurani Chak.*—This village was a little larger than the previous one having an area of about one and a half square miles consisting of mud built huts with tinned or thatched roofs. Daily sandfly catch during the pre-spraying period ranged between 10 to 20 in bed rooms and 25 to 40 in cowsheds. The prevailing species was mostly *P. papatassi*; *P. argentipes* was much less in number and no *P. minutus* could be caught during the season. The breeding places were mainly in the cowsheds, only one was discovered under the stump of a dead banana tree. Sixteen living rooms and 3 cowsheds were sprayed with 2·5 per cent D.D.T. solution during the middle of July. The walls of one of the living rooms were re-painted with mud 5 days after the spray where sandfly appeared the following day.

GROUP D. *Ziadargot.*—This was a small village of an area of about a square mile having 10 mud houses and 4 cowsheds. Out of these, two cowsheds were dilapidated and were not under use. Except in these two broken cowsheds, sandflies were abundant everywhere. The daily collections ranged from 28 to 45 in living rooms and 100 to 200 in cowsheds. *P. papatassi* was the most prevalent species and *P. argentipes* were few. The breeding places were discovered in the cowsheds and only the intact cowsheds were sprayed with 2·5 per cent D.D.T. solution in the middle of August.

GROUP E. *Ramnakal Chak.*—This small village served as a comparison village throughout the investigation. All huts and cowsheds except one hut, which was built isolated in one of the corners of the village with an adjoining cowshed, were left untreated. This isolated hut (living room) was sprayed with 5 per cent D.D.T. solution in the middle of August but the cowshed was left untreated.

RESULTS.

As mentioned above, inspection and collection of sandflies were done every alternate day in the treated villages and daily in the comparison village. It was observed that sandflies disappeared completely both from the living rooms and the cowsheds almost immediately after the spray. It was however difficult to find out whether they died immediately after coming out from their breeding places as no dead sandfly could be recovered from the rough mud floors of the cowsheds, neither they could be recovered from the floors or corners of the living rooms.

In group A (Kaorapooker), the bed rooms remained free from sandflies up to 8 months and the cowsheds up to 7 months. The bed rooms were re-sprayed with 5 per cent solution after that period and they again became sandfly-free for

8 months more. In group B (Midday Chak), the sprayed cowsheds remained free for 6 months and the bed rooms for 6½ months. The cowsheds only were re-sprayed with 5 per cent solution which made them sandfly-free for another 6 months. In group C (Thakurani Chak), the sandflies did not appear up to 5 months in the bed rooms and 4½ months in the cowsheds. The bed rooms here were re-sprayed with 2·5 per cent solution and they were free for 5 months more. In group D (Ziadargot), the cowsheds remained free for 4 months and bed rooms for 4½ months. The cowsheds were re-sprayed after that with 2·5 per cent solution. This kept both the bed rooms and cowsheds free up to 4½ months. Group E (Rammakal Chak), where only one isolated living room was sprayed, remained free up to 7 months. The results are given in Table II.

CONTROL OF SANDFLY WITH GAMMEXANE.

At the request of the Imperial Chemical Industries (India) Ltd., gammexane (B.H.C.) was tried as a spraying solution to control the sandflies and also to compare its efficacy and residual effect with that of D.D.T. A small village about three-quarters of a mile in the north of Kaorapooker was selected and altogether 6 living rooms and 2 cowsheds built of mud were sprayed with gammexane L.G. 140 in a similar way as with D.D.T. Before spray, this L.G. 140 was diluted in kerosene grade III in proportion of 1 to 20 and was sprayed at a dose of 1 pint per 1,000 square feet surface as recommended by the manufacturers. The irritating musty smell of the spraying solution was present to some extent and the inhabitants did not like the spray. Sandflies disappeared immediately thereafter but re-appeared both in the living rooms and in the cowsheds after a month. Very faint odour of gammexane was still present in the mud walls.

PRECIPITIN TEST.

After the daily collection and identification, the blood-fed sandflies from those of the living rooms and from the cowsheds were separately crushed within the folds of a small filter paper and preserved to perform the precipitin tests. In the laboratory, each of these small bits of filter papers were soaked for an hour in small quantity of sterile normal saline and tested separately with concentrated anti-human and anticattle sera by the method described by Roy and Ganguli (1943). Out of a total 778 such tests, it was observed that *P. argentipes* and *P. papatassi* which were caught from the bed rooms gave positive reactions with antihuman serum and those from the cowsheds gave positive reactions with anticattle serum. This clearly shows that both these species of sandflies may become anthropophilic or zoophilic according to the circumstances under which they take their blood feed and they have no particular choice of their hosts.

SUMMARY AND CONCLUSION.

1. Control of adult sandflies, *P. argentipes* and *P. papatassi*, with 5 and 2·5 per cent D.D.T. solution in kerosene has been described. The rise and fall in the incidence of sandflies have been carefully studied and the natural breeding places of *P. argentipes* and *P. papatassi* described.

2. The prevalent species of sandflies in Bengal were *P. argentipes*, *P. papatassi* and *P. minutus* and the most suitable temperature and humidity for their breeding were 82 to 90°F. and 75 to 85 per cent respectively.

3. The quantity of spraying solution used per 1,000 square feet surface, i.e. 6·7 gallons, was much higher than the recommended dose of 1 to 2 gallons, due to the dry mud walls soaking a large quantity of the solution during spraying. (Colorimetric test of recovered D.D.T. from the wall surfaces was not done and total quantity of D.D.T. necessary per square foot of the mud walls was not determined.)

4. The results were striking and a single treatment of walls up to 3 feet height could control the incidence of sandflies for 7 to 8 months with 1,500 mg. per square foot and 4 to 5 months with 750 mg. per square foot.

5. There is not much difference in effective residual periods with groups A and C (where both the rooms and the cowsheds were sprayed) and groups B and D (where the cowsheds only were sprayed). Therefore it is possible to control sandflies by spraying the breeding places and their surroundings only, where these can easily be discovered.

6. Re-painting of walls with mud spoiled the residual action of D.D.T. completely.

7. Precipitin tests with stomach meal of engorged sandflies showed that both *P. argentipes* and *P. papatassi* can feed on man as well as cattle and they have no choice of their host.

8. Gammexane L.G. 110 did not compare favourably with D.D.T. when used with doses recommended by the manufacturers. Probably the dose was too small.

ACKNOWLEDGMENTS.

This investigation was started by the author's predecessor Dr. D. N. Roy who retired after few months and left it to be completed by the author.

The author is grateful to the Mission authorities in Kaorapooker who in spite of shortage of space gave a comfortable room with attached kitchen on a small monthly rent where the working base of the investigation was placed and also for the immense helps rendered.

REFERENCES.

HERTIG, M., and FAIRCHILD, G. B. (1948). *Amer. J. Trop. Med.*, **28**, p. 207.

HERTIG, M., and FISHER, R. A. (1945). *Bull. U.S. Army Med. Deptt.*, No. 88, p. 97.

JACOBIEL, F. (1947) . *Bull. Ent. Res.*, **38**, p. 479.

ROY, D. N., and GANGULI, S. K. (1943). *Ind. Med. Gaz.*, **78**, p. 81.

SMITH, R. O. A. (1925) .. *Ind. J. Med. Res.*, **12**, p. 741.

YOUNG, T. C. M., RICHMOND, A. E., and BRENDISH, G. R. (1926). *Ibid.*, **13**, p. 961.

TABLE I.

Monthly collection of sandflies.

Year and month.	Naorapooker		Midday Chak		Thakrani Chak		Ziabargot		Rammakal Chak	
	Living rooms. 26	Cow-sheds. 16	Living rooms. 9	Cow-sheds. 4	Living rooms. 16	Cow-sheds. 3	Living rooms. 10	Cow-sheds. 2	Living rooms. 11	Cow-sheds. 4
1947										
Mar. (from 15th)	300	470	46	270	283	365	548	700	242	365
Apr. ...	359	550	68	180	358	552	756	1,400	350	475
May ...	585	940	171	311	438	708	926	6,748	415	587
Jun. ...	296 Up to June 8, then sprayed.	778	298	687	648	872	1,076	12,159	490	512
Jul. ...	*Nil*	*Nil*	*Nil*	Sprayed	301 Collected up to 8th, then sprayed.	492	1,817	12,778	729	910
Aug. ...	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	7	Sprayed	836	1,912
Sep. ...	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	712	918
Oct. ...	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	507	837
Nov. ...	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	317	426
Dec. ...	*Nil*	*Nil*	*Nil*	*Nil*	6 (16th to 31st).	52	*Nil*	*Nil* (up to 15th)	115	282
1948										
Jan. ...	*Nil*	*Nil*	10 (after 15th)	20	7	33	12 (after 1st week).	17	48	117

TABLE I - *concld.*

Year and month.	Kaorapookee		Midday Char		Thakuran; Char		Zidargot		Rammakal Char	
	Living rooms. 26	Cow-sheds. 16	Living rooms. 9	Cow-sheds. 4	Living rooms. 16	Cow-sheds. 3	Living rooms. 10	Cow-sheds. 2	Living rooms. 11	Cow-sheds 4
1948—*concld.*										
Feb. ...	2 (after 15th)	11	3	7	4	11	22	48	19	52
Mar. ...	20	37	25	48	32	45	65	107	37	81
Apr. ...	Re-sprayed	38	31	56	24	52	36	109	32	67
May ...	*Nil*	56	81	147	13	35	211	295	89	151
June ...	*Nil*	180	149	209	82	209	440	717	135	263
Jul. ...	*Nil*	343	187	351	Re-sprayed	240	398	788	229	411
Aug. ...	*Nil*	561	22	Re-sprayed	*Nil*	455	572	911	287	510
Sep. ...	*Nil*	301	*Nil*	*Nil*	*Nil*	342	41	Re-sprayed	252	438
Oct. ...	*Nil*	222	*Nil*	*Nil*	*Nil*	201	*Nil*	*Nil*	131	257
Nov. ...	*Nil*	127	*Nil*	*Nil*	*Nil*	162	*Nil*	*Nil*	55	141
Dec. ...	*Nil*	59	*Nil*	*Nil*	*Nil*	103	*Nil*	*Nil*	52	111
1949										
Jan. ...	3	36	*Nil*	*Nil*	2	57	*Nil*	*Nil*	33	82
Feb. ...	*Nil*	17	*Nil*	*Nil*	*Nil*	19	12	36	15	[illegible]
Mar. ...	6	42	*Nil*	6	2	132	27	56	43	79

TABLE II.

Village and place of spray.	Average daily catch of sandfly before spray.	Date of spray and strength of solution used.	Re-appearance of sandfly after spray in bed rooms.	Re-appearance of sandfly after spray in the cowsheds.	REMARKS.
Kaorapooker :					
Living rooms (26) ...	5–20	Jan. 8 to 17, 1947, i.e. 9 days with 5 per cent D.D.T.	Middle of Feb. 1948, i.e. 8 months.	.	Two bed rooms re-painted with mud after the spray treatment and sandflies appeared in both the rooms 2 days after.
		Re-sprayed in April. (1,440–1,680 mg. per square foot.)	Jan. 1949	..	
Cowsheds (16) ...	20–40	Do.		End of Jan. 1948, i.e. 7 months.	
Midday Chak :					
Cowsheds (4) ...	12–18	Jul. 2 and 3, 1947, i.e., 2 days with 5 per cent D.D.T.	Middle of Jan. 1948, i.e. 6½ months.	End of Dec 1947, i.e. 6 months.	
		Re-sprayed in Aug. 1948. (1,440–1,680 mg. per square foot.)	...	After 6 months	
Thakurani Chak :					
Living rooms (16) ...	10–20	Jul. 8 to 12, 1947, i.e. 4 days with 2·5 per cent D.D.T.	Middle of Dec. 1947, i.e. 5 months.	.	One bed room re-painted with mud 5 days after the spray.
		Re-sprayed in Jul. 1948. (720–840 mg. per square foot.)	After 5 months.	..	Sandfly appeared the very next day after re-painting.

TABLE II *concld.*

Village and place of spray.	Average daily catch of sandfly before spray.	Date of spray and strength of solution used.	Re-appearance of sandfly after spray in bed rooms.	Re-appearance of sandfly after spray in the cowsheds.	REMARKS.
Thakurani Chak :—*concld.*					
Cowsheds (3) ...	25–40	Do.	...	End of Nov. 1947, i.e. 4½ months.	
Ziadargot :					
Cowsheds (2) ...	100–200	Aug. 13, 1947, i.e. 1 day with 2·5 per cent D.D.T.	Early Jan. 1948, i.e. 4½ months.	Middle of Dec. 1947, i.e. 4 months.	
		Re-sprayed in Aug. (720–840 mg. per square foot.)	...	After 4 months.	
Rammakal Chak :					
Only 1 living room ...	...	Aug. 15, 1948, i.e. 1 day with 5 per cent D.D.T. (1,440–1,680 mg. per square foot.)	None appeared till Feb. 1949.		

Indian Journal of Malariology, 4, 2, June 1950.

ANTIMALARIAL DRUGS.

BY

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[June 16, 1950.]

UNTIL the beginning of World War II, cinchona alkaloids (especially quinine), atebrin and plasmoquin were recommended for the treatment of malaria patients. As a result of the major sources of cinchona supplies being completely cut off to the Allies, extensive researches on various aspects of chemotherapy of malaria were planned and carried out during the war years both in the United Kingdom and America. A number of potential antimalarials have been synthesized, and results achieved with many of them under a variety of trade names, synonyms and special numbers have appeared in the literature. Information on the more important ones with their chemical structures, dosages, their action against different stages of malaria parasites and toxic manifestations, if any, has been crystallized in the form of a chart indicating most of the relevant details for ready reference and is certainly not intended to be a critical review of the literature on the subject. The dosage regimes included in the chart are representative and it is felt that more definite recommendation cannot be made (especially in the case of recent drugs) due to factors like the varying strains of parasites, host resistance and other special circumstances under which the treatment may be administered. Clinical investigations are still being continued and further improvements on the present dosage recommendations are likely to be expected, particularly with those antimalarials which have not yet become available in sufficient quantities.

Most of the antimalarials are quickly absorbed in the system, and while pentaquine, plasmoquin and quinine are only slightly localized in the tissues, proguanil, atebrin and chloroquin show considerable localization in leucocytes, liver, kidney, spleen, etc. A brief mention of the major toxic reactions has been made in the chart. As regards degradation and elimination of a drug *in vivo*, pentaquin, plasmoquin, paludrine and quinine are more rapidly disposed off when compared to atebrin, chloroquin and cam-aqi. The above factors greatly influence the choice of a particular drug and suitable dosage regimes. Drugs which are markedly localized can be used with advantage in the form of a loading or priming dose in the beginning of treatment. In the case of drugs which are not only markedly localized, but also slowly eliminated, so that they are retained in the body for

longer periods, wider spacing of the drug administration and longer period of protection are possible.

A few relatively important drugs have merely been indicated under column 'Remarks' due to their not being in general use and their great similarity both in chemical structure and activity to those included in the chart. Chemical synthesis and biological evaluation of a large number of compounds derived from various new chemical groups have yielded many potent antimalarial drugs which, although very active against avian and simian malarial infections, have either failed to compete with the well-known drugs (those mentioned in the chart) or the biological data concerning them is yet incomplete and inconclusive. A brief list of such chemical groups has been included in the chart as a footnote for general interest. Certain specialized aspects of the problem like absorption, excretion, localization of the drugs and development of drug-resistant strains have been purposely omitted.

It may be stated here that a safe antimalarial drug displaying the properties of a causal prophylactic against all species has not yet been obtained. Now that early development of sporozoite induced infections in the case of *P. cynomolgi* in monkeys and *P. vivax* and *P. falciparum* in man, prior to the appearance of malaria parasites in the peripheral blood, has been described to occur in the parenchyma cells of liver (Shortt *et al.*, 1948*a*, *b*; 1949), search for a more suitable compound or compounds having action against sporozoites and/or the pre-erythrocytic stages and thus preventing the development of blood infections is likely to be pursued with great interest.

The earlier observations by James, Nicol and Shute (1931) have shown that pamaquin, administered during the first six days after the infection, gave complete protection against malignant tertian malaria and prolonged the incubation period in benign tertian infections. The drug, therefore, is a true prophylactic against *falciparum* infection, but in view of the serious toxic manifestations, it was not recommended for general use. The more recent drugs of the 8-aminoquinoline series like pentaquin and isopentaquin are considered to be less toxic than pamaquin, but are still toxic enough to be recommended for prophylaxis without strict medical supervision.

Paludrine, on the other hand, is claimed to be effective against the pre-erythrocytic forms of *P. falciparum*. Fairley (1946) in his series of experiments has shown that paludrine administered 39 to 131 hours after the exposure to infective mosquito bites afforded complete protection against malignant tertian infection. According to him, the drug destroys the pre-erythrocytic forms of *P. falciparum* due to its direct action on them, whereas in *P. vivax* infection, it has got a deleterious inhibitory effect.

Most of the antimalarial drugs like quinine, atebrin, chloroquin, paludrine and cam-aqi (camoquin) act against the asexual forms of the parasites and are mainly used for effecting a clinical cure. Some of them, for instance, atebrin, paludrine and chloroquin, have been used as suppressants. Quinine, though used extensively in the past for this purpose, is required in large daily doses and is usually not well tolerated. On the other hand, recent experiences have proved the utility of atebrin (mepacrine) in 100 mg daily dosage, paludrine in 300 mg. and chloroquin in 250 mg. weekly dosage.

Curative therapy of malaria, especially *P. vivax*, should mean complete elimination of infections by means of such drugs which act against the persisting tissue phases. Drugs of the 8-aminoquinoline series, viz. pamaquin, pentaquin and isopentaquin, when administered along with quinine or paludrine, are capable of controlling a high proportion of relapses, but are not without toxic manifestations in some cases and should only be given under proper medical supervision.

A list of important references which have been referred to in the preparation of the chart is appended.

The author wishes to record his thanks to Dr. G. Robert Coatney for his very helpful suggestions.

REFERENCES.

FAIRLEY, N. HAMILTON (1946) ...	*Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, 2, pp. 105–151.
JAMES, S. P., NICOL, W. D., and SHUTE, P. G. (1931).	*Lancet*, Aug. 15, p. 341.
SHORTT, H. E., FAIRLEY, N. H., COVELL, G., SHUTE, P. G., and GARNHAM, P. C. C. (1949).	Pre-erythrocytic stage of *Plasmodium falciparum*. *Brit. Med. J.*, No. 4635, November 1949, pp. 1006–1008.
SHORTT, H. E., GARNHAM, P. C. C., COVELL, G., and SHUTE, P. G. (1948*a*).	The pre-erythrocytic stage of human malaria *Plasmodium vivax*. *Brit. Med. J.*, March 20, p. 547.
SHORTT, H. E., GARNHAM, P. C. C., and MALMOS, R. (1948*b*).	*Brit. Med. J.*, **1**, p. 192.

BIBLIOGRAPHY.

AFRIDI, M. K. (1927) ...	A critical review of therapeutic trials on paludrine carried out in India during 1946. *Ind. J. Mal.*, **1**, pp. 347–359.
BAMI, H. L., IYER, B. H., and GUHA, P. C. (1947).	Recent advances in the chemistry of antimalarials. *Science & Culture*, **13**, 1, pp. 18–26.
BISPHAM, W. N. (1949) .	*Malaria: Its diagnosis, treatment and prophylaxis.* Williams & Wilkins Co., Baltimore, U.S.A.
BLACK, R. H. (1949) ..	*Trans. Roy. Soc. Trop. Med. Hyg.*, **42**, 6, pp. 565–568.
BULL. HEALTH ORG. LEAGUE OF NATIONS (1937).	Action of quinine and the synthetic drugs on malaria: Fourth Report, **6**, pp. 895–1153.
CHAUDHURI, R. N. (1948) ...	Treatment of malaria. *Ind. Med. Gaz.*, **83**, pp. 225–230.
CHAUDHURI, R. N., RAI CHAUDHURI, M. N., and CHAKRAVARTY, N. K. (1948).	Chloroquin (SN 7618) in malaria. *Ind. J. Mal.*, **2**, pp. 1–12.
COATNEY, G. R., COOPER, W. C., WHITE, W. C., LISTS, H. A., CELWELL, W. B., and EYLISS, D. (1950).	Studies on human malaria. Protective and therapeutic trials of SN 10751 (Camoquin) against the Chesson strain of *P. vivax*. *J. Nat. Mal. Soc.*, **9**, 1, pp. 67–74.
COATNEY, G. R., RUHE, D. S., COOPER, W. C., JOSEPHON, E. S., and YOUNG, M. D. (1949).	Studies on human malaria. Protective and therapeutic action of chloroquin (SN 7618) against Sb. Elizabeth strain of *vivax* malaria. *Amer. J. Hyg.*, **49**, 1, pp. 49–59.

COOPER, W. C. (1949) . Summary of antimalarial drugs. *Pub. Hth. Rep.*, U.S.A., **64**, 237, p. 717.

COVELL, G. (1947) . A brief review of the history and development of the more important antimalarial drugs. *Ind. J. Mal.*, **1**, 2, p. 231.

Idem (1949) . *Brit. Med. J.*, **ii**, Oct. 8, p. 773.

DAVEY, D. G. (1946) . *Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, 2, pp. 171–182.

EDITORIAL (1949) ... Second thoughts on proguanil. *Brit. Med. J.*, **i**, Jan. 15, pp. 106-107.

FAIRLEY, N. HAMILTON (1949) ... *Brit. Med. J.*, No. 4632, Oct. 15, pp. 825–830.

FIELD, J. W. (1938) ... Notes on the chemotherapy of malaria. *Bull. Inst. Med. Res., Kuala Lumpur, Federated Malay States*, 2.

Idem (1949) .. Modern antimalarial drugs. *Med. J. Malaya*, **3**, p. 173.

GOLDSMITH, K. (1946) ... *J. Mal. Inst. Ind.*, **6**, pp. 311–316.

JASWANT SINGH (1949) ... Recent researches on antimalarials. A review of progress. *Ind. J. Mal.*, **3**, pp. 413–419.

JOHNSTONE, R. D. C. (1946) ... *Ann. Trop. Med. Parasit.*, **40**, p. 330.

LEAGUE OF NATIONS (1937) . Action of quinine and the synthetic drugs on malaria. Fourth General Report. *Bull. Hlth. Orgn.*, **6**, pp. 895–1153.

LOEB, R. F. *et al.* (1946) ... Activity of new antimalarial agent chloroquine (SN 7618). *J. Amer. Med. Assocn.*, **130**, pp. 1069-1070.

MACGRAITH, R. G., ADAMS, A. D. R., KING, J. D., JOTTEY, M. M., RIGBY, J. D., and SLAUDEN, R. A. (1946). *Brit. Med. J.*, **i**, p. 903.

MONK, J. F. (1948*a*) ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **41**, 5, pp. 657–662.

Idem (1948*b*) ... *Ibid.*, pp. 663–668.

PATEL, J. C., and MEHTA, J. M. (1948). *Ind. J. Med. Sci.*, **2**, 11, pp. 675–679.

RAY, A. P. (1948) ... Prophylactic use of paludrine in a tea estate. *Ind. J. Mal.*, **2**, pp. 36–66.

RUSSELL, P. F., WEST, L. S., and MANWELL, R. D. (1946). Practical malariology. W. B. Saunders Co., Philadelphia.

SIMEONS, A. T. W., and CHHATRE, K. D. (1947). *Ind. Med. Gaz.*, **87**, 5, pp. 255–257.

SUMMARY MINUTES OF W.H.O. EXPERT COMMITTEE ON MALARIA (1949). Malaria therapeutics, Section II, Geneva, August 1949 (unpublished).

W.H.O. EXPERT COMMITTEE ON MALARIA (1950). Summary of literature reviewed on camoquin. April 17, 1950.

WISELOGLE, F. Y. (1946) ... *A survey of antimalarial drugs*, 1941–45 : 2 vols. J. W. Edwards, Ann Arbor, Michigan, 1946.

Indian Journal of Malariology, 4, 2, June 1950.

TRYPANOSOMES AND PLASMODIAL SPOROZOITES IN THE SALIVARY GLANDS OF A LABORATORY-BRED *C. FATIGANS* WEID., 1828.

BY

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[June 16, 1950.]

INTRODUCTION.

For many years, large numbers of laboratory-bred *Culex fatigans* Weid., 1828, have been fed on wild-caught house-sparrows (*Passer domesticus* Linnaeus) as a routine measure in this Institute so that gut and gland infections are made available for teaching and demonstration purposes. In April 1950, one specimen of *C. fatigans* on dissection showed the presence of flagellated protozoa in the gland preparation, a phenomenon which had not been previously encountered in these parts.

A brief description of the parasites is given in this paper.

RELEVANT HISTORY OF THE HOST.

A female house-sparrow, No. 2 FTC, was received from the market in the first week of April 1950. Its blood was examined on April 8, 1950, and a scanty infection with gametocytes of *P. relictum* was recorded. On two subsequent examinations on April 17 and 24, increase in the number of gametocytes was observed, there being one gametocyte in every third or fourth field.

THE INFECTION.

On the night of April 24/25, 1950, the sparrow was put in a cage with laboratory-bred *C. fatigans* (over a hundred). The following morning, 60 specimens

were found to have fed on the sparrow, which incidentally died on the following day. All the fed mosquitoes were transferred to another cage and kept in the insectary.

Out of this lot, 22 mosquitoes died and another 20 were used for separate experiments. The remaining 18 were dissected and the results are indicated in the Table.

TABLE.

Progress of 60 *C. fatigans fed on sparrow No.* 2 *FTC on April* 24, 1950.

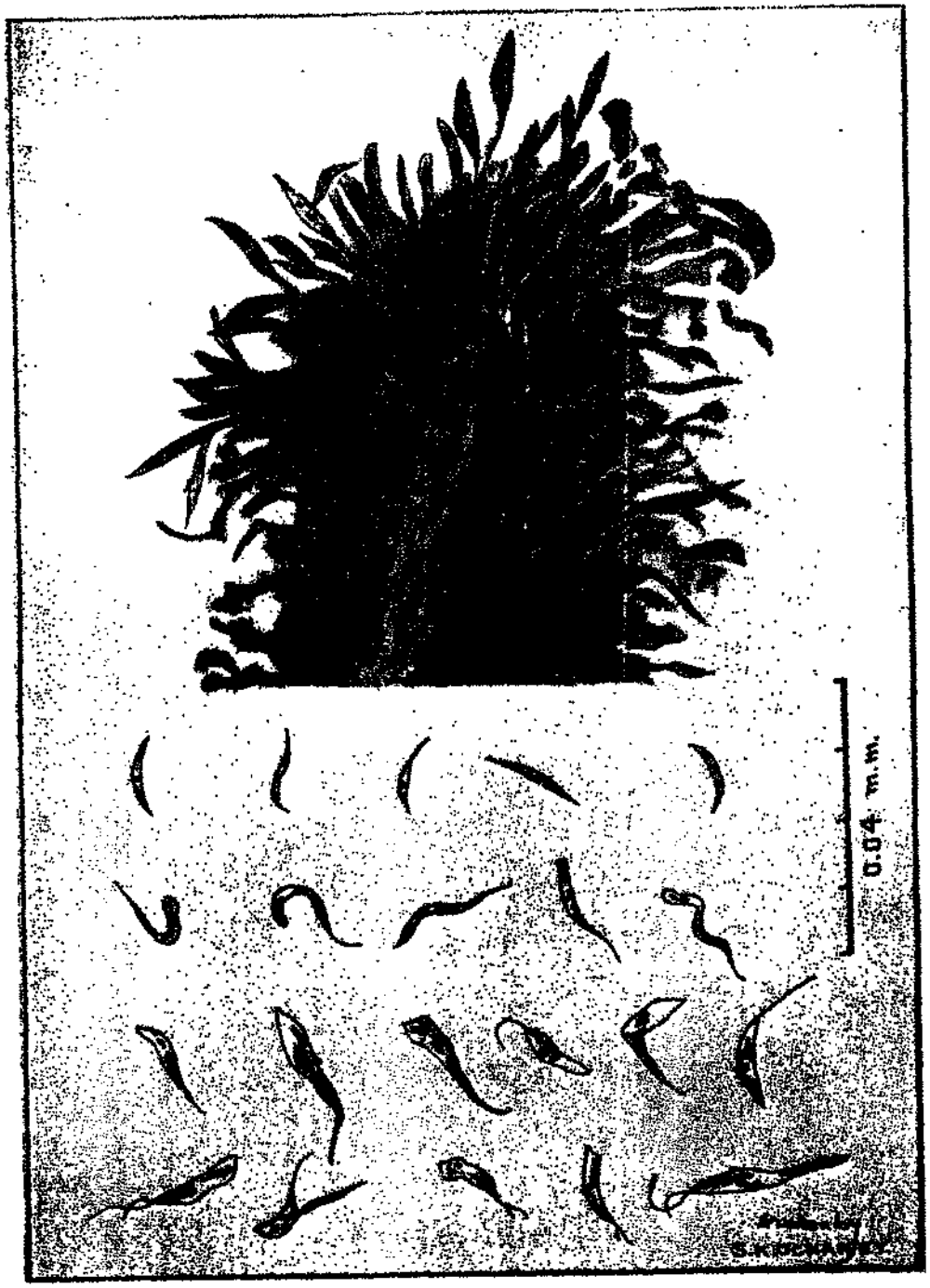
Date.	Number of mosquitoes dissected.	Number with oocysts.	Number with sporozoites.	REMARKS.
1950.				
April 30	2	2	...	Heavy infection.
May 2	7	7	...	" "
6	1	1	1	" "
9	4	...	4	" "
		(A few sporozoites were found in the coelomic fluid.)		(One of the mosquitoes showed the flagellated organisms in the salivary gland.)
10	4	...	4	Heavy infection.
TOTAL	18	10	9	

MORPHOLOGY OF THE FLAGELLATE.

Of the four specimens dissected on April 9, normal sporozoites were visible in and around the salivary glands in all, and in addition in one fresh preparation, large clumps of actively motile organisms were seen both outside and inside the portions of the uncrushed glands. These clumps at first sight looked like masses of agglutinated organisms, still retaining their dynamic activity. A few actively motile flagellated organisms quite unlike the spindle-shaped sporozoites were also seen lying free around the gland preparation. About half the preparation was aspirated into a syringe and was immediately inoculated intracerebrally into a pigeon which is under continuous observation*. The remaining material on the slide was fixed and stained with J.S.B. water soluble stain (Jaswant Singh and Bhattacharji, 1944) in the usual manner.

* Blood smears from the pigeon were examined daily up to June 30 and thereafter once a week up to September 20, 1950. No trypanosomes were seen in any of the preparations. During this period of observation, blood from the pigeon was inoculated into another pigeon and 5 sparrows at different periods, with negative results. *C. fatigans* mosquitoes, fed on the two pigeons as well as the 5 sparrows at different intervals, did not show any trypanosomes.

PLATE VI.



STAINED PREPARATION.

On closer examination of the stained preparation, a large number of trypanosome forms were recognized. Polymorphism was evidenced by the presence of trypanosomal forms of different sizes and shapes. No Leishmania or Crithidial forms could be detected. The polymorphism was exhibited by forms falling under two categories, mainly, (*a*) those which were typical slender-shaped trypanosomes and (*b*) thick long forms (*see* Plate VI). In the former, undulating membrane could be clearly made out, but not in the latter in which only the flagellum was visible. Excepting the plasmodial sporozoites, no other forms without flagella were seen. The average size of the typical trypanosomes was about 9·8 microns (body) and 5 microns (flagellum). The thick long forms showed an average measurement of about 12·9 microns (body) and 7·7 microns (flagellum).

Most of the typical trypanosomes showed the nucleus and the kinetoplast while only in a few of the thick long forms, the two nuclei were clearly visible.

Two clumps of trypanosomes were seen in the preparation and as mentioned already more were seen in the fresh preparation. The staining brought out a uniformly dark smudge at the periphery of which trypanosomes could be seen in large numbers.

Occasionally a few forms which had two flagellæ, presumably the multiplying forms, were also seen.

Sporozoites of *P. relictum* were identified both in the fresh and stained preparations and were typical and uninucleate. They measured on the average about 8 microns in length.

DISCUSSION.

Fortunately the blood slide of sparrow No. 2 FTC, taken on April 24, was preserved and a thorough search of it failed to reveal any trypanosomes or flagellates.

The first obvious factors of any accidental contamination to be examined were the stain, the buffered water and the saline. Such contamination was shown to be unlikely by a thorough check of all the reagents. In all preparations of the 18 mosquitoes dissected, only one showed the flagellates (stain, buffered water and saline used being the same in all cases). As pointed out before, clumps of the flagellates appeared to be inside the salivary gland indicating possible development of this parasite in the insect host. The raisins, from the same stock as were kept on the mosquito cage for their feed during the incubation period, were crushed in saline and examination of the stained preparations gave negative results.

It may seem justifiable to conclude that the infection in the mosquito was derived from sparrow No. 2 FTC. If this conclusion is correct, it is difficult to explain how the trypanosomes were found in the salivary glands of only one of the 18 mosquitoes, particularly in clumped forms.

Viswanathan and Bhatt (1948) have described a similar finding in a specimen of *A. culicifacies* Giles, during their routine dissections of wild-caught mosquitoes, in the course of a malaria survey in Nasik District (Bombay State, India). They discussed the various possibilities and concluded that the trypanosome and the plasmodial sporozoites were of avian origin and suggested the name *Tr. kalwanensis* for the former.

Pleomorphism evidenced by Leshmania and Crithidial forms found by Viswanathan and Bhatt was not observed by the authors.

Mathis (1914) quoted by Wenyon (1926) noted, while dissecting a Culex, an infection of the salivary gland with a flagellate of the Crithidia type and conjectured that these might have been derived from some bird trypanosome. Wenyon came to the conclusion that very little was known regarding transmission of avian trypanosomes, although some evidence of the development of bird trypanosomes in mosquitoes was available.

SUMMARY.

In the course of routine dissection of *Culex fatigans* Weid., 1828, fed on house-sparrow with natural infection of *P. relictum*, one specimen showed trypanosomes as well as sporozoites in its salivary gland.

REFERENCES.

JASWANT SINGH and BHATTACHARJI, L. M. (1944). *Ind. Med. Gaz.*, **79**, 3, pp. 102–104.

VISWANATHAN, D. K., and BHATT, H. R. (1948). *J. Nat. Mal. Soc.*, **7**, 3, pp. 207–211.

WENYON, C. M. (1926) ... *Protozoology*, Vol. 1, Baillière, Tindall & Cox, London.

Indian Journal of Malariology, **4**, 2, June 1950.

ABNORMAL FORMS OF *PLASMODIUM VIVAX* GRASSI AND FELETTI, 1890.

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[June 21, 1950.]

INTRODUCTION.

IT has been the usual practice every year to examine and collect positive blood smears from all suspected cases of malaria from the local hospitals, etc., within the urban area of Delhi. On receipt of a report on December 14, 1948, from a member of the staff of the Institute that his baby, 7 months old, was suffering from fever, blood smears were collected and stained with J.S.B. (Jaswant Singh and Bhattacharji, 1944). During the course of the examination, solid looking malaria parasites of somewhat unusual patterns were seen, some resembling trophozoites and others gametocytes, and surrounded by giant forms of stippled residual blood corpuscles. Numerous such forms in the smear provoked considerable interest, and characteristics of individual parasites were more thoroughly studied.

The baby did not receive any treatment before the blood smears were taken on December 14 and was prescribed 25 mg. of paludrine a day in three divided doses for 10 days. As the child was apparently cured within three days, the parents did not continue the treatment.

The presence of some abnormal forms of parasites was also revealed on the second day, i.e. about 24 hours after the commencement of treatment. On the third day, the smear was negative for malaria parasite and there was no further rise in temperature. The baby was kept under observation, and up to the time of writing this paper no relapse occurred. On two occasions he was reported to be suffering from slight fever but the blood smears on both occasions were negative for malaria parasites.

REPORT ON THE MICROSCOPICAL EXAMINATION OF THE BLOOD SMEARS.

Total parasite count on the first day before drug administration calculated against leucocytes showed 10,100. Differential leucocyte count was polymorphs 24 per cent, lymphocytes 63 per cent, mononuclear 9 per cent and eosinophiles 4 per cent. There was marked anisocytosis and slight poikilocytosis. About 4 per cent of the parasites in the smears collected on the first day resembled typical *P. vivax* and the rest appeared as some aberrant forms of the same species (Plates VII and VIII). A thorough examination of the abnormal parasites in the entire field of the smears collected showed rings, trophozoites, very early schizonts and gametocytes in 5, 34, 1 and 32 per cent respectively. Out of the remaining 24 per cent, 8 per cent of the parasites appeared extracellular and 16 per cent were either growing or sexual forms showing occurrence of giant forms of residual blood corpuscles. Of the free parasites as well as the forms escaping from the red blood corpuscles, 63 per cent resembled gametocytes and 37 per cent trophozoites. Smears collected on the second day also revealed the presence of the same abnormal forms except for the difference that the parasite density was about 50 per cent less and the dividing forms were entirely absent.

DESCRIPTION OF THE ABNORMAL PARASITES.

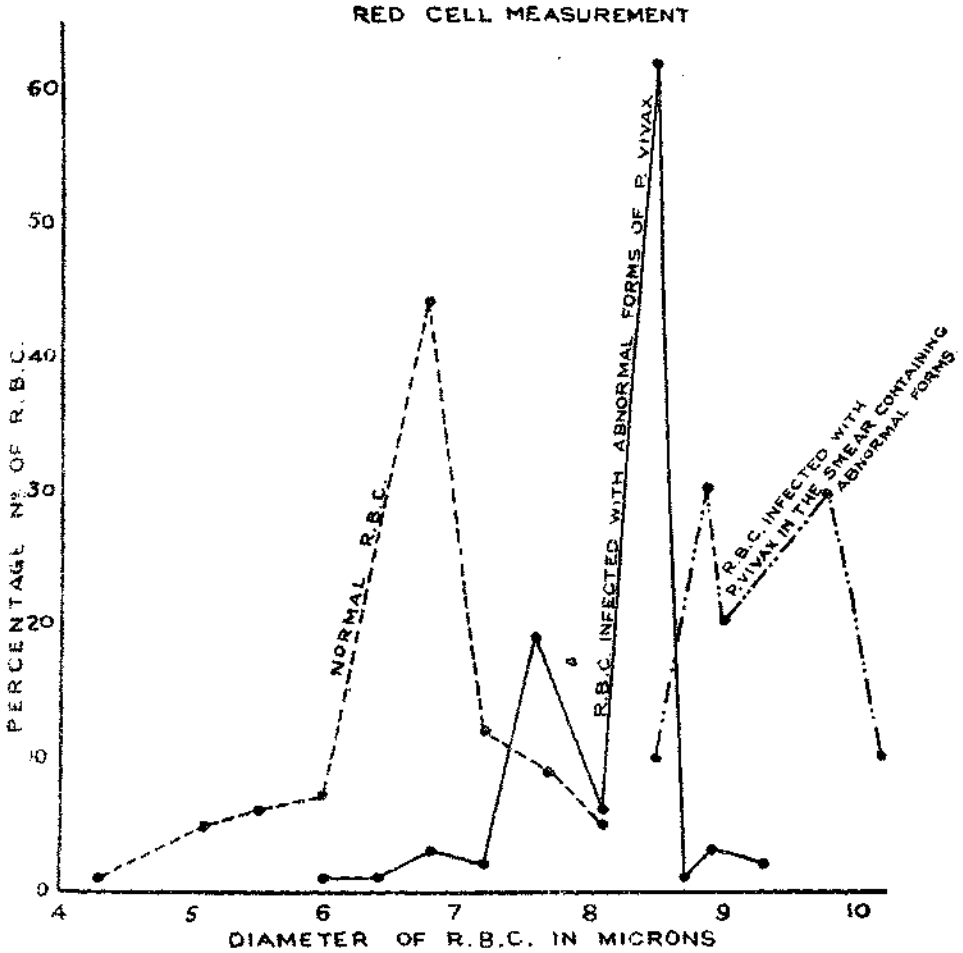
INFECTED CELL.

Size.—The infected corpuscles in all cases were enlarged and were stippled with Schüffner's dots. The enlargement of the cells containing both the young and full-grown trophozoites was however comparatively less than in the case of normal *vivax* infection. Comparative measurements of the R.B.Cs. (red blood corpuscles) infected with parasites which appeared normal and others which appeared as abnormal are given in the Chart along with the measurement of the uninfected R.B.Cs. in the same smear. One hundred cells each of normal and infected with abnormal forms were chosen at random for comparing the sizes, whereas in the case of normal *vivax* infection due to very low number in the smears, the measurement is based on a study of 10 infected cells and converted into the proportionate percentage. The average diameter of the normal cells was $6{\cdot}7 \pm 0{\cdot}734$, of the cells infected with the abnormal forms of *P. vivax* $8{\cdot}231 \pm 0{\cdot}551$, and of those infected with normal *P. vivax* in the same smear $9.28 \pm 0{\cdot}534$. The average diameters of the cells containing normal and abnormal *vivax* parasites divided by the average diameter of the normal red blood cells in the same smear are 1·385 and 1·228 respectively which indicate that the infection with the abnormal *P. vivax* resulted in a comparatively less enlargement of the red cells as compared to the normal *P. vivax* infection.

Shape.—Approximately 9 per cent of the total cells infected with the abnormal forms of the parasite were oval. In another 5 per cent, the cells were oval but they were either pointed at one end or flattened or fimbriated.

Decolorization and stippling.—The decolorization of the red cells was often distinct. Majority of the infected cells were heavily studded with deeply stained and well-defined typical Schüffner's dots.

CHART.



AMŒBOID FORMS.

Parasites of the abnormal forms were as a rule very solid and showed only a slight irregularity in shape. Mostly they were either oval or round in shape, occupying in most of the cases 1/3 to 2/3 of the enlarged cell. The solid nature of the parasite was so marked that, irrespective of the size of the parasite, only in 7 per cent of the total trophozoites, appearance of vacuole was just detectable and not conspicuous.

BAND FORMS.

Equatorial forms were seen in about 4 per cent of the total trophozoites encountered.

DESCRIPTION OF PLATE VII.

1. Gametocyte of normal *P. vivax.*

2 to 21. Abnormal forms of *P. vivax.*

2. Young ring (bird's-eye form) with prominent chromatin dot.

3. Trophozoite illustrating the compact form of the parasite.

4 to 6. Trophozoites showing the intact chromatin lying separate from the cytoplasm of the parasite.

7 and 8. Band forms of the parasite with host cells somewhat flattened on one side.

9 to 14 and 16. Cells infected with abnormal forms of parasites showing different shapes. Some of them are oval: others oval but either pointed or fimbriated at one end.

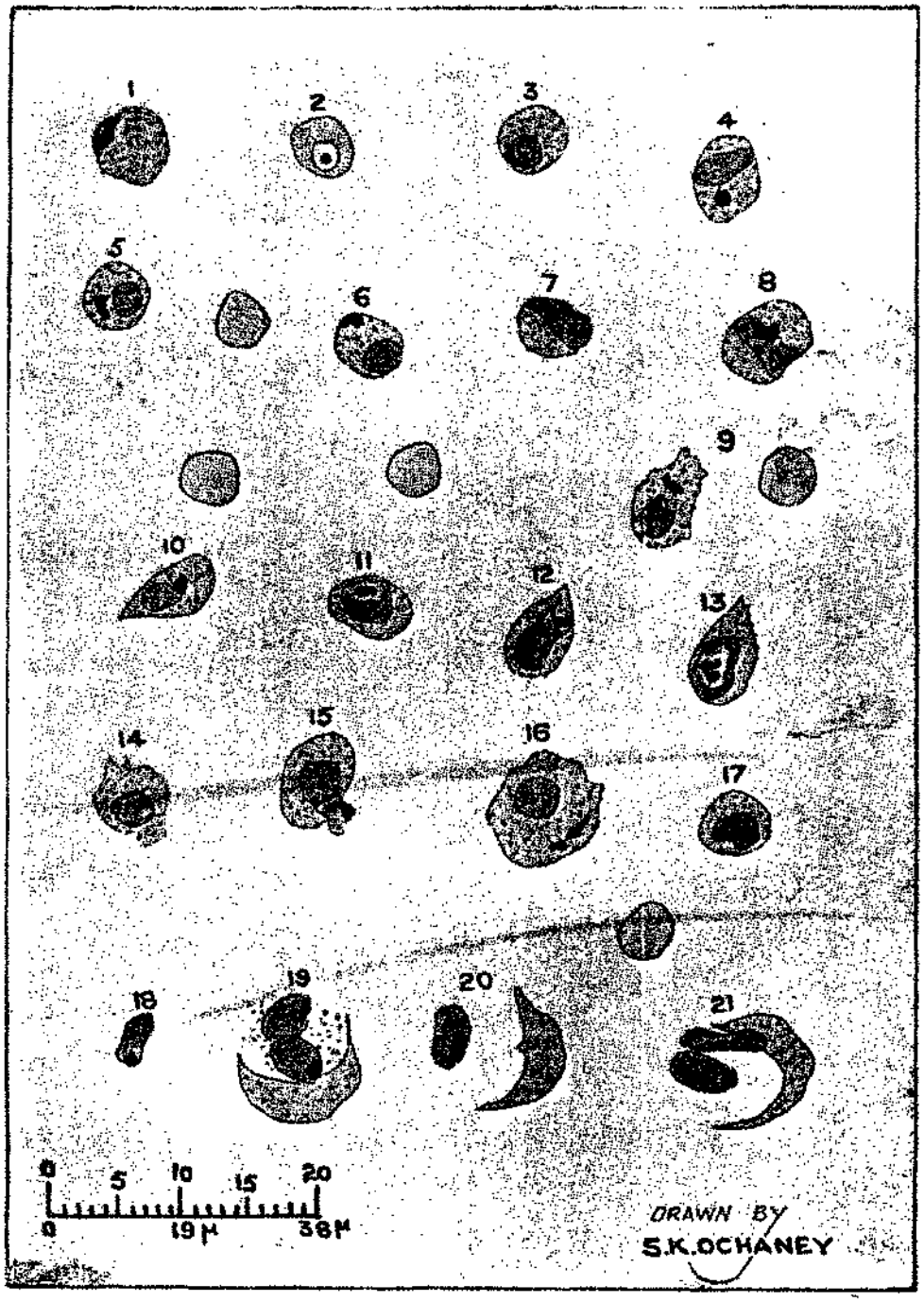
15. A portion of the abnormal parasite lying outside the host cell.

17. Dividing form of the parasite. The chromatin is divided into two and lying well separated.

18. A free parasite lying outside R.B.C.

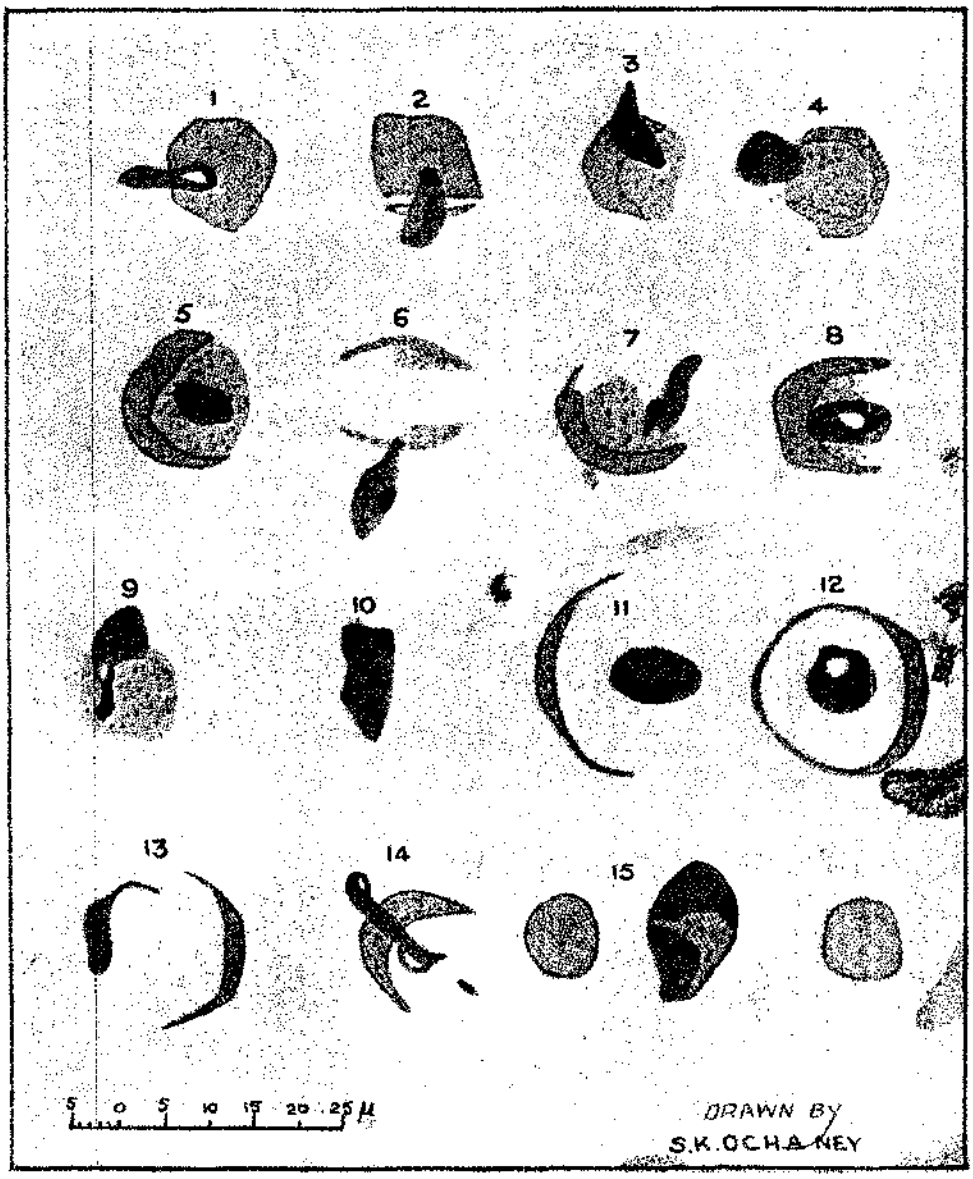
19 to 21. Parasites showing occurrence of giant forms of residual blood corpuscles. 19 and 21 in addition show double infection.

PLATE VII.



Camera lucida drawings of the abnormal forms of *Plasmodium vivax*.

PLATE VIII.



Camera lucida drawings of the abnormal forms of *Plasmodium vivax*.

DESCRIPTION OF PLATE VIII.

1 to 9 and 11 to 14. Abnormal forms of *P. vivax* showing remnants of burst R.B.C. lying in close association with the escaped or escaping trophozoites and gametocytes.

10. Parasite lying outside the R.B.C.

15. Double infection in an intact oval-shaped R.B.C.

DOUBLE INFECTION.

Double infection showing gametocytes was prevalent both among the intact and escaping forms of the parasite and amounted to 4 per cent of the total parasites counted.

CHROMATIN.

At all stages of the parasite, chromatin was abundant and prominent and proportionately greater when compared with the size and stage of the parasite. Another peculiarity was that in about 23 per cent of the trophozoites the intact chromatin was lying separate from the cytoplasm of the parasites.

PIGMENT.

Pigment in the trophozoites and gametocytes was somewhat inconspicuous, dark brown in colour and scattered over the cytoplasm.

DIVIDING FORMS.

These forms were rare. In the very few that were encountered, the parasite was solid looking, occupying 1/2 to 2/3 of the enlarged cell with chromatin divided into two and lying well separated.

GAMETOCYTES.

The distinguishing characters of fully grown up trophozoites and gametocytes were not clear-cut even though the comparative texture of the cytoplasm, the size of the parasite and chromatin, and the features of the pigment were carefully studied for the actual identification between these two stages. They were solid looking with regular shape and occupying about 2/3 to 3/4 of the red blood corpuscles.

DISCUSSION.

Stephens (1922) encountered in East Africa quartan like parasites in a *vivax* like host cell which he named as *P. ovale* due to the frequent oval appearance of the parasite invaded cells. Previous to this, Craig (1900) had described an identical parasite from an American soldier in the Philippines which he then considered as a variety of *P. vivax*. Since Stephens (*loc. cit.*) gave a distinct name for this parasite, Stephens and Owen (1927), Yorke and Owen (1930), James, Nicol and Shute (1932), Muhlens (1934) and de Meillon and Gear (1940) have confirmed the validity of this new species. *P. ovale* is prevalent in many parts of Africa (Blair, 1938; Manson-Bahr and Muggleton, 1937; Tonkin, 1934; Wilson and Wilson, 1935). This species of parasite has also been reported from Egypt (Mageed, 1936); Palestine (Shieber, 1939); U.S.S.R. (Eskin, 1938); and Venezuela (Mendez, 1939). *P. ovale* like parasite has also been described in a patient infected in India by Raman (1940), and in the blood of a native Philippino by Garcia (1941). A Malayan parasite morphologically resembling *P. ovale* contracted in Malaya by an Indian male adult has been reported by Field (1941) where he has not excluded the possibility of the parasite being an abnormal *P. vivax* with peculiar aberration of form produced by prophylactic atebrin. A similar parasite

has also been observed by Wallace (1947) in Kedah. In some respects the abnormal form of the parasites described in this paper resembles *P. ovale* infection but one of the peculiar features of the latter species is the quartan like schizont in enlarged and stippled cells. This phenomenon could not be fully studied in the present case as mature schizonts were absent. Another distinguishing feature of *P. ovale* is the oval shape and ragged edges of the infected red cells. These features though found in the present case, are from morphological considerations alone not enough to label the infection as *P. ovale*.

Roberts (1941) named the 'quartan' type parasites in enlarged decolorized red blood corpuscles which he noticed in patients in North Kenya and Tanganyika territory as *P. wilsoni*. The main features of these parasites according to him were that (1) in parasites that were more than quarter in growth, the enlargement of the cell sometimes amounted to be even greater than is ever seen in *P. vivax*, (2) stippling was frequently absent but when seen it was of the quartan type, (3) small as well as large parasites were compact and very few showed an amœboid appearance, (4) pigments in larger parasites were yellowish black and not aggregated, (5) gametocytes showed pale blue cytoplasm, and (6) parasites were often found lying outside the corpuscles. Field (1942) observed abnormal forms of *P. vivax* in an infant 16 days old in Malaya. The mature schizonts in these cases were compact and occupied only 2/3 of the red cells. The number of merozoites was 8 to 14. The infected cells though heavily stippled were less enlarged than in typical *P. vivax* infections and they exhibited a tendency to assume fimbriated outlines. Wilson (1949) has compared the morphological variations in *P. vivax* in three cases that he observed in the Moshi District, British East Africa, in 1937, with those observed by Field (1942) in Malaya. These parasites as described resembled mostly the forms described by Roberts (1941) except for the multiple invasion of single red cells and the distortion of the host cell in which respect they resembled to some extent the abnormal forms described by Field. Even here the multiple parasites in single cells were practically not vacuolated. Another feature in those abnormal forms was that the chromatin often appeared separate from the cytoplasm. Das Gupta (1939) demonstrated parasites in the peripheral blood of an infant 15 hours after birth, in which especially the schizont stages showed a striking resemblance to *P. ovale*. So it is seen that in some respect or other most of the abnormalities in the morphological appearances of the parasite seen in the child in Delhi have been recorded by other workers namely in Africa and Malaya but description in the present case does not altogether tally with most of the workers.

A striking feature in the morphological variation in the present case that has not so far been recorded is the presence of a large number of forms both trophozoites and gametocytes in the actual process of extrusion from the host cells. The remains of such host cells give an appearance of enormous swelling and of bursting thereafter resulting in characteristic shapes such as small bits of detached membranes, crescentic semilune structures, etc. The residues of these R.B.Cs. enclosing the parasites or remaining in some association or other with them take on pale staining while the stippling remains preserved. The shape of the extruding parasites has been found to be very peculiar and varying in individual cases. According to Nocht and Mayer (1937), if as a result of medicinal action some parasites are destroyed before complete division has taken place or when mature dividing forms

break up, remains of stippled blood corpuscles may persist. In actual practice it is known that crescentic remnants of R.B.C. can be seen sometimes in blood films from cases of *P. vivax* malaria and sometimes from healthy individuals also. But the appearance in large numbers of the remnants of burst R.B.Cs. lying in close association with the escaped trophozoites and gametocytes in untreated cases of human malaria is considered as a very rare phenomenon. It may be argued that such forms might have occurred due to some unknown anomaly in the technique of making the thin smears but it may be mentioned that the technician who prepared the smears from this child is an experienced hand.

The stain used for the staining of the smears from the baby was J.S.B. stain (Jaswant Singh and Bhattacharji, 1944). To rule out the possibility of any particular stain or the staining technique as the cause for the production of the actual abnormality of the parasites or the distortion of the infected cells, three other smears were stained the same day, one in Giemsa stain, and two in J.S.B. stain (one using buffered distilled water and the other using acidulated tap water for washing purposes). On examination all these stained smears showed the same identical morphological appearances of the parasites as obtained in the first instance. One of the smears collected on the first day from the same child and preserved in an unstained condition in the laboratory according to the technique followed in the Institute (Jaswant Singh, Ray and Nair, 1949) was stained in May 1950 and the same also showed all the features recorded on the first day. These simple procedures are considered enough to clear the doubt that the abnormalities of the parasites might have been brought about by an anomaly in the preparation of the smear or during staining.

It is seen that Roberts (1941) found in *P. vivax* infections parasites often lying outside the red blood corpuscles. In the case of *P. berghei*, maintained in white mice, such extra corpuscular situation of the parasites is a common phenomenon. In this respect the morphological features of the parasites described here resemble in a lesser degree to *P. berghei* in white mice. Perhaps the infected cells in these cases become fragile for some reason or other which ultimately results in the extra corpuscular displacement of the parasites.

Field (1948) reports the occurrence of compact forms of *P. vivax* in infections contracted during the war by persons in the civilian internment and prisoners of war camps in Singapore. Such aberrations of the parasites, he believes, are due to the undernourishment of the host with marked specific deficiencies in riboflavin, nicotinic acid and other members of B_2 complex which in turn result in the specific starvation of the parasites. A similar phenomenon perhaps may explain to some extent the cause for the abnormalities observed in the present case as this child was also ill-nourished and anæmic. In such a case it is still a matter of speculation why 3 to 4 per cent of the parasites did not undergo any aberration in the present case. In epidemic and hyperendemic regions, especially among the rural population, many deficiency diseases occur especially of B_2 complex and in them the *P. vivax* infection has not so far been reported as having shown a morphological variation. This perhaps may be due to the fact that detailed studies have not been made so far on this problem. There appears to be a need for elaborate studies on the morphology of parasites in relation to vitamin and mineral deficiencies of the host.

Young and Eyles (1949) have described parasites which resembled in shape and on the reduced number of merozoites per segmenter to *P. ovale* in three strains of *P. vivax*. The segmenters with the small number of merozoites in these cases were found usually about the time of maximum parasitemia which indicated a transient host parasite relationship rather than a permanent one. Moreover, these parasites appeared only for short periods of time in any one patient and were demonstrated only infrequently in patients receiving malaria in serial transfers. This finding undoubtedly has a great significance, but unfortunately the host parasite relationship theory cannot be advantageously applied to the present case as details are lacking regarding the source and course of infection.

Mackerras and Ercole (1947) state that *P. vivax* trophozoites in the presence of paludrine grow at normal rate without the amœboid activity being lessened. But at full size the pigment appears as larger particles than in the case of normal parasites of similar age and arranges itself peripherally. In the dividing forms, the chromatin breaks up and swells into irregular masses due to the lethal action of the drug. Some doubt might arise whether the parasites described in this paper resemble the paludrine forms due to the absence of fully grown schizonts in the smears but the facts that this parasite has many features of its own and some of the other abnormalities peculiar to paludrine forms have not been seen in the present case, and that there was no history of any antimalarials being given to the child before the smears were taken, rule out the possibility of confusing the present one with the paludrine forms of the parasites.

SUMMARY AND CONCLUSIONS.

Abnormal forms of *P. vivax* parasites amounting to 96 per cent of the total parasite and the rest of typical *vivax* morphology were seen in the blood smears of a child of 7 months old in Delhi.

Chief features of these abnormal forms were (1) the presence of some oval and fimbriated infected cells, (2) solid looking small parasites in enlarged stippled cells, (3) extracellular appearance of parasites, and (4) occurrence of 'giant forms' of residual blood corpuscles enclosing partially or completely the trophozoites and gametocytes.

The various possibilities for the occurrence of these abnormal forms are discussed.

REFERENCES.

Blair, D. M. (1938) .. Infection with *Plasmodium ovale* Stephens in Southern Rhodesia. *Trans. Roy. Soc. Trop. Med. Hyg.*, **32**, p. 229.

Craig, C. F. (1900) .. As quoted by Garcia, E. Y. (1941). *Acta Medica Philippina*, **2**, p. 341.

Das Gupta, B. M. (1939) .. Malaria infection in the placenta and transmission to the fœtus. *Ind. Med. Gaz.*, **74**, p. 397.

Eskin, V. A. (1938) .. A case of natural infection with *ovale* in man from the U.S.S.R. *Trop. Dis. Bull.*, **35**, p. 814.

Field, J. W. (1941) .. A Malayan parasite morphologically resembling *Plasmodium ovale* Stephens. *Bull. Inst. Med. Res.* No. 4, Govt. Press, Kuala Lumpur.

FIELD, J. W. (1942) ...	Morphological variation on *Plasmodium vivax* Grassi and Feletti, 1890. *Parasit.*, **34**, p. 82.
Idem (1948) ...	The microscopic diagnosis of human malaria, Part I. *Studies from the Institute for Medical Research Federation of Malaya*, **23**, Govt. Press, Kuala Lumpur.
GARCIA, E. Y. (1941) ...	A *Plasmodium ovale* like parasite in the blood of a native Philippino. *Acta Medica Philippina*, **2**, p. 341.
JAMES, S. P., NICOL, M. D., and SHUTE, P. G. (1932).	*Plasmodium ovale* Stephens: Passage of the parasite through mosquito and successful transmission of their bites. *Ann. Trop. Med. Parasit.*, **26**, p. 139.
JASWANT SINGH and BHATTACHARJI, L. M. (1944).	Rapid staining of malaria parasites by a water soluble stain. *Ind. Med. Gaz.*, **79**, p. 102.
JASWANT SINGH, RAY, A. P., and NAIR, C. P. (1949).	A preliminary report on the preservation of unstained blood smears. *Ind. J. Mal.*, **3**, p. 327.
MACKERRAS, M. J., and ERCOLE, Q. N. (1947).	Observations on the action of paludrine on malarial parasites. *Trans. Roy. Soc. Trop. Med. Hyg.*, **41**, p. 365.
MAGEED, A. A. A. (1936) ...	A case of *P. ovale* in Egypt. *J. Egypt. Med. Ass.*, **19**, p. 229.
MANSON-BAHR, P. H., and MUGGLETON, W. J. (1937).	*Plasmodium ovale* infection contracted in different parts of West and Central Africa. *Brit. Med. J.*, **i**, p. 217.
DE MEILLON, B. D., and GEAR, J. (1940).	*Plasmodium ovale* infection acquired during a short visit to a malarious country. *Trans. Roy. Soc. Trop. Med. Hyg.*, **33**, p. 597.
MENDEZ, C. H. (1939) ...	*Plasmodium ovale* relation del primer case observado en Venezuela. *Publicaciones de la Div de Malariologia*, **3**, Sep. 15, 1939. Caracas, Venezuela.
MUHLENS, P. (1934) ...	*Plasmodium ovale* Stephens. *Arch. f. Schiffs-u. Trop. Hyg.*, **32**, p. 367.
NOCHT, B., and MAYER, M. (1937)	*Malaria*. John Bale Medical Publications, London.
RAMAN, T. K. (1940) ...	*Plasmodium ovale* in India. *J. Ind. Med. Ass.*, **12**, p. 583.
ROBERTS, J. I. (1941) ...	A new malaria parasite of man in East Africa. *East African Med. J.*, **17**, p. 212.
SHIEBER, C. (1939) ...	The presence of *P. ovale* in Palestine. *Trop. Dis. Bull.*, **36**, p. 809.
STEPHENS, J. W. W. (1922) ...	A new malaria parasite of man. *Ann. Trop. Med. Parasit.*, **16**, p. 383.
STEPHENS, J. W. W., and OWEN, D. U. (1927).	*Plasmodium ovale*. *Ibid.*, **21**, p. 293.
TONKIN, H. D. (1934) ...	A case of *Plasmodium ovale* in an East African native. *East African Med. J.*, **11**, p. 166.
WALLACE, R. B. (1947) ...	Personal communication as reported by Field, J. W. (1948).
WILSON, D. B., and WILSON, M. E. (1935).	Infection with *P. ovale* Stephens in Tanganyika territory. *Trans. Roy. Soc. Trop. Med. Hyg.*, **28**, p. 469.
WILSON, M. (1949) ...	Abnormal forms of *Plasmodium vivax*. *Ibid.*, **43**, p. 97.
YORKE, W., and OWEN, D. U. (1930).	*Plasmodium ovale*. *Ann. Trop. Med. Parasit.*, **24**, p. 593.
YOUNG, M. D., and EYLES, D. E. (1949).	Parasite resembling *P. ovale* in strains of *P. vivax*. *J. Nat. Mal. Soc.*, **8**, p. 219.

Indian Journal of Malariology, **4**, 2, June 1950.

STUDIES ON WELTMANN COAGULATION REACTION IN SIMIAN MALARIA.

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INTRODUCTION.

WELTMANN (1930) and Weltmann and Medvei (1933) described that when one part of normal human blood serum was diluted with 50 parts of distilled water containing small amounts of an electrolyte, such as calcium chloride, magnesium chloride and barium chloride, coagulation of the serum protein occurs on boiling (Weltmann coagulation reaction). Accordingly, if 0·1 c.c. of normal human serum is added to 5 c.c. of 0·04 per cent calcium chloride solution and boiled, the protein coagulates. In exudative and inflammatory processes, protein coagulation occurs when higher percentage of calcium chloride than the normal 0·04 per cent is added to the serum, while in fibrotic conditions reverse is the case. Weltmann indicated the use of this test for distinguishing acute inflammatory processes from toxic liver damage, hæmolysis, and chronic proliferative processes especially liver cirrhosis.

The opinion regarding the mechanism of Weltmann reaction is not at present unanimous. Dees (1940, 1943) and Dees and Morton (1942) consider that serum lipoids are an important factor in the coagulation reaction. But Scherles and Levy (1942) show that the reaction is closely related to changes in the percentage of alpha globulin in blood. Various other theories are also put forward to explain the mechanism of the coagulation reaction such as the alteration in the (1) pH of

the calcium chloride solution employed, (2) pH of the serum, (3) serum calcium concentration, (4) total blood globulin, (5) the albumin globulin ratio, (6) the blood fibrinogen, or (7) the combination of any of these factors.

The reaction was studied in malaria cases first by Tschilow (1931), Landeiro (1935), and Levinson and McFate (1943). According to Kleeberg (1945), coagulation occurs nearly always in higher concentrations of calcium chloride in acute inflammations with high fever but in malaria attack, though clinically features of an acute inflammatory process are produced, the flocculation is generally obtained in comparatively lower concentrations which he considered to be due to hæmolysis usually associated with this disease. As a result of this, he suggested that this test might prove to be of special value in blackwater fever and like the sedimentation rate be of use in prognosis. For this purpose, the tests have to be repeated in the course of the disease. Kleeberg (1948) basing the test on alterations in certain serum globulins and taking into consideration the fact that the production of globulin takes place mainly in the liver, interprets prolonged coagulation band with Weltmann coagulation reaction (W.C.R.) as indicative of liver damage. As acute malaria infection under unfavourable conditions such as under-nourishment, hard labour, chronic intestinal diseases or liver damage may exert a heavy strain on the liver, he advocates a special protective liver treatment besides efficient specific chemotherapy in such patients. He observed that though all the patients showing coagulation of calcium chloride at lower concentrations were not anæmic, all anæmic malarial patients whose blood is remarkably sensitive to lysis in bile solution, showed the special feature of prolonged coagulation band, i.e. flocculation with lower concentration of calcium chloride solution.

As malaria infection in monkeys offers an excellent parallel to human malaria and affords a precise follow-up of the course of infection, studies on Weltmann coagulation reaction in *S. rhesus* monkeys of Northern India were undertaken in these laboratories on the same lines as advocated by Kleeberg and the results obtained have been presented in this paper. The observations made were mainly in respect of the reaction (1) in normal monkeys, (2) in monkeys infected with different species of plasmodia, (3) at different periods of the infection such as at the acute stage of infection, during relapse or during latency, (4) after successful treatment or spontaneous cure, and (5) in relation to body temperatures, R.B.C. counts and hæmoglobin percentage.

PROCEDURE.

Ten test tubes (13×100 mm.) are placed in a rack and numbered 1 to 10 from left to right. Measured quantities of 0·1 per cent calcium chloride ($CaCl_2 \cdot 6H_2O$) are added into each tube starting with 5 c.c. in the first tube and decreasing by 0·5 c.c. in each tube in the serial order, so that tube 10 contains only 0·5 c.c. Sufficient quantities of distilled water are added to tubes 2 to 10 to bring the total volume in each tube to 5 c.c. These result in a concentration of calcium chloride ranging from 0·1 per cent in tube 1 down to 0·01 per cent in tube 10. A constant quantity of 0·1 c.c. of unhæmolysed serum obtained from fasting monkeys is added to each tube. The mixtures are shaken and the tubes in the rack kept in a boiling water-bath for 15 minutes. The tubes are removed and readings taken immediately afterwards. The contents of the tubes appear clear, faintly opalescent, opalescent,

turbid or flocculent. The number of the tubes containing the most dilute solution of calcium chloride in which flocculation (coagulation) has occurred gives the reading for the test. If coagulation occurs in tube 1 through 5, the coagulation band is 5 (CB 5 or Weltmann 5).

One hundred and thirty-nine tests were performed in 100 *S. rhesus* monkeys with a view to study the Weltmann reaction in relation to malarial infections. The observations aimed at included the nature of the coagulation band in infected conditions. The infection was produced by inoculating blood from donor monkeys suffering from either *P. cynomolgi*, *P. knowlesi* or *P. inui*. The reactions obtained with the sera of the animals at the time of acute parasitæmia, during latency or chronic infection and after cure (spontaneous or after the administration of drugs), were separately recorded.

Simultaneously, R.B.C. (red blood corpuscles) counts, hæmoglobin percentage and rectal temperature were also recorded in many of the cases with a view to determine whether these factors bear any relation to the W.C.R. (Weltmann coagulation reaction).

RESULTS.

According to Weltmann (1930), Dees and Morton (1942) and Levinson and Klein (1939), the coagulation band in normal human beings is 6 or 7. But Siegel and Kraemer (1948) found the figure to vary from 5 to 7. In 43 normal monkeys, the range was found to commence from a still lower figure of 4 (Table I). The different percentages of the coagulation bands obtained in the different tests are also given in Table I in the various groups, such as those showing coagulation band from 1 through 3 ; 1 through 4 ; 1 through 4 to 7 ; 1 through 5 to 7 and 1 through 8 to 10 in the tubes. The coagulation band in normal monkeys amounted to 74 per cent in the 4 to 7 group out of which 36 per cent occurred in tubes up to 4 alone. 12 per cent of the reaction fell in the group above 7. In no case the coagulation band in these series extended beyond tube 8. In the tests carried out in 56 malarial infections, 82 per cent of the reactions occurred with 5 to 7 groups as against 44 per cent obtained in this group with serum from normal monkeys. The reaction in tubes beyond 7 occurred only in 9 per cent of the cases in the high infection series and in all these the coagulation band was 8 except in one isolated case in which a figure of 9 was obtained with *P. knowlesi* infection. Coagulation band ranging from 1 through 5 to 8 amounted to 88 per cent with *P. knowlesi* infection whereas the same with *P. cynomolgi* and *P. inui* amounted to 76 and 82 per cent respectively. There was therefore no significant difference in the reaction obtained in infections with the three different species.

W.C.R. in 26 monkeys during the stage of latent infection after the initial attack was studied and the results indicated no change in the reaction as from the results seen in the case of acute infections. The test was also applied to 14 monkeys after they were cured of the infection either spontaneously or with the aid of drugs. 57 per cent of the coagulation band in this series fell in the group 1 to 3 and 64 per cent in 1 to 4 group. It is to be remembered in this connection that these animals were in captivity for approximately 6 to 18 months before the actual tests.

In Table II, W.C.R. results are presented in relation to data such as hæmoglobin percentage, R.B.C. count and rectal temperatures recorded at the time of W.C.R. determination. Analysis of the table shows that the maximum

number of reaction comprising coagulation band amounting to 75 to 96 per cent fell in all cases in the groups 4 to 7 and 5 to 7 and as such no correlation seems to exist between W.C.R. and these factors.

TABLE I.

Results of W.C.R. in normal and infected monkeys.

Particulars of the monkey.	Total number in the series.	Percentage coagulation band in different groups.				
		1 through 3.	1 through 4.	1 through 4 to 7.	1 through 5 to 7.	1 through 7 to 10.
Normal	43	14	44	74	44	12
Acute malaria infection (acute parasitæmia all species).	56	4	9	87	82	9
Acute infection (*P. cynomolgi*)	17	0	12	88	76	12
Acute infection (*P. knowlesi*)	16	6	6	88	88	6
Acute infection (*P. inui*) ...	23	4	9	81	82	9
Latent infection ...	26	15	19	81	77	4
After cure	14	57	64	36	29	7

TABLE II.

Particulars of W.C.R. in relation to blood count, haemoglobin percentage and rectal temperature.

Particulars of the monkey used for the test.	Total number in the series.	Coagulation in groups (percentage).			
		1 through 3.	1 through 4 to 7.	1 through 5 to 7.	1 through 8 to 10.
Haemoglobin 40 per cent or below.	21	5	95	86	...
Haemoglobin above 40 per cent.	85	1	94	82	5
R.B.C. less than 4 million ...	14	7	93	79	...
R.B.C. above 4 million ...	45	2	96	26	2
Rectal temperature below 103°F.	52	4	88	75	8
Rectal temperature above 103°F.	65	3	95	86	2

DISCUSSION.

According to the findings of previous workers, the W.C.R. in normal human sera shows flocculation up to tube 6 or 7 (0·05 to 0·04 per cent calcium chloride solution) and in persons suffering from malaria, according to Kleeberg (1945) the W.C.R. either remains normal or flocculation is obtained up to tube 8 or 9 (0·03 to 0·02 per cent calcium chloride solution).

In the present investigations on monkeys, the normal serum shows flocculation up to tube 8. So does the serum obtained from the infected monkeys. But the percentage of infected monkeys showing flocculation up to tubes 5, 6 and 7 is definitely higher than in the case of normal monkeys. Another feature observed is that flocculation occurred in greater percentage in tube 1 through 4 in the normal cases as compared to the infected ones.

It is generally confirmed that a higher percentage of infected monkeys shows flocculation at lower concentrations of calcium chloride but this by itself is not very helpful in the diagnosis of malaria in individual cases as even with normal monkeys such reactions are not uncommon. It may also be mentioned that the reaction with simian sera for differentiating normal from infected cases (malaria) is not as sensitive as obtained with human sera by Kleeberg (1945) because in this experiment no infected monkeys except one on *P. knowlesi* series gave flocculation in a concentration of calcium chloride lower than 0·03 per cent.

With normal human sera, coagulation occurs in the first 5 to 7 tubes, but when the coagulation band is less than 5, it is said that shift to the left has occurred, and when it is more than 7 a shift to the right is described. In the tests with monkey sera, the normal coagulation band occurred in 1 through 4 to 8 tubes, so the shift to the left in simian experiments at least should be considered only when the coagulation band is less than 4.

In the case of human beings, the low reaction is generally considered as indicative of the presence of some exudative or inflammatory disease. In the tests in monkeys completely cured from the disease, it was noticed that 57 per cent of these showed a low Weltmann reaction. In the case of monkeys disease *entities* are not fully understood and it should be admitted that a large percentage of these animals that showed the low Weltmann, died within a few days to a few weeks after the observation, and post-mortem examination revealed pathological lesions similar to consolidation of lungs, miliary tuberculosis, inflammatory conditions of gall bladder, gastro-intestinal tract, etc. Even though the number of monkeys in which corroboration of low Weltmann and pathological lesion indicative of exudative or inflammatory disease has been made is small, it appears safe to conclude that all monkeys showing a low Weltmann reaction suffer from some sort of acute illness.

For any of the laboratory experiments with monkeys or other animals, only healthy ones are used. This selection is generally made by noting the appearance, weight, activity, and appetite of the animals but these observations alone do not reveal the presence of any acute disease until the ailment is much advanced. But if Weltmann test can be performed in such animals, the disease indication may be revealed in a great percentage of cases sufficiently in advance and as such the test appears to have some value in the routine selection of healthy monkeys for any of the laboratory experiments.

In human beings it is reported to be possible to differentiate by Weltmann test malarial fever from other acute fevers caused by exudative or inflammatory lesion, but in monkeys, as the rectal temperature is not of any real value in differentiating normal from sick monkeys and the Weltmann coagulation band is more or less the same in normal and malarial conditions in these animals, the utility of this reaction appears to be somewhat limited.

One of the monkeys in cured series showed highest reaction of CB 10. This probably was a false positive reaction. In the normal and infected groups, 14 and 4 per cent respectively gave a low flocculation reaction in tube 1 through 3. Though these can also be false positive reactions, the question of these particular animals suffering from exudative and inflammatory conditions cannot be ruled out. The finding that a greater percentage of cured monkeys showed a very low reaction, reveals that these animals under captivity extending over several weeks to months, contact various intercurrent diseases and require a great deal of care if they are to be utilized for experimental purposes.

SUMMARY.

Weltmann coagulation reaction was performed with the sera of monkeys infected with malaria parasites as well as with those of normal ones. Normal monkeys sera gave rise to a greater percentage of W.C.R. at a concentration of 0·03 to 0·07 per cent calcium chloride whereas infected sera showed a greater percentage of positive reaction with 0·03 to 0·06 per cent. Thus a greater percentage of simian malaria cases gave W.C.R. at a lower concentration but no clear-cut line of demarcation was obtained in the case of normal monkeys corresponding to that found in healthy human beings.

The disease entities of acute infections in monkeys are not well understood, but indirect evidence shows that greater percentage of acute disease due to exudative and inflammatory conditions give W.C.R. positive at higher concentration (0·1 to 0·08 per cent of calcium chloride solution).

No correlation between W.C.R. and anaemia has been established.

REFERENCES.

DEES, S. C. (1940) ... *J. Pediat.*, **17**, p. 53.
Idem (1943) ... ... *J. Allergy*, **14**, p. 459.
DEES, S. C., and MORTON, H. (1942). *J. Pediat.*, **21**, p. 544.
KLEEBERG, J. (1945) ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **39**, p. 221.
Idem (1948) ... .. *Ibid.*, **41**, p. 555.
LANDEIRO, F. (1935) .. *Lisboa Med.*, **12**, p. 771.
LEVINSON, S. A., and KLEIN, Z. I. (1930). *Ann. Int. Med.*, **12**, p. 1948.
LEVINSON, S. A., and MCFATE, R. P. (1943). *Clinical laboratory diagnosis.* Henry Kimpton, London.
SCHERLES, A., and LEVY, D. S. (1942). *Bull. Johns Hopkins Hosp.*, **71**, p. 24.
SIEGEL, L. H., and KRAEMER, M. D. (1948). *J. Lab. Clin. Med.*, **33**, p. 618.
TSCHILOW, K. (1931) ... *Wien Klin. Wschr.*, **44**, p. 1164.
WELTMANN, O. (1930) ... *Med. Klin.*, **26**, p. 240.
WELTMANN, O., and MEDVEI, C. V. (1933). *Wien. Klin. Wschr.*, **46**, p. 1910.

Indian Journal of Malariology, **4**, 2, June 1950.

CAMOQUIN AND CHLOROQUINE IN THE TREATMENT OF MALARIA.*

BY

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November 19, 1949.]

An account of three years' treatment of malaria cases occurring in tea estates with comparative results of treatment with camoquin, chloroquine and other well-known remedies has been given in this paper.

In view of available literature giving numerous references to papers published on the action of camoquin (Simeons and Chhatre, 1947; Chaudhuri and Chakravarty, 1948) and of chloroquine (Chaudhuri, Rai Chaudhuri and Chakravarty, 1948; Berberian and Dennis, 1948; Smith, Dy., and Cabrera, 1948; Fairley, 1946; Report of the Expert Committee on Malaria, World Health Organization, 1948), a further report at this stage may seem superfluous but the writer wishes to stress that the purport of this article is threefold—first, to give an account of the treatment of 'ambulant patients' attending dispensaries as against hospitalized patients in a malarious district in East Bengal; secondly, to elicit 'relapse rates' after short treatments with camoquin and chloroquine as compared with the other well-known remedies; and thirdly, to assess as far as possible economical aspects and the comparative costs of the different treatments used.

Treatment of ambulatory patients attending the dispensaries is much the same problem as one confronts in treating patients in villages. In each case the main objects are :—

(*a*) Rapid clinical cure.

(*b*) Avoidance of recurrences and relapses.

(*c*) Determining lowest cost per treatment, as evidenced by minimizing sick day attendances and further expenditure on subsequent medical treatment.

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* Camoquin—CAM-AQI—is a quinoline derivative with a formula 4 (3'-diethylaminomethyl-4'-hydroxyanilino)-7-chloroquinoline-di-hydrochloride dihydrate.

Chloroquine—SN 7618; resochin; aralen—is 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline.

In a tea estate population, the relapse rate can be recorded with a fair amount of accuracy, whereas in scattered villages, such a record must be well-nigh impossible to obtain.

The assistant medical officers of the tea estates in the Juri and Lungla valleys were supplied with particular antimalarial drugs with definite instructions. The population of these two valleys is partially immune. One assistant medical officer was given chloroquine tablets and instructed to treat all malaria cases with it. The assistant medical officers of the three other estates were given supplies of camoquin with similar instructions. In the remaining tea estates, the assistant medical officers were required to treat a definite number of malaria patients by administration of drugs which they already possessed and in the usual doses. Instructions to all the doctors were :—

(*a*) to include only the microscopically diagnosed malaria cases in their investigation record ;

(*b*) to keep a careful note of the action of the drug on temperature, disappearance of malaria parasites from the peripheral blood, any toxic symptoms or complications, and to record the number of sick-day attendances in each case ; and

(*c*) to record all relapses or reinfections (microscopically diagnosed) during the entire period of investigation—from January 1 to October 31, 1949—and to follow up the cases for six months or over from the date of administration of drugs.

Tabulated results of paludrine treatment investigation during 1946 with the record of short 'follow-ups' of 50 days (Appendix I), chloroquine series in 1947 (Table V) and a camoquin series (Appendix II) have also been included.

DISCUSSION.

In recording results of treatment of malaria cases, the following factors were constantly borne in mind :—

(*a*) Whether the patients were drawn from an immune, partially immune or non-immune community ;

(*b*) Whether infection and the treatment took place in the same locality ; and

(*c*) The general health of the community from which the patients were drawn.

Further, in estimating the final value of any malarial treatment, especially so, those treatments by drugs now tried over a period of many years, consideration must be given to :—

(*a*) the effects of other chronic diseases already present in subjects suffering from malaria ;

(*b*) the likelihood of acquired resistance to the drug ; and

(*c*) action of the drug in mixed infections.

An account of the present series of cases gives in part an answer to these points and the author feels that much better results would have been obtained had these cases not been so heavily infected by other diseases at the same time ; in this

district practically 100 per cent of the labour population suffer from hookworm infections, a great many from other helminthic infections. Vitamin deficiency is also marked, especially of vitamins A and B. Intercurrent infection amongst paludrine prophylactic subjects is discussed at length by Jeliffe (1949) who considers that the dosage of prophylactic drugs should be increased if patients suffer from intercurrent diseases as well.

Without doubt the question of intercurrent disease is a most important one and often not taken into consideration when questioning the efficacy of antimalarial drugs. Since the war, literature abounds with results of treatment of tropical diseases contracted during the war in the East or Far East, but often these treatments are carried out in the U.S.A. or U.K. and under conditions totally different from those prevailing in the area of infection. It seems fallacious to follow the record of these cases and compare them with similar cases infected and treated in the tropics.

The present series of cases, treated by the various methods now reported on, were drawn from several tea estates. It is naturally impossible to give a clear report on the 'bill of health' on each of these tea estates. However, the following notes made by the assistant medical officers are of interest.

Chloroquine series.

In the chloroquine series, the assistant medical officer reported extremely good results in two serious cases of malaria (one was B.T. and the other M.T.) in advanced pregnancy complicated by severe anæmia. One case had anæmia, bronchitis and nephritis, the other severe anæmia. These cases were treated by divided doses over 72 hours (total 5 tablets, each tablet 0·25 gm. of the base).

Of 170 cases of chloroquine treated, many were selected from the most chronic type of infections. The general condition after treatment is reported as most satisfactory.

Camoquin series.

In these series again, many very chronic cases were selected for treatment, especially benign tertian malaria. All the assistant medical officers carrying out this type of treatment report that it gives great satisfaction in (*a*) a quick return to normal health, (*b*) a very good result in chronic benign tertian relapsing cases.

The author since carrying out the official investigation of the effects of camoquin in the treatment of malaria has been informed of the following sequelæ in the case of 3 ladies treated for what appeared to be clinical malaria (chronic benign tertian). Two ladies were American and one British—all three took 0·50 gm. camoquin once and they all complained of unpleasant effects, one in her own words 'Uterine contractions and nausea', the others nausea and a feeling of giddiness. However, the writer did not see these cases and the blood films were never examined.

In both the camoquin and chloroquine series, reports were received of the marked effect on reduction of the size of enlarged spleens.

Paludrine and other series.

Nothing special was reported by the doctors except frequency of relapses in benign tertian cases. The longer duration of treatment tends to increase the length of time for a complete recovery to normality.

All cases were followed up for three months or over.

Acquired resistance to paludrine and possibly other drugs is another important factor. Though in the present series of cases it has not been proved to have a very great effect, still it has to be taken into consideration. Seaton and Lourie (1949) have discussed this in relation to paludrine and they consider that resistance to the strain of *P. vivax* in treatment with paludrine is very slight. The action of combination of paludrine and camoquin in acute benign tertian malaria seems to be extremely good in this series of cases, though the relapse rate is still a factor to be contended with (Table XI). Admittedly the series of cases is rather small.

Monk (1948) discussed the question of relapses in *P. vivax* malaria, and found a relapse rate of 20 per cent after treatment with paludrine and pamaquin, his relapse rate with quinine and pamaquin was almost identical.

In mixed *falciparum* and *vivax* infections in the series treated by camoquin and chloroquine, the blood clearance of both malignant and benign tertian parasites was quick, but relapses, in the majority of cases, were found to be in the latter only.

Black (1949), when making observations on the treatment of *falciparum* malaria by quinine, gives an extremely interesting temperature chart showing the action of paludrine on a benign tertian infection. He himself is in favour of intravenous quinine therapy in *falciparum* infections. For heavy *falciparum* infection, by far the best immediate treatment is intravenous quinine, which at a later stage can be succeeded by other remedies such as a single dose of 0·5 gm. camoquin; one such case was reported this year and the doctor in question was very pleased with the amazingly quick recovery of an extremely ill patient whose blood film showed heavy *P. falciparum* infection. This was an early case of cerebral malaria and was promptly treated by intravenous quinine 5 grains in 10 c.c. of normal saline followed in 24 hours by a single dose of 0·5 gm. camoquin.

Mepacrine is slightly toxic and though the actual percentage of unpleasant reactions is small, they do occur. In a past Annual Medical Report of the Juri Valley Medical Association, 27 cases of complications after mepacrine treatment for *falciparum* infections were reported, ranging from nausea and dizziness, to severe insomnia, tachycardia and mental disturbances. It is definitely contra-indicated to use the combination of mepacrine followed by pamaquin on a wide scale in villages, and in any case this treatment should only be given under medical supervision and the patients kept at rest. The writer remembers a fatality after treatment of a case of *vivax* infection by this combination—the patient seemed a healthy person, but collapsed and died a day after completion of a full course of mepacrine followed by pamaquin. Further alcoholic addicts, especially those who drink country fermented liquor, are poor subjects for pamaquin therapy.

Follow up.—In the series for the year 1949, every case from the date of infection was followed up to the last day of the investigation, viz. October 31, 1949. Some cases had already been under observation for 10 months, a few more for 8

months and a great number for six months. It is felt that in a *malarious* district such as this, long 'follow-ups' are important in order to assess the efficacy of a treatment; how important it is, can be seen by comparing 1946-47 paludrine and 1947-48 chloroquine series with the 1949 series (Tables I–XIII). In these series, distinction of relapses and reinfections is difficult, but in the opinion of the author, the majority in 1949 were relapses, as a great deal of antimalarial work had been carried out in the district and prophylactic paludrine had also been administered in all the tea estates, though not to the whole population, chiefly to workers. A series of patients under investigation were given prophylaxis, and in this connection the reader is also referred to the comprehensive survey in a tea estate carried out by Ray (1948).

The recorded relapses for mepacrine alone and the combination of mepacrine and pamaquin vary enormously (Nocht and Mayer, 1937). Mepacrine relapses vary from 10·7 to 12 per cent and the combination of mepacrine and pamaquin from 3 to 5 per cent, the army report gave 12 per cent. Thus in this combination of mepacrine and pamaquin, one gains little as far as the relapse rate is concerned, compared to the more recent antimalarial drugs.

Camoquin and chloroquine are useful remedies and even by themselves have a definite place in the treatment of benign tertian infection—the single dose of camoquin 0·5 gm. followed by a fortnightly dose as prophylactic is a powerful therapeutic agent.

Paludrine plus paludrine prophylaxis is equal in its action to any other antimalarial drug in treatment and prevention of *falciparum* infection.

In comparing the action of chloroquine with other drugs, it must be remembered that the 1947 series (4 tablets of 0·25 gm. each) of 108 cases were followed up for a longer period. This makes a difference in the final relapse rate. In the 1949 chloroquine series (dosage: five tablets of 0·25 gm. each) under investigation, the percentage of relapses was reduced from 20 to 8·33 per cent in benign tertian cases in 1947, however the 1949 series 'follow-up' is not yet complete (Tables V–VII).

Cases of interest.—Owing to the extended bi-weekly paludrine prophylaxis, cerebral malaria cases are seldom seen. In a small series in 1949, four cases were treated with intravenous paludrine with three survivals, the fourth was moribund, and beyond all treatment. Intravenous paludrine therapy can be used for cerebral cases, but larger series of trials are required.

A child of two years treated for mixed *falciparum* and *vivax* infections on October 5, 1949, with camoquin 0·1 gm. single dose showed a rapid clinical cure. The blood films after treatment were positive for benign and malignant tertian rings though the result was an apparent clinical cure. A follow-up blood film was taken on November 6 and benign tertian trophozoites were present though she was afebrile. A further treatment was given to clear the *vivax* infection. This shows that the dose of camoquin for a patient of two years of age could be increased by 0·05 gm. In children, a divided dose is recommended at 12 hours' interval.

Three adult cases on single doses of camoquin suffered from nausea and vomiting, and a few children on a proportionate dose to age, i.e. 0·05 gm. per year up to 10 years (adult dose), also complained of slight nausea, but when given in divided doses, no gastric disturbances were recorded. In no case was the drug administered on an empty stomach.

ECONOMIC CONSIDERATIONS IN THE TREATMENT OF MALARIA.

Unfortunately at the present time, prices of drugs are by no means fixed but vary over a wide range, and from month to month. Dollar imports in fact are almost nil, so the assessment of costs of the drugs investigated, becomes a very difficult problem and figures given are only approximate. The prices of drugs quoted in this report have been obtained from commercial sources. Even if costs do vary, it is felt, perhaps, the ratio of costs between the various drugs in the end will not be very wide of the figures given in Appendix III.

The present low price of mepacrine is probably due to its unpopularity and accumulated stocks.

The average number of days under treatment varies a good deal, even in the same series of treatment. This, of course, is due to the general condition of the patient, even more so than the action of the drug itself. for instance, a very debilitated person will have to be treated longer than a strong individual suffering from an uncomplicated attack of malaria. However, in this report, 'averages in days under treatment' have been recorded, which gives one a fairly clear picture of the difference between the 'one dose' treatment of camoquin and the more prolonged treatments. Every doctor working on a tea estate realizes how many different factors enter into 'days under treatment', and this 'record' by itself is not of any great importance, but such information is of value to those who have to treat malarial cases in the villages.

SUMMARY AND CONCLUSIONS.

The treatment of 1,327 microscopically diagnosed positive malarial cases from 1946 to October 1949 in a malarious jungle area has been analysed, and a detailed record with summary of every class of treatment carried out has been recorded.

Efficacy of camoquin and chloroquine has been demonstrated, and a comparison with other remedies has been tentatively drawn. Relapse rates for varying periods have also been recorded.

Comparison between treatment by single large doses of camoquin (1·0 gm. or 0·50 gm.) and the repeated small doses of 0·05 gm. per tablet, is shown in Appendix II.

It has been shown that it is necessary to continue prophylaxis after a full course of treatment, in order to keep relapses and reinfections at their lowest.

Though chemotherapy by itself helps enormously, it is not the complete answer to the immense problem of combating malaria. Antimalarial measures must be continued and mosquito extermination should be the aim of all antimalarial workers. In discussing this great problem, one should bear in mind both the fear of future drug tolerance, and the failure of human beings in carrying out prophylaxis and other antimalarial measures.

ACKNOWLEDGMENT.

The author wishes to thank the assistant medical officers of the Juri Valley Medical Association; Srjt. Abinash Chakravarty, the Senior Laboratory Technician; and the laboratory staff, for their co-operation in carrying out this investigation.

Thanks are also due to Dr. Alan Gilroy, O.B.E., Principal of the Ross Institute, India and Pakistan Branch, and Dr. G. Fraser of Labac (Cachar) for their co-operation in this investigation. Supplies of camoquin tablets were made available through the courtesy of Messrs. Parke, Davis and Co. (Research Department). Colonel Jaswant Singh, Director, Malaria Institute of India, kindly provided supplies of chloroquine/analen.

REFERENCES.

BERBERIAN, D. A., and DENNIS, E. W. (1948).	*Amer. J. Trop. Med.*, **28**, p. 755.
BLACK, R. H. (1949) ...	*Trans. Roy. Soc. Trop. Med. Hyg.*, **42**, p. 565.
CHAUDHURI, R. N., and CHAKRAVARTY, N. K. (1948).	*Ind. J. Mal.*, **2**, p. 115.
CHAUDHURI, R. N., RAI CHAUDHURI, M. N., and CHAKRAVARTY, N. K. (1948).	*Ibid.*, **2**, p. 1.
FAIRLEY, N. H. (1946) ..	*Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, p. 105.
JELIFFE, D. B. (1949) ...	*Lancet*, p. 1052 (June 18, 1949).
MONK, J. F. (1948) ...	*Trans. Roy. Soc. Trop. Med. Hyg.*, **41**, p. 657.
NOCHT, B. N., and MAYER, M. M. (1937).	*Malaria*, p. 62. John Bale Medical Publications, London.
RAY, A. P. (1948) ...	*Ind. J. Mal.*, **2**, p. 35.
SEATON, D. R., and LOURIE, E. M. (1949).	*Lancet*, p. 391 (March 5, 1949).
SIMEONS, A. T. W., and CHHATRE, K. D. (1947).	*Ind. Med. Gaz.*, **82**, p. 255.
SMITH, R. F., DY, F. J., and CABRERA, D. J. (1948).	*Acta. Med. Philippina*, **5**, No. 2 (Oct.-Dec. 1948).
WORLD HEALTH ORGANIZATION. EXPERT COMMITTEE ON MALARIA, WASHINGTON (1948).	*Bull. World Hlth. Org.*, **1**, p. 215.

TABLE I

Treatment with camoquin 0·5 gm. single dose —1948 (without prophylaxis).

Type of infection.	Number of cases treated.	Time required to bring temperature to normal, hours.			Effect on parasites. (Blood films taken after temperature came to normal.)		Relapses/reinfections.		Analysis of relapses/infections (in months). Homologous.						Heterologous.		
		24	48	72	—	..	Number.	Percentage.	1	2	3	4	5	6	1	2	3
M.T. ..	24	8	13	3	22	(1 s) 2 (1 a)	.	..	..	..	..	..	..	..	..	..	..
B.T. ..	52	22	24	6	44	8 (a)	6	11·53	..	4	1	..	..	..	..	1	..
Q. ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Mixed: M.T./B.T.	21	8	11	2	17	4 (a)	2	9·52	..	..	..	..	..	1	..	..	1
TOTAL ..	97	38	48	11	83	14	8	8·24	..	4	1	..	..	1	.	1	1

	Total cases treated.	Number of relapses.	Percentage of relapses.	Type of relapses. B.T.	Mixed: M.T./B.T.
Cases treated 10 months ago	3	1	33·3	..	1
Cases treated 8 months ago	3	*Nil*	..	..	..
Cases treated 6 months ago ..	10	1	10	1	..
Cases treated 4 months ago	27	5	18·51	4	1
Cases treated 3 months ago and under ..	54	1	1·85	1	.
Cases treated over the whole period ..	97	8	8·24	6	2

M.T. = malignant tertian. B.T. = benign tertian. Q = quartan. a = asexual. s = sexual.
+ = malaria parasites found — = malaria parasites not found.

TABLE II.

Treatment with camoquin 0·5 gm. (single dose) followed by prophylaxis of 0·5 gm. fortnightly—1949 (with prophylaxis).

Type of infection.	Number of cases treated.	Time required to bring temperature to normal, hours.			Effect on parasites. (Blood films taken after temperature came to normal.)		Relapses/re-infections.
		24	48	72	+	−	
M.T.	10	4	6	...	10	...	*Nil.*
B.T.	15	4	7	4	12	3(a)	*Nil.*
Total	25	8	13	4	22	3	*Nil.*

	Total cases treated.
Cases treated 10 months ago	..
Cases treated 8 months ago	1
Cases treated 6 months ago	8
Cases treated 4 months ago	15
Cases treated 3 months ago and under	1
Total cases treated over the whole period	25

M.T. = malignant tertian. B.T. = benign tertian. a = asexual.
+ = malaria parasites found. − = malaria parasites not found.

TABLE III.

Treatment with camoquin 1·0 gm. single dose—1949 (without prophylaxis).

Type of infection.	Number of cases treated.	Time required to bring temperature to normal, hours.			Effect on parasites. (Blood films taken after temperature came to normal.)		Total relapses/reinfections.		Analysis of relapses/reinfections (in months). Homologous.					Heterologous.			
		24	48	72	—	+	Number.	Percentage.	1	2	3	4	5	1	2	3	4
M.T.	6	6	..	..	5	1 (s)	1	16·66	..	..	..	..	..	..	..	..	1
B.T.	29	23	3	3	28	1 (a)	3	10·34	..	..	2	..	1	..	..	..	..
Mixed: M.T./B.T. ..	7	5	2	..	6	1 (a)	..	..	..	..	..	..	..	..	..	..	..
TOTAL ..	42	34	5	3	39	3	4	9·52	..	..	2	..	1	..	..	..	1

	Total cases treated.	Number of relapses/reinfections.	Percentage of relapses.	Type of relapses. B.T.
Cases treated 6 months ago ..	11	4	36·36	4
Cases treated 4 months ago	4	..	..	..
Cases treated 3 months ago and under ..	27	.	..	..
Cases treated over the whole period ..	42	4	9·52	4

M.T. = malignant tertian. B.T. = benign tertian. a = asexual. s = sexual.
+ = malaria parasites found. — = malaria parasites not found.

TABLE IV.

Treatment with camoquin 1·0 gm. single dose with 0·1 gm. paludrine bi-weekly prophylaxis—1949 (with prophylaxis).

Type of infection.	Number of cases treated.	Time required to bring temperature to normal, hours.			Effect on parasites. (Blood films taken after temperature came to normal.)		Total relapses/re-infections.		Analysis of relapses/reinfections (in months).					
									Homologous.		Heterologous.			
		24	48	72	—	+	Number.	Percentage.	1	2	1	2	3	4
B.T.	35	35	..	..	35	.	2	5·71	..	1	..	..	..	1
Mixed : B.T./M.T. ..	6	5	..	1	4	2 (1 s) M.T. (1 a)	..	..	..	..	..	..	..	..
TOTAL ..	41	40	..	1	39	2	2	4·87	..	1	..	..	..	1

	Total cases treated.	Number of relapses/re-infections.	Percentage of relapses.	Type of relapses.	
				B.T.	Mixed : M.T./B.T.
Cases treated 4 months ago	22	1	4·54	..	1
Cases treated 3 months ago and under ..	18	1	5·5	1	..
Total cases treated over the whole period ..	41	2	4·87	1	1

M.T. = malignant tertian. B.T. = benign tertian. a = asexual. s = sexual.
+ = malaria parasites found. — = malaria parasites not found.

TABLE V.

Treatment with 1·0 gm. chloroquine initial dose of 0·5 gm. followed by two doses of 0·25 gm. each at 6–8 hours' interval in 24 hours—1947 (without prophylaxis). One full year's observation.

Type of infection.	Number of cases treated.	Time required to bring temperature to normal, hours.			Effect on parasites. (Blood films taken after treatment.)		Relapses/re-infections.		Analysis of relapses/reinfections (in months). Homologous.										Heterologous.									
		24	48	72	—	+	Number.	Percentage.	1	2	3	4	5	6	7	8	9	10 or over.	1	2	3	4	5	6	7	8	9	10 or over.
M.T. ..	83	31	52	..	76	7	14	16·86	1	..	..	4	..	..	..	..	1	..	..	1	3	..	..	..	1	1	..	2
B.T. ..	25	7	18	..	24	1	5	20·00	..	1	1	..	1	..	..	..	..	1	..	..	..	..	..	..	..	..	..	1
TOTAL ..	108	38	70	..	100	8	19	17·5	1	1	1	4	1	..	..	..	1	1	..	1	3	..	..	..	1	1	..	3

	Total cases treated.	Number of relapses.	Percentage of relapses.	Type of relapses. B.T.	Type of relapses. M.T.
Cases treated 10 months ago	1	..	..	..	..
Cases treated 8 months ago	14	5	35·71	2	3
Cases treated 6 months ago	47	8	17·02	2	6
Cases treated 4 months ago	45	6	13·33	1	5
Cases treated 3 months ago and under ..	1	..	..	..	..
Cases treated over the whole period ..	108	19	17·59	5	14

M.T. = malignant tertian. B.T. = benign tertian. + = malaria parasites found. — = malaria parasites not found.

Table VI.

Treatment with 1·25 gm. chloroquine initial dose of 0·5 gm. followed by 0·25 gm. after 6–8 hours ; 0·25 gm. on 2nd day morning and 0·25 gm. on 3rd day morning—1949 (without prophylaxis).

Type of infection.	Number of cases treated.	Time required to bring temperature to normal, hours.			Effect on parasites. (Blood films taken after treatment.)		Relapses/re-infections.		Analysis of relapses/reinfections (in months). Homologous.											Heterologous.									
		24	48	72	+	−	Number.	Percentage.	1	2	3	4	5	6	7	8	9	10 or over.	1	2	3	4	5	6	7	8	9	10 or over.
M.T.	11	6	5	..	8	(1 s) 3 (2 s)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
B.T.	24	10	14	..	22	2 (a)	2	8·33	..	..	1	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..
Mixed : M.T./B.T.	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Total ..	36	16	20	..	31	5	2	5·55	..	..	1	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..

	Total cases treated.	Number of relapses/re-infections.	Type of relapses. B.T.	Mixed : M.T./B.T.	Percentage of relapses
Cases treated 6 months ago	4	..	..	..	..
Cases treated 4 months ago	12	..	..	..	..
Cases treated 3 months ago and under ..	20	2	1	1	10
Total cases treated over the whole period ..	36	2	1	1	5·5

M.T. = malignant tertian. B.T. = benign tertian. a = asexual. s = sexual.
+ = malaria parasites found. − = malaria parasites not found.

TABLE VII.

Treatment with 1·25 gm. chloroquine : initial dose of 0·5 gm. followed by doses of 0·25 gm. after 6-8 hours, 2nd day morning and 3rd day morning and paludrine (bi-weekly prophylaxis dosage of 0·1 gm.)—1949 (with prophylaxis).

Type of infection.	Number of cases treated.	Time required to bring temperature to normal, hours.			Effect on parasites. (Blood films taken after treatment.)		Relapses, reinfections.	
		24	48	72	+	−	Number.	Percentage.
B.T. ...	23	23	...	...	22	1(a and s)	...	...
Mixed : M.T./B.T.	3	3	..	...	3	...	...	...
TOTAL ...	26	26	..	...	25	1	...	...

	Total cases treated.
Cases treated 6 months ago	24
Cases treated 4 months ago	2
Total cases treated over the whole period ...	26

M.T. = malignant tertian. B.T. = benign tertian. a = asexual. s = sexual.
+ = malaria parasites found. − = malaria parasites not found.

TABLE VIII.

Treatments with paludrine and paludrine plus pamaquin without prophylaxis; and quinine with paludrine prophylaxis.

Treatment given.	Types of infection.	Number of cases treated.	Relapses/re-infections.		Analysis of relapses/reinfections (in months).						
					Homologous.					Heterologous.	
			Number.	Percentage.	1	2	3	4	5	1	2
Paludrine 0·3 gm. a day for 10 days without prophylaxis.	M.T. ..	24	2	8·33	..	..	1	..	..	..	1
Paludrine 0·3 gm. for 10 days followed by pamaquin 0·03 gm. a day for 5 days without prophylaxis.	B.T. ..	34	6	17·64	..	4	..	1	1	..	..
	Mixed: M.T./B.T.	9	2	22·22	..	2	..	..	..	..	..
	TOTAL ..	43	8	18·60	..	6	..	1	1	..	..
Quinine bihydrochloride, 10 grains intramuscularly for 2 consecutive days followed by 15 grains daily for 5 days by mouth with 0·1 gm. paludrine prophylaxis bi-weekly.	M.T. ..	8	..	..	..	..	..	..	..	..	..
	B.T. ..	3	..	..	..	..	..	..	..	..	..
	Mixed: M.T./B.T.	3	..	..	..	..	..	..	..	..	..
	TOTAL ..	14	..	..	..	..	..	..	..	..	..

M.T. = malignant tertian. B.T. = benign tertian.

TABLE IX.

Treatments with paludrine : paludrine plus quinine ; mepacrine and quinine with prophylaxis.

Treatment given.	Types of infection.	Number of cases treated.	Relapses/re-infections.		Analysis of relapses/reinfections (in months).									
					Homologous.				Heterologous.					
			Number.	Percentage.	1	2	3	4	1	2	3	4	5	6
Paludrine 0·2 gm. b.d. for 5 days with paludrine bi-weekly prophylaxis of 0·1 gm.	M.T.	60	7	11·66	..	1	..	1	1	2	..	1	..	1
Paludrine 0·2 gm. b.d. for 5 days ; quinine sulphate 10 grains b.d. for 4 days followed by 0·1 gm. bi-weekly paludrine prophylaxis.	B.T.	18	3	16·66	..	..	1	1	..	..	1	..	..	..
Mepacrine 0·3 gm. daily for 5 days followed by 0·1 gm. bi-weekly paludrine prophylaxis.	M.T.*	40	1	2·5	..	..	..	..	..	1	..	..	..	..
Quinine sulphate 8 grains b.d. for 5 days followed by 0·1 gm. bi-weekly paludrine prophylaxis.	B.T.	21	3	14·28	1	1	1	..	..	..	..	..	..	..
	Mixed : M.T./B.T	3	*Nil*	*Nil*										
	TOTAL	24	3	12·5	1	1	1	..	..	..	..	..	..	..

M.T. = malignant tertian. B.T. = benign tertian. b.d. = twice a day.

* Special trial on suggestion of author (C. S. P. H.).

TABLE X.

Treatment with paludrine (with and without prophylaxis) and paludrine plus plasmochin (without prophylaxis).

Treatment given.	Types of infection.	Number of cases treated.	Relapses/re-infections.		Analysis of relapses/reinfections (in months).													
					Homologous.										Heterologous.			
			Number.	Percentage.	1	2	3	4	5	6	7	8	9	10 or over.	1	2	3	4
Paludrine 0·4 gm. daily for 3 days without prophylaxis.	M.T. ..	19	11	57·89	..	2	1	..	..	..	..	..	..	..	2	3	2	[illegible]
Paludrine 0·4 gm. daily for 3 days followed by plasmochin 0·02 gm. daily for 3 days without paludrine prophylaxis.	B.T. ..	9	3	33·33	1	1	..	1	..	..	..	..	..	..	..	..	..	
	Mixed : M.T./B.T.	3	3	100·00	..	..	..	..	..	..	..	..	..	..	..	1	1	[illegible]
	TOTAL ..	12	6	50·00	1	1	..	1	..	..	..	..	..	..	..	1	1	[illegible]
Paludrine 0·4 gm. for 3 days with 0·1 gm. bi-weekly paludrine prophylaxis.	M.T. ..	61	8	13·11	2	..	1	1	..	..	2	..	..	1	1	..	..	
	B.T. ..	22	1	4·54	1	..	..	..	..	..	..	..	..	..	..	..	..	..
	Mixed : M.T./B.T.	4	1	25·00	..	..	..	..	..	..	..	..	..	..	..	1	..	..
	TOTAL ..	87	10	11·49	3	..	1	1	..	..	2	..	..	1	1	1	..	..

M.T. = malignant tertian. B.T. = benign tertian.

TABLE XI.

Types of treatments adopted for malaria with their relapses for the year 1949 (all cases are diagnosed microscopically).

Treatment given.	Total number of cases.	Type of infection.			Total relapses.	Relapse rate, per cent.	Reference to table.
		M.T.	B.T.	Mixed: M.T./B.T.			
Paludrine 0·2 gm. b.d. for 3 days followed by paludrine 0·1 gm. bi-weekly prophylaxis.	87	61	22	4	10	11·49	X
Paludrine 0·2 gm. b.d. for 5 days followed by paludrine 0·1 gm. bi-weekly prophylaxis.	60	60	...	...	7	11·66	IX
Paludrine 0·2 gm. b.d. for 5 days followed by 10 grains quinine sulphate b.d. for 4 days with bi-weekly paludrine prophylaxis.	18	..	18	...	3	16·66	IX
Mepacrine 3 tablets daily for 5 days with bi-weekly paludrine prophylaxis.	40	40		...	1	2·50	IX
Quinine sulphate 8 grains b.d. for 5 days followed by bi-weekly paludrine prophylaxis.	24	...	21	3	3	12·50	IX
Quinine bihydrochlor 10 grains intramuscularly for 2 consecutive days followed by 15 grains quinine sulph. daily for 5 days with bi-weekly 0·1 gm. paludrine prophylaxis.	14	8	3	3	*Nil*	*Nil*	VIII
Camoquin 0·5 gm. single dose followed by fortnightly 0·5 gm. camoquin prophylaxis.	25	10	15	...	*Nil*	*Nil*	II
Camoquin 1·0 gm. single dose followed by bi-weekly paludrine prophylaxis.	41	...	35	6	2	4·87	IV
Chloroquine 2 tablets (0·25 gm. each) and 1 tablet after 6 to 8 hours: then 1 tablet each on the 2nd and 3rd morning (total 5 tablets) followed by 0·1 gm. bi-weekly paludrine prophylaxis.	26	..	23	3	*Nil*	*Nil*	VII

b.d. = twice daily. M.T. = malignant tertian. B.T. = benign tertian.

TABLE XII

Types of treatments adopted for malaria with their relapses for the years 1949 and 1947 (all cases are diagnosed microscopically)—Without prophylaxis.

Treatment given.	Total number of cases.	Type of infection.			Total relapses.	Relapse rate, per cent.	Reference to table.
		M.T.	B.T.	Mixed; M.T./B.T.			
Paludrine 0·2 gm. b.d. for 3 days	19	19	...	...	11	57·89	X
Paludrine 0·2 gm. b.d. for 3 days followed by plasmochin 0·02 gm. daily for 3 days.	12	...	9	3	6	50·0	X
Paludrine 0·3 gm. daily for 10 days	24	24	...	...	2	8·33	VIII
Paludrine 0·3 gm. daily for 10 days followed by pamaquin 0·03 gm. daily for 5 days.	43	...	34	9	8	18·60	VIII
Camoquin 1 gm. single dose treatment	42	6	29	7	4	9·52	III
Camoquin 0·5 gm. single dose treatment	97	24	52	21	8	8·24	I
Chloroquine experiment in 1947—0·5 gm. initial dose; 0·25 gm. 6 to 8 hours after; followed by another dose of 0·25 gm. 6 to 8 hours after. Total 1 gm. in 24 hours.	108	83	25	...	19	17·50	V
Chloroquine experiment 1949—0·5 gm. initial dose; 0·25 gm. 6 to 8 hours after; 0·25 gm. 2nd day morning and 0·25 gm. on 3rd day morning. Total 1·25 gm.	36	11	24	1	2	5·55	V
Paludrine 0·3 gm. daily for 10 days followed by a course of camoquin, 0·5 gm. single dose.	11	..	10	1	*Nil*	*Nil*	XI

b.d. = twice daily. M.T. = malignant tertian. B.T. = benign tertian.

TABLE XIII.

Total number of cases treated with camoquin and chloroquine with their effect on temperature, parasites and toxicity.

Treatment given.	Total number of cases.	Effect on temperature. Temperature came to normal in hours.			Effect on parasites. Blood films taken after temperature came to normal.			Remarks.
		24	48	72	Negative.	Positive.	Percentage positive.	
Camoquin 0·5 gm. single dose (1949) ...	122	46	61	16	105	17	16·18	
Camoquin 1·0 gm. single dose (1949) ...	83	74	5	4	77	5	6·4	
Chloroquine 0·25 gm. divided dosage; total 1·25 gm.	36	16	20	..	33	3	8·33	Blood film taken after completion of treatment.
Chloroquine 0·25 gm. divided dosage; total 1·00 gm. in 24 hours' time.	108	38	70	...	100	8	7·5	

In the above Tables, the action of camoquin and chloroquine on temperature and parasites have been recorded. It is noticed that 0·5 gm. dosage of camoquin takes a longer period to bring down the temperature to normal, whereas with 1·0 gm. dosage it is quicker.

In case of clearing the parasites from peripheral blood, 1·0 gm. dosage is more active than that of 0·5 gm. Very few cases of sexual form of parasites both benign and malignant tertian were found in these series of investigations. However, it was noted that 4 to 5 days' time is required to clear the blood of benign tertian gametocytes and in case of crescents, practically with no action. In pregnancy cases, the untoward result with a single dose 1·0 gm. was in a severe anæmia case which was treated for acute *falciparum* infection and she aborted a fortnight after. It is felt at such long a period after taking the dose, camoquin cannot be the case. In 1·0 gm. dosage, vomiting and nausea were recorded in 2 cases. No other toxic symptoms were met with.

In the chloroquine series, the action on temperature and parasites seems quite good. It has no action or very little action on sexual forms of parasites. No signs of toxicity were noticed whatsoever amongst the treated cases in divided dosage.

APPENDIX I.

Paludrine treatment return—1946.

Routine or special treatment.	Class of treatment.	Total number of cases treated during the investigation.	Total number of relapses during the investigation.	Relapse rate, per cent.	REMARKS. (Follow up 2 months).
Routine treatment	A	57	13	22·80	
Routine treatment	B	156	29	18·58	
Routine treatment	C	197	18	9·13	
Special treatment	B(1)	35	4	11·42	But no relapse occurred since placing them on 0·1 gm. per week from 2nd week of September.
Special treatment	C(1)	43	2	4·65	Relapses occurred after placing them on prophylaxis of 0·1 gm. per week.
Special treatment	C(2)	22	3	13·63	Only one relapse occurred since starting 0·2 gm. per week from October 4.
Special treatment	C(3)	15	1	6·66	This class was only carried out since November 1946.
TOTAL ...	..	525	70	13·33	

A = 0·1 gm. once.
B = 0·3 gm. once.
C = 0·2 gm. twice daily for 3 days.
B(1) = B plus prophylaxis 0·1 gm. per week.
C(1) = C ,, ,, 0·1 gm. per week.
C(2) = C ,, ,, 0·2 gm. per week.
C(3) = C ,, one day's treatment more, i.e. total 1·6 gm.

APPENDIX II.

Treatment with camoquin (1947 and 1948 investigation cases)—Copy of report submitted to the Ross Institute in June 1948.

The manufacturers had recommended the following different types of dosages of camoquin of which only the first three have been tried :—

Class A. An initial dose of 2 tablets (0·1 gm.) of the drug followed by one tablet every twelve hours for seven doses, i.e. a total course of nine tablets of 0·05 gm. each.

Class B. An initial dose of 2 tablets followed by 2 tablets every 12 hours for 3 doses only, i.e. a total of 8 tablets (0·05 gm. each).

Class C. An initial dose of 3 tablets followed by 3 tablets after 12 hours' interval and then 2 tablets every 12 hours for 2 doses, i.e. a total of 10 tablets (0·05 gm. each).

Class D. An initial dose of 4 tablets followed by 4 tablets after 12 hours, i.e. a total of 8 tablets.

Cases treated under different classes.

Class of treatment.	Type of infection.	Number of cases treated.	Number of relapses.	Analysis of relapses. With homologous infection, within days.							Analysis of relapses. With heterologous infection, within days.						
				10	20	30	40	50	60	Over 60.	10	20	30	40	50	60	Over 60.
A	B.T. ...	17	1							1							
	M.T. ...	11	...														
	Total ...	28	1	i.e. 3·57 per cent.													
B	B.T. ...	6	1							1							
	M.T. ...	10	...														
	B.T./M.T. ...	2	...														
	Total ...	18	1	i.e. 5·55 per cent.													
C	B.T. ...	16	...														
	M.T. ...	13	3							1 *2	* (One after 80 days and one after 105 days.)						
	Total ...	29	3	i.e. 10·34 per cent.													
	Grand Total	75	5	i.e. 6·66 per cent.													

B.T. = benign tertian. M.T. = malignant tertian.

APPENDIX II—contd.

Five cases were treated in May 1948 and as this report was submitted in June 1948 and these cases have not yet relapsed, they are excluded from the final figures when recording relapses. The record will read as follows :—

Total cases treated 70
Relapses/reinfection rate ... 7·14 per cent.

Effects on temperature :—

In these three classes of treatment it has been noticed that the temperature came to normal after administration of the tablets (each tablet contains 0·05 gm.) as follows :—

Number of tablets required to reduce temperature to normal.

Class of treatment.	Total cases treated.	Temperature came to normal after administration of tablets.								
		2 tabs.	3 tabs.	4 tabs.	5 tabs.	6 tabs.	7 tabs.	8 tabs.	9 tabs.	10 tabs.
A	28	5	4	8	5	2	3	1	...	...
B	18	...	...	5	...	9	..	4	...	...
C	29	...	6	...	...	13	..	10	...	...

From the above analysis it has been observed that not a single case required more than 8 tablets (0·4 gm.) to bring down the temperature to normal whereas some of them reacted even after administration of 2 tablets (0·1 gm.).

Effect on parasites (asexual form) :—

Blood films from all the cases were examined before starting the treatments and also after completion of treatments. Amongst all these treated cases only in one case of *vivax* infection, parasites were found after treatment and without giving any further treatment this case was observed, and the blood films taken after a week, when it was found to be negative. This is the only case which showed slight delayed action on the parasites.

Relapses/reinfections :—

Out of 75 cases treated, there were only 5 relapses—two had *vivax* infection (one relapsed after 50 days and the other after 69 days) and three *falciparum* which relapsed after 80, 105 and 18 days of the t eatment and the last was treated again with a second course of camoquin. Since then, during the last 3 months he had no further attack of malaria. It must be remembered that some of these relapses may be reinfections especially those over 60 days.

Toxic effect :—

No toxic symptoms were observed during and after treatment of the cases in the three grades. In this series of treatment, there were three pregnant cases but none of them had any ill effects during and after the treatment. It has been noticed that there is a good reduction of spleen after 3 to 4 weeks of treatment.

Conclusion :—

Various kinds of antimalarial drugs are available now but very few are of curative value in *vivax* infections. This preparation seems to have promising results in the treatment of benign tertian malaria.

APPENDIX III.

Economic table.

Types of treatment.	Cost at the rate of	Approximate cost per 100 cases.*	Expected relapse rates without prophylaxis, per cent.	Average sick days.	REMARKS.
Camoquin, single dose treatment, dosage 1·0 gm.	Re. 1/4/- for 0·6 gm.	Rs. 215/-	9-10	4	.....
Camoquin, single dose treatment, dosage 0·5 gm.	Re. 1/4/- for 0·6 gm.	,, 105/-	8–10	4	Percentage may be more as most of the cases 'followed up' for 3 months only.
Paludrine, 30 tablets (0·1 gm. each) in 10 days.	Rs. 20/- per 1,000 tablets.	,, 63/-	8-9	10	Only M.T.
Paludrine, single dose treatment, dosage 0·3 gm. tablet.	Rs. 52/- per 1,000 tablets.	,, 6/4/-	18-19	4	Observation for 60 days only.
Paludrine 12 tablets (0·1 gm. each) in 3 days.	Rs. 20/- per 1,000 tablets.	,, 25/-	57-58	6	.....
Paludrine, 30 tablets (0·1 gm. each) in 10 days followed by pamaquin 15 tablets (0·01 gm. each) in 5 days.	Re. 1/3/- per 15 tablets of pamaquin.	,, 182/-	18-19	15	.....
Paludrine, 12 tablets (0·1 gm. each) in 3 days followed by pamaquin 6 tablets (0·01 gm. each) in 3 days.	Re. 1/3/- per 15 tablets of pamaquin.	,, 72/-	50	6	...
Paludrine, 30 tablets (0·1 gm. each) in 10 days followed by camoquin 0·5 gm. single dose.	...	,, 167/-	...	11	Only 11 cases treated.
Quinine sulphate 100 grains in 5 days.	Rs. 65/- per pound.	,, 94/-	20–30	7	... ..
Mepacrine 15 tablets (0·1 gm. each) in 5 days.	Rs. 9/- per 1,000 tablets.	,, 14/-	10–12	7	The present low price of mepacrine is probably due to its unpopularity since the war, and accumulation of army surplus supplies.
Mepacrine 15 tablets (0·1 gm. each) in 5 days followed by 5 days' course of 15 tablets of pamaquin.	Re. 1/3/- for 15 tablets of pamaquin.	,, 133/-	8–12	10	
Chloroquine, 5 tablets (each 0·25 gm. of the base).	$33·33 per 1,000 tablets.	Cost not yet available in India and Pakistan.	5-6	5	.....

* Export, import, sales tax, duties will vary. (The cost of paludrine bi-weekly prophylaxis 2 tablets each 0·1 gm. for 9 months Rs. 162-8-0 for 100 cases.)

Indian Journal of Malariology, **4**, 3, June 1950.

STUDIES IN SULPHA-BIGUANIDES: SOME N^1-ARYL-N^5-p-(2-PYRIDYL)PHENYLSULPHONAMIDE-BIGUANIDES.

BY

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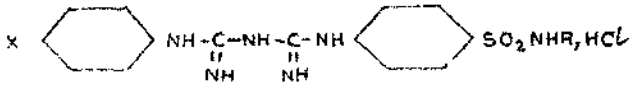
[July 30, 1950.]

THE biguanide molecule, as starting point for the synthesis of new potential antimalarials, has received considerable attention since the discovery of potent antimalarial properties of paludrine, which is a substituted biguanide derivative. During the course of systematic investigations in the field of synthetic antimalarials, a number of substituted biguanide derivatives have previously been reported (Bami, Iyer and Guha, 1947, 1948*a*, 1948*b*; Bami and Guha, 1949*a*, 1949*b*), and some of these have shown encouraging antimalarial activity when tested against *P. gallinaceum* in chicks (Bami *et al.*, 1949), and *P. knowlesi* in *rhesus* monkeys (Bami *et al.*, *loc. cit.*; Jaswant Singh, Ray, Nair and Basu, 1949).

Previously a number of sulpha-biguanides of type 'A' derived from sulphanilamide, sulphathiazole, sulphadiazine, sulphamerazine and sulphamethazine (Bami, Iyer and Guha, 1947, 1948*a*), as well as those from N^1 benzoylsulphanilamide [personal communication from Mr. P. R. Gupta, Fellow, Indian Council of Medical Research] have been prepared and some of these have displayed definite antimalarial activity against experimental malaria (especially those derived from sulphadiazine and sulphamerazine, viz. type 'A' where R=2-pyrimidyl and 4-methyl-2-pyrimidyl respectively) (Bami *et al.*, *loc. cit.*; Jaswant Singh, Ray, Nair and Basu, *loc. cit.*).

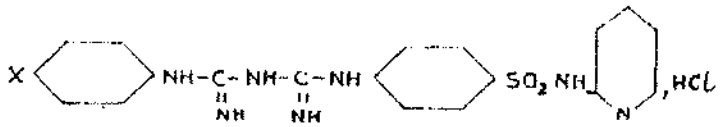


TYPE 'A'

X=H, Cl, Br, I, CH_3 CH_3O R=H, 2-thiazolyl 2-pyrimidyl; 4-methyl-2-pyrimidyl; 1:6-dimethyl-2-pyrimidyl and benzoyl.

In continuation of this work, it was considered of interest to complete this series by synthesizing some sulpha-biguanides of type 'B' which are derived from

sulphapyridine. Sulphapyridine (M. & B. 693) is the first of the series of potent heterocyclic sulphanilamides and has previously shown some promise as antimalarial both in experimental (Jaswant Singh and Harwant Singh, 1939; Manwell, Counts and Coulston, 1941) and human malarial infections (Chopra, Hayter and Sen, 1939; Curd, 1943).



TYPE 'B'

m.p. in °C. for compounds of type 'B':—

When x=H, 174 decomp; x=Cl, 238; x=Br, 240 decomp; x=I, 204 decomp; x=CH_3, 236; x=C_2H_5O, 241.

Respective aryleyanoguanidines have been condensed with sulphapyridine hydrochloride in boiling aqueous dioxane and the resulting biguanides have been isolated and characterized through their hydrochlorides. All these compounds are white crystalline powders soluble in dilute alcohol, acetone and dioxane but insoluble in water excepting the phenyl analogue, (type 'B' where x=H) which is soluble in hot water. They could also be isolated through their sodium salts which are stable and fairly soluble in cold water. Details about the present work and results of their antimalarial screening tests will be reported later.

ACKNOWLEDGMENT.

The author wishes to express his grateful thanks to Lieut.-Colonel Jaswant Singh, Director, Malaria Institute of India, for his encouragement and keen interest in these investigations.

REFERENCES.

BAMI, H. L., and GUHA, P. C. (1949*a*). *J. Ind. Inst. Sci.*, **31A**, p. 1 (cf. *Current Science*, 1948, **17**, p. 272).

Idem (1949*b*). *Ibid.*, **31A**, p. 9 (cf. *Current Science*, 1948, **14**, p. 386).

BAMI, H. L., IYER, B. H., and GUHA, P. C. (1947). *Ibid.*, **29A**, p. 15 (cf. *Current Science*, 1947, **16**, p. 252).

Idem (1948*a*). *Ibid.*, **30A**, p. 1 (cf. *Current Science*, 1948, **16**, p. 90).

Idem (1948*b*). *Ibid.*, **30A**, p. 9 (cf. *Nature*, 1948, **162**, p. 146).

BAMI, H. L., NATRAJAN, S., RAMASWAMY, A. S., DE, N. N., IYER, B. H., and GUHA, P. C. (1949). *Current Science*, **18**, p. 50.

CHOPRA, R. N., HAYTER, R. T. M., and SEN, B. (1939). *Ind. Med. Gaz.*, **74**, p. 658.

CURD, F. H. S. (1943) ... *Ann. Trop. Med. Parasit.*, **37**, p. 115.

JASWANT SINGH and HARWANT SINGH (1939). *J. Mal. Inst. Ind.*, **2**, p. 181.

JASWANT SINGH, RAY, A. P., NAIR, C. P., and BASU, P. C. (1949). *Ind. J. Mal.*, **3**, p. 405.

MANWELL, R. D., COUNTS, E., and COULSTON, F. (1941). *Proc. Soc. Expl. Biol. Med.*, **46**, p. 523.

Indian Journal of Malariology, **4**, 2, June 1950.

ABSTRACT.

FRESH-WATER FORM OF *A. SUNDAICUS*.*

BY

V. VENKAT RAO,

AND

V. RAMAKRISHNA.

[April 19, 1950.]

In the Chilka Lake area in Orissa and Vizagapatam Coast, *A. sundaicus* has been found to breed more often in fresh waters (salinity less than 0·1 per cent) than in saline waters. The epidemiological evidences, collected from the area under study, suggested the possibility of the existence of two races of *A. sundaicus* in these areas; the available evidence indicating that the fresh-water form was a more potent vector than the saline form (Senior White *et al.*, 1947).

Attempts were, therefore, made to determine whether the two forms could be separated by differences in their morphological characters.

It was noted that the fresh-water form was closely allied to *A. ludlowi*, a fresh-water species of *ludlowi-sundaicus* group found in the Philippines.

The fresh-water form differed from the brackish-water form in the number and shape of the leaflets of the phallosome. The leaflets of the fresh-water form were six in number and were broad and blade-like, whereas the leaflets of the salt-water form were five in number and were narrower and shorter than in the fresh-water form.

The variation in the leaflets of the phallosome, however, is not a fixed character and at times very difficult to see. Moreover, these observations are by no means complete, but this short note is published to stimulate further work on the question of any new variety or varieties of *ludlowi-sundaicus* occurring along the Coast of India, and whether or not the fresh and salt-water forms can be separated validly into distinct subspecies or varieties.

REFERENCE.

Senior White, R., Ramakrishna, V., and Venkat Rao, V. (1947). *Ind. J. Mal.*, **1**, pp. 81–122.

* Copy of the original manuscript (4 pages, 7 references and 1 text-figure) has been placed in the library of the Malaria Institute of India, and will be available on loan to workers who may wish to consult it. —*Editor.*

REVIEWS.

MALARIOLOGY.

Malariology. By Mark F. Boyd. (Volumes I and II ; pp. 1643). Published by W. B. Saunders Company, Philadelphia and London. 1949.

WE do not as a rule review books in these columns, but we make no apology in drawing our readers' attention to Mark F. Boyd's book which is a comprehensive survey of all aspects of malariology from a global standpoint. As Colonel Sir Rickard Christophers puts it in his foreword 'the volume is an integration of modern malaria research and practice' it will be of immense value to those engaged in the practice of malariology. The editor's intention has been stated to be to make this volume a comprehensive working manual of malariology, and we have no hesitation in saying that he has succeeded in this remarkably well.

There is a quality about this work, apart from its technical excellence, which will make it a favourite with the students, the research worker as well as the man responsible for organizing malaria control measures. Instead of being merely a store-house of scientific data, it seems to reflect the enthusiasm and personality of its distinguished authors. This impression is conveyed not only in the vivid and concise text, but in the abundant illustrations. Some 75 contributors have co-operated in producing this work and most of them are recognized authorities in their respective fields throughout the world. The book has been divided into five sections. Section I is a historical review which, amongst others, is Boyd's own contribution and is admirable. Section II deals with parasitology and has been contributed by well-known authorities like M. L. Brumpt, Robert Coatney, Sir Rickard Christophers and others. Section III deals with the definitive host and here again authorities like Christophers, Buxton and Wigglesworth have made their contributions. The entomological data has been dealt with on a zoo-geographical basis. Section IV deals with the intermediate host and is an excellent presentation of such different problems as the epidemiology of the disease, immunity and antimalarials. The last section on the control and eradication deals with such problems in various regions of the world, and as such is very helpful. The concept of eradication of the disease from the globe has been put into the forefront and mere prevention and control is no longer the objective of the modern malariologists. The engineering aspects of malaria, drainage, etc., find a special place and have been dealt with by sanitary engineers of renown.

We commend this book to all our readers. The two volumes cost about Rs. 150/-, and this will in our opinion be a sound investment.

JASWANT SINGH.

Indian Journal of Malariology, **4**, 3, September 1950.

MALARIA SURVEY OF KYAUKPYU, RAMREE ISLAND, BURMA.

BY

T. W. TYSSUL JONES

(*Lieutenant Entomologist, R.N.V.R.*).

(April 4, 1950.)

INTRODUCTION.

When the Arakan was ceded to the British in 1826, Kyaukpyu, previously a small fishing village, became the chief military station, but in 1850 was abandoned for military purposes.

The embankment was built about 1847 by the labour of about 500 prisoners from Akyab and Kyaukpyu jails. It may be assumed that these prisoners did not introduce malaria fever since Kyaukpyu is reported to have been a healthy military station for some years.

In the year 1882, systematic working of the oilwells on Ramree Island was first started. For this work about 500 coolies were imported, of these 150 came from Chittagong and Orissa and were housed at Ngalapwe. At the same time about 140 coolies from Madras were brought in to build the road from Kyaukpyu to Ngalapwe. A few years later the embankment between Pyinfumaw and Ngalapwe was repaired and raised by two feet. This work was carried out by 150 to 200 coolies from Calcutta.

Towards the year 1895, the town was highly malarious and had assumed such serious proportions that the military police brought in were stricken with the disease within a very short time.

Itinerant coolies are as a class notoriously malaria-stricken. There is no doubt that the introduction of these coolies into a tropical area like Kyaukpyu, where the malaria-carrying mosquitoes abound, was the cause of the introduction of this disease.

The malaria situation deteriorated so rapidly after this that the provincial malaria committee in 1911 chose Kyaukpyu as the first centre at which malaria investigations were carried out. This work was carried out by Colonel Lalor in 1912. As a result of his investigations he recommended that the swampy area

within the municipal limits of Kyaukpyu be reclaimed and that paddy cultivation be prohibited and substituted by the growing of fruits and vegetables.

In 1918, Colonel Williams of the Indian Medical Service recommended a drainage and reclamation scheme (Williams, 1922). This scheme which aimed at reducing malaria in Kyaukpyu was admitted to be a partial one, as it provided only for the reclamation of the marshy land to a distance of one or two furlongs at the most from the boundaries of the town. Yet beyond that limit still larger marshes surrounding inhabited villages situated on slightly higher ground existed. To these villages, which according to Lalor were highly malarious, Williams recommended that his scheme of drainage and land reclamation be extended to remove the stigma of unhealthiness.

In 1923, Major Jolly of the Indian Medical Service inspected the area. He remarked that much land reclamation had been carried out and that paddy cultivation within the municipal limits of Kyaukpyu had been compulsorily restricted and dry cultivation instituted. He again stressed the importance of the continuance of the scheme as outlined by Lalor and Williams (Jolly, 1927).

The survey reported in this paper was carried out during the early months of 1945 and indicates the picture as it then existed. The question of the anopheline vector or vectors in Kyaukpyu is also discussed.

TOPOGRAPHY.

Kyaukpyu (Map 1) is situated on a low-lying tongue of land which forms the north-east point of Ramree Island, and is some 30 miles from the Arakan coast from which it is separated by other smaller islands. This tongue of land is bounded on the west by a low range of hills which are thickly wooded, and on the north and the east by Kyaukpyu Harbour and the south by the Ngalapwe Creek. The Kyaukpyu area is roughly 4 miles eastward by 2 miles at its western base. The low-lying parts to the east and the south would be flooded by the sea at the spring tides were they not protected by the embankment.

RAINFALL.

The first 4 months of the year are dry with little more than an inch of rain. The monsoons commence in May and continue up to September with approximately 150 inches of rain. There is some rain in October after which it peters out to the dry month of December. Table I gives the average monthly rainfall for the years 1906 to 1910, the only figures available.

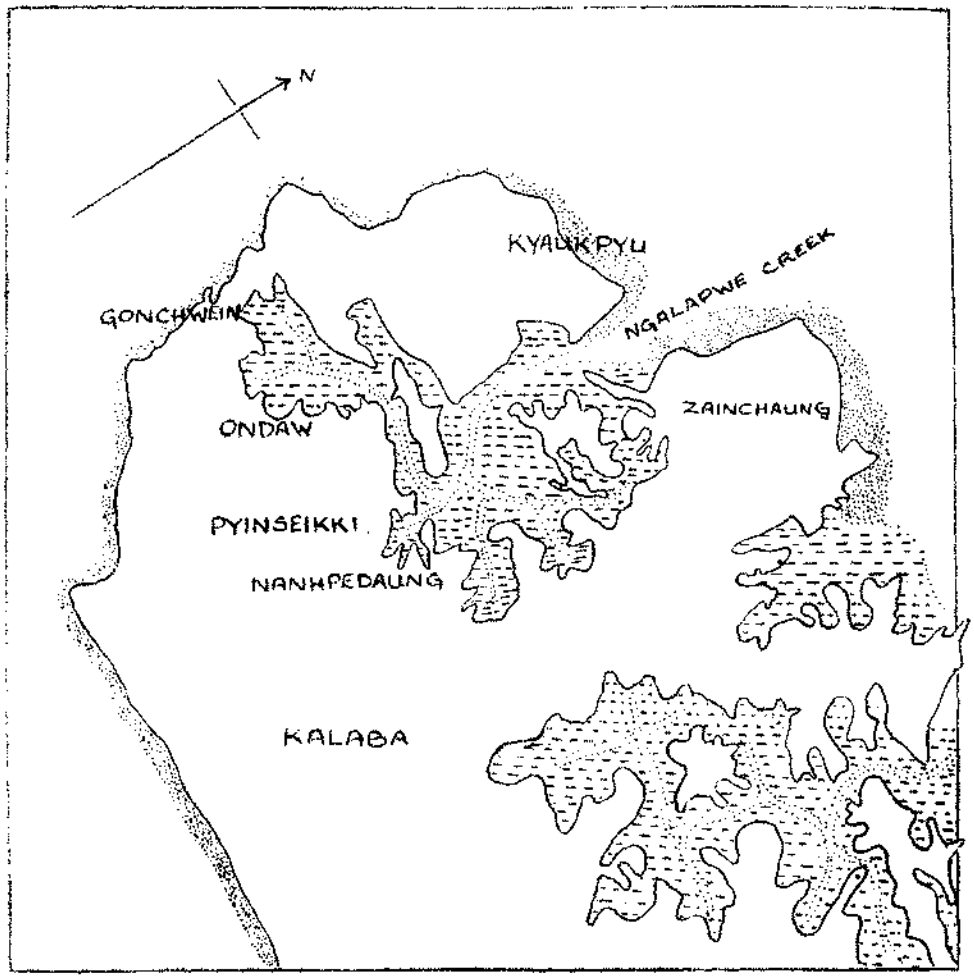
TABLE I.

Average monthly rainfall for the years 1906 *to* 1910.

Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
0	0	1	1	13	39	43	41	16	9	1	0

MAP 1.

MAP OF NORTH RAMREE ISLAND.



REFERENCE.
Scale 1 inch = 2·79 miles.

TEMPERATURE.

At no time of the year is the temperature below that necessary for the continuous development of the malaria parasite within the mosquito. Table II gives the average maximum and minimum temperatures for the years 1906 to 1910,

TABLE II.

Average maximum and minimum temperatures for the years 1906 *to* 1910.

	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Maximum ...	76	76	82	87	86	85	85	84	85	85	81	78
Minimum ...	70	70	70	73	78	79	78	80	72	75	72	70

TIDE TABLES.

The tides rise and fall rapidly. The average rise during the spring is between 7 and 8 feet. In certain areas, particularly north-east of Ngalapwe, there is a serious danger of overflow into the surrounding low lands during the monsoon. The relationship between the tides and the moon phases is given in Table III.

TABLE III.

Height of the tides in relation to the moon phases.

Month.	New moon.	Tides.		Full moon.	Tides.	
		Highest wave.	Lowest wave.		Highest wave.	Lowest wave.
Jan. ...	12	6·1	0·4	27	6·0	0·2
Feb. ...	14	7·2	1·3	26	6·9	0·0
Mar. ...	12	8·0	1·1	27	7·4	0·2
Apr. ...	11	8·6	0·3	27	8·1	0·9
May ...	11	8·6	0·3	27	8·1	0·9
Jun. ...	10	9·2	0·6	25	8·5	1·5
Jul. ...	9	9·4	1·5	23	9·0	1·5
Aug. ...	8	9·6	1·7	23	9·3	1·3
Sep. ...	6	8·6	1·6	21	9·0	0·5
Oct. ...	6	8·1	1·3	21	8·9	0·2
Nov. ...	4	7·5	1·3	19	8·1	0·2
Dec. ...	4	6·5	1·2	19	7·1	0·1

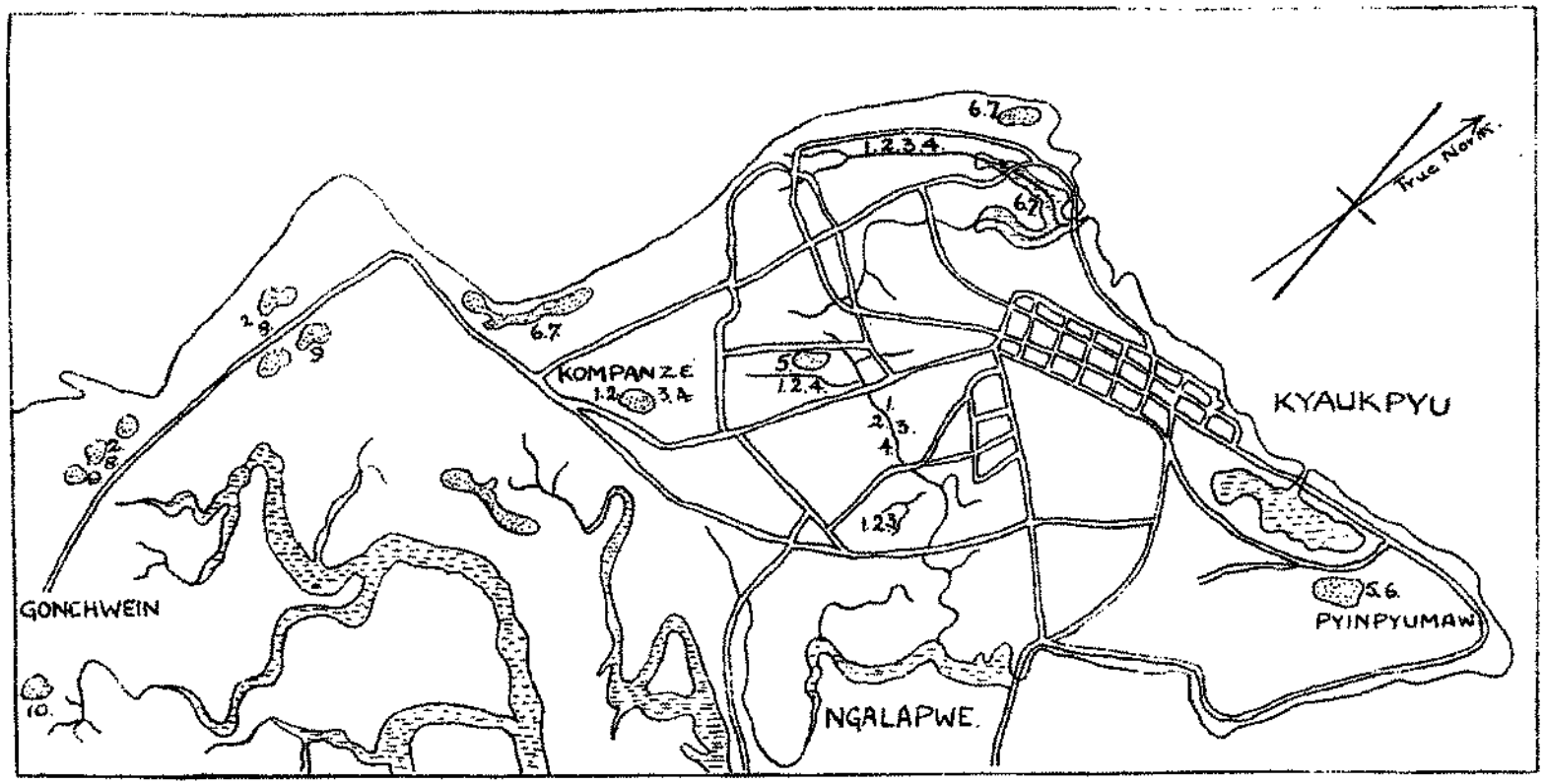
PARASITE RATE.

The parasite rate of only one village, Kalaba, was taken. Of the 45 films examined, 5 were positive, i.e. 11 per cent. and all were *vivax* infection.

SPLEEN RATES.

A large number of villages (Maps 1 and 2) were visited and the spleen rate ascertained among children between the ages of 1 and 10 years. Table IV compares the results with those of Lalor (1912*a*).

MAP OF KYAUKPYU.



DISTRIBUTION OF LARVÆ.

1. *A. aconitus.*
2. *A. annularis.*
3. *A. hyrcanus.*
4. *A. jamesi.*
5. *A. vagus.*
6. *A. subpictus.*
7. *A. sundaicus.*
8. *A. philippinensis.*
9. *A. pallidus.*
10. *A. kochi.*

REFERENCE.

Scale 1 inch = 286 yards.

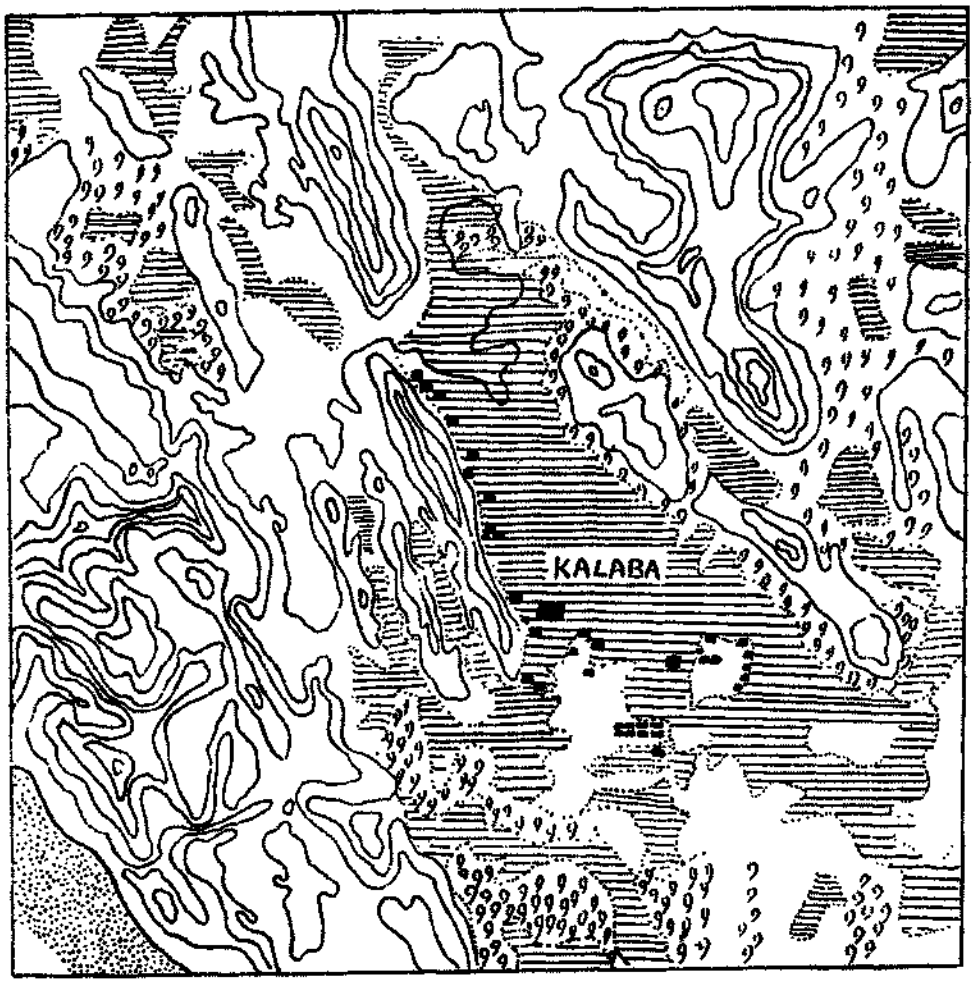
Roads

Chaung, pools

Streams

MAP 3.

MAP OF KALABA.



REFERENCE.

Scale 5·48 inches = 1 mile.

- Water tanks.
- Sand.
- Paddy.
- Woodland.
- Open ground.

TABLE IV.

Village.	1945 SURVEY.			LALOR, 1912.
	Number examined.	Number with enlarged spleen.	Percentage.	Percentage.
Saiboke ..	101	45	45	...
Gonchwein	39	18	46	...
Ondaw	36	17	48	...
Ondaw (western) ..	58	14	24	...
Passi (Kyaukpyu) ...	22	13	59	64
Pyinfumaw	55	24	44	60
Kanyindaw	53	26	49	64
Kalabadaung ...	37	26	70	86
Ngalapwe	46	24	52	87
Taungyin	39	13	34	79
Kompanize ...	55	33	60	70
Zainchaung	Record lost	Record lost	70	...
Pyinseikki	,,	,,	34	...
Nanhpedaung .	,,	,,	12	...
Kalaba	,,	,,	15	...

LARVAL IDENTIFICATION.

Throughout the whole of this survey, living larvæ only were identified. This is considerably quicker and affords complete and accurate identification.

To ascertain a representative picture of the anopheline population, 1,172 larvæ were collected and identified. The findings are given in Table V.

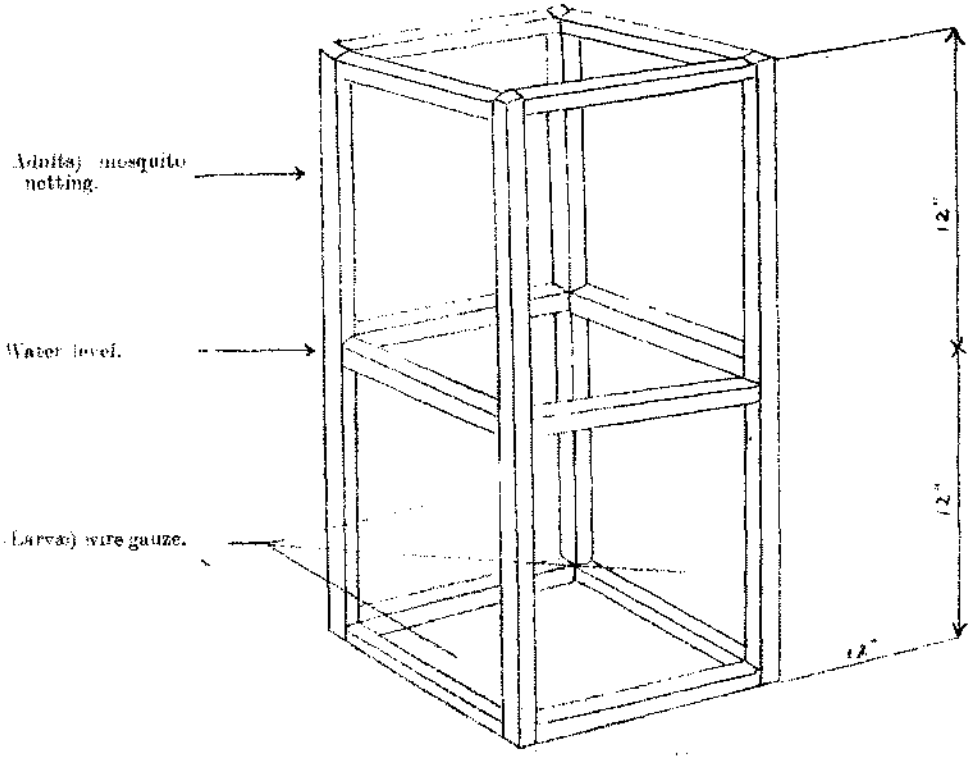
TABLE V.

Anopheline larvæ collected during the survey.

Species.	Number.	Percentage.
A. aconitus ...	135	11·5
A. annularis ...	144	12·4
A. hyrcanus ...	129	10·9
A. jamesi	82	6·9
A. kochi	12	1·1
A. pallidus ...	79	6·7
A. philippinensis ...	63	5·4
A. subpictus ...	267	22·8
A. sundaicus ...	86	7·4
A. vagus	175	14·9
	1,172	

On February 23, 110 larvæ for six dips, using an ordinary soup ladle in some brackish water, were encountered, and were identified as 74 *A. subpictus* and 36 *A. sundaicus*. However, no confidence was felt in this result because the larval identification of these two species is by no means certain as the distinguishing characteristics are not clearly defined. It could never be decided with certainty whether hair number 5 on the mesothorax was split in the basal or distal half. Again many larvæ were found in which the post spiracular hair had six branches on one side and seven on the other. To check these identifications, efforts were made to breed out the larvæ in the laboratory, but these failed. A simple field method was therefore adopted. The apparatus (Fig. 1) consisted of a wooden frame 2 feet high by 1 foot square, the bottom half as well as the base was covered with wire netting of a mesh fine enough to prevent larvæ from passing through. The upper half was covered with a piece of mosquito netting drawn into a sleeve on one side to allow of access into the interior. This was placed in the pond, a number of larvæ introduced and allowed to breed out under more or less natural conditions. This proved highly successful. In the brackish water mentioned above, the adults bred out showed a higher percentage of *A. sundaicus* which confirmed the lack of confidence in larval identification.

FIG. 1.



Lalor (1912*a*) remarks that *A. annularis* was the most numerous species. He must obviously have been referring to the potential vectors. It will be seen from Table V that *A. annularis* comprises 12·4 per cent of the species identified during the dry months in which this survey was carried out with 7·4 per cent of *A. sundaicus*. However, in view of the preceding remarks regarding the larval identification of *A. subpictus* and *A. sundaicus*, the true figure for the latter will probably lie between 7·4 and 30·2 per cent and may in fact be much higher than the figure given for *A. annularis*. It is submitted that during the rains these figures will increase proportionately.

Referring to the rainfall and tide tables it is important to note that the high tides occur during the months June to September, which correspond with the maximum rainfall. The author has noticed in other areas where pools of water, separated from the sea by complete bunds, were nevertheless brackish owing to seepage through the bunds. It is therefore distinctly possible owing to the coincident times of highest rainfall and high tides that breeding places for *A. sundaicus* will be quite excessive within the bund for the above reason, and in the low-lying areas to the south around Ngalapwe this tendency will be enhanced.

In the Zainchaung area on the other side of Ngalapwe Creek, where there is no bund protection, the salinification of the fresh water by the sea water permeating inland along the *chaungs* will undoubtedly take place, again giving numerous breeding places for *A. sundaicus*.

VECTOR SPECIES.

Lalor as a result of dissection concluded that *A. annularis* was the vector. The sporozoite rates for *A. annularis* and *A. sundaicus* are given in Table VI.

TABLE VI.

Sporozoite rates for A. annularis *and* A. sundaicus *in* 1912.

Species.	Date of dissection.	Number dissected.	Results.
A. annularis ..	Jan. 23–Oct. 15	562	Sporozoites in two.
A. annularis ...	Dec. 15–Feb. 27	31	Negative.
A. annularis .	Mar. 29–May 10	148	Sporozoites in three.
A. sundaicus .	Jan. 15–Oct. 15	110	Negative.
A. sundaicus .	Dec., Feb.	229	,,
A. sundaicus ..	Mar.–May	71	,,

These results are of fundamental importance as indicating that *A. sundaicus*, a confirmed carrier in most other areas, was not the vector in Kyaukpyu. In

order to corroborate these findings, a number of night catches were undertaken with a view to obtaining anophelines for dissection, however the number caught was so small that dissection would give no indication of the true position, in addition to which the survey had to be carried out in the earlier dry months and not during or just prior to the malaria season.

Since it was not possible to carry out dissections for the sporozoite rate, it was decided to try to obtain indirect evidence to confirm Lalor's findings. The map of Ramree Island was studied to find villages where there was little possibility of *A. sundaicus* breeding and where the vector would only be a fresh water breeder. Villages surrounded by paddy and open land were sought in view of Lalor's recommendation that paddy cultivation be discontinued because the fields offered ideal breeding grounds for *A. annularis*. Three villages meeting these requirements were selected.

Pyinseikki (Map 1) is about 8 miles from Kyaukpyu and is surrounded by woodland. The paddy area is well above the *chaung* and it is not considered likely that there would be any overflow into the paddy or because of the height of any seepage. The spleen rate was 35 per cent.

Nanhpedaung (Map 1) is at least 50 feet above sea-level and situated on the western side of a hill 120 feet in height. The spleen rate was 12 per cent.

The village of Kalaba (Maps 1 and 3) is 12 miles from Kyaukpyu. It is separated from the coast by a long range of hills and has also hills surrounding it to the north and east. To the south is more open country but there is no *chaung* within 2 miles. The village itself consists of a long straggling line of huts completely surrounded with paddy or open ground which during the rains would hold much water and form ideal breeding places for *A. annularis*. The spleen rate was 15 per cent.

It was expected that these three villages, all within 12 miles of Kyaukpyu and situated among breeding areas favourable to a fresh water breeding vector, would have given much higher spleen rates. It seems inconceivable that a species carrying malaria in Kyaukpyu would not also be responsible for transmission 12 miles away.

Endemic malaria tends to decrease with an increase in the human population. This is particularly true in areas where the vector has a low sporozoite rate. Both Kalaba and Kyaukpyu are similar in respect of their areas being suitable for *A. annularis* breeding and it would be expected that the spleen rates would have been higher among a scattered rural population than in a dense urban one, because the number of anophelines per person would be greater. The very reverse was found. The spleen rates in these three villages were the lowest recorded during the whole survey.

If *A. annularis* is the only vector, or even the main carrier of malaria in Kyaukpyu, it is difficult to understand why the spleen rates in villages 12 miles away should be so low. There is no doubt that it does transmit malaria, though the sporozoite rate is low.

Comparison of the spleen rates in 1912 and 1945 (Table IV) illustrate that, with the exception of Taungyin, there has been no significant reduction. It is of interest to note that the high rates found during this survey were around Zainchaung, where it has been noted that there is a marked tendency for considerable *A. sundaicus*

breeding areas during the rains. It is also of interest to note the high spleen rate in Kompanize, within a mile of Kyaukpyu where, even during the dry season in which this survey was carried out, there was a considerable amount of *A. sundaicus* breeding. It is contended that during the rains this breeding would increase quite considerably.

From the above considerations, though of an indirect nature, the possibility remains, in spite of the negative findings of Lalor, that *A. sundaicus* is a vector in Kyaukpyu.

SUMMARY.

1. A survey of Kyaukpyu has been carried out and attention has been paid to the degree of breeding of the potential vector species.

2. It has been found that, of the potential fresh water breeding vector species, *A. annularis* was the most common.

3. The difficulty of differentiating the larvæ of *A. sundaicus* and *A. subpictus* has been noted and attention has been drawn to the possibility that the former was breeding more prolifically than *A. annularis* during the dry period in which this survey was carried out.

4. Spleen rates for the years 1912 and 1945 have been compared.

5. Indirect evidence has been put forward that *A. sundaicus* is a vector in this area.

REFERENCES.

JOLLY, G. G. (1927) ... *Report on the malaria problem at Kyaukpyu.* Official report by the Director of Public Health, Burma, Rangoon.

LALOR, N. P. O. (1912) ... *Investigation of malaria at Kyaukpyu.* Supdt., Govt. Printing, Burma, Rangoon.

Idem (1912a) ... *Spleen census report for Burma.* Supdt., Govt. Printing, Burma, Rangoon.

WILLIAMS, C. E. (1922) ... *Prevention of malaria at Wuntho and other stations in the Katha District* (MS.).

Indian Journal of Malariology, **4**, 3, September 1950.

SOME ASPECTS OF MALARIA IN JAMMU AND KASHMIR STATE.

BY

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(*Armed Forces Medical College, Poona, India.*)

[May 30, 1950.]

INTRODUCTION.

THE stationing of troops in Kashmir made it necessary to carry out a malaria survey which was commenced in the autumn of 1948 and was completed in October 1949. The results thereof are reported here.

PHYSIOGRAPHICAL FEATURES.

Jammu and Kashmir State is roughly rectangular in shape and has for its boundaries Russia and China in the north, Tibet in the east, Punjab (India) in the south and West Pakistan and Afghanistan in the west. It lies between 32° 17′ and 36° 58′ north latitudes and 73° 26′ and 80° 30′ east longitudes. The State is approximately 84,500 square miles in extent and consists of the administrative provinces of Jammu, Kashmir and Ladakh and the Jagir districts.

The entire region is mountainous with the exception of a small strip in the south which is continuous with the plains of the Punjab (India). It is traversed by four important ranges of mountains, dividing the area naturally into four regions. Between the Karakorum in the north and the Great Himalayan Range lies the north-eastern portion of the State. The central plateau is situated between the Great Himalayan Range and the Pir Panjal Range. South of Pir Panjal Range is the southern region gradually merging into the plains of the Punjab. The general direction of the mountains referred to above is east-westerly. A fourth or the western region is separated from the rest of the State by two offshoots of the Pir Panjal Range, Shurzawan and Shumshabari, both of which in general have a north-south direction. The valleys formed by the great mountains are important water sheds of the rivers Indus, Jhelum and Chenab and their numerous tributaries.

Thus the Kashmir State consists of plains and foothill areas, the central plateau, and snow-clad ranges of hills reaching up to 22,000 feet, with valleys in

between. Consequently, the State has almost every description of climate in the world ranging from the tropical to the arctic.

The general slope of the land in the State is westerly and north-westerly in the northern areas and is towards the south in the remaining areas.

METEOROLOGICAL CONDITIONS.

The plains and the foothills of the south have extremes of climate, being very hot in the summer and cold in the winter. The central plateau has a temperate climate during the greater part of the year with a cold winter. The deep narrow valleys of Jhelum and Kishenganga in the west are very hot and humid while the northern region of Ladakh is extremely cold. Dras in the north is claimed to be the second coldest inhabited place in the world with minimum temperatures reaching to 10°C. in January and February*.

The rainfall in Kashmir is varied, being the lowest in the extreme north (Leh, below 5 inches a year). The plateau receives on an average 15 to 30 inches, while the northern reaches of the same region get about 60 inches a year. The semi-mountainous area in the south and west gets about 40 inches or more during the year.

IRRIGATION AND AGRICULTURE.

The main water sheds of Kashmir, as stated above, are the middle reaches of River Indus with its tributaries in the north-east, the Jhelum and Kishenganga in the central region, and River Chenab and tributaries and River Ravi in the west. A fairly comprehensive irrigation system exists in Kashmir by means of canals from the rivers and their tributaries.

The main crops in the irrigated area are rice and wheat. In the foothill regions of the south, rice is grown in terraced plots. A certain amount of maize is grown along hill slopes which are also rich in forests of great economic value.

In Ladakh on the north, the Indus River is of little value for irrigation. A few channels from the glacial streams which join the river are utilized to grow barley on the slopes.

MAJOR DISEASES IN KASHMIR.

On the occasion of the first health conference of Jammu and Kashmir in 1949, the Chief Medical Officer of the State pointed out that epidemics of cholera, small-pox, typhus and malaria were a great source of trouble in the State, and that the last two diseases were more or less endemic. Kalra and Rao (1949) described typhus as the major epidemic disease in the State.

A perusal of hospital registers indicates that malaria morbidity is very high in most places. As was pointed out by Rao and Kalra (1949), it is possible that malaria is mistakenly diagnosed as relapsing fever and *vice versa*, since the latter had remained an unrecognized entity till February 1948.

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* Tables depicting the average monthly mean temperatures, relative humidity, and rainfall for 40 years have been kept in the files of the Malaria Institute of India for those who wish to consult them.

PLATE IX.

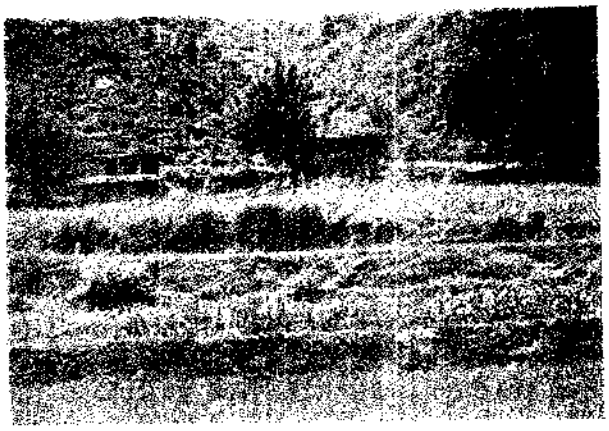

FIG. 1. A typical Kashmir hut by the side of paddy fields.

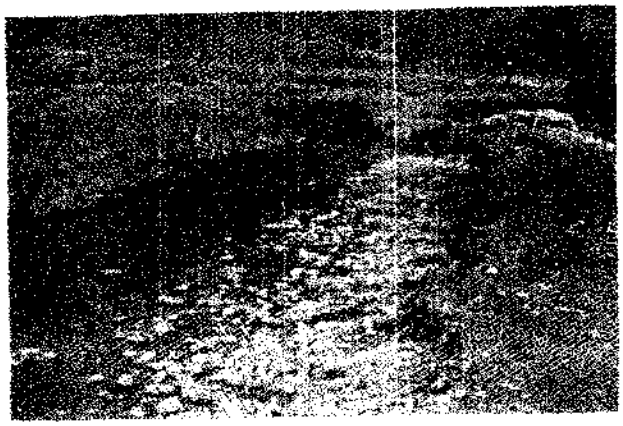

FIG. 2. Hill streams in Kashmir breeding *A. fluviatilis.*

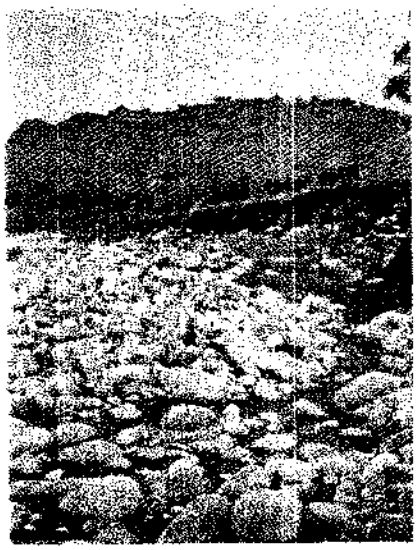

FIG. 3. Stream with large pebbles breeding *A. culicifacies.*

The State Civil Hospital at Srinagar has recorded 18 cases of malaria (microscopically diagnosed) in indoor patients and 1,797 cases (clinically diagnosed) as out-patients out of a total of 70,000 patients that attended the hospital during 1947 and 1948. The monthly figures available in the records show a definite rise in the summer months. It should also be borne in mind that Srinagar in the central plateau attracts a large number of visitors from the plains and other parts of India during the summer*.

PREVIOUS SURVEYS.

Apart from exhaustive records of the anopheline fauna of Kashmir (James, 1902; Adie, 1905; Adie and Adie, 1913; Perry, 1912; Christophers, 1931; Covell and Puri, 1936; Puri, 1948), the only available record of a malaria survey is a manuscript report of data collected in 1946. The survey was far from complete and was confined to Jammu Province and recorded an average spleen rate of 13 per cent for the whole State. A parasite rate of 3 per cent was recorded for the northern zone and 10 per cent for the central zone. While *P. falciparum* was the predominant species in the former, *P. vivax* predominated in the latter and southern zones.

Of the 1,091 specimens of *A. culicifacies* dissected in 1946, two showed gland infections and 3 oöcysts in the gut. No other species was incriminated as vector.

THE PRESENT SURVEY.

As stated above, the present survey was commenced in August 1948 and was completed late in 1949. Some of the areas surveyed were visited twice, once before the fever season, and subsequently during the height or the decline of the fever season.

SPLEEN AND PARASITE RATES.

Collection of children for examination presented considerable difficulties in most cases due to the unsettled conditions prevailing in the area. A most careful individual inquiry was necessary to fix the nativity of the children. In many cases, house-to-house visits were necessary to examine them. Table I depicts the spleen rates according to sectors and altitudes of the areas where the examinations were carried out. Map shows spleen rates recorded in a few selected zones.

ENTOMOLOGICAL DATA.

All types of habitations were searched for adult mosquitoes, and the best collections were made from discrete huts near cultivated areas with a stream or irrigation channel nearby. In most houses, animals were also present and it was not possible to differentiate collections as from human habitations and animal sheds. Plate IX, fig. 1 shows a typical Kashmir rural habitation. Larval collections were made in stream and channels near human habitations. Figs. 2 and 3 show typical breeding places of *A. fluviatilis* and *A. culicifacies* respectively.

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* Monthly hospital figures for malaria during 1947 and 1948 from different hospitals in the State are in the files of the Malaria Institute of India for reference purposes.

MAP

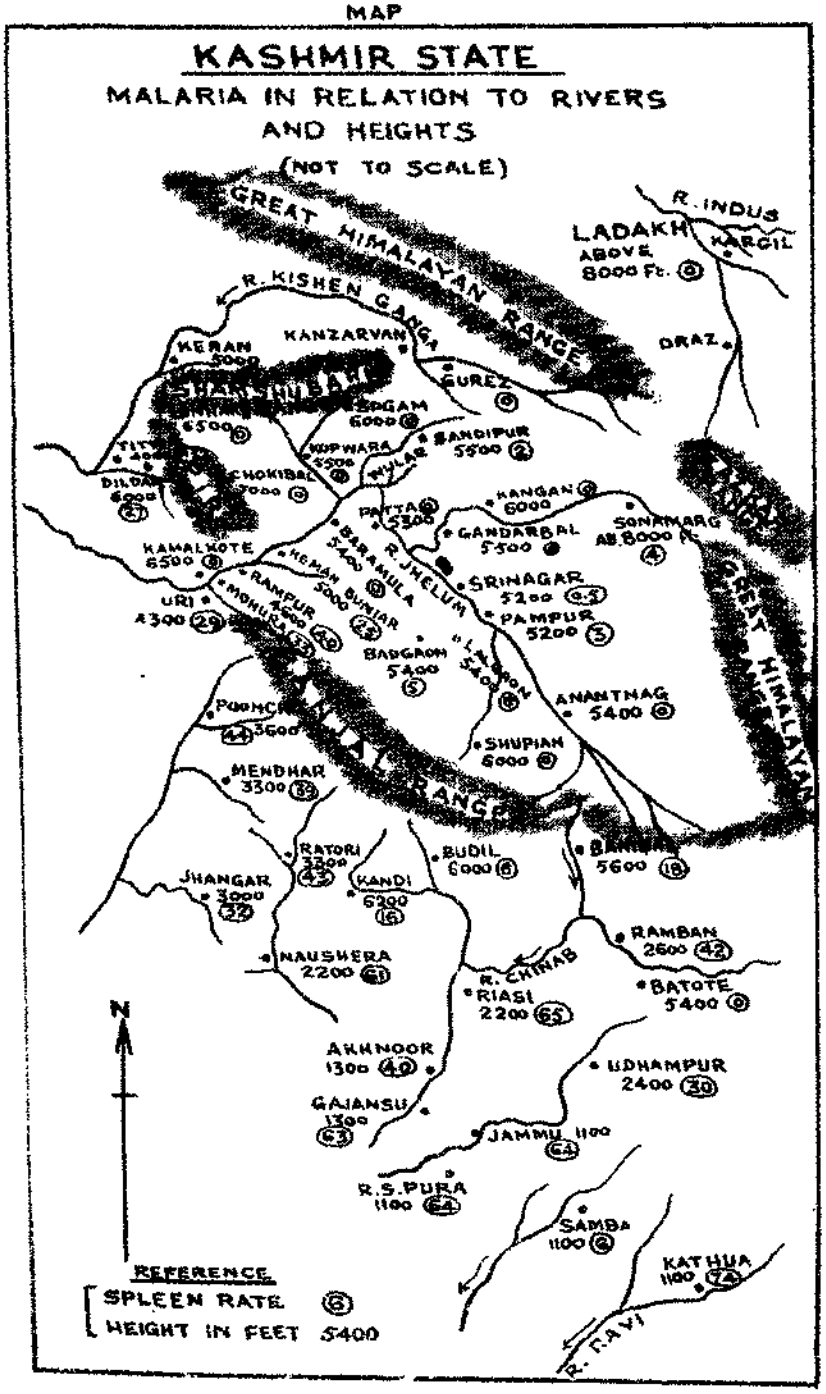
KASHMIR STATE
MALARIA IN RELATION TO RIVERS AND HEIGHTS
(NOT TO SCALE)
GREAT HIMALAYAN RANGE
R. INDUS
LADAKH ABOVE 8000 Ft.
KARGIL
DRAZ
R. KISHEN GANGA
KANZARVAN
KERAN 5000
GUREZ
SOPGAM 6000
SANDIPUR 5500
KUPWARA 5500
WULAR
CHOKIBAL 7000
DILDAR 6000
PATTA 5500
KANGAN 6000
GANDARBAL 5500
SONAMARG AB. 8000
KAMALKOTE 6500
HEMAN 5000
BARAMULA 5400
R. JHELUM
SRINAGAR 5200
PAMPUR 5200
URI 4300
RAMPUR 4500
MOHURA
BADGAM 5400
LAKROON 5400
ANANTNAG 5400
POONCH 3600
SHUPIAN 6000
MENDHAR 3300
PIR PANJAL RANGE
GREAT HIMALAYAN RANGE
RATORI 3300
KANDI 6300
BUDIL 6000
5600
JHANGAR 3000
RAMBAN 2600
NAUSHERA 2200
R. CHINAB
RIASI 2200
BATOTE 5400
N
AKHNOOR 1300
UDHAMPUR 2400
GAJANSU 1300
JAMMU 1100
R.S.PURA 1100
SAMBA 1100
KATHUA 1100
R. RAVI
REFERENCE
SPLEEN RATE
HEIGHT IN FEET 5400

TABLE I.

Spleen rates determined in Kashmir State in different places.

Sector.	Section.	Altitude above sea level in feet.	Aug.–Sep. 1948. Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Apr.–Oct. 1949. Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Villages.
Kashmir	Baramula-Khazigund	5,000–6,000	1,058	6	...	119	0	0	Khanapura, Baramula, Dilna, Pattan, Srinagar, Badgaon, Lalagaon, Pampur, Shupian, Kannabal, Anantnag, Khazigund.
	Baramula-Chokibal	5,000–6,000	105	0	0	32	0	0	Sopur, Kopuwara, Sogam.
		6,000–7,000	75	2	2	65	0	0	Lenhari, Panzgam, Chokibal.
	Ganderbal-Sonamarg	5,000–6,000	140	0	0	...	...	...	Ganderbal, Kangan.
		8,000–9,000	...	...	..	25	1	4	Sonamarg.
	Bandipur-Gurez ...	5,000–6,000	153	3	2	...	...	...	Bandipur.
		8,000–9,000	60	0	0	...	...	...	Kanzarwan, Gurez.
	Jhelum Valley ...	4,000–5,000	136	29	21	14	1	7	Naushera, Hemar Bumiar, Rampur, Mohura, Bandi, Bhraman, Uri, Jabla.
		5,000–6,000	30	1	3	...	...	...	Sherinarvaw.
		6,000–7,000	...	...	...	21	9	43	Kamalkote.

TABLE I—*concld*

Sector.	Section.	Altitude above sea level in feet.	Aug.-Sep. 1948. Number examined.	Aug.-Sep. 1948. Number with enlarged spleen.	Aug.-Sep. 1948. Spleen rate, per cent.	Apr.-Oct. 1949. Number examined.	Apr.-Oct. 1949. Number with enlarged spleen.	Apr.-Oct. 1949. Spleen rate, per cent.	Villages.
Kashmir ...	Kishenganga ...	4,000–5,000	...	...	...	32	11	34	Dringula, Gandishad, Chitrakote, Chumkote.
		5,000–6,000	...	...	...	28	6	20	Keran, Dildar, Kandipain.
	Ladakh Valley ...	9,000–11,000	...	...	...	216	...	...	Dras, Kargil, Gurgurdo, Leh.
Jammu ...	Kathua-Banihal ...	1,000–2,000	...	...	...	273	96	35	Kathua, Samba, R. S. Pura, Jammu.
		2,000–3,000	168	50	30	543	328	60	Riasi, Udhampur, Ramban.
		5,000–6,000	60	12	21	107	13	12	Batote, Banihal.
	Jammu-Poonch	1,000–2,000	...	...	...	60	33	55	Gajansu, Akhnoor.
		2,000–3,000	...	...	...	101	49	49	Naushera, Jhangar.
		3,000–4,000	...	...	...	282	133	48	Rajauri, Mendhar, Poonch.
		6,000–7,000	...	...	...	65	5	8	Kandi, Budil.

No anophelines, either adults or larvæ, were encountered in Ladakh, Sonamarg, Gurez and Kanzarwan. The deserted houses in Gulmarg were searched in August 1948 with negative results. But in the grass-covered springs in the meadows, *A. gigas* as well as *A. moghulensis* were breeding.

In the central plateau, specimens of five anopheline species (*A. annularis*, *A. splendidus*, *A. stephensi*, *A. subpictus* and *A. fluviatilis*) were collected as adults. Of the last, a single specimen was found in September 1949. *A. maculatus* was found only as larvæ.

The western portion of the State formed by the valleys of Jhelum and Kishenganga was found to be rich in anopheline fauna. In addition to the species mentioned above, specimens of *A. lindesayi*, *A. d'thali*, *A. turkhudi* and *A. culicifacies* were collected. The density of *A. fluviatilis* and *A. maculatus* was very high.

The foothill and the plains area, south of the Pir Panjal Range, were similar to the western zone in anopheline fauna. Table II depicts the details of mosquito collections according to species and altitudes at which collected.

A. culicifacies was common in the Jammu sector with a wide distribution and was found to occur up to an elevation of 3,500 feet above sea level. The density of the species was high in July to September. In a previous survey, as stated above, this was proved to be a vector in Jammu. This species was not found in the central plateau, or in the western valleys and foothills above an altitude of 4,000 feet. A few adult specimens were collected late in July and early August 1949 from Uri.

A. d'thali is recorded for the first time in Kashmir during the present survey. Three adults were collected from Rajaori and Jhangar in the Jammu Province, while 47 females and 1 male were found in Uri in the Kashmir Province. No larvæ of this species were found anywhere.

A. fluviatilis has an interesting distribution and from indirect evidence it is possible that this may be a vector in certain parts of the State. Both adults and larvæ of the species were collected in areas above 6,000 feet (Table II). Larvæ were found in a variety of breeding places, such as pebbly streams, springs, irrigation channels and terraced paddy fields. In the foothills of the south, the species was breeding intensely in May and June, while in August the larval density was low. In the foothills, west of the central plateau, the density was found to be high in July. The species does not appear to have established itself in the central plateau, as only a single specimen was found in Anantnag at the eastern limit of the zone in September 1949.

Although no adult specimens of *A. gigas* and *A. moghulensis* were found during the survey, their larvæ were found to be breeding, as already stated, in pools in Gulmarg. *A. moghulensis* is recorded in Kashmir for the first time during the present survey.

A. stephensi is more common in the south. This species was collected at Banihal which is 5,500 feet above sea level.

DISCUSSION.

Previous to the survey, little was known about the incidence of malaria in Kashmir State. It is now evident that hyperendemic malaria exists in the State,

TABLE II.

Anopheline species collected at different altitudes in Kashmir from August 1948 to October 1949.

Sector.	Altitude above sea level in feet.	*A. annularis.*	*A. culicifacies.*	*A. d'thali.*	*A. fluviatilis.*	*A. gigas.*	*A. lindesayi.*	*A. maculatus.*	*A. moghulensis.*	*A. splendidus.*	*A. subpictus.*	*A. turkhudi.*	*A. stephensi.*	REMARKS.
Kashmir	4,000–5,000	+	+	+	+	...	+	+	...	+	+	+	+	Uri, Lagama, Titwal.
	5,000–6,000	+	...	...	+	...	+	+	...	+	...	...	+	
	6,000–7,000	+	...	...	+	...	+	+	...	...	...	...	...	Tangdar, Chokibal, Kamalkote.
	8,000–9,000	...	...	...	...	+	...	...	+	...	...	...	...	Gulmarg.
Jammu ...	1,000–2,000	+	+	...	+	...	...	+	...	+	+	+	+	
	2,000–3,000	+	+	...	+	...	...	+	...	+	+	+	+	
	3,000–4,000	+	+	+	+	...	...	+	...	+	+	...	+	
	5,000–6,000	...	...	...	+	...	+	+	...	+	...	...	+	Banihal, Batote*.
	6,000–7,000	...	...	...	+	...	+	+	...	...	...	...	...	Kandi, Budil.

+ = collected.

* *A. fluviatilis* was not collected.

although its distribution is patchy. In certain areas, in September, many of the houses visited in some sectors had almost every occupant down with fever. In general, the period of transmission seems to be July, August and September.

The consensus of opinion at present is that autochthonous malaria is absent in India above an altitude of 5,000 feet. In the present survey a few children with enlarged spleens were found in villages situated much above this altitude. It was not always possible to elicit the information with certainty that malaria at these high altitudes was acquired locally.

On receipt of a report of fever cases from Kamalkote, a village situated between 6,000 and 7,000 feet above sea level facing the Jhelum Valley, blood smears were obtained from four individuals suffering from fever. Much to the author's surprise, all the four showed *P. vivax*. Subsequently, a spleen and parasite examination of children of the village revealed a spleen rate of 43 per cent (21 children examined) and a parasite rate of 35 per cent (17 blood films examined). *P. vivax* was the only species found.

In view of the fact that Kamalkote is about 6,000 feet high, great care was taken to eliminate the possibility of members of the population having immigrated into the village recently. The most careful questioning revealed that the population examined had been static in Kamalkote for years, and especially the children had not seen any place outside Kamalkote. Thus under the circumstances it is concluded that the infection met with at this high altitude is autochthonous. Contrary to expectations, the place was hot and humid during the visit. During the fever season, specimens of *A. fluviatilis* were found in the houses.

In Banihal, situated between 5,500 and 6,000 feet above sea level, 12 out of 60 (20 per cent) children examined were found to have enlarged spleens.

The central zone or the Kashmir plateau was found to be non-malarious and only 6 out of 1,314 children examined in various sectors of the plateau had enlarged spleens. As stated previously, the plateau is a very popular tourist resort and attracts a large number of visitors from outside the State every year.

No mosquito dissections were carried out during the present survey. As mentioned previously, *A. culicifacies* was proved as the vector in Jammu area. During the present survey, this species was not encountered at heights above 4,000 feet. If malaria found above this elevation is autochthonous it raises the question of vectors in these localities. At Budil, Kandi, Banihal and Kamalkote along the Jhelum and Kishenganga valleys, all situated between 5,500 and 6,500 feet above sea level, a certain amount of malaria probably autochthonous was found. The only mosquito species found in any appreciable density in these areas was *A. fluviatilis*. *A. culicifacies* was absent in these villages. The former, which is an efficient vector in the foothills of South India, has not been recorded to be breeding at altitudes higher than 4,000 feet above sea level (Russell and Jacob, 1942). The presence of this species in abundance at altitudes higher than 4,000 feet, up to which *A. culicifacies* is the vector, strongly suggests it as a possible vector at higher altitudes in Kashmir.

SUMMARY.

The results are reported of a malaria reconnaissance survey of Kashmir State carried out in 1948 and 1949.

From the data collected it appeared that malaria in Kashmir State is endemic as well as hyperendemic and is of a patchy distribution.

The central zone or the Kashmir plateau was found to be free from malaria. Certain amount of evidence is presented to indicate that malaria transmission may be possible at altitudes over 6,000 feet.

The possibility of *A. fluviatilis* being the vector at high altitudes in Kashmir is discussed.

Two species of mosquitoes, *A. d'thali* and *A. moghulensis*, have been recorded from Kashmir for the first time.

ACKNOWLEDGMENTS.

The author is grateful to Brigadiers B. M. Rao and S. Narain who initiated the survey and afforded the opportunity to do it.

His thanks are also due to Major S. L. Kalra of the typhus team for his help and encouragement throughout the survey, and to the civil medical officers of the State for their co-operation.

REFERENCES.

ADIE, J. R. (1905) ... *Ind. Med. Gaz.*, **40**, pp. 5–12.
ADIE, J. R., and MRS. ADIE, J. R. (1913). *Ibid.*, **48**, pp. 341–342.
CHRISTOPHERS, S. R. (1931) ... *Rec. Mal. Surv. Ind.*, **4**, pp. 305–332.
COVELL, G., and PURI, I. M. (1936). *Health Bulletin, No.* 17 (*Malaria Bureau*, No. 8).
JAMES, S. P. (1902) ... ... *Sc. Mem. Govt. India*, No. 2.
KALRA, S. L., and RAO, K. N. A. (1949). *Ind. J. Med. Res.*, **37**, pp. 395–400.
PERRY, E. L. (1913) ... Distribution of Anopheles in the Punjab (MS.).
PURI, I. M. (1948) ... *Ind. J. Mal.*, **2**, pp. 67–107.
RAO, K. N. A., and KALRA, S. L. (1949). *Ind. J. Med. Res.*, **37**, pp. 385–394.
RUSSELL, P. F., and JACOB, V. P. (1942). *J. Mal. Inst. Ind.*, **4**, pp. 349–392.
Report of malaria survey by the Epidemiology Dept., Jammu (1946) (MS.).

Indian Journal of Malariology, **4**, 3, September 1950.

RAIL TRANSPORTATION OF MOSQUITOES.*

BY

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[July 8, 1950.]

RAIL transportation of mosquitoes has been referred to by several workers. Sergent once watched a specimen of *A. maculipennis* in his compartment during a journey all the way from Algiers to Oran (personal communication). The Medical Research Institute, Federated Malay States (Report for 1928) recorded the results of searching trains from Singapore and Penang for *A. maculatus*, to account for an outbreak of malaria in the brickfields area at Kuala Lumpur, a most carefully controlled locality. The trains were searched at the carriage sidings near the brickfields after the passengers had alighted at the main station, and in consequence of the disturbance caused by this, the searches were not very fruitful. The cause was subsequently found to be local, due to the fact that *A. hyrcanus* was not then suspected of being a vector (Report, Med. Res. Inst., 1929, *vide* Ann. Rept., Mal. Advisory Board, 1929).

Senior White (1937) and Sen (1941) have given an account of train importation of *A. sundaicus* into Calcutta, many of the specimens being found infected.

Vel'tishchev (1943) has described the capture of *A. pulcherrimus* in the station of Kazalinsk (Turkestan), and the capture of a few *A. superpictus* at various points along the Trans-Caspian Railway, and attributes these findings to accidental importation by train.

Wilson (1946) has briefly referred to the danger of the train importation of *A. gambiæ* into MacKinnon Road Station on the Kenya-Uganda Railway. The subject of the transportation of three species of *Glossina* by trains of that

* The authors wish to record their thanks to the Chief Medical Officer of the Bengal-Nagpur Railway for permission to publish this paper.

railway into stock-farming areas beyond the fly-belt has recently been dealt with at length by Lewis (1950). Lewis' observations on the very much larger numbers which are transported by goods rather than passenger trains open up a new aspect of the matter under consideration.

Ayroza Galvao (1948) has given some very interesting data from South America. He quotes Levi-Castillo (1944) as describing the frequent transportation of *A. pseudo-punctipennis*, *A. albimanus* and *A. punctimacula* as larvæ in rail-transported water-containers in Ecuador.* He quotes Coutinho and Ferraz (1946) on the capture of 595 Anopheles sp. (among them three *A. darlingi*, one of which was infected) inside night trains in the Brazilian State of Minas Gerais: also Deane (1947) as finding *A. darlingi* in trains in Amazonia.

As a routine measure in checking mosquito level, weekly catches have for many years been made in various catching stations within the general mosquito control of the Bengal-Nagpur Railway at Khargpur (West Bengal), Adra (South Bihar) and Tatanagar (Chota Nagpur Division, Bihar). In each case, one of the catching stations is the station waiting room, and the differences between the waiting room and the other catching station results are striking. In every case the railway station is situated at the centre of the controlled area, and thus its mosquito population should be the lowest of any catching station. Table I shows that, in respect of many species, it is not. Table II shows that, in waiting room catches, there are a number of species found that are not represented in the catches from the other local catching stations. The only explanation that can be advanced for these phenomena is that numerous mosquitoes are brought into the areas by train, and, disturbed by passenger movements, take refuge in the nearest suitable shelter, the station buildings. The figures cover four years of weekly catches. Only common species are listed in Table I. Those present in waiting rooms in large numbers are :—

A. culicifacies, *annularis*, *C. fatigans*, *vishnui*, *tritæniorhynchus* and *gelidus*, and all are mostly pool breeders.

Turning now to Table II it will be seen that the total catches include thirty-nine species of which only eight have been captured in the waiting rooms. The four anophelines in this list are species of hilly country and are most unlikely to breed in the vicinity of any of the three stations considered.

The capture of a single ♀ of *Theobaldia longiareolata* at Khargpur is inexplicable The nearest recorded locality to Khargpur for this species is Naini Tal (7,000 ft.) (Barraud, 1934) in the Western Himalayas. Naini Tal is some ten miles from the rail head at Kathgodam. Kathgodam is on a metre gauge line, whereas all the three railway stations considered in this paper are on the Indian standard (5'-6")

* Coastal Ecuador appears to be largely desert. West of the Andean, the streams originating from the snows are used for irrigation (and produce malaria) in the upper parts of their courses, but frequently do not reach the sea for months together. This would explain the train transportation of water (and larvæ) to the coast. Train transportation of water also occurs in India in the dry season from temporary pumping stations established near permanent rivers to stations and track gang huts, the wells of which often run dry. It has never occurred to the authors to check whether larvæ are transported by this procedure. Even if they are, and if they survive, the disturbance in the carriers (400 gal. G. I. tanks in India) and the low saturation deficiency into which the adults would emerge, precludes any fear of their living to transmit malaria. The position may be different on the Coast of Ecuador, where the sea fog caused by the meeting of the hot air of the land and the cold waters of the Humboldt current may possibly raise saturation deficiency to transmission level.

gauge lines. No possibility of direct train importation therefore exists. Even on the assumption that the specimen changed trains at the gauge junction at Bareilly, there is no through passenger train between Bareilly and Khargpur. Had the specimen made the journey in a goods wagon, the goods station at Khargpur is not adjacent to the passenger station, and if disturbed by unloading of the wagon, there are much closer shelters available than the passenger station. How otherwise did this specimen make the journey, which is nearly 680 miles in a straight line from Naini Tal area? The event occurred in 1934, so aeroplane transportation can be ruled out. Wind-borne or train transportation from a nearer locality can be invoked as one of the explanations.

SUMMARY AND CONCLUSIONS.

Certain species of mosquitoes are transported by trains in significant numbers. These include several malaria vectors (two of which were actually found infected in trains) and a filariasis vector. At the three Indian railway stations seven species which could only have been train-imported were found in four years. The capture of an eighth species many hundred miles from its nearest known breeding ground is recorded and discussed.

REFERENCES.

AYROZA GALVÃO, A. L. (1948) ... *Proc. 4th. Int. Cong. Trop. Med. Mal.*, **1**, p. 656, Washington.

BARRAUD, P. J. (1934) ... *Fauna Brit. India. Dipt.*, **5**, p. 91. Taylor and Francis, London.

COUTINHO, J. O., and FERRAZ, D. (1946). *Rev. Bras. Med.*, **3**, p. 277.

DEANE, L. M. (1947) ... *Rev. Serv. Espec. Saude Publica*, **1**, pp. 3–60.

LEVI-CASTILLO, R. (1944) ... *Biol. Abstracts*, **21**, p. 494.

LEWIS, E. A. (1950) ... *Bull. Ent. Res.*, **40**, pp. 511–534.

MALARIA ADVISORY BOARD, FEDERATED MALAY STATES, (1930). *Annual Report*, 1929. Govt. Press, Kuala Lumpur.

MEDICAL RESEARCH INSTITUTE, FEDERATED MALAY STATES, (1929). *Annual Report*, 1928. Govt. Press, Kuala Lumpur.

Idem (1930) ... *Annual Report*, 1929. Govt. Press, Kuala Lumpur.

SEN, P. (1941) ... ... *J. Bombay Nat. His. Soc.*, **42**, p. 593.

SENIOR WHITE, R. (1937) ... *Ind. Med. Gaz.*, **72**, pp. 307–312.

VEL'TISHCHEV, P. A. (1943) ... English translation in *Rev. App. Ent.* (1947), **35**, part B, p. 111.

WILSON, D. B. (1946) ... *East Africa Med. J.*, **23**, pp. 258–272.

TABLE I.

Female catches in waiting rooms compared with average catches in other catching stations.
(Number of latter in brackets.)

♀♀ only 4 yrs. figures combined.	Waiting rooms.			Average of other catching stations.			4 Years.		
	Khargpur.	Adra.	Tatanagar.	Khargpur (11).	Adra (3).	Tatanagar (3).	Waiting room total.	Average others total.	Waiting room per cent compared to others.
A. subpictus ...	194	240	337	148	643	163	771	954	−12·4
A. vagus	86	113	117	44	293	69	316	306	+ 3·4
A. culicifacies ...	...	15	85	...	31	14	100	45	+45·0
A. annularis ...	...	...	...	5	53	...	0	58	− 0·0
C. fatigans ...	488	196	635	204	389	267	1,319	860	+34·8
C. vishnui ...	212	47	57	68	110	15	316	193	+38·9
C. tritæniorhynchus ...	218	24	20	88	71	6	262	165	+37·1
C. gelidus	36	9	...	10	25	...	45	35	+22·2
C. bitæniorhynchus ...	...	12	...	...	37	...	12	37	−59·2

TABLE II.

Species taken in all catching stations at Kharagpur, Adra and Tatanagar.

	Total.			Total.	
	♂	♀		♂	♀
A. hyrcanus ..	...	...	*Fic. hybrida* ...	...	...
A. barbirostris	...	...	*Aed. piperatatus* ...	...	...
A. subpictus ..	...	...	*Aed. pallidostriatus* ...	...	...
A. vagus	...	...	*Steg. fasciata* ..	...	...
A. culicifacies	..	..	*Arm. obturbans* ...	...	...
A. varuna ...	..	...	*Aedom. venustipes* + ...	0	2
A. minimus +	0	1	*Bank. lineatopennis* + ...	0	1
A. aconitus ...	.	.	*Lutz. fuscanus* ..	...	...
A. jessellatus ..	.	...	*Culex epidesmus* + ...	0	1
A. splendidus + ..	0	1	*Culex fatigans* ...	...	...
A. stephensi ...	..	.	*Culex cisloni* ..	...	...
A. maculatus + ..	1	0	*Culex tritaeniorhynchus*	...	...
A. karwari +	0	1	*Culex bitaeniorhynchus* ...	...	...
A. ramsayi ...	..	..	*Culex gelidus* ...	...	...
A. annularis	...	...	*Culex whitmorei* ...	...	...
A. pallidus ..	...	...	*Culex fuscocephalus* ...	...	...
A. philippinensis ..	...	...	*Loph.* sp.	...	...
Man. annuliferus ...	...	...	*Loph. minutissimus* ...	...	...
Man. uniformis ..	..	...	*Theo. longiareolata* + ...	0	1
Man. indiana ..	..	...			

+ denotes species only taken in a waiting room at any of these stations.

Indian Journal of Malariology, **4**, 3, *September 1950*.

SOME CONSIDERATIONS ON INDOOR RESIDUAL SPRAYING FOR MALARIA CONTROL IN RURAL INDIA.

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[July 10, 1950.]

INTRODUCTION.

THERE has never been a malaria control programme easier to promote, finance and execute than the residual spraying of dwellings. Where such programmes are properly employed, in most cases significant and immediate reduction of malaria morbidity is being obtained.

Nevertheless, the technical problems of this new approach are far from solved. Research on specific problems is best met in the field where knowledge of the area, biology of the mosquito vector and of local conditions, play an important rôle in guiding the study. The purpose of this report is to direct attention to problems inherent in the use of residual sprays in India.

A survey of about 1,000 rural houses in South India revealed 88 per cent having mud or clay wall construction with thatch roof (Russell and Menon, 1942). An average home usually occupied by six persons has two rooms and about 1,000 sq. ft. of surface to be treated. Wall openings for doors and windows are kept to a minimum and natural illumination is poor. The wall construction is quite similar in many respects to abodes found in tropical America and may be classified into three general types: (1) the 'Pipse' or 'mud wall' formed of balls of clay laid into a thick wall with doorways and windows framed with bamboo; (2) the 'Jhatimati' or 'wattle wall' framed with bamboo posts between which is secured split bamboo or 'coppice' and the exterior and interior surface plastered to a thickness

of 1 inch with clay ; and (3) walls made of bamboo posts supporting straw or grass matting which is covered with mud plaster. Upon drying, these walls crack and it is customary to surface the wall with mud, clay or ' cowdung-clay ' plaster. This plastering is renewed as needed and when it becomes too thick, is removed completely before replastering. Surface treatment with whitewash or lime is not too common in rural India. The roofing is almost always thatch supported on bamboo frames or matting. Rice straw and local grasses such as ' Bena ' and ' Inkar ' are more common than palm leaves. The walls are about 7 feet high and the ridge of the roof may extend some 3 to 4 feet higher with a 1 to 1½ slope to the overhanging eave. The thatch is normally replaced each year just before the rainy season.

Most rural homes have no ceiling, save a rough flooring or planks to facilitate overhead storage. Cooking of food is done indoors over an open fire, the smoke being allowed to drift through the thatch roof and wall openings. The thatch soon becomes black with greasy carbon deposits and normally contains many spiderwebs.

The difficulty of obtaining a uniform deposit of D.D.T. on the above described surfaces is quite apparent. A spray solution capable of wetting clay wall surfaces would most likely run or drip off the non-wetting surface of the thatch. The lack of natural lighting inside the house, coupled with the difficulty of seeing where spray is applied on the thatch, would require more supervision than is feasible for a programme of this type.

Early in the use of residual insecticides, many workers reported that the plain clay wall was unable to retain an effective residual deposit while the palm and grass thatch appeared to maintain effective deposit for several weeks depending upon the dosage of D.D.T. Much speculation as to the cause of the rapid deterioration on clay surfaces included possible chemical deterioration or combination of D.D.T. with the elements in the clay. Later work by Cutkomp (1947) and Maier *et al.* (1948) seems to favour the theory of physical absorption of solution and emulsions of D.D.T. by porous clay surfaces which made the insecticide unavailable to the mosquito. The latter theory is confirmed by the use of various sizing or covering materials, such as whitewash, decreasing permeability and resulting in much longer residual effect. Further it was found by several investigators (Puri and Krishnaswami, 1947 ; Maier *et al.*, 1948 ; Sundararaman and Pefly, 1949) and from our own experience, that suspensions of water-wettable D.D.T. are far superior to emulsions or solutions when applied to mud or clay surfaces.

In India, however, emulsions are used predominantly and dosages employed are usually below 100 mg. per sq. ft. with expected duration of a half to two months, whereas in the United States dosages greater than 100 mg. per sq. ft. are used and emulsions generally give complete protection for three to four months. Reasons for the difference in experience in the United States and in India are to be sought in the different types of houses encountered. Poor housing in the United States is mostly constructed of lumber with walls and ceiling either uncovered, papered or painted, while the most characteristic building materials for rural India are mud and thatch.

For the most part, experiments have been concentrated on the use of D.D.T. as the most impressive residual insecticide. A few workers have been making

preliminary tests on clay surfaces with chlordane and benzene hexachloride. Metcalf and Wilson (1945) pointed out the residual toxicity of pyrethrins and suggested its use for surface treatment of dwellings. The cost of pyrethrins in the United States has limited its use in this manner although it still remains the most effective ingredient of space sprays. However, since India is one of the principal producers of pyrethrum, investigations of any locally obtainable insecticide should be considered before adopting D.D.T. as the only insecticide economically justified. Pyrethrins are light-labile, but it was thought that the absence of daylight in the typical rural house would justify trials of various formulations with some synergist to keep the dosage to a minimum.

Laboratory investigations were therefore undertaken to determine what practical measures could be suggested to enhance the residual effect on clay wall surfaces, to investigate the possibilities of locally produced pyrethrum formulation as a substitute for D.D.T. and to determine what effect the smoking of the thatch has upon the residual toxicity of the insecticide.

METHOD AND MATERIAL.

Preparation of test panels.—In laboratory studies plywood or masonite panels 6 × 7 inches were made. Soil from the brickyard in Baltimore (Maryland, U.S.A.) was moulded on to the surface of these panels to a thickness of about 1/2 inch and 6 × 6 inches surface area. An analysis of the soil was made; it was found to be clay 46 per cent, sand 29 per cent, silt 29 per cent, liquid limit 26 per cent, plastic limit 26 per cent and shrinkage 17 per cent. These panels were allowed to dry thoroughly. In cases where clay panels were surface-treated, sufficient time was allowed for thorough drying before applying the insecticide. Simulated thatch was prepared from dried strands of rice straw. The straw was secured on the plywood panels in mats of 1/2 inch thickness by narrow strips of cardboard tacked at both ends of the board. The ends of the thatch were trimmed to measure a 6 × 6 inches surface area.

Mode of spraying insecticide.—The insecticide required for each panel was weighed separately and made up to a volume of 2 to 4 ml. of diluent which was then applied to the test panel with a small sprayer. An effort was made to obtain a fairly uniform coverage of the panel with the dosage used.

General procedure.—The tests had to be conducted under room temperature and humidity conditions with insectary-reared *Anopheles quadrimaculatus*. All results were corrected for controls by the method suggested by Fay *et al.* (1947):

$$\text{Mortality per cent} = \frac{D - E}{T - E} \times 100$$

Where D = number of mosquitoes dead in test.

T = total number of mosquitoes used in test.

E = number of mosquitoes dead in control of size T.

The tests were run with as many replicates as the insectary colony could supply. At times six replicates of about 20 individuals could be run and never less than 2 replicates per panel were used. Efforts were made to check the residual effect each week. Experiments were repeated when mortality in controls was significantly high.

The handling of mosquitoes was kept to a minimum. About 30 pupæ were allowed to emerge into a glass lamp chimney cage. The mosquitoes were allowed to age for 3 to 4 days after emergency and were fed continuously on a mixture of honey and water. Both sexes were represented in each batch*. These mosquitoes were anæsthetized in a gentle stream of CO_2, collected on a clean card and transferred under a clean petri dish cover (90 mm. diameter). About 15 to 20 minutes were allowed for recovery from CO_2 before the specimens were exposed to the surface of the test panels. After a 30-minute exposure time, the panels were inverted and the mosquitoes again anæsthetized with a gentle stream of CO_2 and collected in the dish and transferred gently to clean fine wire mesh cages before they could recover. The mosquitoes were fed water and honey mixtures and mortality recorded at the end of 24 hours. Confining mosquitoes for long periods of time to insecticide residues often bears little relationship to field exposure. Repeated experiments (Metcalf *et al.*, 1945) have shown considerable repellency of D.D.T.-treated surfaces. After a resting time of 15 to 30 minutes, depending upon the age of the residue, *A. quadrimaculatus* becomes restless and movements are unnaturally frequent. It was, therefore, thought best to limit the exposure to 30 minutes to more closely approximate the reaction pattern anticipated in the field.

Insecticidal materials.—For preliminary study, a number of conventional insecticidal formulations were employed and from the experience gained it appeared that comprehensive testing should be limited to the water-wettable powders. Geigy Gesarol†, a 50 per cent D.D.T. wettable powder, is available in India and was employed for study of D.D.T. residues. Several water-wettable pyrethrum powders containing different synergists were first screened for effectiveness on clay panels. Most of these preparations were made up by the U. S. Industrial Chemicals‡ under the name of 'pyrenone'. One compound, pyrenone T-194A, proved to be the most economical and was selected for extensive testing. Pyrenone T-194A is a wettable powder containing 10 per cent piperonyl butoxide synergist and 0·5 per cent pyrethrins.

COMPARATIVE RESIDUAL TOXICITY OF D.D.T. AND PYRETHRUM FORMULATIONS ON AIR-DRIED CLAY WITH VARIOUS SURFACE TREATMENTS.

Any material suggested for surface treatment of clay or mud walls should be cheap, easy to apply and readily available in rural India. Three coating materials were selected; waste starch from boiling rice, coconut charcoal from grinding burnt shells, and cowdung. The starch was applied in three coatings with a 7 per cent w/v solution. The powdered coconut charcoal was rubbed on to the wetted surface of the clay at a rate of 2 grammes per sq. ft. The cowdung was mixed

* The resistance of male and female mosquitoes to insecticides is variable and, therefore, for such tests only female mosquitoes are generally used; if the proportion of the female and male mosquitoes in each batch is not constant, the results are not comparable.—*Editor.*

† Supplied by the Geigy Co., Inc., New York.

‡ U. S. Industrial Chemicals Inc., Technical Development Laboratories, Baltimore, Maryland.

with water and plastered over the surface of the clay to about 1/4 inch thickness in the customary manner employed in some houses in India. On some of the panels, powdered coconut charcoal was mixed with cowdung before plastering.

Suspensions of water-wettable D.D.T. were applied at a rate of 100 mg. D.D.T./sq. ft. to one set of panels and residual testing was inaugurated one week later. The results of these experiments have been summarized in Table I and in Chart 1. It may be seen that with D.D.T. suspensions on plain clay the deterioration of toxicity is progressive with no significant effectiveness remaining after the third week. It should be noted too, that emulsions of D.D.T. on plain clay were never effective, even a day after spraying*. Clay panels surfaced with starch, cowdung or cowdung-carbon delayed the deterioration of the residual effect. Coconut charcoal did not enhance the residual effect as compared to plain clay and the tests conducted seem to indicate the carbon is detrimental to the toxicity.

Three weeks after spraying there was very little significant difference between the residual effects on starch, cowdung and cowdung-carbon surface coatings. At the end of the first month, the cowdung surface began to deteriorate while the starch and cowdung-carbon surfaced panels continued to maintain a good residual up to the 6th week after spraying. While the residual duration appeared to be somewhat larger on the cowdung-carbon coating, all panels lost toxicity rapidly often at the end of two months. The very rapid breakdown of surface treatments after a period of good residual may be explained in part by flaking, although care was exercised in handling panels. Generally, the surface coatings of starch, and cowdung-carbon compared favourably with the standard glass panel treated with D.D.T. emulsion at 100 mg./sq. ft. One can only speculate at this stage about the action of cowdung and coconut charcoal, neither of which are observed to be toxic independently. Perhaps here is a good combination of cheap materials which effectively check the physical penetration or absorption of the insecticide.

Were it economically possible to compare pyrethrins with D.D.T. on an equal weight basis (i.e., 100 mg./sq. ft.) there would be no doubt of the outstanding usefulness of pyrethrins as a residual insecticide. In an effort to approach a cost comparison, the application of water-wettable pyrenone was limited to 10 mg. pyrethrins and 200 mg. of piperonyl butoxide/sq. ft. of surface. These panels were kept well protected from daylight, but no special effort was made to keep the surface free of normal dust deposits occurring in the room. The results of these tests are given in Table II and in Chart 2. On most of the surfaces the pyrethrum preparations were not as effective as the D.D.T. One surface treatment, the cowdung and carbon, compared favourably with D.D.T. It should be noted that one panel surfaced with cowdung-carbon lost toxicity unusually early because of inadvertent exposure to light during one day. Where cowdung-carbon panels were kept in comparatively dark storage they remained significantly toxic up to 9 weeks. A similar panel stored in light lost all residual effectiveness at the second week.

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* According to some workers (Afridi and Bhatia, 1947; Puri and Pal, 1947; Viswanathan and Rao, 1947) emulsions have proved effective on clay surfaces for periods extending to a few weeks.—*Editor.*

TABLE I.

Average 24-hour percentage mortality of Anopheles quadrimaculatus *adults after a 30-minute exposure to residues of 100 mg. D.D.T./sq. ft. on air-dried clay with various surface treatments, by age of residual.*

Surface material.	Time in weeks after spraying.											
	1	2	3	4	5	6	7	8	9	10	11	12
Glass surface, D.D.T. emulsion.	100	82	84	80	72	68	65	60	57	52	50	49
Plain clay surface, D.D.T. emulsion.	15	3	0	...	...	...	...	...	...	...	...	...
D.D.T. suspension (water wettable).	90	59	47	19	20	2	0	...	...	...	...	...
Suspension of water-wettable D.D.T. on :—												
Starch-surfaced clay ...	100	100	100	100	80	66	46	35	15	3	0	...
Cowdung-surfaced clay	100	88	82	65	40	40	36	22	11	0	...	...
Carbon-surfaced clay ...	100	75	5	0	...	...	...	...	...	...	...	...
Cowdung and carbon-surfaced clay.	82	95	85	82	80	82	80	78	20	15	2	5

CHART I.

Mortality of Anopheles quadrimaculatus *adults after a 30-minute exposure to residues of 100 mg. D.D.T./sq. ft. of known age on air-dried clay with various surface treatments.*

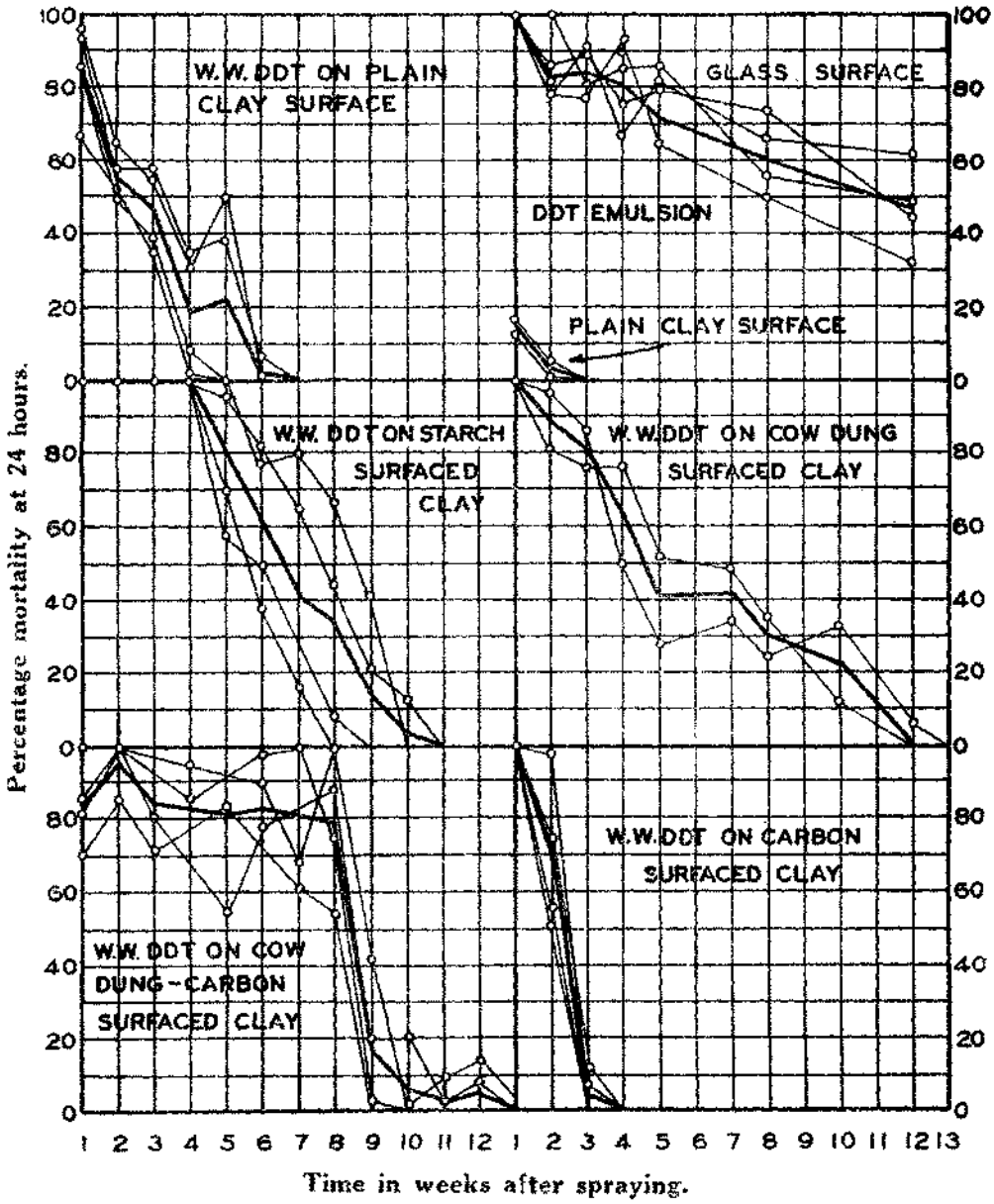


TABLE II.

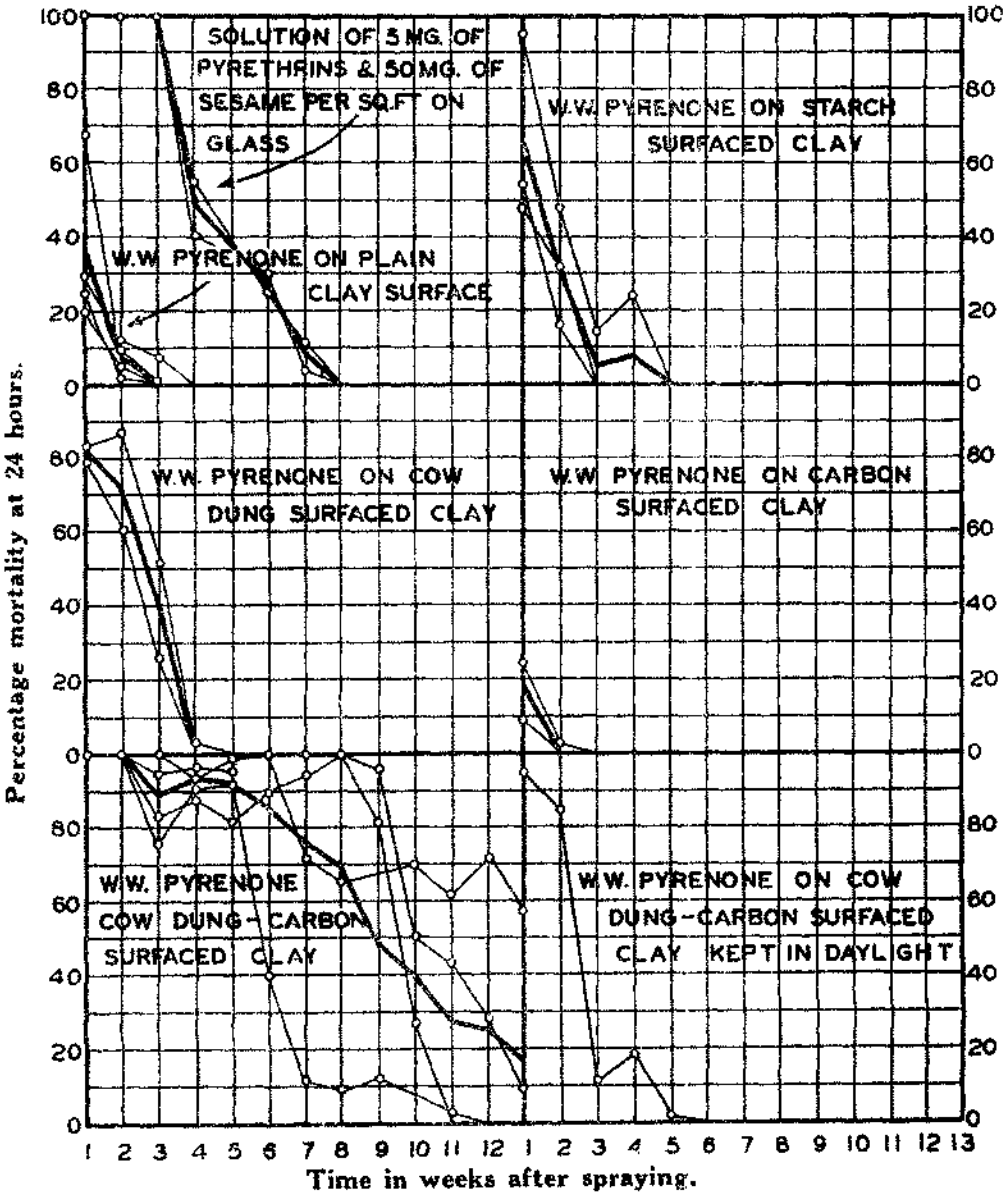
Average 24-hour percentage mortality of Anopheles quadrimaculatus *adults after a 30-minute exposure to residues of water-wettable pyrenone applied at 10 mg. pyrethrins and 20 mg. piperonyl butoxide/sq. ft. to air-dried clay surfaces, by age of residue film*.*

Surface material.	Time in weeks after spraying.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Plain clay surface	35	10	0	...	...	...	...	...	...	...	...	...	...
Starch-surfaced clay	65	32	5	8	0	...	...	...	...	...	...	...	...
Carbon-surfaced clay	18	2	0	...	...	...	...	...	...	...	...	...	...
Cowdung-surfaced clay	81	72	40	3	0	...	...	...	...	...	...	...	...
Cowdung and carbon-surfaced clay ...	100	100	90	93	92	87	75	70	48	40	27	25	18
Ditto (kept in daylight)	95	85	11	10	2	0	...	...	...	...	...	...	...

*All test panels kept in dark unless otherwise noted.

CHART 2.

Mortality of Anopheles quadrimaculatus *adults after a 30-minute exposure to residues of 10 mg./sq. ft. of pyrethrins and 200 mg./sq. ft. of piperonyl butoxide of known age on air-dried clay surfaces*.*



* Kept in dark unless noted.

RELATIONSHIP OF SMOKE DEPOSITS TO THE RESIDUAL TOXICITY OF D.D.T. ON STRAW THATCH.

Several investigators (Maier *et al.*, 1948; Sundararaman and Peffly, 1949) have shown the superior ability of D.D.T.-sprayed roof thatching materials to retain an effective toxicity to anopheline mosquitoes for long periods of time varying from 15 to 20 weeks. In these laboratory experiments the thatching materials were not subjected to the smoke from the cooking fire which may tend to shorten the duration of the D.D.T. residual. In Indian houses the thatch soon becomes blackened with soot and while mosquitoes are repelled by the smoke, they may be found resting on the sooty thatch after the fire dies out.

Thatch panels were treated with suspensions of water-wettable D.D.T. at rates of 50 and 100 mg. D.D.T./sq. ft. At the end of one week one set of panels was clamped in a 'V' position and held for 30 minutes over a smouldering fire of cotton soaked in oil until the thatch was darkened. Care was taken not to heat the surface of the thatch beyond 110–120°F. All panels were checked for residual toxicity and the process repeated at weekly intervals until the residual became exhausted. The results of these examinations are shown in Table III. It is believed that these tests do not show the true residual efficiency of the straw thatch. A great deal of difficulty was experienced in conducting these tests on the surface of the straw. The unusually rough handling of the panels in sealing holes to prevent mosquitoes from escaping and dislodging specimens caught in the straw, did much to mechanically remove the D.D.T from the surface of the straw. It is difficult to assess the effect of the smoking on the residual duration other than indicating that when a sufficiently heavy cover of soot is applied the residual is destroyed. It would be quite feasible to prolong the smoking periods from 30 minutes to several hours and destroy the residual toxicity of the thatch at a very early age regardless of the dosage of D.D.T. applied. (A 200 mg. D.D.T./sq. ft. straw panel was so rendered ineffective one week after spraying.)

SUMMARY AND CONCLUSIONS.

The principal surfaces upon which residual insecticides are to be applied in rural India are clay walls and thatch roofs. While the surface of the thatch remains toxic unusually long, the mud or clay wall, uncoated, presents an extremely rapid loss in toxicity. Evidence is presented which confirms findings by others that water-wettable suspensions of D.D.T. are vastly superior to emulsions of D.D.T. on mud wall surfaces. The use of suspensions should be further encouraged because the uniformity of application may more easily be checked in the field by observing the residues of inert materials. Also, additional economies may be expected through simpler mixing procedures, ease in handling and transporting the powders in contrast to solution or emulsion. Modifications in equipment will be required to provide agitation of the suspension. Data is presented to show that a cheap, simple wall surfacing with a mixture of cowdung and coconut charcoal will significantly enhance the duration of toxicity. The prevailing opinion of lack of residual effect of pyrethrum and synergist seems to be questionable especially when applied on cowdung and coconut charcoal-treated surfaces and protected from direct light. The laboratory results with water-wettable pyrenone justify further investigation of pyrethrins and synergist for use in residual house spraying. Evidence

TABLE III.

Average 24-hour percentage mortality of A. quadrimaculatus *adults after a 30-minute exposure to residues of water-wettable D.D.T. of known age on natural and smoked straw thatch.*

Material.	Time in weeks after spraying.										
	1	2	3	4	5	6	7	8	9	10	11
Smoked straw thatch—											
50 mg./sq. ft.	100	74	18	0	...	...	...	...	...	...	...
100 mg./sq. ft.	100	100	67	0	...	...	...	...	...	...	...
Natural straw thatch (comparison)—											
50 mg./sq. ft.	100	100	100	88	52	38	43	25	10	0	0
100 mg./sq. ft.	100	93	90	100	78	65	60	57	32	40	12

is presented to show that deposits of soot from an open fire can coat the thatch sufficiently to partially or completely destroy the residual toxicity of D.D.T.

ACKNOWLEDGMENTS.

Acknowledgment is given to Dr. Lloyd E. Rozeboom and members of the Department of Parasitology of the Johns Hopkins School of Public Health for their contribution of insectary facilities for laboratory experiments. The authors are particularly grateful to Dr. W. E. Dove, Director of Entomological Research at the U. S. Industrial Chemicals Inc., who provided valuable advice in the use of pyrethrum. To Mrs. Loretta Peterson of the Department of Sanitary Engineering, acknowledgment is made for assistance in conducting the experiments; to Dr. Abel Wolman of the Department of Sanitary Engineering and Dr. John Maier of the Rockefeller Foundation for suggestions in the preparation of this report.

REFERENCES.

AFRIDI, M. K., and BHATIA, M. L. (1947). Malaria control of villages around Quetta (Baluchistan) with D.D.T. *Ind. J. Mal.*, **1**, pp. 270–287.

BRADLEY, G. H., and FRITZ, R. F. (1946). Entomological evaluation of D.D.T. residual spraying for malaria control. *J. Nat. Mal. Soc.*, **5**, pp. 141–145.

Idem (1947) ... Entomological evaluations of results of residual D.D.T. spraying during 1946. *J. Nat. Mal. Soc.*, **6**, pp. 117–121.

CUTKOMP, L. K. (1947) ... Residual sprays to control *A. quadrimaculatus*. *J. Econ. Ent.*, **40**, pp. 328–333.

FAY, R. W., SIMMONS, S. W., and CLAPP, J. M. (1947). Extended laboratory investigations on the toxicity of D.D.T. residues to adults of *A. quadrimaculatus*. *Pub. Hlth. Rep.*, **62**, pp. 149–158.

HESS, A. D., and KEENER, G. G. (1947). The effectiveness of D.D.T. residual house spray in controlling *Anopheles quadrimaculatus*. *Amer. J. Hyg.*, **45**, pp. 133–143.

MAIER, J., RENDTORFF, R. C., and SUAREZ, M. (1948). The duration of residual effect of D.D.T. sprays on building materials used in rural Venezuela. *Amer. J. Trop. Med.*, **28**, pp. 889–894.

METCALF, R. L., HESS, A. D., SMITH, G. E., JEFFERY, G. M., and LUDWIG, G. W. (1945). Observations on the use of D.D.T. for the control of *A. quadrimaculatus*. *Pub. Hlth. Rep.*, **60**, pp. 753–774.

METCALF, R. L., and WILSON, C. (1945). The residual toxicity of pyrethrins to *A. quadrimaculatus*. *J. Econ. Ent.*, **38**, p. 499.

PURI, I. M., and KRISHNASWAMI, A. K. (1947). Studies on some insecticides against anopheline adults and larvæ. *Ind. J. Mal.*, **1**, pp. 159–181.

PURI, I. M., and PAL, R. (1947) Studies on some insecticides against anopheline adults and larvæ. *Ind. J. Mal.*, **1**, pp. 133–158.

RUSSELL, P. F., and MENON, M. K. (1942). A malaria economic survey in rural South India. *Ind. Med. Gaz.*, **77**, pp. 167–180.

SUNDARARAMAN, S., and PEFFLY, R. L. (1949). Effectiveness against flies and mosquitoes of D.D.T. to clay, palm and straw surfaces. *J. Nat. Mal. Soc.*, **8**, pp. 267–269.

VISWANATHAN, D. K., and RAO, T. R. (1947). Control of rural malaria with D.D.T. indoor residual spraying in Kanara and Dharwar districts, Bombay Province: First year's results. *Ind. J. Mal.*, **1**, pp. 503–542.

WEATHERSBEE, A. A., ARNOLD, F. T., and HOPKINS, J. P. (1948). Observations on the duration of toxicity of D.D.T. to *Anopheles quadrimaculatus* say under field conditions. *J. Nat. Mal. Soc.*, **7**, pp. 138–147.

Indian Journal of Malariology, **4**, 3, September 1950.

NOTES ON THE SEASONAL PREVALENCE OF ANOPHELES MOSQUITOES IN SOUTHERN FORMOSA.*

BY

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[July 22, 1950.]

INTRODUCTION.

DURING the 50 years of Japanese rule of Taiwan (Formosa), extensive studies of malaria and of Anopheles mosquitoes were made. These studies were not much concerned with the bionomics of anophelism; for the most part, they described mosquito collections from various parts of the island. With the establishment of our first field station for malaria studies at Ch'ao Chow in December 1946, routine collection of anopheline larvæ and adults was instituted and has been carried on to date. This paper is a study of the prevalence of the more important Anopheles species in the vicinity of Ch'ao Chow for the three-year period ending December 1949. Correlation of anophelism with malaria prevalence and related studies will be reported in other papers.

* The studies and observations upon which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation, in co-operation with the Taiwan Provincial Malaria Research Institute and the National Institute of Health, China.

FIELD CONDITIONS.

Topography of Southern Formosa.—The coastal plain of Southern Formosa is a continuation of the western coastal plain. Sweeping down from the north around the barrier of the central mountain range and its foothills, the southern plain, broad at its western end, becomes increasingly narrow as it progresses south-eastward, finally becoming pinched off between the mountains and the South China Sea. The southern plain is thus roughly triangular and the topography of this triangle is nearly uniform.

The southern coastal plain is nearly level sloping gently toward the sea. Its soil is a fertile, sandy clay which is underlain by gravel. The ground water table varies in height with the rainy season, but is under considerable pressure so that wells driven into it will flow for most of the year. These flowing wells are used extensively for irrigation of rice and other crops. The plain is cut from north to south by two large rivers and by many smaller streams which become torrents in the rainy season and always contain water. Other bodies of water include numerous large and small irrigation canals, seepage water collection from springs (especially at the foothills) and water in man made excavations.

The population of the area lives in discrete villages, and isolated houses are rarely seen. The villages are often near a stream or an irrigation canal, surrounded by a grove of bamboo and plantains; very often ricefields are immediately contiguous to houses. Rice has been the principal crop and two crops are grown every year; but rice has been replaced to a considerable extent by sugarcane in recent years.

Meteorological factors.—Rainfall data shown in Table 1 were collected by the authors at Ch'ao Chow. The other data are from a weather observatory at Heng-chuan, about 75 kilometres south-east of Ch'ao Chow; they are probably representative of temperature and humidity throughout the southern plain.

While there is considerable variation in rainfall from month to month, there is a definite rainy season from June to September, caused by the south-west monsoon. This is usually preceded by lesser rains in April and May. The rest of the year is relatively dry. This situation is almost exactly the reverse of conditions in the north, which is not much affected by the summer monsoon and derives most of its rains from the north-east winter monsoon.

There is a well-marked cool season during the winter months, but temperatures never fall low enough to prevent the active propagation of anophelines or the maturation of malaria infections in the mosquito host.

While rainfall is, of course, an important factor in the propagation of anophelines in that surface water area is directly related to rainfall, it will be shown later that anophelism responsible for malaria transmission is due largely to agricultural practices.

Methods.—Soon after field studies were established at Ch'ao Chow it was found that the two anophelines thought to be of most importance, *A. hyrcanus* var. *sinensis* and *A. minimus*, could not be found in significant numbers in dwellings during the day time. On the other hand, diurnal collections from stables used for oxen and water-buffaloes yielded numbers in keeping with estimates of adult densities from larval collections. Consequently, such stables were used routinely for diurnal

TABLE 1.

Meteorological data from the vicinity of Ch'ao Chow, Southern Formosa. (The rainfall data are from Ch'ao Chow Town, the other data from Heng-chuan observatory, 75 kilometres south-east of Ch'ao Chow.)

Month.	TEMPERATURE (°C.) (1940–49).					Average relative humidity, per cent (1940–49).	RAINFALL (MM.).	
	MAXIMUM.		MINIMUM.		Average.			
	Absolute.	Mean.	Absolute.	Mean.			1948.	1949.
Jan. ...	28·2	23·4	12·1	16·7	19·6	69	0·0	30·5
Feb. ...	29·6	25·2	14·4	17·5	20·7	71	1·3	75·4
Mar. ...	31·0	26·3	19·4	17·9	21·5	74	1·8	3·6
Apr. ...	31·9	28·2	16·2	19·9	23·4	78	148·6	8·1
May ...	32·6	30·8	20·9	23·0	25·9	82	65·8	39·4
Jun. ...	33·7	31·6	23·1	24·5	27·2	87	168·2	698·8
Jul. ...	32·7	30·7	22·0	24·8	27·1	88	623·8	833·3
Aug. ...	33·1	31·2	22·5	24·0	26·9	84	482·9	606·0
Sep. ...	32·6	31·0	21·7	23·5	26·5	83	763·8	223·8
Oct. ...	32·5	30·1	19·1	22·8	25·8	78	10·4	177·6
Nov. ...	31·9	27·9	15·8	21·5	24·1	71	12·9	3·6
Dec. ...	30·0	26·3	14·0	18·2	21·8	67	15·2	11·9

collection of mosquitoes. These collections were made by the same person at weekly intervals from January 1947 to December 1949. The number of mosquitoes collected in 15 minutes with a chloroform tube was used to compute density in terms of man-hours. These collections were supplemented by routine collections of larvæ at weekly intervals from fixed stations that had been selected upon the basis of their conformity to optimum habitat for the various species found at Ch'ao Chow.

Collections of all the adults were also made daily, except Sundays and holidays, from a ground pit which was dug near a village. This pit measured approximately 215 × 110 × 120 cm.; it was covered by bamboo matting topped by a layer of earth and leaves. The interior, which was entered through a small opening, was relatively dark and humid.

Finally, night collections of mosquitoes were made from the skin surface of a water-buffalo which was tied in a village compound. These collections were made

by two persons working simultaneously on opposite sides of the animal. All the mosquitoes offering to feed upon the buffalo were caught throughout alternate half-hour intervals from sunset to sunrise twice monthly for the three-year period. This method permitted the collection of data on *A. annularis*, which is not found in substantial numbers in the diurnal shelters mentioned.

The discussion of seasonal prevalence for individual species is based upon collections by the method which gave best results for each species.

RESULTS AND DISCUSSION.

Of the 16 Anopheles species found in Formosa (Chow, 1949), *A. hyrcanus* var. *sinensis* is the most prevalent and is thought to be the most important malaria vector in the plains throughout Formosa. For the hilly regions, the most important species is considered to be *A. minimus*. Anazawa (1931), however, reports the finding of malaria parasites upon dissection of other mosquitoes, as follows, with infection rates reported for each species: *A. maculatus*, 0·4 per cent; *A. sundaicus (bulloni)*, 0·6 per cent; *A. annularis*, 0·2 per cent; *A. tessellatus*, 1·9 per cent; *A. splendidus*, 2·3 per cent; *A. hyrcanus* var. *sinensis*, 0·8 per cent; and *A. minimus*, 2·4 per cent.

Anopheles hyrcanus var. *sinensis*.—From Chart I it is seen that this mosquito may be found in fairly large numbers throughout the year. Its annual prevalence, however, is marked by two well-defined seasonal peaks, in February-March and September-October. These peaks are due to the circumstance that *sinensis* propagation occurs principally in irrigated ricefields and its seasonal density is a result of agricultural practices.

Two crops of rice are grown each year at Chiao Chow and elsewhere in Southern Formosa. Planting occurs during January and July. Water is turned into the paddy to facilitate ploughing and remains on the fields until the transplanted rice is well set and growing. The water is then drawn off to permit cultivation and weeding, after which the fields are inundated again and water remains in the paddy at almost constant level until flowering is completed and the grain is maturing. The fields are then de-watered and remain dry until the rice is harvested. The first period of inundation does not result in significant propagation of *sinensis*; the water is too much disturbed and remains in the fields for too short a time. The second period of inundation apparently offers optimum conditions for propagation and results in increasingly high densities of the mosquito. Unlike the case of *A. culicifacies* (Russell and Rao, 1942), the height of rice *per se* does not appear to offer mechanical obstruction to oviposition of *sinensis* and does not interfere with *sinensis* propagation.

The spring peak of *sinensis* anophelism was greater than the autumn peak in 1948 and 1949, lower than the autumn peak in 1947 (Chart I). The higher spring peak for this species is believed to be representative of average years. Larval density in ricefields is consistently lower during the second crop and the autumn peak of *sinensis* prevalence would probably be lower still were it not for the many breeding places other than ricefields which are produced by the summer rains. The authors believe that the lower density of larvæ in the fields during the second crop is due to fluctuation of water levels in the fields. The heavy rains of

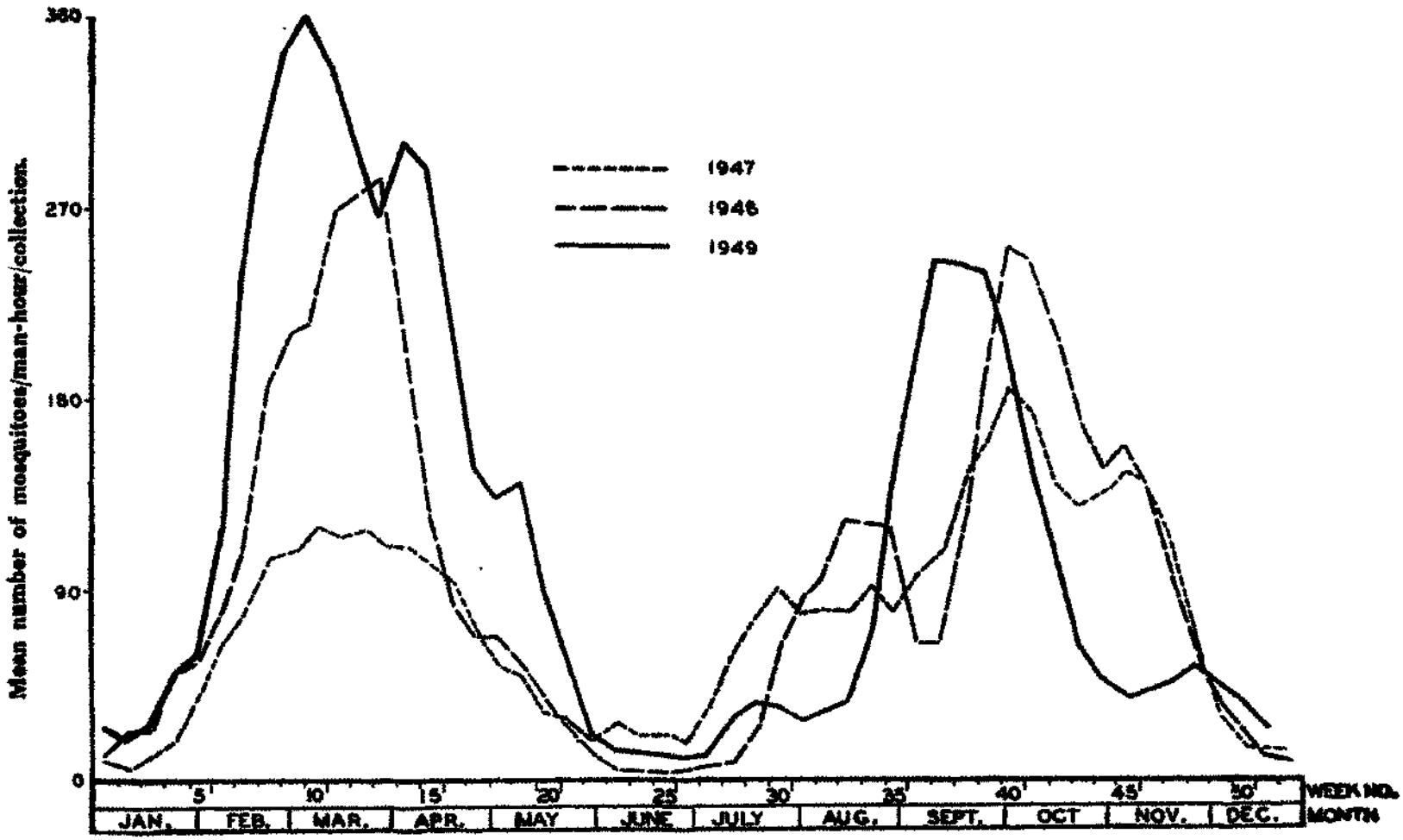

Chart 1.—Seasonal prevalence of *Anopheles hyrcanus* var. *sinensis*: three weeks' moving mean of weekly collections from seven cattlesheds.

summer require that the fields be de-watered from time to time. The pelting rain may actually kill larvæ and almost certainly interferes with their development.

Koidzumi (1926) also reports two peaks of *sinensis* prevalence, in May and in October, the spring peak being higher, for the general vicinity of Taipei, in Northern Formosa. His observations were based upon daily collections of *sinensis* from houses and animal shelters for eight years, 1915–22. Koidzumi thought that these peaks of prevalence were principally influenced by temperature. He reports that the larval stage was much prolonged during February (the coldest month) and March, but active propagation with the short larval periods was established during April. He attributed the decline in prevalence which he noted for July and August to high summer temperatures, and the autumn peak to re-establishment of temperatures favourable to optimum *sinensis* propagation. Katagai (1931), reporting on work in the same locality during 1927–30, confirmed Koidzumi's results and agreed with his explanations.

While Northern Formosa is much colder than the southern part during winter, the authors believe that the influence of these temperatures upon *sinensis* propagation is more indirect than direct. Temperature influences the time of planting the first rice crop in the north, which is about one and a half months later than in the south. The spring peak of *sinensis* anophelism is about one and a half months later in the north than in the south. Similarly, the second crop in the north is about one month later than the second crop in the south and the autumn peak prevalence about a month later. Summer temperatures in Formosa are not considered to be a likely factor in deterring *sinensis* propagation.

Anopheles tessellatus.—After *sinensis*, this is the species most commonly found at Ch'ao Chow; it is present throughout the year but its density is low except during the autumn months (Chart 2). The high autumnal prevalence is caused by the fact that typical breeding places of *tessellatus* are pools formed in sugarcane fields during the rainy season. As these pools dry out after the rains stop, *tessellatus* density diminishes. The relationship between rainfall and seasonal prevalence may be noted by comparing Chart 2 with Table I. In 1949, the continuation of the rains through October deferred peak production of this species until mid-October, while in 1948, peak prevalence was a month earlier, the rainy season being a month shorter.

Anopheles annularis.—Ricefields are usually considered to offer the optimum conditions for the propagation of this species but there is no evidence to support this view in Formosa. At Ch'ao Chow, *annularis* larvæ are commonly found in pools associated with larger number of *tessellatus* larvæ. Thus the seasonal prevalence of this species is quite like that of *tessellatus* (Chart 3).

Anopheles sundaicus (*ludlowi*).—The favourite breeding places of this species are small pools with sandy or stony bottoms, without vegetation and exposed to direct sunlight. Such pools are commonly found in the margin of streams during the dry season. When these breeding places are destroyed by the full streams of the wet season, *sundaicus* larvæ may be found in small numbers in ricefields, usually associated with floating aquatic vegetation such as *Lemna* sp. Thus maximum prevalence of this species is found during the dry season (Chart 4), though small numbers are also found during the second rice crop.

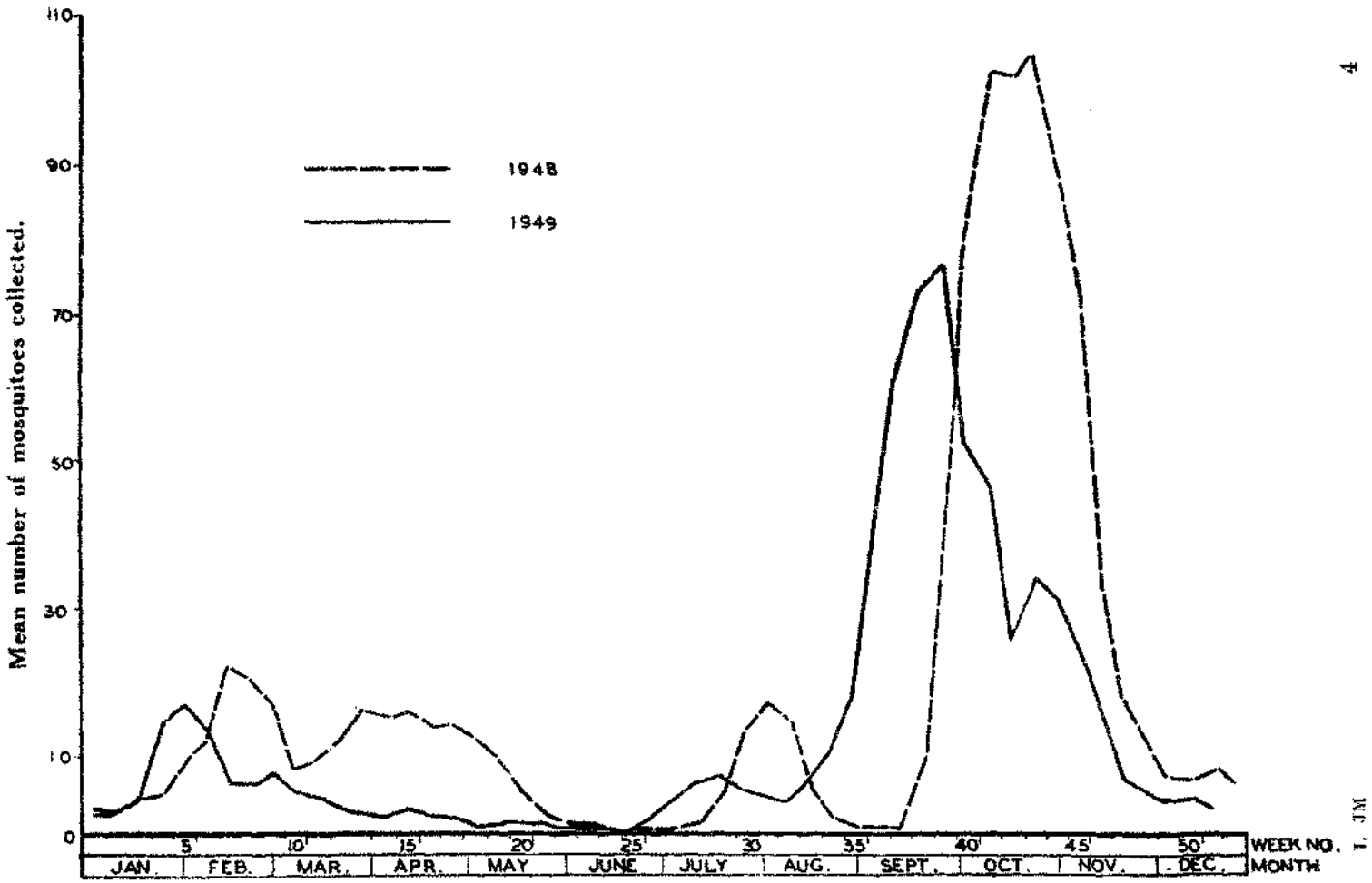

Chart 2.—Seasonal prevalence of *Anopheles tessellatus* : three weeks' moving mean of daily collections in an underground pit.

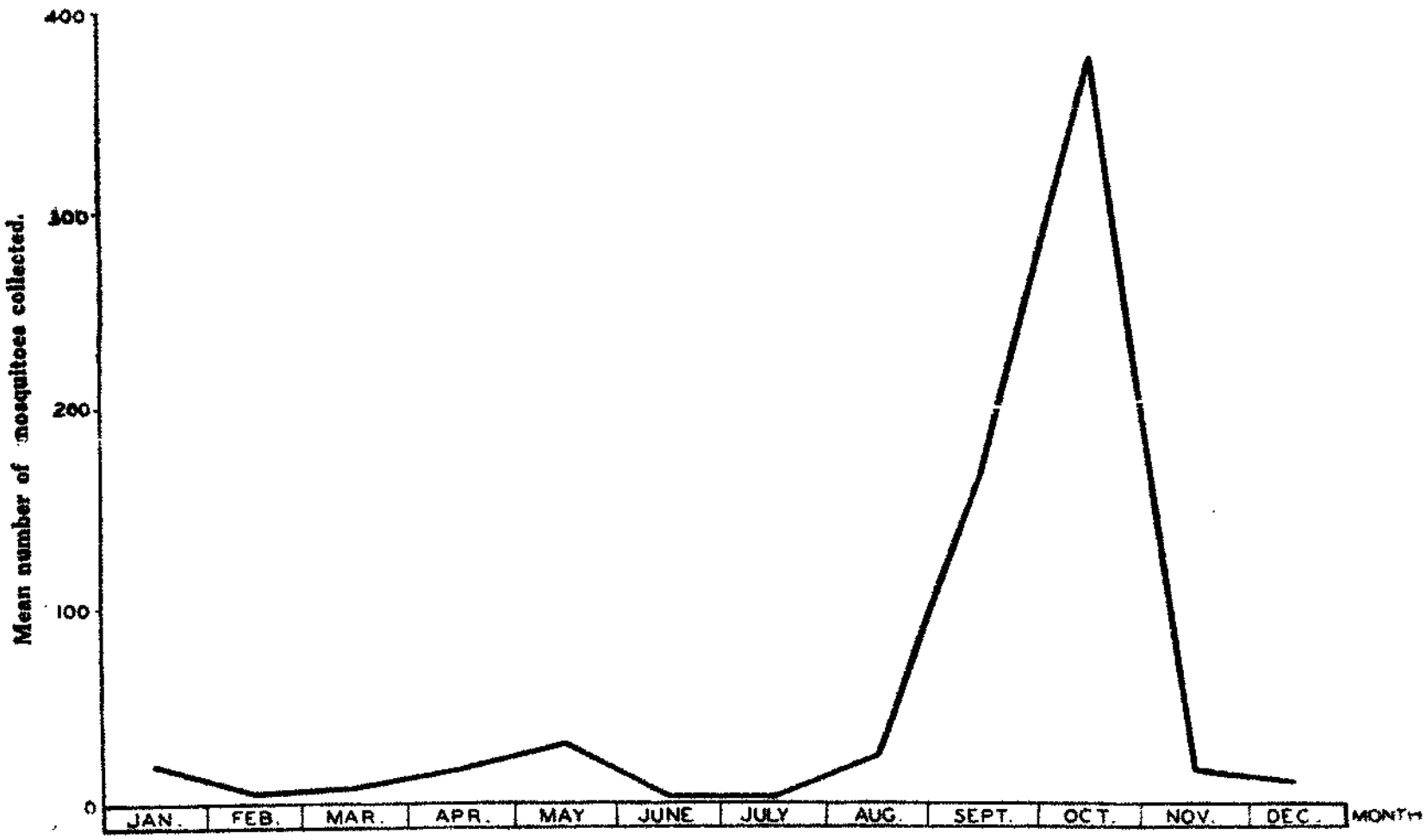

Chart 3.- Seasonal prevalence of *Anopheles annularis* : mean number of mosquitoes from bimonthly night collections from the skin surface of a water-buffalo.

Anopheles minimus.—This important anopheline is not found in large numbers at Ch'ao Chow (Chart 5). As it is an efficient malaria vector, we made special efforts to determine its density and have concluded that circumstances obtaining at Ch'ao Chow mitigate against its prolific propagation. Elsewhere in Formosa, particularly in the foothill regions, this mosquito is found in much higher densities, is easily collected in houses and cattlesheds and is known to be an important vector.

It is well known that *minimus* is quite sensitive to changes in its aquatic environment; this fact has been the basis for control work elsewhere through the use of periodic flushing of streams. At Ch'ao Chow, *minimus* propagates along the margins of small streams and irrigation channels. During the late winter and early spring, the water in these channels is not subject to wide or sudden level fluctuations and conditions are relatively favourable for *minimus* production. Peak production thus is usually noted in May, before the onset of the rainy season. The flushing of the channels by the rains inhibits propagation; nevertheless, one would expect greater production of *minimus* during the autumn months than evidently occurs. In South China, which has conditions comparable to those at Ch'ao Chow, an autumnal rise in *minimus* density is commonly found.

Other species.—Larvæ of *A. maculatus* may be found during the dry season, especially during April and May, in stream bed pools, particularly in foothill streams. While some of these larval collections have been made near houses, this species has never been collected from a house. In three years only 20 adults have been captured: eight were from the underground pit, two from the buffalo bait and 10 from stables. *A. subpictus* var. *indefinitus* larvæ have been collected from brackish pools along the coast (about 13 kilometres from Ch'ao Chow) and sometimes from ponds. A small number of this species was collected from stables during night catches, principally during April and May. A very few *A. splendidus* were also captured from the buffalo bait.

Larvæ of *A. aitkeni* var. *bengalensis*, *A. insulaflorum* and *A. lindesayi* were found from time to time, but never in large numbers. Larvæ of the first two species were found in shaded pools in mountain stream beds; of *A. lindesayi* from the shaded margins of mountain streams, 2,000 feet or higher above sea level. No adult of these species was captured. One specimen of *A. leucosphyrus* was collected from a stable.

Relative effectiveness of the three collecting methods.—The total numbers of nine anopheline species captured by the three collecting methods employed are shown in Table II. From these data it may be seen that no one method of collection would have given a true picture of anophelism at Ch'ao Chow. Stable collections, however, apparently give a good notion of the prevalence of *sinensis* and *minimus*, the species known to be of importance in malaria transmission. The best collecting method from all standpoints seems to be the use of buffalo bait: the average collection was larger and only the prevalence of *minimus* and *ludlowi* was underestimated by this method as compared with the other methods.

SUMMARY.

Three methods were used to study the prevalence of Anopheles mosquitoes at Ch'ao Chow in Southern Formosa. These methods were weekly collections from

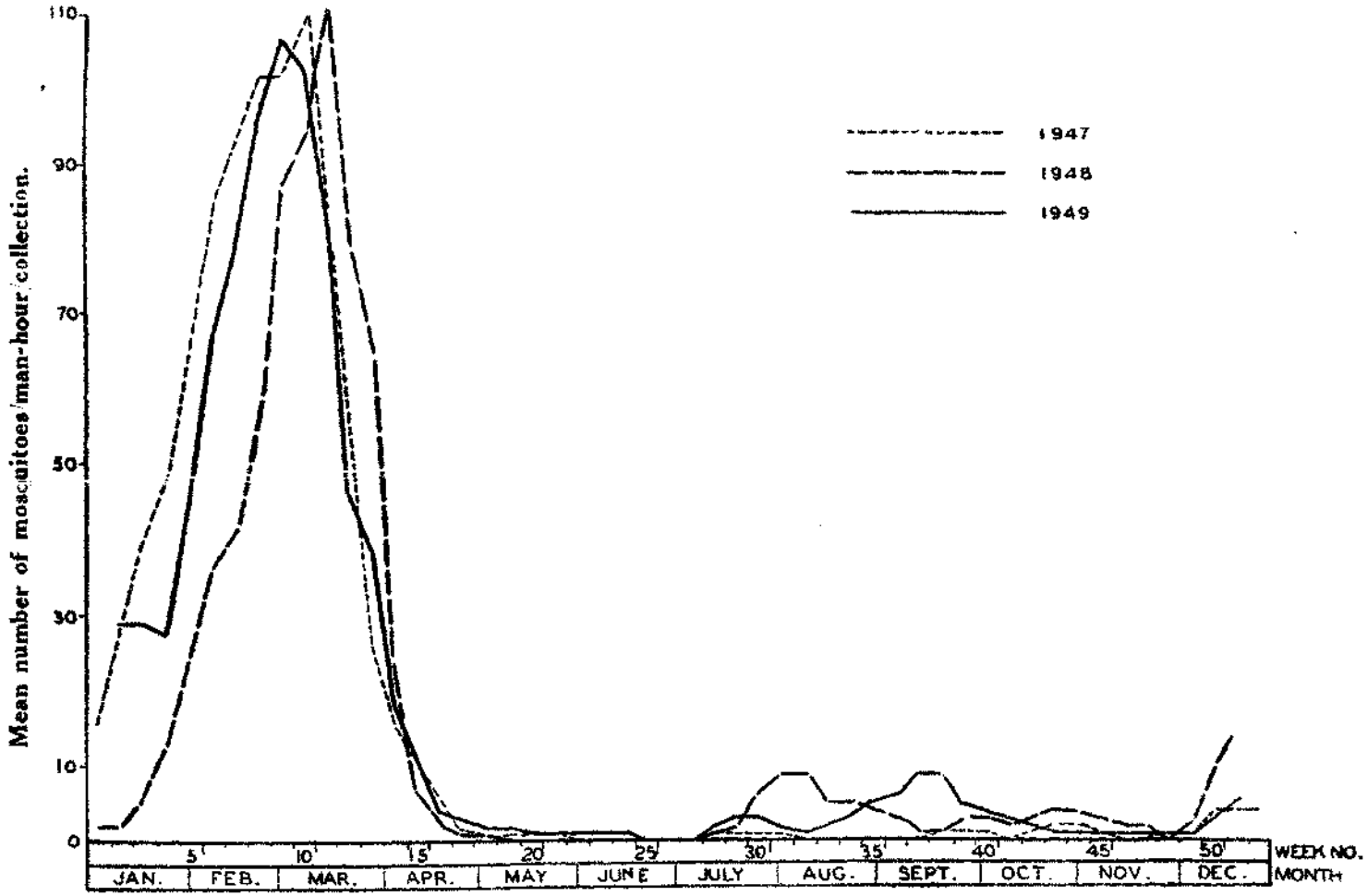

Chart 4.—Seasonal prevalence of *Anopheles sundaicus* (*ludlowi*): three weeks' moving mean of weekly collections from seven cattlesheds

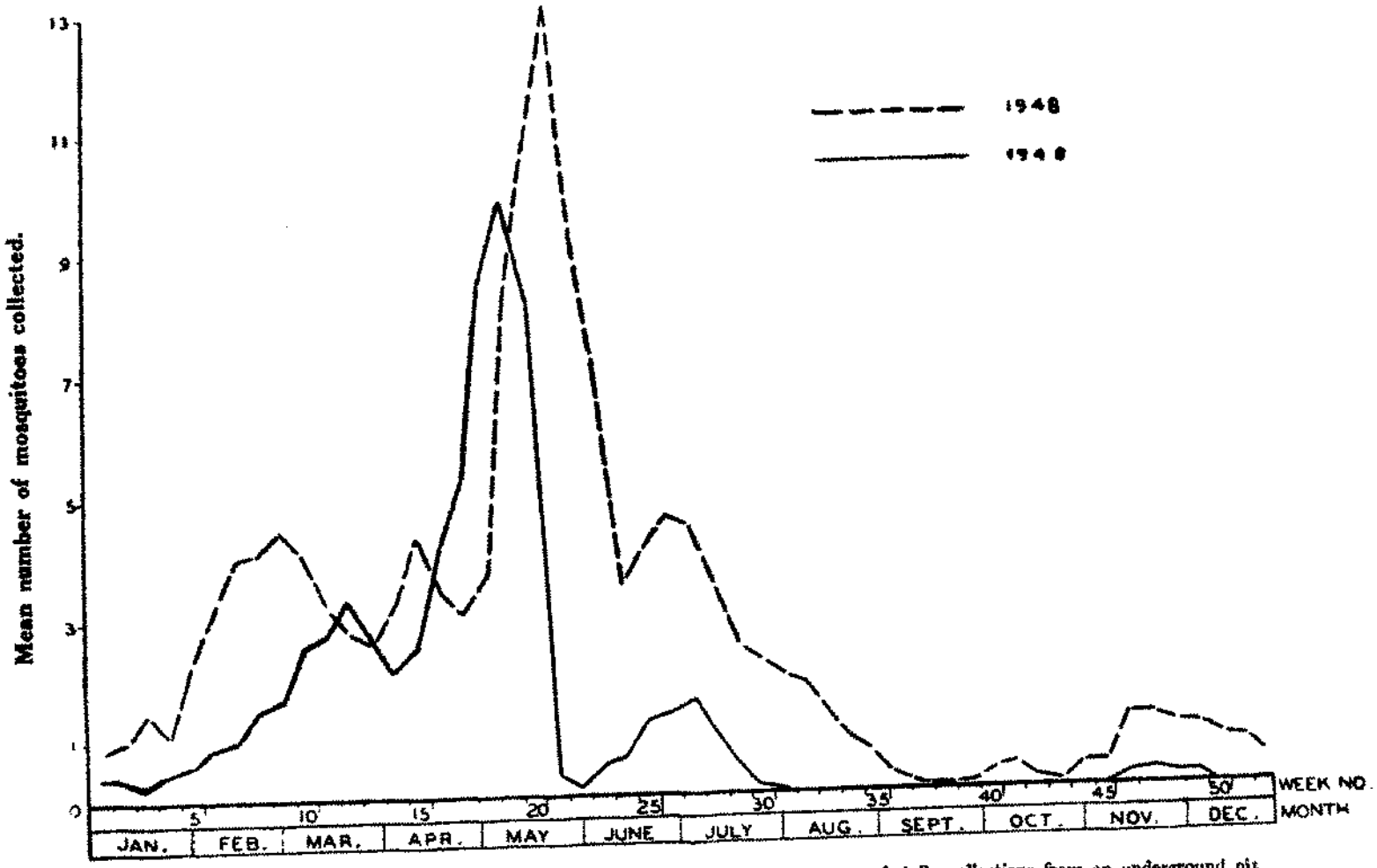

Chart 5.—Seasonal prevalence of *Anopheles minimus*: three weeks' moving mean of daily collections from an underground pit

TABLE II.

Total number of anopheline mosquitoes captured by three collecting methods.

Method of collection.	Weekly collections from cowsheds.	Daily collections from underground pit.	Bimonthly buffalo bait catches.
Total number of collections ...	1,189	627	42
Species :—			
A. hyrcanus var. *sinensis* ...	43,332	1,090	11,605
A. tessellatus	62	11,773	11,565
A. sundaicus	5,587	2,858	2,450
A. minimus	1,819	1,050	595
A. annularis	38	10	2,674
A. subpictus var. *indefinitus*	29	89	142
A. splendidus	6	98	261
A. maculatus	19	8	2
A. leucosphyrus ...	1	...	...
Mean number of mosquitoes per collection.	45	27	691

cattlesheds, night catches of mosquitoes biting a buffalo and collections from an underground pit.

A. hyrcanus var. *sinensis* is the most prevalent species found at Ch'ao Chow. It is present throughout the year and has two peaks of prevalence associated with the two rice crops. It is believed to be the principal vector of malaria in the southern plain of Formosa.

A. minimus was found in smaller numbers than would be expected upon the basis of work in mainland China. This species, while found throughout the year, has a peak of prevalence in April and May. It is thought to play a relatively minor rôle in malaria transmission in the plains of Formosa.

A. tessellatus and *A. annularis* are found in fairly large numbers during about three weeks in September and October at the end of the rainy season. Large numbers of *A. sundaicus* are found at the height of the dry season. These three species are not commonly found in appreciable numbers except at the times noted. They have not been incriminated as vectors by the authors.

REFERENCES.

ANAZAWA, K. (1931) .. Observations on natural infection of various Taiwan anophelines with reference to the critical value of each species from the malaria epidemiological point of view. *J. Med. Assoc. Formosa*, **30**, pp. 1027–1049.

CHOW, C. Y. (1949) .. The anopheline mosquitoes of Taiwan, China. *Quart. J. Taiwan Museum*, **2**, pp. 1–9.

KATAGAI, T. (1931) .. Anopheline fauna in Taihoku, with notes on its numerical change. *J. Med. Assoc. Formosa*. **30**, pp. 1276–1287.

KOIDZUMI, M. (1926) Eight years' observation of the seasonal prevalence of *Anopheles sinensis* in Formosa, with some remarks on the influence of climatic conditions and on the habits of the mosquitoes. *Ibid.*, No. 256, pp. 631–672.

RUSSELL, PAUL F., and RAO, T. RAMACHANDRA (1942). On the swarming, mating and ovipositing behaviour of *Anopheles culicifacies*. *Amer. J. Trop. Med.*, **22**, pp. 417–427.

Indian Journal of Malariology, **4**, 3, September 1950.

NATURAL INFECTION OF ANOPHELINE MOSQUITOES WITH MALARIA PARASITES IN FORMOSA.*

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November 17, 1950.]

MALARIA is perhaps the most prevalent communicable disease in Formosa, yet there are few reports of studies on the natural infection of Formosan anophelines with malaria parasites. Anazawa (1931*a*) reported seven species of Anopheles from central Formosa infected with Plasmodium, as shown in the following tabulation :—

Anopheles species.	Number dissected.	Infection rate, per cent.
A. hyrcanus var. *sinensis* ...	2,462	0·8
A. minimus	4,208	2·4
A. ludlowii (*sundaicus*) ...	949	6·6
A. maculatus	246	0·4
A. annularis	946	0·2
A. tessellatus	104	1·9
A. splendidus	44	2·3

* The studies and observations upon which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation, in co-operation with the Taiwan Provincial Malaria Research Institute and the National Institute of Health, China.

The first three species listed were reported as having stomach and gland infections, the remainder gland infections only. Most of the positive specimens were collected from cattlesheds with the exception of *minimus*, all of which were caught in human dwellings.

Experimentally, Anazawa (1931*b*) was able to infect the species listed and *A. lindesayi*; he concluded that all the eight species might be involved in the transmission of malaria in Formosa.

At Tainan, in Southern Formosa, Shimomura (1935) found 15 infected specimens among 2,812 *A. hyrcanus* var. *sinensis* (0·53 per cent), and one infected specimen among 264 *A. minimus* (0·38 per cent) dissected. He does not mention the origin of the mosquitoes or the site of the infections.

This paper records the results of dissections of Anopheles mosquitoes done during a three-year period (1947–49) in three localities in Formosa: Keelung, Shui-Li-K'eng and Ch'ao Chow. These places are located in extreme northern, west central and southern Formosa, respectively. The mosquitoes dissected were collected from cattlesheds, human dwellings, underground pits and traps. They were stored for seven days prior to dissection in bobbinet cages measuring 10 × 10 × 17 cm. The stored mosquitoes were kept in a moist chamber and were fed by means of cotton pads soaked with a 10 per cent solution of cane syrup in water. The mosquitoes were killed with chloroform vapour and dissected under a microscope. The salivary glands and mid-guts were examined for malaria parasites and the malpighian tubules, thorax, abdomen, proboscis and head for filariæ.

RESULTS

Anopheline mosquitoes of eight species were dissected, but of these only *A. hyrcanus* var. *sinensis* and *A. minimus* were found infected with malaria parasites (Tables I and II). *A. hyrcanus* var. *sinensis* (45,458) comprised 74 per cent and *A. minimus* (6,498) 10 per cent, approximately, of the 60,915 total dissections. The gross infection rate (sporozoites and oöcysts) for *A. hyrcanus* var. *sinensis* was 0·0197 per cent and for *A. minimus* 0·169 per cent. The results for the three localities were as follows:

Keelung.—The dissections were done from July to December 1947. Of 887 *A. hyrcanus* var. *sinensis* dissected, 691 (78 per cent) were collected from cattlesheds. The only infected specimen was found in August for a rate of 0·11 per cent. In contrast, of 2,365 *A. minimus* dissected, 2,270 (96 per cent) were collected from houses, but two of the eight infected specimens were collected from cattlesheds. Six of the eight infected specimens were found in August, one each in July and September. The infection rates for *minimus*, for the months when infected specimens were found, were: July, 0·48 per cent; August, 2·07 per cent; and September, 0·27 per cent.

Shui-Li-K'eng.—The dissections were done from July 1947 to June 1948, a total of 5,437 anophelines of six species being examined. Nearly all (97 per cent) of the *sinensis* were collected from stables, about half of the *minimus* from houses. No infected specimen of *sinensis* was found. Two infected *minimus* were found, one each in August and November; both specimens came from cattlesheds. The infection rate for all *minimus* (1,313) was 0·15 per cent; for August dissections (111), 0·90 per cent and for November dissections (262), 0·38 per cent.

TABLE I.

The results of dissections of 45,458 A. hyrcanus *var.* sinensis *and* 6,498 A. minimus, *from three localities in Formosa,* 1947–49.

Place.	Source of mosquitoes.	*A. hyrcanus* var. *sinensis.*				*A. minimus.*			
		Number dissected.	Gl+	G+	Total.	Number dissected.	Gl+	G+	Total.
Keelung ...	Stables ...	691	0	1	1	95	1	1	2
	Houses ...	196	0	0	0	2,270	0	6	6
Shui-Li-K'eng ...	Stables ...	3,728	0	0	0	667	2	0	2
	Houses ...	126	0	0	0	646	0	0	0
Ch'ao Chow ...	Stables ...	39,666	8	0	8	1,096	0	1	1
	Houses ...	159	0	0	0	1,340	0	0	0
	Pits or traps	892	0	0	0	384	0	0	0
TOTAL ..	...	45,458	8	1	9	6,498	3	8	11

Gl+ = salivary glands infected. G+ = guts infected.

TABLE II.

Record of anopheline dissections in which no malaria infections were found. 1947–49.

Anopheles species.	PLACE.		TOTAL.
	Ch'ao Chow.	Shui-Li-K'eng.	
A. tessellatus	4,765	26	4,791
A. sundaicus (*ludlowii*) ...	3,594	0	3,594
A. annularis	222	232	454
A. subpictus var. *indefinitus* ..	61	0	61
A. splendidus	45	1	46
A. maculatus	2	11	13

Ch'ao Chow.—During three years (January 1947 to December 1949), 52,226 specimens of eight Anopheles species were examined. As elsewhere, most (97 per cent) of the *sinensis* were collected from cattlesheds. Of the 40,717 specimens dissected, eight (0·019 per cent) were infected. Three of the infected specimens were found in February, one in March and four in December, for infection rates of 0·09, 0·02 and 0·20 per cent respectively. About half of the 2,820 *minimus* dissected were from houses, the rest from cattlesheds. One infected specimen was found in June, for a gross rate of 0·035 per cent and a rate for that month of 0·21 per cent.

Filariæ infections.—Larval filariæ were found on several occasions in the head, proboscis, thorax and abdomen of mosquitoes but none was found in the malpighian tubules. The larvæ resembled those of *Wuchereria* but were not positively identified. The authors have never found microfilariæ in thick blood films.

DISCUSSION.

The gross infection rates obtained for *minimus* and *sinensis* are substantially lower than those reported for these mosquitoes by Anazawa (1931*a*) and by Shimomura (1935). These findings probably underestimate somewhat, perhaps considerably, the true infection rates of these species. The data herein reported were collected as a routine by technicians, trained by the authors, whose technical proficiency presumably increased with experience. After their training period, these technicians were under nominal supervision only. They are excellent technicians, but they are not research workers. Their positive findings were confirmed, but it is likely that they failed to find some infected specimens among the mosquitoes examined. It is also possible that the mosquitoes dissected by Anazawa (*loc. cit.*) and by Shimomura (*loc. cit.*) were collected from highly endemic situations, where the chances of an anopheline becoming infected were greater than for the mosquitoes dissected in the present case.

One of the authors (Chow) has recently found flagellates, tentatively identified as *Crithidia* sp., in the salivary glands of anophelines collected near Taichung in west central Formosa. These flagellates resemble sporozoites and could be mistaken for them on superficial examination. It is suggested that such a mistake may have been made by former malaria workers in Formosa.

The findings reported in this paper confirm our opinion, reached upon epidemiological grounds, that *A. hyrcanus* var. *sinensis* and *A. minimus* are the most important if not the only vectors of malaria in Formosa. Our data suggest that *minimus* in Formosa is infected with malaria parasites about nine times more frequently than *sinensis*. The authors believe this means that *minimus* is a more dangerous vector than *sinensis*, not because of some inherent physiological difference but because of its habits. Almost all specimens of *sinensis* examined were collected from cattlesheds, a very few from houses, which suggests that this species is primarily zoophilic. Sixty-five per cent of the *minimus* examined came from houses, while 28 per cent came from stables. But five of the 11 infected specimens were found in stables, which may mean that *minimus* is more anthropophilic than the number collected from houses indicates.

As previously noted (Chang, Watson and Chow, 1950), *sinensis* is the most prevalent anopheline of the plains regions of Formosa, while *minimus* is found principally

in the foothills regions. Seasonal prevalence of malaria and increasing *sinensis* anophelism in the plains regions are correlated with each other and with the two rice crops grown annually (Watson and Liang, to be published), though *sinensis* is found throughout the year. Other factors being equal, the authors believe that a much higher level of *sinensis* anophelism than of *minimus* anophelism is required to produce the same level of malaria prevalence in a given Formosan situation.

While the numbers of *sundaicus*, *tessellatus*, *annularis*, *subpictus* var. *indefinitus* and *splendidus* dissected by the authors were relatively small, the negative findings confirm the view of the relative unimportance of these mosquitoes as malaria vectors in Formosa. If Anazawa's (*loc. cit.*) data are taken at face value, all of these species *except indefinitus* are potentially important malaria vectors. It is possible that *sundaicus* is a vector for a short time in late winter, *tessellatus* and *annularis* for a short time in the autumn, when their densities are temporarily high (Chang, Watson and Chow, 1950). But it has never been possible to collect *subpictus* var. *indefinitus* and *splendidus*, by any means anywhere, in densities that suggest these mosquitoes are of any importance as malaria vectors.

The authors are not satisfied with the studies on *A. maculatus*. Larvæ of this mosquito, which is so important a vector in Malaya, can be found in very large numbers during the dry season in river bed pools in southern and central Formosa. Adults are rarely taken from usual collecting places and in three years only 13 specimens could be collected for dissection. Investigations on this mosquito are continuing.

SUMMARY.

From January 1947 to December 1949 dissections were made for 60,915 Anopheles mosquitoes of eight species: *hyrcanus* var. *sinensis*, *minimus*, *tessellatus*, *annularis*, *subpictus* var. *indefinitus*, *splendidus*, *sundaicus* and *maculatus*. These mosquitoes were collected from diurnal resting places and traps and stored for seven days before dissection.

Infections of the salivary glands or stomach with malaria parasites were found only in specimens of *sinensis* and *minimus*. Of 45,458 *sinensis* examined, nine (0·0197 per cent) were infected, while of 6,498 *minimus* examined, 11 (0·169 per cent) were infected. These findings confirm the importance of these species as malaria vectors in Formosa.

Larval filariæ resembling *Wuchereria* sp. were sometimes found in the abdomen, thorax, proboscis and head of anophelines. A flagellate resembling *Crithidia* sp., which might be confused with sporozoites, was sometimes found in salivary glands.

REFERENCES.

ANAZAWA, K. (1931*a*) Observations on the natural infection of the various Taiwan anophelines with reference to the critical value of each species from the malaria epidemiological point of view. *J. Med. Assoc. Formosa*, No. 319, pp. 1027–1049.

ANAZAWA, K. (1931*b*) ... Experimental studies on the infectivity of anopheline mosquitoes of Taiwan. *J. Med. Assoc. Formosa*, Nos. 312–315, pp. 269–285, 381–393, 531–542, 609–632.

CHANG, T. L., WATSON, R. B., and CHOW, C. Y. (1950). Notes on the seasonal prevalence of Anopheles mosquitoes in Southern Formosa. *Ind. J. Mal.*, **4**, pp. 281–293.

CHIMOMURA, H. (1935) ... Features of anopheline distribution in the prefecture of Taiwan. *J. Med. Assoc. Formosa*, **34**, pp. 51–55.

WATSON, R. B., and LIANG, K. C. (1950). Seasonal prevalence of malaria in Southern Formosa. (*Under publication.*)

Indian Journal of Malariology, **4**, 3, *September 1950*.

FIELD TRIAL OF CHLOROQUINE (SN-7618-5) FOR MALARIA CONTROL IN CENTRAL TAIWAN (FORMOSA).*

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[November 10, 1950.]

INTRODUCTION.

THIS paper is a report on the use of chloroquine as a suppressive and therapeutic agent for the control of malaria in a group of industrial workers in Central Taiwan (Formosa).

In May 1947, a field station for malaria studies was established at Shui-Li-K'eng Village, situated in the western foothills of the Central Mountain Range near the centre of the island. Quarters were furnished by the Taiwan Electric Company, the management of which solicited the authors' aid in the control of malaria among its employees. Employees of the power company had suffered severely from malaria for several years. There had been a number of cases of blackwater fever and some deaths, and a high prevalence of malaria infection had reduced the efficiency of employees and had resulted in considerable absenteeism.

* The studies and observations on which this paper is based were conducted under the auspices and with the support of the International Health Division of The Rockefeller Foundation in co-operation with the Taiwan Provincial Malaria Research Institute of the Taiwan Provincial Health Department.

The authors had no D.D.T. in 1947, but did have a supply of several anti-malarial drugs. Among these was a supply of chloroquine which had been given for field trial. Employees and their families of two generating plants of the power company, located near Shui-Li-K'eng, were treated with the drug for six months from June to December 1947. Excellent control of malaria was secured when appropriate doses were employed.

STUDY CONDITIONS.

General.—The area in which Shui-Li-K'eng is situated is about 250 metres above the mean sea-level. The topography is hilly and the very little level land is along the main roads and in river valleys. Only about one-fourth of the rice requirement of the population is grown locally. The chief agricultural products are bananas and pineapples, and much of the population is engaged in small industries and in timber work. The town of Shui-Li-K'eng is located on the Tsang Shui River, which pursues a precipitous westward course, in a broad rocky channel. During the rainy season, this river is quite large and at all seasons carries a heavy load of black silt, hence its name, which means 'dirty water'.

Climate.—Meteorological data are shown in Table I. These data are from the weather station at Sun-Moon Lake, about 25 kilometres east of Shui-Li-K'eng and at an altitude of about 800 metres above sea-level. The temperatures are therefore somewhat lower than at Shui-Li-K'eng, but there is a definite cold season at the latter place; during December to February, mean temperatures probably would prevent maturation of the definitive cycle of malaria parasites.

TABLE I.

Meteorological data for the Shui-Li-K'eng area, from the Sun-Moon Lake weather station, 1937–1946.

Months.	Temperature, C.		Rainfall.	
	Maximum.	Minimum.	Total mm.	Rainy days.
Jan. ...	69·3	51·2	45·23	7·4
Feb. ...	69·7	54·2	86·29	9·0
Mar. ...	72·1	61·4	166·98	13·9
Apr. ...	75·7	63·2	186·81	12·3
May ...	79·9	66·4	389·71	19·2
Jun. ...	81·1	68·8	602·38	22·4
Jul. ...	82·4	69·3	422·12	22·7
Aug. ...	85·3	71·4	417·93	22·6
Sep. ...	82·3	68·1	219·99	14·4
Oct. ...	79·5	64·7	46·68	6·3
Nov. ...	76·5	60·7	25·87	5·2
Dec. ...	70·0	54·7	63·86	7·0

During the study the only significant variation from these average values was unusually heavy rainfall at Shui-Li-K'eng during June: 1,385 mm.

Anophelism.—Because of its efficiency in transmitting malaria, *Anopheles minimus* is possibly the most important vector in the area. Its breeding places are found in the numerous streams between the hills, in the margins of small rivers and in the irrigation channels. This mosquito is readily found in considerable numbers in houses, in contrast to the situation in the southern plains region of Formosa. *A. hyrcanus* var. *sinensis* is found seasonally in much higher densities than *minimus* and is certainly a vector of importance. The mean densities of these two mosquitoes, by weeks, are shown in Chart 1 for a year, the six months of the study and the first six months of 1948. During the dry season, pools in river-beds offer breeding places for *A. maculatus*, which the Japanese malaria workers thought was an important vector in the area. The authors consider that this mosquito is certainly of secondary importance to *minimus* and *sinensis*, though it may be a seasonal vector. The authors collected very small numbers, and only from March to June. Of eight other anophelines collected in the area, only *A. annularis* occurs in sufficient densities to be of importance as a vector but it is doubtful whether it is of much importance because of its zoophilic habits.

Malaria prevalence.—Records of the former malaria treatment centre of the Japanese Government at Ch'i-Ch'i, about 15 kilometres from Shui-Li-K'eng, reveal that malaria was a serious problem in the vicinity of Shui-Li-K'eng for some years. Since percentage data on parasitism from these records are based upon the examination of all blood films, which include blood films from people who had just finished treatment, the data are weighted in favour of underestimation of malaria prevalence. This underestimation is reckoned to be about 50 per cent. Chart 2 shows mean annual parasite rates for three villages in the Shui-Li-K'eng area for the 15 years prior to closing the centre in 1939. There is a suggestion from the Shui-Li-K'eng curve of a cycle of prevalence with maximum values about every 10 years.

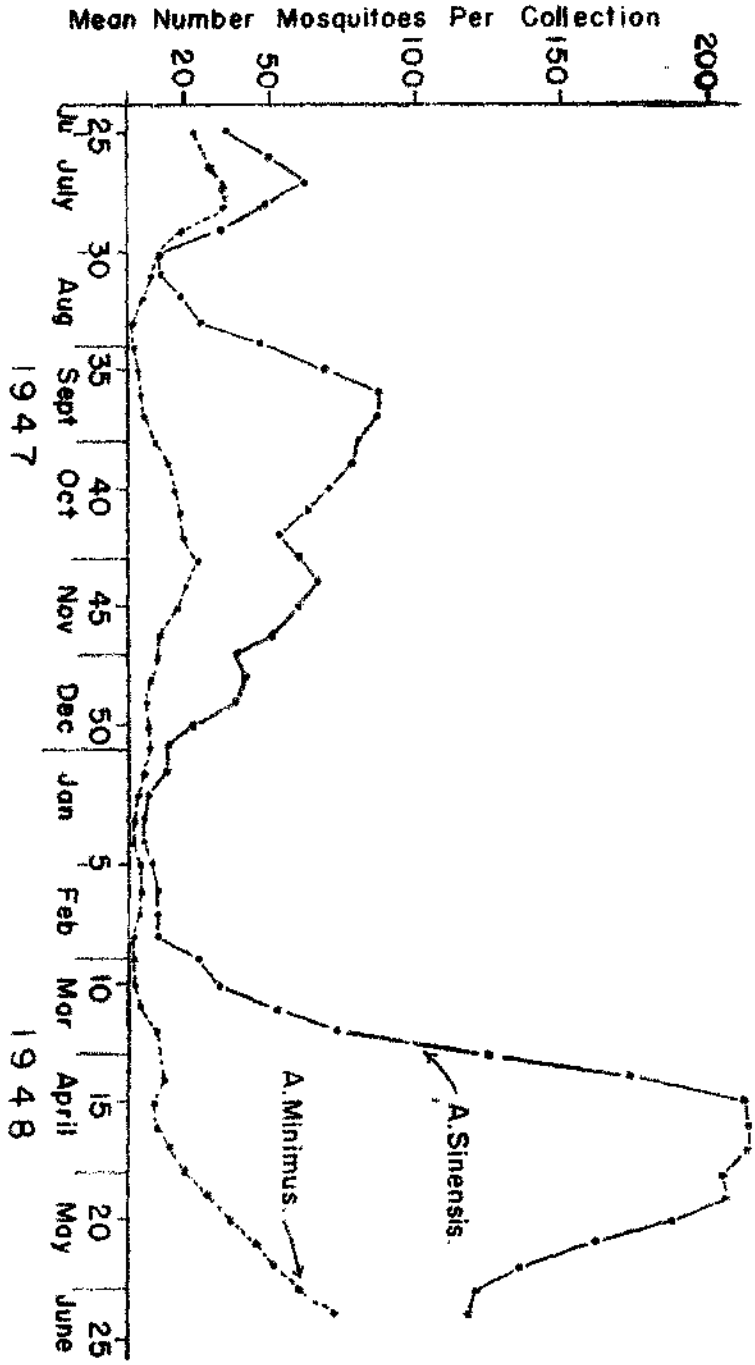
Chart 3 gives mean values for parasitism per month for the 15-year period. There is a definite increase of malaria prevalence from early summer to the end of November, but there is no evidence of the well-marked spring-early summer, late summer-autumn peaks of prevalence observed in areas of Formosa where *sinensis* from ricefields is the principal vector.

Table II gives the monthly variation of spleen and parasite rates, and the average enlarged spleen size, among the children of Shui-Li-K'eng Primary School from June 1947 to May 1948. It is to be noted that parasite rates increased during the summer months, reaching a peak (38·7 per cent) in September. Thereafter, they decreased to a low point (22·5 per cent) in March. Infections with *P. vivax* were most prevalent during the 12 months and in all months but three when *falciparum* infections were greater in number. Infections with *P. falciparum* were next most common and *P. malariæ* infections next; multiple infections were often found.

These data all serve to indicate that the general area of Shui-Li-K'eng suffered from malaria of hyperendemic proportions in past years and during the period of this study as well.

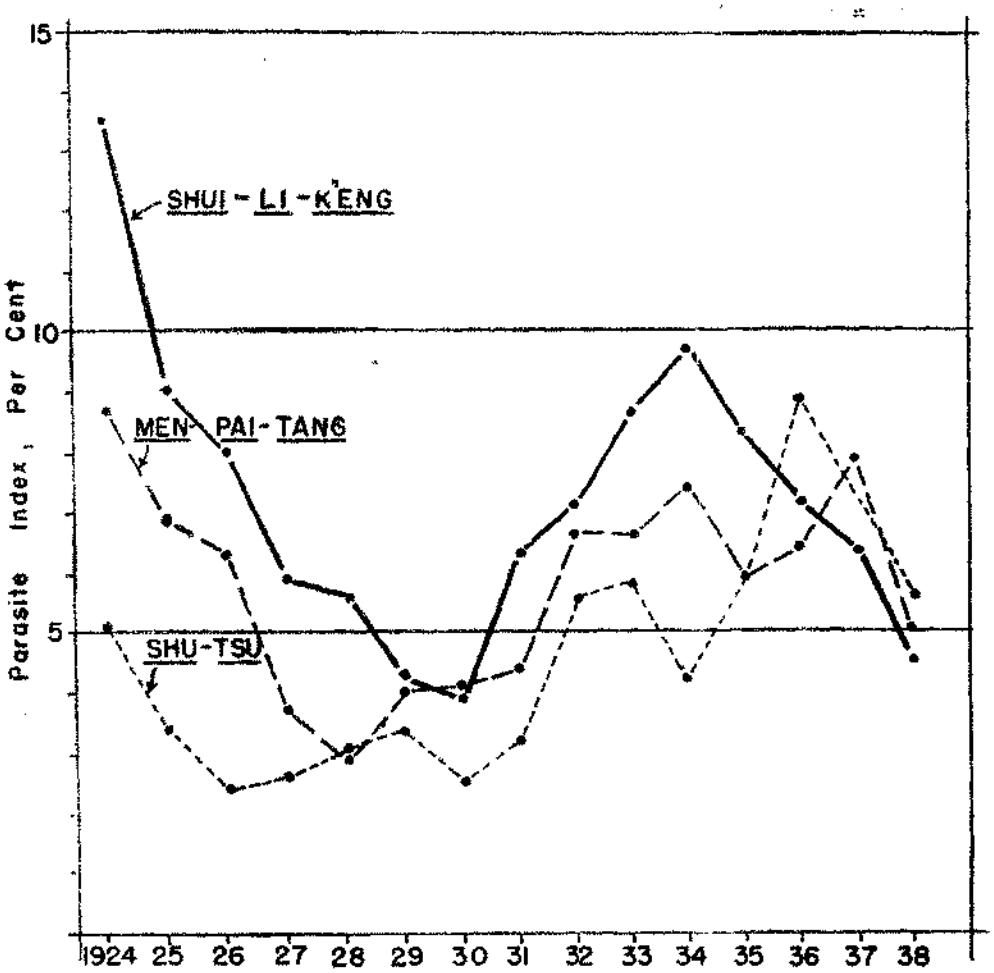
Study population.—Employees of the power company and their families at two hydro-electric generating plants located at Men-Pai-Tang and at Shui-Li-K'eng were treated with chloroquine.

CHART I.



Three weeks' moving mean of the numbers of *Anopheles hyrcanus* var. *sinensis* and *A. minimus* collected from nine catching stations in the vicinity of Shui-Li-K'eng, by weeks, June 1947 to May 1948. The spring increase in *sinensis* density is associated with the rice crop.

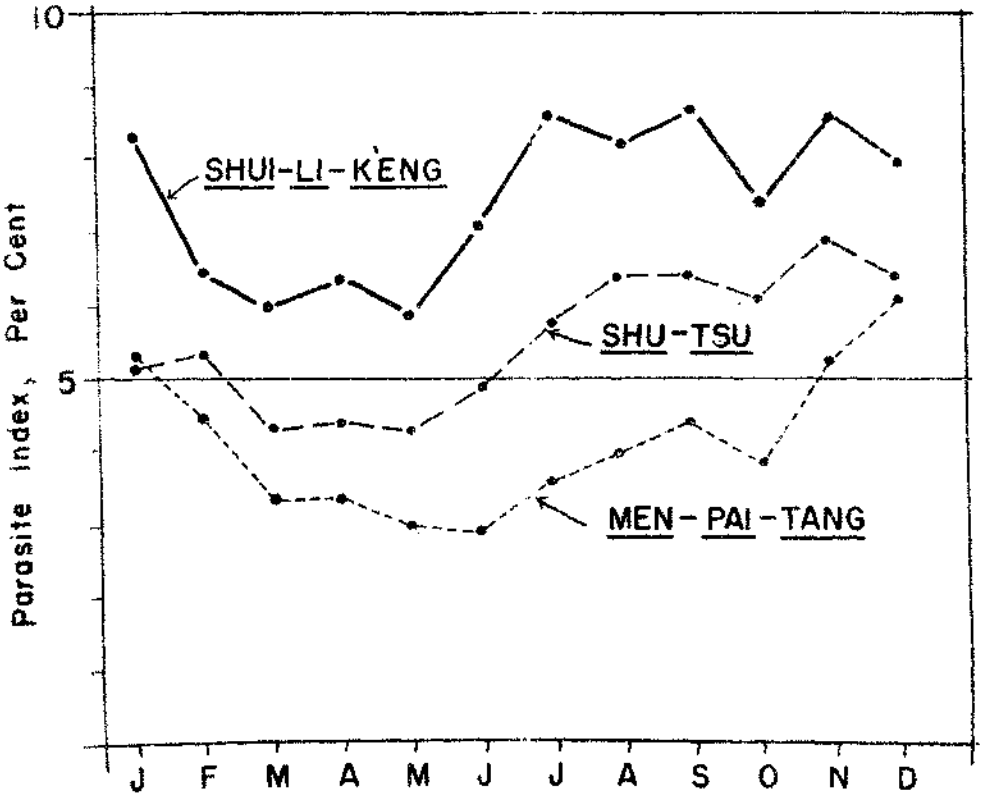
CHART 2.



The mean annual parasite rate for three villages in the Shui-Li-K'eng area of Central Formosa, as computed from the records of the malaria treatment centre at Ch'i-Ch'i, 1924–1938.

Men-Pai-Tang is about seven kilometres from Shui-Li-K'eng and most of its inhabitants work for the power company. These workers, with their families, numbered 251 individuals in 61 families. Of these, 43 families lived in poorly screened quarters and 18 had no screening; however, 55 families used mosquito nets. The houses were all in close proximity to breeding places of *sinensis* and *minimus* and these mosquitoes could be found in the houses throughout the study.

CHART 3.



The seasonal prevalence of malaria in the Shui-Li-K'eng area of Central Formosa. The parasite rates for this composite year were computed from the records of the malaria treatment centre at Ch'i-Ch'i, 1924–1938.

Records of the power company medical office show that an explosive epidemic of malaria followed the bombing of the power plant by the United States Air Force in October 1944. At that time nearly all houses were destroyed and the workers and their families had lived in a tunnel for some weeks. Later, the families moved to neighbouring farm houses and toward the end of 1945 were removed to their present location. During this period there were many deaths from cerebral malaria and from blackwater fever, six deaths in August 1945 alone. From 1943–1945, there was an average of 29·5 cases of malaria per month. During 1946, there were six recorded cases of blackwater fever and one death. During the latter part of 1946, supplies of quinine and mepacrine were secured and afforded temporary relief; nevertheless, an average of 37·3 cases of malaria were treated per month. Drug supplies were exhausted by January 1947 and an average of 21·4 cases of malaria were treated per month through May. There were no deaths nor any blackwater fever during this time. The authors' first survey of this group in June

TABLE II.

Seasonal variation of parasite and spleen rates and of spleen size in the children of Shui-Li-K'eng Primary School, as determined by monthly surveys, June 1947 to May 1948.

Months.	Number examined.	Spleen rate, per cent.	Average enlarged spleen.	Blood films positive for				Parasite rate, per cent.
				vivax.	*falciparum.*	*malariæ.*	Mixed.	
1947								
Jun.	434	76·5	2·1	59	74	13	0	33·6
Jul.	196	80·6	2·2	24	15	16	4	30·1
Aug.	335	71·0	2·2	46	25	41	7	35·5
Sep.	416	69·5	2·4	64	41	41	15	38·7
Oct.	614	70·4	2·4	74	54	51	15	31·6
Nov.	507	71·2	2·2	60	62	18	10	29·6
Dec.	575	68·1	2·1	52	71	26	17	28·9
1948								
Jan.	327	59·3	2·1	38	27	16	7	26·9
Feb.	539	63·0	2·2	67	25	23	9	23·0
Mar.	644	55·1	2·1	70	28	39	8	22·5
Apr.	614	57·3	2·1	57	41	43	7	24·6
May	640	54·5	2·0	71	52	49	3	27·3
All months mean.	487	66·4	2·18	56·8	43·2	31·3	8·5	28·7

1947 included individuals of all age groups among 217 examinations. The spleen rate was 40·5 per cent with an average enlarged spleen of 2·0; the parasite rate being 14·3 per cent.

The personnel of the power plant at Shui-Li-K'eng and their families live in a group of houses on a hill about 300 metres above the level of the river and village. The population of approximately 200, comprising 43 families, were in poorly screened houses; 26 families used bed nets. The site had been selected by the Japanese management of the company in the belief that it was less malarious than Shui-Li-K'eng Village. Any such benefit was partly lost through the fact that many of the residents spent their evenings in the village.

There were no records of malaria cases for this group except for the six months preceding June 1947, when there was an average of 43 cases per month. Our first survey of this group in June 1947 included 194 individuals of all ages. The spleen rate was 41·8 with an average enlarged spleen of 1·7; the parasite rate being 18·5 per cent.

METHODS.

The primary objective of this study was to determine the minimum dose of chloroquine which would effect satisfactory suppression of clinical malaria, while the secondary one was to determine an effective dosage regime for clinical malaria. At the time of the study, no record of the drugs having been used on Chinese was available. There were some misgivings on this score because instances of severe toxic reactions following the administration of atabrine (mepacrine or quinacrine) to Chinese were known. The maximum single dose given (0·500 gm.*) was based partly upon the fact that this dose of chloroquine could be taken safely by adult Europeans, partly because of supply of the drug was in tablets containing 0·250 gm. of the base. These tablets were scored so that they could be broken easily into halves; smaller doses were obtained by cutting the halves with a razor blade, or by reducing the halves to powder and dividing into smaller doses.

At the first survey, everyone was weighed and a suppressive dose was given according to the following schedule; subsequent doses were given at weekly intervals.

Weight in kg.		*Dosage in grammes.*
50 or more	...	0·250
30 to 49	..	0·125
15 to 29	..	0·063
10 to 14	..	0·030
9 or less	..	0·020

All persons demonstrating parasitaemia at the first survey or subsequently were given a therapeutic course of the drug with doses at six-hour intervals; one week after the last dose the suppressive regime was resumed. This dosage schedule, in grammes, was as follows:—

Weight in kg.		*First dose.*	*Second dose.*	*Subsequent doses.*	*Total drug.*
50 or more	...	0·500	0·250	0·250	2·00
30 to 49	...	0·250	0·250	0·125	1·50
15 to 29	..	0·250	0·125	0·125	1·00
10 to 14	...	0·050	0·025	0·025	0·25
9 or less	...	0·025	0·015	0·015	0·10

Table III gives the approximate dosage in milligrammes per kilogram body weight for age-weight groups according to these treatment schedules.

* All dosages of chloroquine noted in this paper are expressed in terms of the base of the drug.

TABLE III.

Approximate dose of chloroquine in milligrammes per kilogram body weight by age-weight groups of the study population, for suppressive and for curative treatment.

Age group.	Number.	Mean weight, kg.	Average suppressive dose, mg./kg.	Average total therapeutic dose, mg./kg.
0–2	20	7·5	2·65	12·1
3–5	30	12·7	2·37	19·6
6–10	41	17·4	3·62	57·4
11–15	36	25·6	2·46	39·0
16–20	66	43·3	2·88	36·9
21–30	117	51·1	4·89	39·1
31–40	55	53·8	4·64	37·1
41–50	34	51·2	4·88	39·6
51 and over	16	49·7	5·23	40·2

The values for the suppressive dose were computed from the schedules for the original regime.

Blood films were taken at 24-hour intervals from persons receiving therapy until parasites disappeared from the blood stream.

The first therapeutic dose and all suppressive doses were taken under the supervision of a physician. It was not feasible to supervise the full course of therapy; the requisite drug supply was given to a responsible person with full directions for its administration. It is considered that these directions usually were followed exactly.

At the end of the sixth week of the study, a second survey was done and only one positive blood film (*P. falciparum* gametocytes) was found, so the suppressive dosage was reduced. One-half of the people (Group 1) were given the original dosage at intervals of two weeks; the other half (Group 2) were given half the original dosage at weekly intervals.

At the end of the 12th and 16th weeks, other surveys were made and cases of parasitæmia were determined; also cases of clinical malaria had occurred with increasing frequency from the eighth week. Accordingly, beginning with the 17th week, Group 1 was given double the original suppressive dose at intervals of two weeks, while Group 2 was returned to the original dosage schedule at weekly intervals. This regime was continued to the end of the study.

Excellent co-operation was secured from the people throughout the study.

RESULTS.

Suppression of malaria parasitism.—At the first survey there were 30 cases of malaria illness among the 411 people examined: 11 *falciparum* infections; 13 *vivax* infections; 4 *malariæ* infections; and 2 mixed infections (*malariæ-vivax* and *malariæ-falciparum*). There were also some cases of illness without parasitæmia (which may have been malaria) and 38 instances of asymptomatic parasitæmia: 6 *falciparum*; 25 *vivax*; 5 *malariæ*; and 1 *vivax-falciparum* infection. These 68 cases of parasitæmia cleared up promptly under treatment.

During the following six weeks (week 2 to week 7), when the people were under the influence of the original suppressive regime, only four instances of parasitæmia occurred. These were all *falciparum* infections and only one case had previously demonstrated an infection (*falciparum*): three were clinically ill and one, with gametocytes only, was asymptomatic.

During the following 10 weeks (week 8 to week 17), under the second regime, there were 38 cases of parasitæmia. People in Group 1, receiving the original dose at intervals of two weeks, furnished 18 instances of which 16 were ill: 9 *vivax* and 8 *falciparum*. People in Group 2, receiving half the original dose at weekly intervals, had 13 cases of malaria illness: 8 *vivax* and 5 *falciparum*. There were 7 instances of asymptomatic parasitæmia: 3 *vivax* and 4 *falciparum*.

During the last 12 weeks of the study, there were 27 cases of parasitæmia. People in Group 1, receiving double the original suppressive dose at intervals of two weeks, furnished 18 cases of which 11 were sick: 3 *vivax* and 8 *falciparum*: the asymptomatic cases were *vivax* infections. Group 2, on the original regime, had two cases of malaria illness (one *vivax* and one *falciparum*): and 7 asymptomatic infections: 2 *vivax* and 5 *falciparum*.

There was no instance of a *malariæ* infection after the 11 cases found at the original survey had cleared up under treatment.

Of the 69 cases of parasitæmia that developed during the study, 41 cases (59 per cent) occurred in persons less than 16 years old.

Response of infections to therapy.—In addition to the cases noted above, new employees and persons who had broken their suppressive discipline for various reasons furnished cases of parasitæmia which were treated. Table IV is a record of 81 cases of malaria illness and 39 asymptomatic cases which were examined at 24-hour intervals until parasites disappeared from their blood films. Of the sick cases, those with *vivax* infections seemed most sensitive to therapy: 30 out of 37 cases were parasite-free within 24 hours. Of the three *malariæ* infections, one required longer than 72 hours to become free of parasites, the only instance of any infection which did not clear up in three days. No record is given for cases where only gametocytes of *P. falciparum* were found: these were resistant to treatment and there are several instances where these forms persisted in the blood after 96 hours. The asymptomatic cases cleared up much more quickly than the clinical cases: 38 of the 39 infections had disappeared within 24 hours.

Toxic effects presumably due to chloroquine.—The only complaints which might have been due to toxic effects of the drug following suppressive therapy occurred in persons more than 50 years old among the group which received the double dose from the 7th to the 16th week. This dose amounted to about 10·5 mg. per kg.

TABLE IV.

Clearance of parasites from the blood stream following administration of chloroquine.

Parasite species.	Hours required for blood films to become negative.									
	Malaria illness.					Asymptomatic parasitæmia.				
	Number of cases.	24	48	72	96	Number of cases.	24	48	72	96
P. falciparum	37	22	8	7	0	7	7	0	0	0
P. vivax	37	30	5	2	0	25	24	1	0	0
P. malariæ	3	1	1	0	1	6	6	0	0	0
Mixed infections	4	3	1	0	0	1	1	0	0	0
All infections	81	56	15	9	1	39	38	1	0	0

body weight. Of 80 persons in this group, there were 16 complaints of dizziness only; 1 of dizziness with nausea and vomiting; 1 of dizziness with abdominal pain; 1 of dizziness with diarrhœa; and 1 of diarrhœa only. These complaints developed shortly after taking the drug and soon disappeared. There were no complaints among the children or other adults.

Of 427 persons who took the original suppressive dose at weekly intervals, there were only 15 complaints, many of which may not have been due to the drug. These complaints were: dizziness alone, 4; dizziness with backache, 1; dizziness with diarrhœa, 1; headache and epigastric pain, 4; diarrhœa alone, 5.

Persons taking the therapeutic regime gave a higher proportion of complaints: of 132 persons treated, 26 (19·7 per cent) complained of discomfort that may have been due to the drug. Persons aged 30 or more furnished 21 instances, and the age group 15 to 29 years the remaining 5 instances. There were 11 cases of dizziness only; 3 of headache only; 3 of diarrhœa only; 4 of dizziness with anorexia, nausea or headache; 3 of headache and abdominal pain; 2 of anorexia only; and 1 case of abdominal pain.

The frequency of the complaint of dizziness in all groups, either alone or associated with some other complaint, suggests that this may be an authentic symptom of drug toxicity. In no instance was a complaint of sufficient importance to warrant withholding the drug. Ten pregnant women received the drug and eventually came to term and delivered normally; there were no cases of miscarriage or abortion during the study.

Changes in spleen rates and size.—Table V gives the data from the seven surveys of the people at the two plants. There has been no adjustment of the rates for

persons who came into the study at the time of a survey after the first survey, or for persons who may have broken suppressive discipline. These rates are therefore weighted slightly in favour of high values, but are a better indication of malaria prevalence than adjusted rates would be. The parasite rate fell from 16 per cent to less than 1 per cent during the study. The spleen rate declined progressively from 41 to 23 per cent; the average enlarged spleen size from 1·9 to 1·6.

TABLE V.

Changes in parasite rates, spleen rates and spleen size, in the population receiving chloroquine, as determined by eight surveys, 1947-48.

Survey date.	Number examined.	Spleen rate, per cent.	Average enlarged spleen.	Parasite rate, per cent.
1947				
Jun. 5	411	41	1·9	16·3
Jul. 17	377	35	1·8	0·5
Aug. 28	362	32	1·7	4·1
Sep. 25	372	30	1·8	3·5
Oct. 23	373	27	1·6	2·4
Nov. 29	352	22	1·6	1·7
Dec. 18	353	23	1·6	0·6
1948				
Feb. 1	287	27	1·65	10·1

Attention is invited particularly to the data from the survey of February 1, made six weeks after chloroquine had been discontinued in both population groups. The parasite rate rose to 10 per cent; of the 29 infections, 27 were *vivax* and 2 *falciparum*. These are not considered new infections, but they may have been. The spleen rate and the average enlarged spleen size show increase, but not a great deal.

After this survey, all employees of the power plants were placed upon the original suppressive regime with good results. A supply of the drug was made available to the management of the power company for this purpose and for the treatment of women and children.

Malaria prevalence in unprotected populations.—In Table II are shown the data on malaria among children of the Shui-Li-K'eng Primary School, based on monthly surveys from June 1947 to May 1948. In Table VI are data from surveys of all

people living in the village of Men-Pai-Tang, who were not included in the study. The small number of persons examined makes these rates unstable, but it is evident that the people of this village suffered severely from malaria during the period of the study. There would appear to be little difference between the prevalence of malaria at Shui-Li-K'eng and its prevalence at Men-Pai-Tang during the latter half of 1947.

TABLE VI

Malaria parasitism and splenomegaly in the unprotected population of Men-Pai-Tang village, 1947.

Survey date.	Number examined.	Spleen rate, per cent.	Average enlarged spleen.	Parasite rate, per cent.
Aug. 19 ...	63	87·3	2·52	31·7
Sep. 9 ...	61	85·2	2·73	29·5
Oct. 29 ...	37	83·7	2·54	37·8
Nov. 21 ...	39	97·4	2·71	38·5
Dec. 22 ...	60	90·0	2·64	33·3

There is other evidence of the seriousness of the malaria problem in the general vicinity of Shui-Li-K'eng during the time of this study. A regiment of Chinese soldiers from the mainland was stationed at Shui-Li-K'eng on June 17. A survey of these men, about 500 persons, was made by a technician from the malaria treatment centre at Ch'i-Ch'i; malaria parasites were found in only two blood films. At the end of two months this regiment was removed, having suffered severely from malaria during its stay. No exact data on malaria prevalence in these men is available, but immediately before they left, 53 soldiers who were reported to have had malaria were examined, 31 were found infected with malaria parasites: 23 *falciparum*, 7 *vivax* and 1 *malaria-falciparum*.

On September 27, replacement troops arrived and were stationed at the two power plants. They were said to be free of malaria on arrival but on October 3 one case of malaria was reported. The second case was reported on October 19 and the authors were asked to examine the sick and to suggest control measures. At Men-Pai-Tang, 44 men who claimed to have malaria were examined; 14 were infected, 10 with *falciparum* and 4 with *vivax* parasites. Of 84 men examined at Shui-Li-K'eng, 13 were infected with *falciparum* and 6 with *vivax* parasites.

DISCUSSION.

This study was begun at a time of the year when malaria parasitism was increasing in the general population of the Shui-Li-K'eng area. One should be mindful of this fact when considering the effectiveness of the suppression programme with chloroquine. The parasite rate of the people treated was 16 per cent at the start

of the study, about half the rate for the Shui-Li-K'eng school children examined at the same time. The malaria prevalence of the study population was probably somewhat lower than that in the general population when the study started. If so, this is considered to be due mostly to the treatment received by the study population up to about six months before the programme was commenced.

It is considered reasonable to suppose that parasite rates in the study population would have risen to levels proportional to those in the associated unprotected population; say, to about 20 to 22 per cent in September. The reduction of rates actually recorded must therefore have been due to the influence of the drug.

The authors believe that their primary objective, to find the minimum satisfactory suppressive regime with chloroquine under their working conditions, may have been gained. In general, the authors think that the original schedule which provided approximately 5 mg. per kg. of the drug to persons weighing about 50 kg., at weekly intervals, would be adequate in most of Formosa. This dosage was attained by giving one tablet containing 0·25 gm. of the base and it is noted that the manufacturers are now recommending this dosage for general use. The effectiveness recorded for the original dosage schedule may have been due in part to treating all cases of parasitæmia before starting suppression; in part to the lower rate of malaria transmission at the beginning of the study. In hyperendemic places a higher dosage and shorter intervals might be desirable; say, 6 mg. per kg. every five days.

It is believed that the dosage for the children was too low and would favour upward adjustment on a weight basis to provide about 5 mg. of drug per kg. body weight.

It is evident that adequate suppression is not afforded by doses smaller than those employed in the original schedule. Also that intervals between doses longer than seven days gave poor results, even when the original dose was doubled. While excretion rates of the drug are slow, Berliner *et al.* (1948) have shown that plasma concentrations drop at the rate of about 60 per cent per week after administration of the last dose of a treatment regime. Some protection may have been afforded at the end of the first week, but very little towards the end of the second week after the single suppressive dose.

The therapeutic schedule employed seemed to us to give effective results. The authors do not think that it would be safe to use a regime which would provide for much less than 40 mg. per kg., total dosage, where one has to deal with a virulent *falciparum* strain. Therefore, the doses employed for children less than six years old are considered dangerously low and should have been increased. This view has been strengthened by the case of a *falciparum* infection in the child (an infant) of one of the junior authors. This child received chloroquine at the rate of approximately 12 mg. per kg. and had a stormy illness for two days.

It is noted that the manufacturers of chloroquine are recommending a total dose of 2·5 grammes for adults; this would provide 50 mg. per kg. for 50 kg. people and in general should be adequate. But in the treatment of *falciparum* malaria, the authors hold to the Osleriana admonition that the sinews of understanding are developed by being sober and distrustful. This should apply to any rule-of-thumb treatment regime, particularly in the absence of good diagnostic facilities and the presence of virulent *falciparum* strains.

It has been remarked that the people being treated were quite co-operative: this is a relative term and does not mean that everyone reported promptly every week for his suppressive dose. Some people had to be hunted down and required to take the drug. There was evident lack of interest toward the end of the study as compared with its start: it is remarkable how a state of relative well-being produces an amnesia to the causes which brought about this state. This fact should be reckoned within any programme that seeks to control malaria by the use of drugs alone, or that is otherwise dependent upon the active co-operation of people to be protected.

SUMMARY.

The employees of two power plants in a highly malarious area of Central Formosa and their families, about four hundred people, were treated with chloroquine during the last six months of 1947. The drug was administered as a suppressive and as a curative agent to persons with parasitæmia, with or without clinical illness.

Suppressive doses of 5 mg. per kg. body weight at weekly intervals gave good results in adults. Doses as low as 2·5 mg. per kg. to infants and children also gave good suppression of parasitæmia. Smaller doses per week and increasing the interval between doses to two weeks, even when the dose was doubled, did not give adequate protection.

The authors believe that suppressive programme using chloroquine should be based upon the administration of 5 mg. per kg. body weight to all persons at weekly intervals.

Treatment of parasitæmia cases with a total dosage of 40 mg. per kg. over a two-day period was followed in most instances by disappearance of parasitæmia in 24 hours. In infants and children, doses as low as 12 mg. per kg. gave satisfactory results.

When chloroquine is used to treat malaria cases, a total dose of 50 mg. per kg. should be used for all age groups, with the initial dose twice the size of subsequent individual doses.

Some of the adults complained of symptoms that may have been due to toxic effects of the drug. The most common symptom was "dizziness".

REFERENCE.

Berliner, R. W., Earle, D. P., Jr., Taggart, J. V., Zubrod, C. V., Welch, W. J., Conan, N. J., Bauman, E., Schnider, S. T., and Shannon, J. A. (1948). Studies on the chemotherapy of the human malarias. VI. The physiological disposition, antimalarial activity and toxicity of several derivatives of 4-aminoquinoline. *J. Clin. Invest.*, **27**, No. 3, part 2, pp. 98–107.

Indian Journal of Malariology, 4, 3, September 1950.

FURTHER OBSERVATIONS ON TRANSMISSION EXPERIMENTS WITH *P. KNOWLESI*.

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[July 20, 1950.]

IN a previous paper the authors reported their success in completing the sporogony cycle of *P. knowlesi* in *A. annularis* and *A. stephensi* (type) and subsequently transmitting the infection to healthy *S. rhesus* monkeys of northern India (Jaswant Singh *et al.*, 1949). During the course of the investigations they had noticed that the prepatent period in the monkey receiving inoculation of glands from three infected mosquitoes was much shorter than that in the animal which received inoculation from one mosquito only. Two out of the three animals which received inoculation from one mosquito each failed to show patent infection. Similar observations have been made by Boyd (1940), who had shown that when the dosage is small the proportion of unsuccessful inoculations is high. Jaswant Singh *et al.* (*loc. cit.*) had also indicated that the intensity of infection, in both the monkeys in which patent infection was established after sporozoite inoculation was low, and the infection ran a chronic course. Due to the scanty infection, detailed study had not been possible.

The present paper reports further observations made during the spring and the early summer of 1950, and records the findings on the possible relationship of the dose of the inoculum (*sporozoites and trophozoites*), the prepatent period, the intensity and the course of the infection. Morphological variations encountered in the sporozoite-induced infections are also noted.

MATERIALS AND METHODS.

Host.—All monkeys, *M. rhesus*, used for these investigations were young adolescents and obtained from the neighbourhood of Delhi.

Vector.—*A. annularis* and *A. stephensi* (*mysorensis*) were mainly utilized for feeding on the infected animals. A few *A. fluviatilis* from South India were also fed, but they were so few that no inference should be drawn on the results obtained with that species. *A. annularis* were bred from larvæ collected in the field in the neighbourhood of Delhi. In this connection it may be mentioned that this species breed extensively in and around Delhi during early winter and spring. Collection of *A. annularis* larvæ after the middle of May, though not impossible, is highly improbable, as practically all breeding places dry up in early summer months. *A. stephensi* (*mysorensis*) was obtained partly from the colony maintained at this Institute and partly from larvæ collected from wells in the city of Delhi.

The mosquitoes were fed from glass chimneys covered with mosquito netting on both sides, on shaved abdominal wall of the infected animal tied to a wooden cross for 30 minutes at a time.

Number of feeds and maintenance thereafter.—As the infectivity rates in the mosquitoes in the authors' previous experiments were found to be very low, comparative study of repeated feeding based on observations of James (1926), that mosquitoes which at first seemed refractory to infection could be forced, so to speak, to become infective by repeated feeding in suitable cases, was also undertaken. Many mosquitoes were fed only once but in other series the mosquitoes were grouped into three batches, the first were fed only once, the second twice and the last batch three times at intervals of 2 to 3 days. Infectivity rates were apparently higher in those fed twice or thrice, and the results of these observations will appear later along with further studies on *P. knowlesi*.

As a rule the mosquitoes were starved overnight before feeding on infected monkeys.

After the infective feeds, the mosquitoes were kept on glucose or raisins for the rest of the period, pending infective bites and dissection thereafter.

Temperature and humidity of the insectarium where the mosquitoes were kept in Barraud's cages were recorded daily and the average monthly figures are given in Table I.

TABLE I.

Temperature and humidity of the insectarium.

	Temperature, F.		Humidity, per cent.	
	Maximum.	Minimum.	8 a.m.	4 p.m.
March	72·8	71·0	68·0	68·5
April	79·7	75·5	76·5	73·7
May	85·3	83·1	60·0	66·4

Dissection of the mosquitoes.—From the experience gained in our previous studies the mosquitoes were not dissected until the 3rd week. Prior to dissection, each batch of insects which had survived this period, were allowed to bite healthy *rhesus* monkeys. Serum from healthy monkey with half the quantity of 0·9 per cent sodium chloride solution was used during dissection. The emulsion of infected glands in serum-saline were inoculated intravenously in most cases. On one occasion inoculation was given subcutaneously while in another intraperitoneally (Table II).

Parasites.—Total parasite and gametocyte counts were carried out prior to feeding the mosquitoes on the infected animals. Fowl cell suspension technique (Sinton, 1924) was adopted throughout for parasite count. Blood smears, thick and thin (on the same slide), were stained with J.S.B.* stain (Jaswant Singh and Bhattacharji, 1944).

In all cases at least 100 microscopical fields of thick films were examined before recording 'no parasite detected'. For study of the schizogony cycle, blood films were taken at an interval of 4 hours in most cases and 3 in some.

Prior to feeding, exflagellation tests were carried out in a large number of cases, though exflagellation was not always encountered.

Subinoculations.—Unless specifically mentioned, subinoculations were carried out with 5 million parasites.

Observations and results.—Out of a total number of 3,991 *A. annularis* fed on infected monkeys showing gametocytes varying from two hundred to fifteen thousand per c.mm., the number available for infective bites on healthy animals and subsequent dissection was only 590, i.e. 14·8 per cent. The long incubation period that is necessary for the sporogony cycle in this plasmodium is the main factor for such high mortality. The total number of mosquitoes found infected (gland) was 19, i.e. the overall infectivity rate during the period of observation was 3·2 per cent. The detailed observations are recorded in Table II. The number of mosquitoes dissected as shown in the table were fed in March, April and early May.

The number of *A. stephensi* fed and subsequently dissected after bites on healthy animals was 1,860 and 386, respectively. Neither oöcysts nor sporozoites were detected in any of them. Similarly 5 out of 7 *A. flaviatilis* showed negative result.

Sporozoite dose, prepatent period and the intensity of infection.—Mention has been made in the previous studies that the maximum dose of sporozoites inoculated to a healthy monkey was from three mosquitoes, and though the patent infection was established after 7 days, the infection ran a chronic course. In the present series the animals, all except one, received graded dosage of inoculum in the form of suspension of infected glands, in serum diluted one-half with 0·9 per cent sodium chloride. As previously stated, the inoculation was given subsequent to the infective bites. In Series VII (Table III), the animal did not receive any inoculation but received bites from a batch of 20 *A. annularis* mosquitoes out of

* It may be mentioned here that due to the overwhelming advantages over other stains, for the past 6 years, no other stain had been in use in these laboratories.

It has not been uncommon to encounter fine stipplings (finer than seen in *P. cynomolgi* infections) in some of the R.B.C. infected with *P. knowlesi* in well-stained films.

TABLE 11

Details of feeding & maintaining, dissections and inoculations of infected glands

Series number.	Monkey number (Ali.)	Total parasite count per c.mm.	Total gametocytes per c.mm.	Number of A. sinensis fed from different batches	Date of feeding	Number dissected.	Date of dissection.	Gut infections.	Gland infections	Remarks.
					1950		1950			
I	1801	29,500	2,000	10	Mar. 14	3	Apr. 3		1	Injected to monkey number 1845 i.v. on Apr. 3, 1950.
	1801	21,600	2,500	30	Mar. 18	12	Apr. 3		2	Injected to monkey number 1845 i.v. on Apr. 3, 1950.
	1801	61,000	[illegible]	16	[illegible]		Apr. 4			Injected to monkey number 1847 i.v. on Apr. 4, 1950.
	1801	92,000	6,000	50	Mar. 16	6	Apr. 5		2	Injected to monkey number 1845 i.v. on May 5, 1950.
	1802	33,500	5,500	83	Mar. 14	28	Apr. [illegible]		1	Injected to monkey number 1845 subcutaneously on May 5, 1950.
II	1857	48,000	6,000	70	Apr. 29	7	May 15	...	1	Injected to monkey number 1896 i.v. on May 15, 1950.
	1861	58,000	8,000	60	May 2	9	May 16	...	1	Injected to monkey number 1896 intraperitoneally on May 16, 1950.
	1862	38,000	2,000	27	May 5	4	May 20	...	1	Injected to monkey number 1896 i.v. on May 20, 1950.
III	1856	92,000	3,000	36	Apr. 27	3	May 9	...	1	Injected to monkey number 1884 i.v. on May 9, 1950.
	1868	86,000	2,000	30	Apr. 21	2	May 11	...	1	Injected to monkey number 1884 i.v. on May 11, 1950.

IV ...	1829	25,000	1,000	120	Apr. 5	20	May 1	...	1	Injected to monkey number 1875 i.v. on May 1, 1950.
V ...	1829	32,800	3,600	50	Apr. 3	5	Apr. 30	...	1	Injected to monkey number 1876 i.v. on Apr. 30, 1950.
VI and VII	1814	40,000	2,000	90	Mar. 29	19	Apr. 25	...	1	Infective feed on monkey number 1846, but gland suspension injected to monkey number 1869 i.v. on Apr. 25, 1950.

* Sporozoites in the glands of 4 other *A. annularis* of different batches were also detected. These were utilized in connection with some other investigation and also for staining and preserving. For camera lucida drawings of the sporozoites (*see* Plate X).

TABLE III.

Dose of inoculum, prepatent period and course of infection.

Series number.	Monkey number.	Infective bites.	Inoculum.				Prepatent period in days.	Day of death from the date of patent infection.	Tendency to chronicity.
			Number of infective mosquitoes involved.	Degree of gland infection.					
				Heavy.	Medium.	Light or scanty.			
I	1845	+	5	2	1	2	11	7	.
II	1896	+	3	1	1	1	18	15	...
III	1884	+	2	...	1	1	Died during the prepatent period.		
IV	1875	+	1	...	1	...	38	...	Chronic throughout.
V	1876	+	1	...	1	...	Died during the prepatent period.		
VI	1869	No infective bites	1	...	...	1	44	...	Chronic throughout.
VII	1846	..	...	No inoculation of gland			53		Do.

which one was subsequently found infected. The gland suspension was inoculated to one monkey in Series VI. The various dosage regimes adopted and the results obtained are shown in Table III and have been described under Series I to VII, classified according to the number of infected mosquitoes involved in each series.

Series I.—Glands from five infected mosquitoes from different batches were inoculated to one monkey on three consecutive dates; from two mosquitoes each on April 3 and 5 and from one on April 4, after they had fed on the same animal. The prepatent period was 11 days after the first inoculation or 9 days after the last, and there was a rapid and progressive rise in the infection culminating in the death of the animal when the infection was at its peak. Prior to the death of the animal, subinoculation was carried out in two normal monkeys (1856 and 1859) and in both cases patent infection was established in one day after a dose of 5 million erythrocytic parasites in each. The course of the infection was rapid though not to the same degree as was noticed in M. 1845. While one monkey died after 12 days (just after the peak) of the first appearance of the parasites in the peripheral blood, the other died on the 18th day, a few days after the peak. Post-mortem report in case of these animals showed enlargement of spleen and signs of severe anæmia. These and subsequent subinoculations are shown in Chart I.

Monkey 1868 subinoculated from M. 1856 showed patent infection the following day and died after 5 days when infection was very heavy (+ + + +)*. Due to sudden death, subsequent subinoculation could not be done.

Monkey 1870 was subinoculated from M. 1859 (subinoculation 2); patent infection was established after one day and the animal died 16 days thereafter. On post-mortem, the spleen was found to be enlarged and signs of severe anæmia were detected. Subinoculation (3) from this animal was carried out into two monkeys, one receiving the usual dose, i.e. 5 million of erythrocytic parasites, and the other one million parasites. While the first died just after the peak of the parasitæmia on the 10th day after patent infection was established, the infection in the other (M. 1872) took a chronic course after a slow but progressive rise in parasitæmia. It is interesting to note that the patent infection after receiving a dose of 5 million parasites was established in M. 1888 after 2 days, whereas in all the previous cases which had the same dose, prepatent period was only one day; prepatent period was 3 days in M. 1872 which received inoculum of 1 million parasites. As the course of infection appeared to be very slow, no further subinoculation was done from this animal, though subsequent subinoculation (4) was made in respect of M. 1901 from M. 1888. The dose of inoculum was 5 million and the prepatent period was only one day. The infection, however, ran a chronic course after an initial rise in the parasitæmia (up to + + +)†. Results of subsequent subinoculations showed similar chronic course and the patent infection was established in M. 1904 which received 2·5 million parasites from M. 1901 in 3 days. In this connection it is worth while to mention that when erythrocytic parasites of strain I‡ are inoculated in 5 million dosage, the prepatent period is 3 days.

* 26 to 50 parasites per field of thin film.

† 11 to 25 parasites per field of thin film.

‡ Strain I refers to the blood-passaged strain in which attenuation of the virulence has been noticed from 1947 (Jaswant Singh *et al., loc. cit.*). This strain is being maintained for years in these laboratories. Strain II refers to sporozoite-induced strain. Strain IIa parasites in the animals subinoculated from strain II.

CHART I.

Series I.

Monkey 1845 was inoculated with gland suspension from 5 infective *T. annularis*, from two each on April 3 and 5 and from one on April 4.

1845

Patent infection was established on April 14.
Died on April 21.

1856

SUBINOCULATION (1) on April 14.
Inoculum—5 million.
Patency established—April 15.
Died—May 3.

1868

SUBINOCULATION (2) on April 21.
Inoculum—5 million.
Patency established—April 22.
Died—April 27.

1883

SUBINOCULATION (3) on May 6.
Inoculum—5 million.
Patency established—May 8.
Died—May 16.

1901

SUBINOCULATION (4) on May 15.
Inoculum—5 million.
Patency established—May 16.
Chronic after infection had attained +++.

1904

SUBINOCULATION (5) on May 26.
Inoculum—2·5 million.
Patency established—May 29.
Chronic after the infection had attained +++.

1859

SUBINOCULATION (1) on April 19.
Inoculum—5 million.
Patency established—April 20.
Died—May 2.

1870

SUBINOCULATION (2) on April 26.
Inoculum—5 million.
Patency established—April 27.
Died—May 14.

1872

SUBINOCULATION (3) on April 28.
Inoculum—1 million.
Patency established—May 1.
Chronic after infection had attained +++.

Series II.—Gland suspension from three infected mosquitoes, all from different batches, was inoculated on three different dates. The prepatent period calculated from the date of the first injection of the sporozoites appeared to be 18 days. The infection was progressive though not as rapid as was seen in case of the monkeys up to the 3rd subinoculation in Series I. The animal, however, died after 15 days, several days after the peak was over and at a time when the parasitaemia was very low, parasites being detected *only* in the thick film. Post-mortem examination showed enlargement of the spleen and signs of extreme anaemia. Subinoculation to M. 1905 was carried out with 15 c.c. of blood shortly before the death of the animal. The patency was established after 7 days. The infection ran a very chronic course subsequent to an initial rise up to '+' *. The prepatent period was however only one day in the subinoculation Series II in M. 1939, when 5 million parasites were inoculated. But the course of infection appeared to be extremely chronic. The infection at no stage reached over 'scanty' ‡ stage.

Series III.—The animal which received inoculation from two infected mosquitoes (Table III, Series III) unfortunately died of acute dysentery during the prepatent period on the 5th day after the sporozoite inoculation.

Series IV and V.—Suspension of infected glands from one mosquito was inoculated in M. 1875. Similar dose was given to another, M. 1876, which died during the prepatent period due to intercurrent infection (post-mortem examination showed extensive lung lesions). The prepatent period in M. 1875 was observed to be 38 days. The infection ran very low, parasites being detected only in thick film preparations.

Series VI and VII.—Monkey 1846 (Series VII) was subjected to infective bites from a batch of 20 mosquitoes, out of which one showed sporozoite of low density in the salivary glands. The suspension of the glands was inoculated to another, M. 1869 (Series VI). While the prepatent period in the latter was 44 days that in the former was 53 days. The course of infection ran extremely low in both, parasitaemia never attained over 'v.v.s.' ‡ stage. The animals subinoculated from these also showed a very low grade infection. Parasites were detected mainly in the thick film preparation though on a few occasions parasites in the thin film were observed in respect of M. 1869 and 1921 (subinoculated from M. 1869).

Morphology.—As previously mentioned, patent infection was established in M. 1845 in Series I after 11 days from the date of the 1st inoculation of the sporozoites. The parasites were detected both in the thick and thin film on the same day (1 parasite per 50 fields of thin film). Curiously enough a few gametocytes were also detectable.

Certain amount of morphological changes were noticed from the very early stage, particularly amongst the early developmental forms. The trophozoites showed marked aberrations and appeared to deviate from the accepted descriptions on *P. knowlesi* (Franchini, 1927; Knowles and Das Gupta, 1932; Sinton and Mulligan, 1932; Mulligan, 1935; Russell *et al.*, 1946) (Plates X and XI).

Rings.—Besides the typical forms, many of the ring stages were found to be oval, pear-shaped or irregular. Accolé forms and multiple infection were frequently

* 1 to 5 parasites per field of thin film.
† One parasite per field of thin film.
‡ One parasite per 10 or more fields of thin film.

EXPLANATION OF PLATE X.*

1. Sporozoites of *P. knowlesi*.
2. Rings.
3. Normal R.B.C.
4. Early trophozoites.
5. Developing trophozoites.

6 and 7. Segmenting forms and mature schizonts.

8. Gametocytes.

* Explanations against items 1 to 8 above also include the respective drawings below them.

PLATE X.

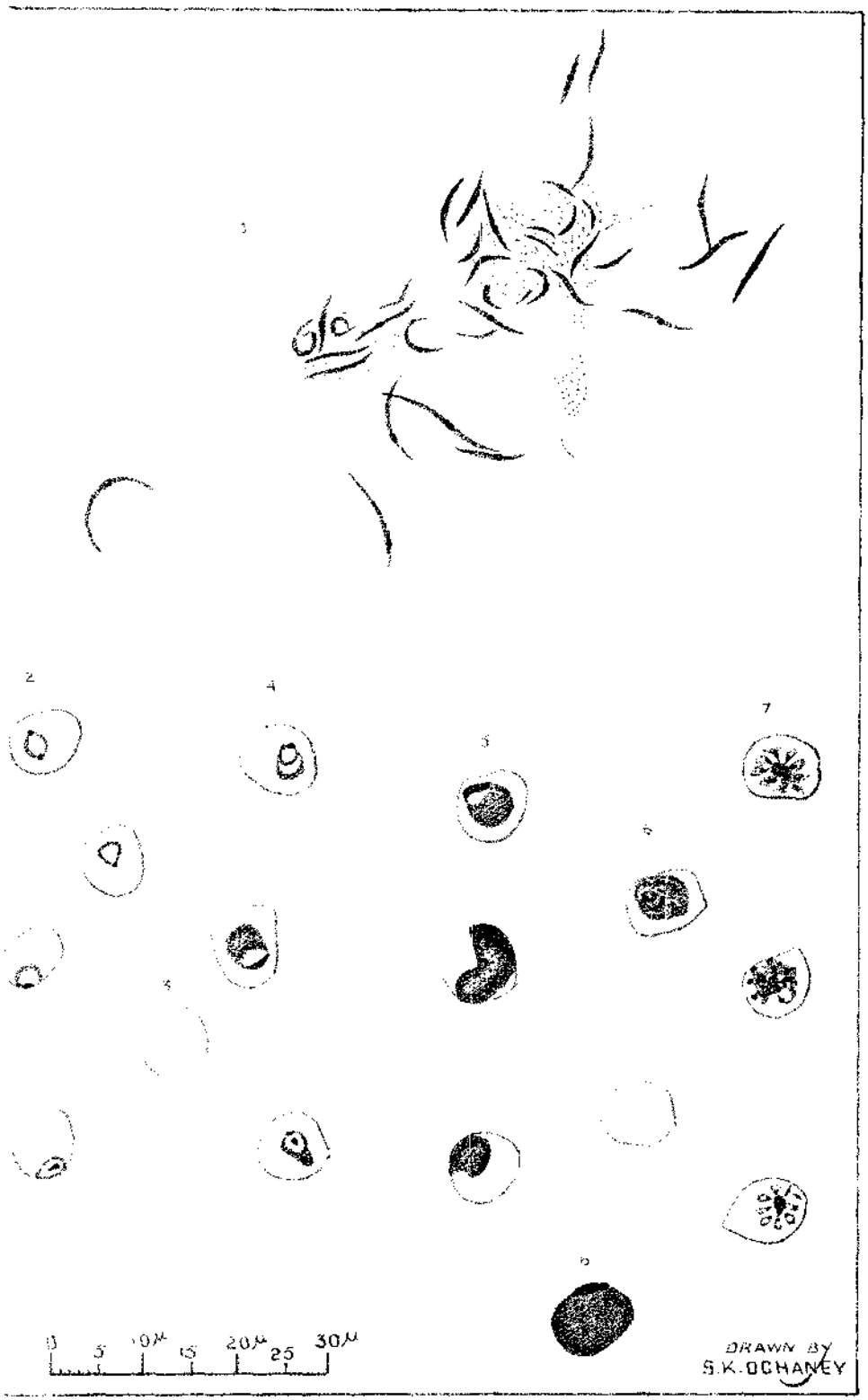
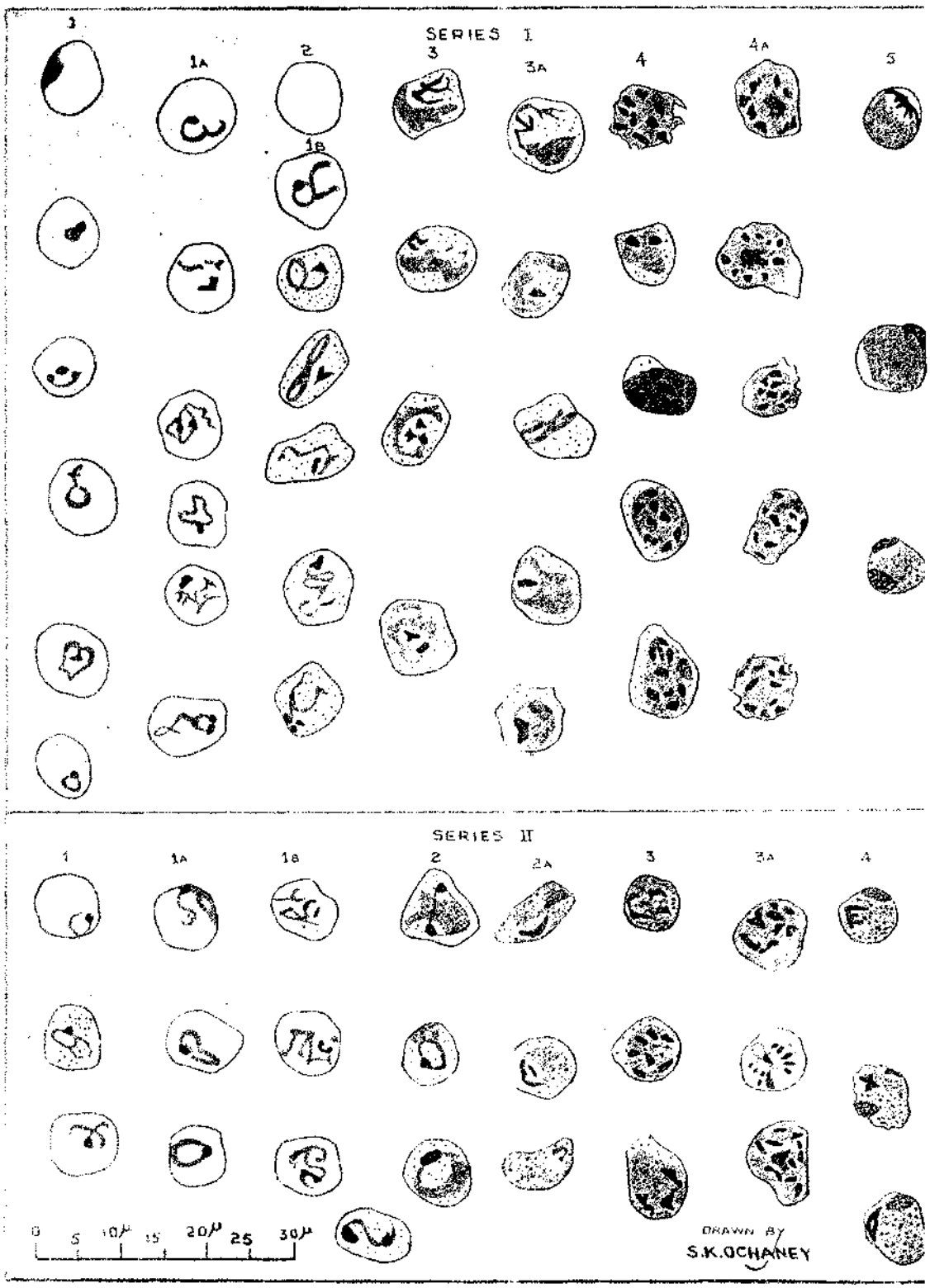


PLATE XI.


SERIES I
1
1A
2
1B
3
3A
4
4A
5
SERIES II
1
1A
1B
2
2A
3
3A
4
0 5 10μ 15 20μ 25 30μ
DRAWN BY
S.K.OCHANEY

EXPLANATION OF PLATE XI.*

SERIES I.

1, 1*a* and 1*b*. Rings and early amœboid trophozoites.
2. Normal R.B.C.
3 and 3*a*. Developing trophozoites.
4 and 4*a*. Segmenting forms and mature schizonts.
5. Gametocytes.

SERIES II.

1, 1*a* and 1*b*. Rings and early amœboid trophozoites.
2 and 2*a*. Developing trophozoites.
3 and 3*a*. Segmenting forms and mature schizonts.
4. Gametocytes.

* Explanations against items 1 to 5 in Series I, and 1 to 4 in Series II above, also include the respective drawings below them.

encountered. The chromatin was usually prominent in some cases and stood out of proportion to the body. Very often it appeared to be linear, or like rods, bars, forks or fan-shaped. Presence of the chromatin body inside the ring, apparently free from cytoplasm, was not uncommon. Accessory chromatin dots were usually present and appeared either close to the main body laterally or on the side opposite to the chromatin.

Cytoplasm.—Varied from a mere wisp, or a round disc to ring forms. More often than not the cytoplasm appeared to be thin and hair-like resembling *P. falciparum.* Slightly thickening of the ring at the side opposite the chromatin was also encountered.

Vacuoles.—These were seen to be more persistent and prominent in those animals where the infection ran a chronic course, though in most cases the vacuoles had disappeared at the preschizont stages.

Early trophozoites.—Many of the rings, at this stage, appeared to have tail-like processes. In some cases the ring had completely disappeared and fine filamentous structure with a chromatin dot at one end was seen, while in others the protoplasm spread like web across the cell with chromatin either at the periphery or inside the web. Various other forms, like band forms, *tenue* forms (Sinton, 1922), starfish forms and forms which baffle descriptions, were encountered in films taken at 3 or 4-hourly intervals.

These features were well marked in monkeys belonging to Series I and II.

Late trophozoite.—By this time the parasite had well developed and in many the cytoplasm appeared thick and amœboid resembling *P. cynomolgi* or *P. vivax.* These features were more marked in Series I. In some, the vacuoles were observed to be large and persistent. This characteristic was commonly encountered more in Series II than in the others.

Presegmenter and segmenting forms.—The parasites appeared to be similar to those encountered in strain I, with the exception that some of them were larger. They were usually associated with distorted or oval cells.

Mature schizonts.—These were found to be similar to those encountered in strain I, though quite a few were found to be larger than the rest. The average merozoite count was 10·7.

Gametocytes.—Mostly appeared normal, a few larger than others.

Infested R.B.C.—A certain proportion of the R.B.C. appeared larger in size than normal R.B.C. Sinton and Mulligan (1932) dealing with blood-passaged strain observed that in *P. knowlesi* infection the enlargement of R.B.C. was seldom more than 8μ, but the present authors have noticed enlargement of cells infested with parasite of sporozoite-induced strain well beyond 8μ up to 9·4μ. During the trophozoite stage the size of the R.B.C. varied from 7·5 to 9·4μ and the average size observed to be 8·5μ.

Stippling.—It has been previously mentioned that with the use of J.S.B. strain, it is not uncommon to encounter stippling in blood-passaged strain of *P. knowlesi* infection specially when the slides are deeply stained. With the sporozoite-induced strain, stippling was encountered more frequently, particularly in the late trophozoite stages. Stippling was also observed in the early developmental stages as well, specially in those where amœboidicity was marked.

Shape of R.B.C.—After the parasite had developed into the preschizont or schizont stage, many of the R.B.C. appeared distorted, oval, polygonal, pear-shaped, fimbriated and irregularly crenated. In this aspect the sporozoite-induced strain appeared very much like the blood-induced strain, even though in some cases the red blood cells were very much enlarged.

Pigment.—Pigment of brown colour appeared early, and developed into dark brown or dark at later stages. More often than not, pigment was abundant and seen at the periphery rather than in the centre. This feature was commonly encountered at the preschizont stage (Plate XI, Series I, last 3 drawings under 4 and 4*a*).

Schizogony cycle.—Sporulation at interval of 24 hours was observed in the monkeys in Series I and II. In most cases 200 parasites were counted in each film (Charts 2 and 3). Due to the extremely low grade infection in the other series, this study was not possible.

To sum up therefore morphological aberrations from strain I were very much noticeable in the monkeys in Series I up to the 3rd subinoculation. Although amœboid character was retained throughout (though much less in the later subinoculations), the incidence of cell enlargement diminished in frequency after the 3rd subinoculation. Amœboid features and cell enlargement appeared to be common only during the early phase of the initial infection in those animals in which the infection ultimately became chronic. In Series II at the stage when the infection became chronic and after subinoculation, vacuoles remained prominent and persistent in some cases till a very late developmental stage, a feature characteristic of *P. inui*. Stippling was frequently observed.

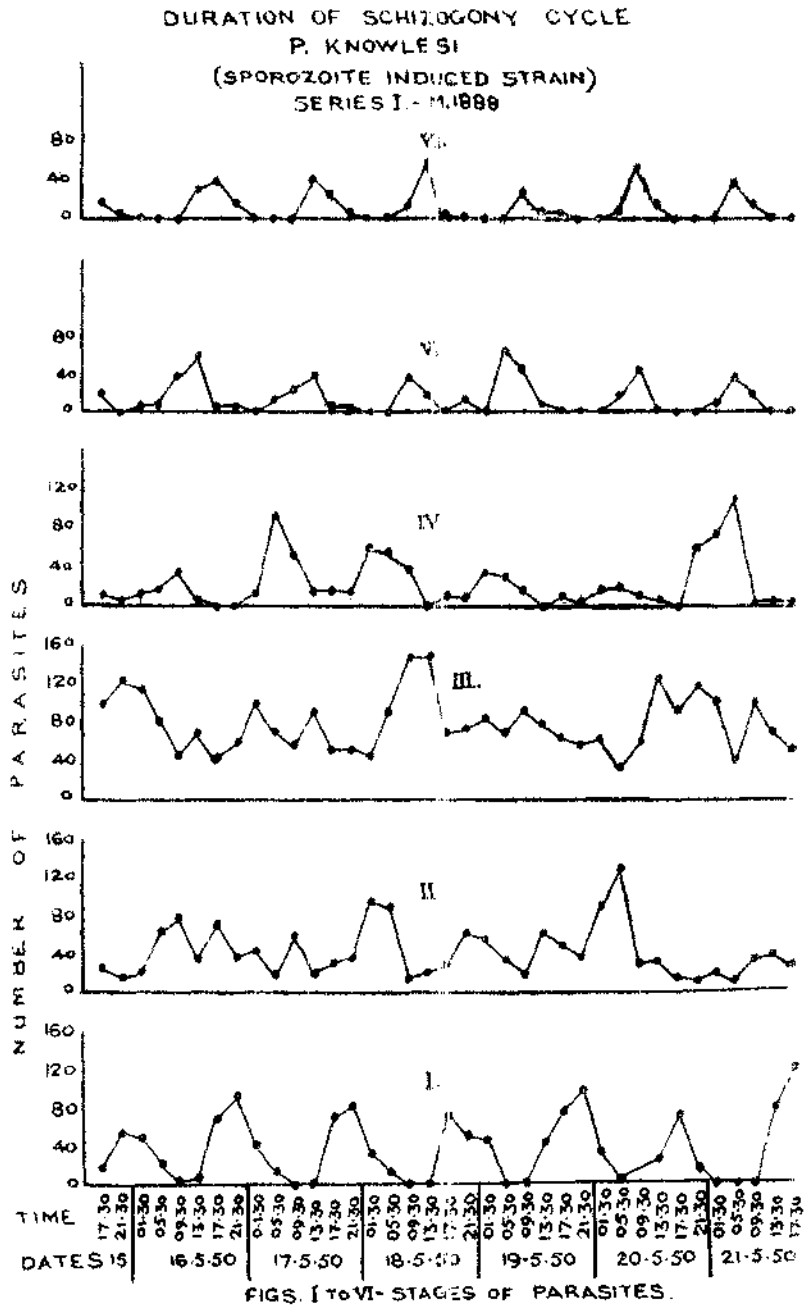
DISCUSSION.

The main features noticed during these studies are the relationship of the dose of sporozoite (in the form of inoculation of gland suspension or bite of infected mosquitoes) to the length of the prepatent period; also the difference in the length of the prepatent periods in blood-induced infection in respect of strain I on the one hand and strains II and II*a* on the other; the apparent influence of the dose of sporozoites on the intensity of infection. Lastly, the obvious morphological variations of the parasites observed in the monkeys particularly so in Series I and II.

Sporozoite-induced infection, prepatent period and intensity of infection.—In the sporozoite-induced infections, the prepatent period varied from 11 days in Series I to 53 days in Series VII.

In the absence of sporozoite counts, it is not possible to indicate the approximate number of sporozoites that were introduced in each case. But from Table III, a general idea may be formed about the degree of infection in each case. Where the inoculum consisted of 5 infected glands, the prepatent period was much shorter than in the case in Series VII where the animal was subjected to only one infective bite. Similarly in Series II, where the dose consisted of infected glands from 3 mosquitoes, the prepatent period was longer than Series I and shorter than the other series, *i.e.* the prepatent period varied inversely with the dose of the inoculum. These observations are in conformity with those studied with *P. vivax* infection in man where the dose of sporozoite inoculation regulated the length of the prepatent period (James, 1931; Boyd and Kitchen, 1938; Boyd, 1940, 1949; Craige *et al.*, 1947).

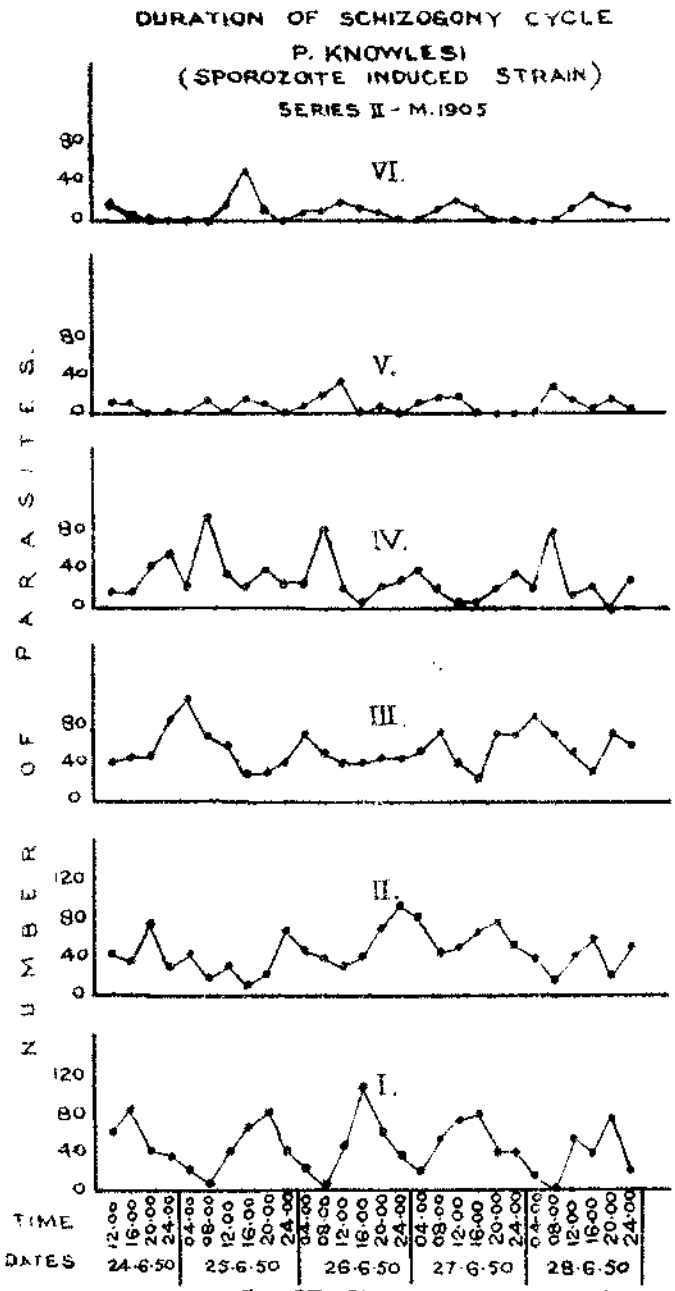
CHART 2.



FIGS. I TO VI - STAGES OF PARASITES.

I. Rings.
II. Early trophozoites.
III. Developing trophozoites.
IV. Fully developed trophozoites.
V. Segmenters.
VI. Schizonts (mature).

CHART 3.



FIGS. I TO VI - STAGES OF PARASITES.

I. Rings.
II. Early trophozoites.
III. Developing trophozoites.
IV. Fully developed trophozoites.
V. Segmenters.
VI. Schizonts (mature).

The results of the previous observations where the prepatent period was 7 days after inoculation of infected glands from three mosquitoes seem to differ from those seen in Series I and II in the present series, the prepatent period being 11 and 18 days after inoculation of 5 and 3 glands respectively. In one of his earlier works, James (1926) had observed that the incubation period was not significantly altered by the amount of sporozoite dose, nor the intensity of infection affected. This, he attributed to individual susceptibility. On the other hand, according to Boyd and Stratman-Thomas (1933), the prepatent period was longer in those cases where the infection was transmitted by mosquitoes with low sporozoite density ('poor lots') than those which received dose of sporozoites from the same number of mosquitoes with moderate ('fair') number of sporozoites and longer still than the series which had bites from heavily ('good') infected mosquitoes. This, then, may be the reason for the observations made by James (1926) and by Jaswant Singh *et al.* (1949) in which the latter workers had found the prepatent period to be of 7 days in M. 1355 after inoculation of glands from three mosquitoes, whereas 11 and 18 days in Series I and II in the present series, thus confirming the general view that dose of sporozoites regulates the prepatent period to some extent and it is likely the number of sporozoites in the glands of the two mosquitoes in the previous series was larger than those in the present series.

This, however, does not explain the low intensity of infection seen in M. 1355 as compared to M. 1845 in Series I. Prior to 1947, working with the virulent strain I, it had all along been observed that without therapeutic interference the animals died invariably within 5 to 7 days after patent infection was established following inoculation with erythrocytic parasites. Knowles and Das Gupta (1932) had reported that the plasmodium infection in *rhesus* monkey which was later confirmed as *P. knowlesi* by Sinton and Mulligan (1932) proved invariably fatal in *rhesus* monkey within a week or less of the first appearance of parasites. Similar observations have been made by Mulligan (1935) and Wats and Harbhagwan (1937) where the survival rates were observed to be less than 1 and 1·5 per cent respectively. High mortality was also observed by Krishnan *et al.* (1933) (though their series were small) and Coggeshall and Kumm in 1937 (quoted by Richardson *et al.*, 1946). The last named workers have themselves made similar observations. As previously reported, blood-induced infection with strain I does not now prove fatal nor the intensity is so acute as it used to be prior to 1947-48. Further, there is a tendency of the infection becoming chronic; but with the sporozoite-induced strains II and IIa in Series I and II, the infection appeared to be rapid and progressive specially in Series I, where it proved fatal in M. 1845 in 7 days and within 5 to 18 days in M. 1856, 1859, 1868, 1870 and 1888 up to the subinoculation Series III (Chart 1). Neither heavy infection nor any mortality was encountered in cases of subsequent subinoculations (4 and 5). In most of these cases after a transient rise to (++)*, the infection became chronic and remained so throughout the period of observation. The intensity of infection was noticed to be low in respect of M. 1921, second subinoculation in Series II, and extremely so in respect of Series IV, VI and VII.

In the present studies, the close relationship of the dose of sporozoite and the intensity of infection is apparent, and the intensity continued to remain so up to the 3rd subinoculation in Series I.

* 6 to 10 parasites per field of thin film.

Trophozoite-induced infection and prepatent period.

Further, it has been mentioned previously that, while dealing with strain I, the patent infection is established in 3 days after inoculation of 5 million erythrocytic parasites, which is almost similar to that observed after inoculation of 1 to 2·5 million parasites of strains II and II*a* in Series I. In this series the prepatent period is only one day after a dose of 5 million parasites in almost all cases of subinoculation and 2 days in one case in the 4th subinoculation. This obvious difference in the change in the prepatent period may be of some significance and may indicate biological changes as compared to strain I.

Morphological variations.—These variations have been mentioned before. The chief features studied were that the parasites were of highly amœboid nature, there was enlargement of the cells in many of the parasitized R.B.C. and, thirdly, stippling of the cells appeared to be common and more than that normally encountered with strain I infection, when films are stained with J.S.B. stain. Curiously enough, these features which are normal for *P. cynomolgi* were more noticeable in the early developmental stage of the parasites than the preschizont or schizont stage, which were not morphologically dissimilar to those of strain I. Moreover, not all the parasites in a film which were at the same stage of development appeared to show such variations. Again these characteristics were more noticeable in animals up to the stage of 3rd subinoculation in Series I and 2nd in Series II. In the subsequent subinoculation in the above 2 series and in IV, VI and VII, where the infection was chronic throughout, the parasites were similar in appearance to strain I, though persistent and prominent vacuole, a feature characteristic of *P. inui*, were noticeable in some of the parasites.

Thus it may be summarized by stating that compared to Series IV, VI and VII (Table III), where the dose of sporozoites was small, with larger doses of sporozoite inoculation in respect of Series I and II, not only the intensity of infection was severe with shorter prepatent period but there had been considerable morphological variations as well. The morphological features somewhat resembled *P. cynomolgi* in some cases where the dose of inoculum was large and the infection proved fatal, and to *P. inui* in a few cases where the dose was smaller and the infection became chronic. At this stage it is important to emphasize on the following points: The schizogony cycle in respect of strain I studied before and after 1947 (since when the attenuation of the virulence of the parasite has been observed) has all along been 24 hours. The cycle in respect of strains II and II*a* (Series I and II) was also observed to be of 24 hours. The characteristic deformities of the infested cells by older parasites are common in both the strains. Sinton and Mulligan (1932) considered that a 24-hour schizogony and occurrence of such deformities of infested cells by older parasites are the two features which at once separate *P. knowlesi* from all plasmodia of lower monkeys of the old world. Further, based on the investigations on large number of specimens by Knowles and Das Gupta (1932) and Sinton and Mulligan (1933*a*, 1933*c*), malarial infection in nature in northern India *rhesus* monkey is unknown. In these laboratories, hundreds of monkeys have been dealt with during the course of years and so far no plasmodium has ever been encountered. Instances when plasmodial infections in monkeys in India have been reported are *P. semnopitheci* in *Pygathrix entellus* by Knowles (1919) and *P. inui* in *S. sinicus* by Donovan (1920) in the Nilgiri Hills, South India. Chimisso (quoted by Sinton and Mulligan, 1933*b*), while working in Italy,

discovered a plasmodium in the blood of a monkey identified as *S. rhesus*, believed to be brought from India but it was not certain if it was so. According to Sinton and Mulligan (1933*b*), this parasite is probably identical with *P. semnopitheci* of Knowles, though Wenyon (1926) thought it to be *P. inui*. Schizogony cycle in none of these cases had been determined.

Knowles and Das Gupta (1932) had noticed morphological variation of a parasite in different hosts resembling *P. vivax* in *Cercopithecus pygerythrus*, which they called '*Cercopithecus* type' and quite different in *Macacus rhesus* the '*rhesus* type'. Further they had established that the morphology of the parasite remained constant for the same host species. But Sinton and Mulligan (1932) observed that Knowles and Das Gupta had been dealing with mixed infection of *P. cynomolgi* and *P. knowlesi*. Clark (1930, 1931) while working with *P. brasilianum* in red spider, white-throated and howler Panama monkeys had observed that the parasites were basically of two types, one quartan like, in white-throated monkey, while the other showing enlargement of R.B.C. in red spider monkey resembling *P. vivax*. Taliaferro (1932), however, concluded after detailed study that there is only a single species of malaria parasite in Panamanian monkeys, which varies in morphology depending upon the species of animal in which it is grown. On the other hand, Sinton and Mulligan, on the basis of their observations as to the constant features encountered and maintained by the parasite of Knowles and Das Gupta in *rhesus* monkeys, established the new species of *P. knowlesi*. It must, however, be remembered that these workers had all along been working with blood-induced strain. Neither Sinton and Mulligan (1933*a*) nor Mulligan *et al.* (1949) had reported of any morphological variations in respect of sporozoite-induced infections with *P. inui* and *P. cynomolgi* respectively.

Morphological variations in the sporozoite-induced *P. knowlesi* have, however, been observed, and such variations like increased amœboid features of the parasite seem to be associated in some way with the intensity or virulence of the infection. There is still considerable amount of controversy whether *P. tenue* is a separate species at all, and Stephens (1914) in describing *P. tenue* had observed extreme amœboid character of the parasite similar to the *P. knowlesi* seen in this series. He considered it to be a new species. Craige (quoted by Stephens, 1915) described the same as atypical forms of *P. vivax*, whereas Balfour and Wenyon (quoted by Stephens, 1915) considered them to be amœboid forms of *P. falciparum*. But they were not sure if this meant increased activity or metabolism on the part of the parasite or diminished resistance on the part of the host (Sinton, 1922). Blood films of two patients observed by them showed parasites identical with *P. tenue*.

Although none of the authors mentioned above discussed about the virulence or intensity of the infection, Wenyon (quoted by Stephens, 1915) had mentioned that one of the patients died soon after the blood films were taken. Again, Sinton (1927), quoting Christophers, mentioned that the latter had observed *P. tenue* in Orissa—a blackwater fever area. These might lead one to believe that *P. tenue* or abnormal forms of *P. falciparum* (with morphological variations) are likely to prove fatal as has been observed in the sporozoite-induced infection in Series I and II in which the morphological features have considerably deviated from strain I.

In every textbook on protozoology, one of the factors responsible for stipplings seen in various plasmodial infection has been attributed to the amœboid activity

of the parasite and are considered to be points of erythrocytic membrane to which pseudopodial processes of the parasite have been attached which have been subsequently withdrawn (Knowles and Senior White, 1927; Das Gupta, 1944), though Hingst (1936) considers them to be derived from reticular granules in the host cell. If the former theory is to be accepted it would not be difficult to explain the stipplings seen in the sporozoite induced strain more than those usually encountered in strain I of *P. knowlesi*. The trophozoites of strain I of this laboratory are not amoeboid and the same has been reported by all other observers like Russell *et al.* (1946), Sinton and Mulligan (1932), etc., whereas the parasites in strains II and IIa of these series have been found to be extremely so.

SUMMARY.

During the present investigations it has been possible to transmit *P. knowlesi* successfully both by inoculation of salivary glands and bites of infected *A. annularis*.

A. stephensi (*mysorensis*) proved refractory to infection.

Where comparatively larger number of infected glands (from five mosquitoes) were inoculated in an animal, patent infection was established after comparatively shorter prepatent period than in those series where smaller dose of inoculum was introduced. In all the series taken together, the dose of inoculum seemed to regulate the prepatent period which varied inversely with the dose.

The infection in the animal which received the heaviest inoculum progressed rapidly with the intensity formerly seen in the virulent form of old *P. knowlesi* strain I. The course of infection ran similar to the above though to somewhat lesser intensity in the animals up to subinoculation 3. Taking all the series together, it appeared that the intensity varied directly with the dose of the inoculum. Tendency to chronicity was marked when bites or gland inoculations from one mosquito were instituted.

Morphological variations in the form of amoeboidicity, enlargement of certain percentage of the infected R.B.C. and more than normal amount of stippling were noticed and well marked in those cases where the intensity was high and proved fatal. Whether and how these changes are linked up with the increased virulence of the parasite, it is not possible to definitely assess the reason at the moment and these must await further studies.

ACKNOWLEDGMENTS.

The authors wish to record their thanks to Captain P. C. Basu for his assistance in the conduct of these investigations; also to Messrs. K. S. Krishnan and M. L. Mammen of the Entomology Division of the Institute for supply of mosquitoes.

REFERENCES.

Boyd, M. F. (1940) . *Amer. J. Trop. Med.*, **20**, pp. 279–286.

Idem (1949) .. *Malariology*. W. B. Saunders Co., Philadelphia and London.

Boyd, M. F., and Kitchen, S. F. (1938). *Amer. J. Trop. Med.*, **18**, pp. 723–728.

BOYD, M. F., and STRATMAN-THOMAS, W. K. (1933).	*Amer. J. Hyg.*, **17**, pp. 666–685.
CLARK, H. C. (1930) ...	*Amer. J. Trop. Med.*, **10**, pp. 25–41.
Idem (1931) ...	*Ibid.*, **11**, pp. 11–20.
CRAIGE, B., ALVING, A. S., JONES, R., WHORTON, C. M., PULLMAN, T. N., and EICHELBERGER, L. (1947).	*J. Inf. Dis.*, **80**, pp. 228–236.
DAS GUPTA, B. N. (1944) ...	*Knowles's introduction to medical protozoology*, p. 129. U. N. Dhar and Sons Ltd., Calcutta.
DONOVAN, C. (1920) ..	*Ind. J. Med. Res.*, **7**, pp. 717–721.
FRANCHINI, G. (1927) ...	*Arch. Ital. Sci. Med. Colon.*, **8**, pp. 187–190.
HINGST, H. E. (1936) ...	*Amer. J. Trop. Med.*, **16**, pp. 679–684.
JAMES, S. P. (1926) ...	*Trans. Roy. Soc. Trop. Med. Hyg.*, **20**, pp. 143–165.
Idem (1931) ...	*Ibid.*, **24**, pp. 477–538.
JASWANT SINGH and BHATTACHARJI, L. M. (1944).	*Ind. Med. Gaz.*, **79**, pp. 102–104.
JASWANT SINGH, RAY, A. P., and NAIR, C. P. (1949).	*Ind. J. Mal.*, **3**, pp. 145–148.
KNOWLES, R. (1919) ...	*Ind. J. Med. Res.*, **7**, pp. 195–202.
KNOWLES, R., and DAS GUPTA, B. M. (1932).	*Ind. Med. Gaz.*, **67**, pp. 301–320.
KNOWLES, R., and SENIOR WHITE, R. (1927).	*Malaria. Its investigation and control.* Thacker, Spink & Co., Calcutta.
Idem (1930) ...	*Ind. Med. Res. Mem.* No. 18, pp. 385–398
KRISHNAN, K. V., CHIRANJI LAL and NAPIER, L. E. (1933).	*Ind. Med. Gaz.*, **67**, pp. 68–80.
MULLIGAN, H. W. (1935) ...	*Archiv für Protistenkunde*, **84**, pp. 285–314.
MULLIGAN, H. W., SOMMERVILLE, T., and LLOYD, O. C. (1949).	*Ind. J. Mal.*, **3**, pp. 211–234.
RICHARDSON, A. P., HEWITT, R. I., SEAGER, L. D., BROOKE, B. B., MARTIN, F., and MUDDUX, H. (1946).	*J. Pharm. Exper. Therap.*, **87**, pp. 203–215.
RUSSELL, P. F., WEST, L. S., and MANWELL, R. D. (1946).	*Practical Malariology*, pp. 71–78. W. B. Saunders Co., Philadelphia and London.
SINTON, J. A. (1922) ...	*Ind. J. Med. Res.*, **10**, pp. 215–235.
Idem (1924) ...	*Ibid.*, **12**, pp. 341–346.
Idem (1927) ...	*Trans. Roy. Soc. Trop. Med. Hyg.*, **20**, pp. 417–418.
SINTON, J. A., and MULLIGAN, H. W. (1932).	*Rec. Mal. Surv. Ind.*, **3**, pp. 357–380.
Idem (1933*a*) ...	*Ibid.*, pp. 381–443.
Idem (1933*b*) ...	*Ibid.*, pp. 719–767.
Idem (1933*c*) ...	*Ibid.*, pp. 769–808.
STEPHENS, J. W. W. (1914) ...	*Ann. Trop. Med. Parasit.*, **8**, pp. 119–128.
Idem (1915) ...	*Ibid.*, **9**, pp. 169–171.
TALIAFERRO, W. H. (1932) ...	*Amer. J. Hyg.*, **16**, pp. 429–449.
WATS, R. C., and HARBHAGWAN (1937).	*Rec. Mal. Surv. Ind.*, **7**, pp. 179–183.
WENYON, C. M. (1926) ...	*Protozoology*, **2**, p. 969. Baillière, Tindall & Cox, London.

Indian Journal of Malariology, **4**, 3, September 1950.

THERAPEUTIC ACTION OF PALUDRINE LACTATE* (INTRAVENOUS) IN SIMIAN MALARIA.

BY

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[August 14, 1950.]

INTRODUCTION.

COMPARATIVE studies on modern antimalarials have shown that proguanil (paludrine chlorguanide) is one of the drugs of choice in routine suppression (Maegraith *et al.*, 1945 ; Fairley, 1946, 1949 ; Afridi, 1947 ; Lomax, 1947 ; Robertson, 1947 ; Ray, 1948 ; Monk, 1948 ; Klopper *et al.*, 1948 ; Klopper, 1949 ; Covell, 1949 ; Cooper, 1949) and therapeutic treatment of malaria (De and Dutta, 1947 ; Clavero *et al.*, 1947 ; Chaudhuri and Rai Chaudhuri, 1947 ; Afridi *et al.*, 1947 ; Sen, 1947 ; Viswanathan and Baily, 1947 ; Jafar, 1947 ; Ghosh, 1947 ; Rognoni and Puyol, 1947 ; Schneider and Mechali, 1948 ; Van Riel, 1948 ; Jones *et al.*, 1948 ; Ansari, 1948 ; Ciuca *et al.*, 1948 ; Sandicchi, 1948 ; Giovanardi *et al.*, 1948). With oral administration of paludrine, various workers have found that dosages varying from 100 to 1,200 mg. daily for 10 to 14 days are sufficient to control or radically cure both natural and experimental *P. falciparum* infection (Maegraith *et al.*, *loc. cit.* ; Fairley, 1946). It has also been claimed that paludrine has the added advantage of its value in the causal prophylaxis of *P. falciparum* infection (Fairley, 1946,

* Samples of paludrine lactate were obtained through the courtesy of Imperial Chemical Industries (India) Ltd.

1949; Gunther, 1946; Packer, 1947; Geigy and Rahm, 1949) and prevents the development of gametocytes of *P. falciparum* into mature oöcysts in the stomach of the mosquito (Fairley, 1946, 1949; Mackerras and Ercole, 1947; Shute and Maryon, 1948). Further, that it is the least toxic of all the antimalarial drugs, as the margin of safety between the therapeutic and the toxic dose is considerably wide. Except for individual cases of idiosyncracy, no report of significant toxic symptoms are available (Fairley, 1946: De and Dutta, *loc. cit.*; Chaudhuri and Rai Chaudhuri, *loc. cit.*; Parekh and Boghani 1947; Woodruff, 1947; Srivastava, 1947; Jones *et al.*, *loc. cit.*; Covell, *loc. cit.*; Halawani *et al.*, 1948).

Following the satisfactory results obtained with oral administration of paludrine, parenteral administration of its various salts has also been tried in the treatment of infection with *P. vivax* and *P. falciparum*, including cases of cerebral malaria (Mullick and Gupta, 1947; Black, 1949; Chaudhuri and Chakravarti, 1949; Field, 1949). Field (1949) administered paludrine acetate parenterally to 17 and paludrine lactate to 2 patients (intravenous 9 and intramuscular 10). Intramuscular administration of paludrine lactate has been reported from Belgian Congo (Peeters, 1949). It was administered to children suffering from acute attacks of malaria but no firm conclusions of its advantages could be drawn, as all the recipients had paludrine orally after the injections. Apart from these observations, no further published information seems to be available.

The present paper deals with the results of observations on preliminary trials with paludrine lactate administered intravenously as a therapeutic measure in northern Indian *rhesus* monkeys (*S. rhesus*) infected with *P. knowlesi*, *P. cynomolgi* and *P. inui*. Minimum effective, maximum sub-lethal and the lethal doses in these animals have also been assessed.

MATERIALS AND METHODS.

Host.—Adolescent northern Indian *rhesus* monkeys obtained from the neighbourhood of Delhi were utilized for these investigations. In all cases, the weight of the animal, R.B.C. counts and the hæmoglobin percentages were recorded prior to inoculation and weekly thereafter throughout the period of observation extending up to about 3 months. Rectal temperature of all experimental animals was taken twice a day during parasitæmia.

Parasite.—Each of the healthy monkeys was inoculated intravenously with *P. knowlesi* or *P. cynomolgi* or *P. inui*, 50 million erythrocytic parasites per kg. body weight. Thick and thin blood smears were taken twice daily at 10 a.m. and 5 p.m. and were stained with J.S.B. (Jaswant Singh and Bhattacharji, 1944). The parasite densities were determined in terms of the number of parasites per c.mm. of blood by using Sinton's fowl cell suspension method (Sinton, 1924).

Drug.—Paludrine lactate is supplied as a 5 per cent aqueous solution containing 50 mg. of paludrine salt per c.c. (37 mg. paludrine base). The solution has a pH of 5·5 to 6·0, is stable at normal temperature and does not deteriorate on keeping. The recommended route of administration is by slow intravenous injection. If required, it may be diluted with distilled water. With physiological saline it forms a precipitate and as such should never be diluted with it. The dosage recommended for human beings is 2 c.c. every 3 hours until oral administration is possible.

The drug was administered when the infection reached 0·4 per cent of the R.B.C. count and was given intravenously either alone or diluted with distilled water taking not less than 5 to 10 minutes in the process of injection. The different dosage regimes followed were :—

(1) Single intravenous injection varying from 4 mg./kg. base to 90 mg./kg. base (5·4 to 121·5 mg. salt).
(2) 46 mg./kg. base (62·1 mg. salt) in two divided doses for 2 days.
(3) 15 mg./kg. base (20·25 mg. salt) daily for 3 days.
(4) 25 mg./kg. base (33·75 mg. salt) daily for 7 days.

In the test against *P. knowlesi*, all the regimes were employed, but against *P. cynomolgi* and *P. inui*, only a single injection with 33 mg./kg. base was tried.

Experiments were also undertaken along with the therapeutic studies to elucidate the acute lethal effect following intravenous injection of paludrine lactate in 71 monkeys. The drug diluted with equal volume of sterilized distilled water was administered intravenously into the saphenous vein as a single injection calculated on the basis of mg./kg. body weight of paludrine base. The injection was given slowly in not less than 5 to 10 minutes. Objective symptoms were noted and a record of the time was kept from the onset of injection to any fatality that occurred.

At the expiry of the period of observation, the animals were subjected to various tests with a view to activating latent infection, if any. The tests were (*a*) intravenous injection of 1/2 c.c. of 1 in 1,000 adrenaline hydrochloride solution and taking blood smears every 15 minutes up to 2 hours, (*b*) subinoculation of normal animals, (*c*) splenectomy followed by subsequent observation for a minimum period of two months. If after these tests no parasites were detected, it was assumed that the animals were completely sterilized.

OBSERVATIONS AND RESULTS.

(*a*) *Therapeutic tests.*—The details of the results of therapeutic trials are given in Tables I and II.

In the single intravenous injection series against *P. knowlesi* it was noticed that the dosage of 5 mg./kg. base and less showed no activity. Dosages 7 mg./kg. base to 33 mg./kg. base were active and parasites were cleared within 144 to 192 hours (average 169 hours). In the latter dosage regimes it was observed that the asexual forms disappeared within 144 hours as against 180 to 192 hours required for the clearance of sexual stages of the parasites. Dosages of 42 mg./kg. base and above as a single injection proved to be toxic and precipitated death within 2 to 8 minutes. Repeated intravenous injections were tried in dosages of 46 mg./kg. base daily in two divided doses for 2 days, 15 mg./kg. base daily for 3 days and 25 mg. daily for 7 days but these did not show better results in the matter of clearance of parasites from peripheral blood over the single dosage regime. Two transient relapses occurred after a long interval of 28 and 40 days respectively in 2 and 3-day regimes but none in the 7-day regime.

In the trials against *P. cynomolgi* and *P. inui*, where the dosage used was 33 mg./kg. base as a single injection, though asexual forms alone disappeared within 18 to 78 hours in both the infections, the total disappearance of all stages of parasites

took 72 to 100 hours (average 86 hours) in *P. cynomolgi* and 156 hours in *P. inuus*. Relapses occurred during the period of observation in all the monkeys in this series.

(*b*) *Toxicity tests.*—The maximum tolerated dose of paludrine lactate by intravenous injection was found to be 33 mg. of the base per kg. body weight. 48 mg. of the base per kg. body weight resulted in 50 per cent deaths and with 56 mg. dosage all the animals died. The details are given in Table III. Neither 25 mg. of the base per kg. body weight injected for 7 days at 24 hourly intervals nor 4 injections of 23 mg. of the base per kg. body weight given at 12 hourly intervals produced any additive toxic effect (Table I).

TABLE I.

Therapeutic tests with paludrine lactate against P. knowlesi *(intravenous injection).*

Number of animals used.	Dosages (mg./kg. base).	Time taken for disappearance of parasites from the peripheral blood (hours).	Relapses.	REMARKS.
1	98 (single dose)	...	.....	Died within 3 minutes.
1	62 (single dose)	...	.....	Died within 2 minutes.
1	42 (single dose)	...	.....	Died within 8 minutes.
2	33 (single dose)	180–192	Relapsed thrice. First relapse after 15 days.	Asexual forms disappeared after 144 hours.
1	27 (single dose)	192	Relapsed after 15 days.	.....
2	19 (single dose)	144 (both)	Relapsed after 10 and 14 days.	.....
1	9 (single dose)	168	Relapsed after 7 days.	.....
2	7 (single dose)	144	Frequent relapses after 3 days.	.....
1	5 (single dose)	...	.....	No activity.
1	4 (single dose)	...	.....	No activity.
1	46 (daily in two divided doses for 2 days).	168	Two transient relapses; first one after 40 days.	.....
1	15 daily for 3 days	192	Transient relapse after 28 days.	.....
1	25 daily for 7 days	192	.....	.....

TABLE II.

Therapeutic tests with paludrine lactate against P. cynomolgi *and* P. inui *(intravenous injection).*

Species of parasites.	Number of animals used.	Dosages (mg./kg. base).	Time taken for disappearance of parasites from the peripheral blood (hours).	Relapses.	REMARKS.
P. cynomolgi	2	33 (single dose)	72–100	Twice	Asexual forms disappeared between 48 and 78 hours.
P. inui ...	2	33 (single dose)	156	Repeated	Do.

TABLE III.

Toxicity test with paludrine lactate in monkeys.

Dosage in mg. of the base per kg. body weight.	Number of monkeys used for the test.	Number of monkeys died.	Lethal dose.
7	3	...	...
15	2	...	...
19	1	...	...
25	2	...	...
33	10	...	0 per cent.
37	10	1	...
41	10	3	...
44	10	3	...
48	10	5	50 per cent.
52	4	2	...
56	4	4	100 per cent.
63	4	4	...
89	1	1	...

In the cases in which fatality occurred following the injection of paludrine lactate intravenously, restlessness was observed during the process of injection. Hurried respiration was observed in a few cases and the animals became drowsy and gradually passed on to comatose stage. In some cases bouts of coughing and vomiting preceded this stage. During the comatose stage the respiration became irregular, jerky and laboured with depth of inspiration very much prolonged and the rate became as low as one per minute. Death occurred within 1 to 37 minutes after the completion of injection except in one which occurred 20 hours later. Post-mortem examination revealed congestion of all internal organs and dilatation of the right side of the heart.

DISCUSSION.

The parenteral administration of antimalarial drugs is indicated in a case of emergency in which one cannot afford to risk the delay that may intervene between the oral administration and absorption of the drug and the desired effect. These emergencies may occur (1) in heavy *P. falciparum* infection associated with hyperpyrexia or cerebral involvements and (2) in cases in which it is doubtful whether the patient is in a position to swallow, retain or absorb the drug.

In cerebral malaria the pathological changes are associated with presence of parasitized red cells at the pre-schizont stage of development in the capillaries of the brain which in heavy infections are usually blocked, while the arterioles and larger vessels contain younger forms, including the young ring forms (Marchiafava and Bignami, 1900; Rigdon, 1944; Black, 1949). As such it is obvious that any drug to be effective must have quick and powerful schizonticidal action against all stages of the asexual parasites.

Black (1946) in his *in vitro* trials with paludrine in *P. falciparum* has shown that the parasites continue to develop in parallel with those in control cultures without paludrine until they reach the early schizont stage, when in the former, development stops and parasites become vacuolated and degenerate. On the basis of these studies he observed that paludrine exerts its action only at the early schizont stage.

In clinical studies also it has been observed that paludrine hydrochloride administered orally takes longer time for the disappearance of asexual forms of plasmodia compared to other older antimalarials such as quinine and atebrin (Fairley, 1946; Field, 1949). The same has been observed by Jaswant Singh *et al.* (1949) dealing with *rhesus* monkeys infected with *P. knowlesi*.

In the authors' studies with intravenous paludrine lactate against *P. knowlesi* in *rhesus* monkeys it has been established that with the maximum tolerated dose of 33 mg. base per kg. body weight the time required for clearance of asexual forms of the parasite was 144 hours. The same was observed with a dose of 23 mg./kg. base twice daily for 2 days. That shows that the action of paludrine lactate is definitely slow.

Seaton as quoted by Black (1949) reports the case of a patient who went into coma some time after the administration of 100 mg. paludrine hydrochloride given orally over a period of 2 days. He has also reported that death may occur in some heavy *falciparum* infections despite the intravenous injection of paludrine.

Chaudhuri and Chakravarti (*loc. cit.*) in their studies with intravenous paludrine acetate found that in a large number of cases a dosage of 200 to 400 mg. controlled the temperature and cleared the peripheral blood of asexual parasites but they did not get satisfactory results in two *P. falciparum* cases, one with typical cerebral malaria and the other with delirium and hyperpyrexia. The authors however viewed that it was difficult to judge the effect of the drug in the latter case as the patient was in a moribund state before treatment.

In view of the observations by most of the workers as mentioned above, it appears that paludrine when administered parenterally may not be helpful in the treatment of acute malaria complicated by cerebral involvement.

Quinine which is commonly used for emergencies when given intravenously has been found to precipitate fatalities and may act as a contributory factor for the onset of black water fever (Brahmachari *et al.*, 1932 ; Gaskell, 1920 ; Strahan, 1948 ; Covell, *loc. cit.*). Chopra *et al.* (1934) working with quinine acid hydrobromide given intravenously in man and monkey found the average maximum concentration in blood in both cases after 15 minutes of its administration but against this has to be considered the fall in blood pressure that accompanies such injection. Fernando and Sandarasagara (1935) on their clinical study of 647 patients treated for malaria during Ceylon epidemic of 1934-35 found that intravenous quinine was contra-indicated when the systolic blood pressure was below 90 mm. of Hg. However, intravenous quinine by drip method may be found to be safer for the treatment of cerebral malaria (Strahan, *loc. cit.*).

Mepacrine sulphonate when given intravenously has a low margin of safety, toxic side-effects, and does not sterilize gametocytes. It does however arrest the development of *P. falciparum* early in the schizogonous cycle (Covell, *loc. cit.*).

Camoquin hydrochloride and chloroquin diphosphate in aqueous solution have been experimentally tried by intramuscular injection against *P. vivax* trophozoite and sporozoite induced infections. Payne *et al.* (1949) found *P. vivax* to disappear from peripheral blood in 24 to 192 hours with camoquin hydrochloride and it was free from any untoward local reaction. Similarly, Culwell *et al.* (1948), Coatney (1949) and Spicknall *et al.* (1950) have recommended 200 mg. base of chloroquine by intramuscular injection. Though these have been suggested as emergency measures in pernicious malaria for initiation of treatment, no further results are yet forthcoming regarding their routine applicability.

Chaudhuri and Chakravarti (*loc. cit.*) observed no untoward side-effect such as fall in blood pressure with paludrine acetate when given by intravenous injections. In this respect, therefore, parenteral paludrine may be found useful in treatment of malaria in conditions other than pernicious malaria. On the other hand as previously mentioned, Field (1948), while treating 7 cases with paludrine acetate and 2 cases with lactate administered intravenously, had observed thrombosis of cephalic vein in one case treated with the acetate and almost instantaneous death in the other treated with lactate.

In the authors' experiments in monkeys, the maximum sub-lethal dose of intravenous paludrine lactate is observed to be 33 mg. of the base per kg. body weight. This is more or less in agreement with the results obtained by Butler *et al.* (1947) with paludrine monoacetate in chicks which is 32·4 mg. of the base per kg. body weight. However, when dosages which cause 50 per cent and 100 per cent

mortality are considered, paludrine lactate appears to be slightly more toxic in monkeys than in chicks receiving monoacetate. 50 per cent mortality is caused by 48·6 to 64·8 mg. of the base per kg. body weight of monoacetate in chicks as compared to 48 to 52 mg. of the base per kg. of paludrine lactate in monkeys. The corresponding figures for 100 per cent mortality are 81 and 56 mg. respectively for chicks and monkeys. No additive toxic effect is noted with repeated injection of paludrine lactate in monkeys in the dosages tried. In monkeys, intravenous paludrine lactate in dosages much exceeding the corresponding recommended dosage in man (calculated according to body weight) did not give rise to any toxic effect. In this connection it may be mentioned that Mullick and Gupta (*loc. cit.*) used approximately 5 mg. of paludrine hydrochloride per kg. intravenously in 50 patients and found no untoward side-effects except anorexia in 4 and insomnia in 3 cases but even this could not be ascribed to paludrine directly.

SUMMARY.

1. Intravenous paludrine lactate is found effective in dosages from 7 mg. to 33 mg. of the base per kg. body weight in simian malaria when given as a single dose. The average time required for clearance of parasites in *P. knowlesi* infection was 169 hours, in *P. cynomolgi* 86 hours and in *P. inui* 156 hours.

2. Lethal dose (zero per cent) of paludrine lactate by intravenous injection in monkeys is 33 mg. of the base per kg. body weight. Lethal dose (50 per cent) was obtained with 48 to 52 mg. of the base per kg. body weight, whereas lethal dose (100 per cent) was obtained with 56 mg. of the base per kg. body weight. No additive toxic effect was noticed with repeated injection of paludrine lactate in dosages varying from 15 to 23 mg./kg. base.

REFERENCES.

AFRIDI, M. K. (1947) .. *Ind. J. Mal.*, **1**, pp. 347–359.
AFRIDI, M. K., RAMAKRISHNAN, S. P., GOSWAMI, A., and MENON, M. K. (1947). *Ibid.*, **1**, pp. 221–229.
ANSARI, M. Y. (1948) ... *Ind. Physician*, **7**, pp. 177–184.
BLACK, R. H. (1946) ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, pp. 163–170.
Idem (1949) ... *Ibid.*, **42**, pp. 565–568.
BLANC, F. (1949) ... .. *Med. Trop.*, Marseilles, **9**, pp. 143–172.
BRAHMACHARI, U., BRAHMACHARI, P., and BANERJEA, R. (1932). *Amer. J. Trop. Med.*, **12**, pp. 117–122.
BUTLER, R., DAVEY, D. G., and SPINKS, A. (1947). *Brit. J. Pharm. Chem.*, **2**, pp. 181–188.
CHAUDHURI, R. N., and CHAKRAVARTI, H. (1949). *Brit. Med. J.*, Jan. 15, pp. 91–93.
CHAUDHURI, R. N., and RAI CHAUDHURI, M. N. (1947). *Ind. Med. Gaz.*, **82**, pp. 247–253.
CHOPRA, R. N., ROY, A. C., and DAS GUPTA, B. M. (1934). *Ibid.*, **69**, pp. 560–566.
CICCA, M., SOFLETE, A., CONSTANTINESCO, P., and TERITEANU, N. (1948). *Bull. World Hlth. Org.*, **1**, pp. 301–307.
CLAVERO, G., ROMEO VIAMONTE, J. M., and LOZANO, A. (1947). *Kuba*, Havana, **3**, pp. 183–185.
COATNEY, G. R. (1949) ... *Bol. Oficina San. Pan Amer.*, **28**, pp. 27–31.

COOPER, W. C. (1949)	*Pub. Hlth. Rept.*, Washington, **64**, pp. 717–732.
COVELL, G. (1949) ...	*Brit. Med. J.*, Oct. 8, pp. 773–777.
CULWELL, W. B., COOPER, W. C., WHITE, W. C., LINTS, H. A., and COATNEY, G. R. (1948).	*J. Nat. Mal. Soc.*, **7**, pp. 311–315.
DE, M. N., and DUTTA, P. N. (1947).	*Ind. Med. Gaz.*, **82**, pp. 257–260.
FAIRLEY, N. H. (1946) ...	*Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, pp. 105–162.
Idem (1949) ...	*Brit. Med. J.*, Oct. 22, pp. 891–897.
FERNANDO, P. B., and SANDARASAGARA, A. P. (1935).	*Ceylon J. Sci.* (Sec. D), **3**, pp. 195–233.
FIELD, J. W. (1948) ...	*Second interim report, Malaria Sub-Committee of the Colonial Medical Research Committee on prophylactic and therapeutic trials in Malaya of paludrine chloride and atebrin*, pp. 24 to 25.
Idem (1949)	*Med. J. Malaya*, **3**, pp. 173–190.
GASKELL, J. F. (1920) ...	*Brit. Med. J.*, Jul. 24., pp. 122–123.
GEIGY, R., and RAHM, U. (1949)	*Acta Tropica*, Basle, **6**, pp. 153–157.
GHOSH, B. N. (1947) ...	*Ind. J. Mal.*, **1**, pp. 369–371.
GIOVANARDI, A., SEPULCRI, P., and DE NEGRI, U. (1948).	*Riv. di Mal.*, **27**, pp. 191–200.
GUNTHER, C. E. M. (1946) ...	*Med. J. Austral.*, **1**, pp. 510–511.
HALAWANI, A., BOZ, I., and MORCOS, F. (1948).	*Ann. Trop. Med. Parasit.*, **42**, pp. 304–311.
JAFAR, M. (1947)	*Ind. J. Mal.*, **1**, pp. 365–368.
JASWANT SINGH, and BHATTACHARJI, L. M. (1944).	*Ind. Med. Gaz.*, **79**, pp. 102–104.
JASWANT SINGH, RAY, A. P., and NAIR, C. P. (1949).	*Ind. J. Mal.*, **3**, pp. 387–403.
JONES, R., JR., PULLMAN, T. N., WHORTON, C. M., CRAIGE, B., JR., ALVING, A. S., and EICHELBERGER, L. (1948).	*J. Clin. Investi.*, **27**, Pt. 2, pp. 51–55.
KLOPPER, S. (1949) ...	*Documenta Neerlandica et Indonesica de Morbis Tropicis*, Amsterdam, **1**, pp. 50–54.
KLOPPER, S., SLOF, D., and OP'T LAND, C. (1948).	*Nederl. Tijdschr. v. Geneesk.*, **92**, Part IV, pp. 3922–3929.
LOMAN, P. H. (1947) ...	*Ind. J. Mal.*, **1**, pp. 389–393.
MACKERRAS, M. J., and ERCOLE, Q. N. (1947).	*Trans. Roy. Soc. Trop. Med. Hyg.*, **41**, pp. 365–376.
MACGRAITH, B. G., ADAMS, A. R. D., KING, J. D., TOWNSEND, R. H., DAVEY, T. H., and HARVARD, R. E. (1945).	*Ann. Trop. Med. Parasit.*, **39**, pp. 232–236.
MARCHIAFAVA, E., and BIGNAMI, A. (1900).	*Twentieth Century Practice of Medicine* (Edited by Thomas L. Stedman). Sampson Low, Marston & Co., London.
MONK, J. P. (1948) ...	*Brit. Med. J.*, June 26, pp. 1221–1225.
MULLICK, K. B., and GUPTA, J. C. (1947).	*Ind. Med. Gaz.*, **82**, pp. 660–668.
PACKER, H. (1947)	*J. Nat. Mal. Soc.*, **6**, pp. 147–154.
PAREKH, J. G., and BOGHANI, B. P. (1947).	*Ind. Med. Gaz.*, **82**, pp. 253–255.
PAYNE, E. H., SHARP, E. A., and NICKEL, K. C. (1949).	*Amer. J. Trop. Med.*, **29**, pp. 353–368.
PEETERS, E. M. E. (1949) ...	*Ann. Soc. Belge de Med. Trop.*, **29**, pp. 225–229.
RAY, A. P. (1948)	*Ind. J. Mal.*, **2**, pp. 35–66.
RIGHOS, R. H. (1944) ...	*Southern Med. J.*, **37**, pp. 687–694.
ROBERTSON, J. D. (1947) ...	*Zanzebar Med. San. Rept. for the Year* 1947 and *Trop. Dis. Bull.*, **45**, p. 972.
ROGNONI, M., and PEVOT, L. M (1947).	*Arch. Hosp. Santo Tomas.*, Panama, **2**, pp. 391–396.
SANDICCHI, G. (1948) ...	*Riv. di Mal.*, **27**, pp. 123–129.

SCHNEIDER, J., and MECHALI, D. (1948). *Bull. Soc. Path. Exot.*, **41**, pp. 274–282.
SEN, A. (1947) ... ... *Calcutta Med. J.*, **44**, pp. 170–174.
SHUTE, P. G., and MARYON, M. (1948). *Parasit.*, **38**, pp. 264–270.
SINTON, J. (1924) ... ... *Ind. J. Med. Res.*, **12**, pp. 341–346.
SPICKNALL, C. S., TERRY, L. L., and COATNEY, G. R. (1950). *Trop. Dis. Bull.*, **47**, p. 318.
SRIVASTAVA, R. S. (1947) ... *Ind. J. Mal.*, **1**, pp. 361–363.
STRAHAN, J. H. (1948) ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **41**, pp. 669–676.
VAN RIEL, J. (1948) ... *Ann. Soc. Belge de Med. Trop.*, **28**, pp. 85–95.
VISWANATHAN, D. K., and BAILY, J. D. (1947). *Ind. J. Mal.*, **1**, pp. 307–318.
WOODRUFF, A. W. (1947) ... *Trans. Roy. Soc. Trop. Med. Hyg.*, **41**, pp. 263–267.

Indian Journal of Malariology, **4**, 4, September 1950.

PRELIMINARY STUDIES ON 'EX-SHEATHING' OF *MF. BANCROFTI.*

BY

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[November 9, 1950.]

NORMALLY *microfilariæ bancrofti* are found within their sheaths, *in vivo* in the human body, or *in vitro* in drops of blood on glass slides with or without a cover-slip. In the blood meal of *C. fatigans*, some microfilariæ are seen within their sheaths and in some cases empty sheaths with no trace of the larvæ that have escaped, are observed. Abe (1937) described the following types of ex-sheathing in *C. fatigans* :—

(1) by a longitudinal tearing off of the sheath about its middle, or

(2) through a cap formed at the anterior end.

PRESENT WORK.

Similar types of 'ex-sheathing' as described by Abe (*loc. cit.*) have been noted in blood containing *Mf. bancrofti*, to which hetrazan had been added and kept under reduced atmospheric tension for about 45 minutes and exposed to air thereafter. Preliminary results of the studies carried out are set forth in this note.

DETAILS OF WORK AND RESULTS.

Equal parts of blood infected with *Mf. bancrofti* were taken from a pricked finger on four glass slides (numbered 1-4) at 7 p.m. To slides 1 and 2, hetrazan solution (100 mg. in 100 c.c. saline buffered to pH 7·4) in the same volume as blood, was added. Similar quantities of saline only, buffered to pH 7·4 were added to slides 3 and 4. Slides 1 and 3 were kept open and to each of slides 2 and 4, a cover-slip ringed with vaseline was applied. The slides were examined at hourly intervals

till 11 p.m. and left overnight in the laboratory. The microfilariæ were noted to be alive and actively motile during these examinations.

In the morning, the microfilariæ were still found to be alive in all slides; in slide 2, however, they were found to be markedly sluggish.

Cover-slips were removed from slides 2 and 4 and cleansed of vaseline. Smears were made from blood in all the four slides and stained with J.S.B. solution I (blue solution only) (Jaswant Singh and Bhattacharji, 1944) for 4 minutes. On an examination, all the microfilariæ in slides 1, 3 and 4 were found within their sheaths. In slide 2, however, most of the microfilariæ were seen in various stages of ex-sheathing (Plate XII, figs. 1 to 4).

These experiments were repeated with blood from different volunteers with varying microfilarial contents, with identical results.

The blood hetrazan mixtures were also kept in Thunberg tubes and air pressure in the tubes was reduced by means of a water pump. After keeping the reduced atmospheric pressure for some time, air was let in. Soon after, smears were made from the resultant blood-drug mixture. On staining and examining, the same phenomena mentioned before (as in slide 2) were noted. The experiments were repeated with identical results.

Further experiments were carried out to determine the minimum period for which the blood-drug mixture had to be kept under a cover-slip or period for which evacuation must be kept up in Thunberg tubes, to get a positive 'ex-sheathing' of *Mf. bancrofti* when exposed to air. This period appeared to be about 45 minutes. This phenomena did not occur in the blood to which no hetrazan had been added.

Further work is in progress.

CONCLUSION.

1. *Mf. bancrofti* try to get out of their sheaths, if kept in contact with hetrazan for a minimum period of 45 minutes, under reduced atmospheric pressure, and thereafter exposed to air.

2. The types of ex-sheathing are similar to those observed in the stomach of *C. fatigans* as described by Abe (*loc. cit.*).

ACKNOWLEDGMENT.

Help rendered by Laboratory Assistant Mr. S. Cornelius is acknowledged.

REFERENCES.

ABE, S. (1937) ... ... *J. Med. Assoc., Formosa*, **36**, pp. 516-519.
JASWANT SINGH, and BHATTACHARJI, L. M. (1944). *Ind. Med. Gaz.*, **79**, pp. 102-104.

Plate XII.

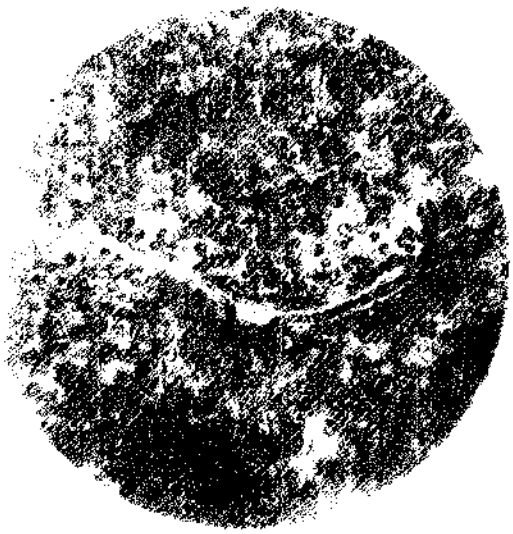

Fig. 1.

Fig. 2.

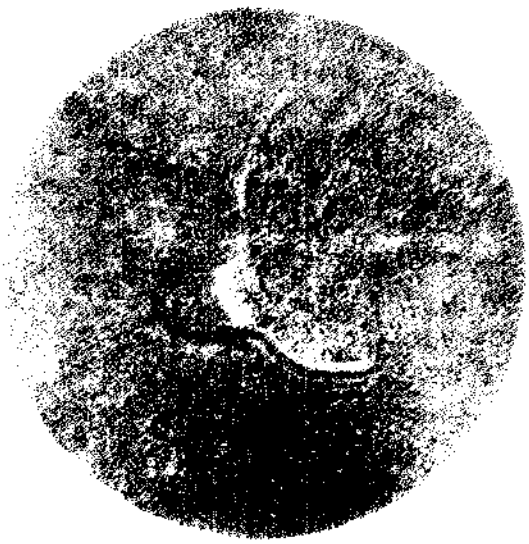

Fig. 3.

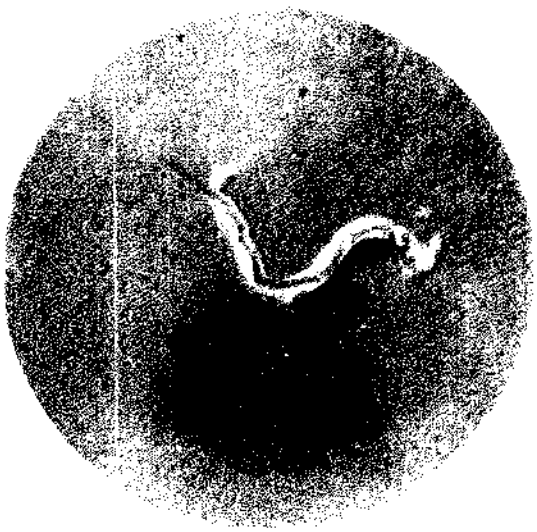

Fig. 4.

Indian Journal of Malariology, 4, 3, September 1950

TECHNIQUE OF MAKING BLOOD SMEARS AND THEIR STAINING IN DIAGNOSIS OF MALARIA.

BY

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[September 30, 1950.]

DIAGNOSIS of enteric group of fevers, typhus fever, syphilis, etc., by serological tests is a standard procedure. Serological tests for kala-azar, like aldehyde test of Napier (1922), urea stibamine test of Chopra (Chopra *et al.*, 1927) and capillary tube test of Raghavan (1949), are of definite diagnostic use, but for early diagnosis, these methods are of little value and the importance of direct method, e.g. detection of L.-D. bodies, has been stressed in almost all textbooks on the subject (Low and Fairley, 1933; Blacklock and Southwell, 1935; Beaumont, 1948; Hutchison and Hunter, 1949, and others).

There is evidence to show that serum of some malaria cases gives a 'false positive test' for syphilis (Kent *et al.*, 1948). Serological test like the complement-fixation test has been found useful in the identification of well-developed or recently subsided *P. vivax* infections. Though it is of little value in primary attacks, serological reactions seem to develop a week or so after patent parasitæmia or fever (Rein *et al.*, 1949). Hormann (1949) considers that serological reactions are of greater value in determining the immunity responses of individual subjects rather than in the diagnosis of malaria. Harris and Reidel (1948) are of the opinion that complement-fixation test in its present form is no substitute for the finding of malaria parasite in the blood films. Importance of microscopical diagnosis of malaria still holds the ground and having due regard to this the Expert Committee of the World Health Organization on malaria recommended that trials on antimalarials should be limited to microscopically diagnosed cases of malaria and results analysed by species of parasites (W. H. O., 1950).

Direct diagnostic measure in malaria by examination of blood is, therefore, of prime importance specially during the early stage of the disease. Such examination may be carried out—

(*i*) in fresh blood preparations (unstained),
(*ii*) by concentration method (e.g. centrifuging)
(*iii*) in thick and thin films (stained).

Fresh blood preparations.—Laveran, who was the first to discover the malaria parasite in fresh blood, himself admitted that dried blood stained with colouring agents are advantageous in diagnosis of malaria (Field, 1948). However, fresh blood preparation can still be used, though not as a routine, for the study of living parasites, gyroscopic movements of the crescents as described by van Rooyen (1941), pigment particles and their distribution, etc.

A fresh film may be prepared by taking a small drop of blood on the centre of a cover glass which is then gently dropped on a slide. The amount of blood taken should be small or else the blood will spread beyond the edges of the cover glass. A central transparent zone, an intermediate slightly opaque and an outer thick opaque zones are characteristics of a good preparation.

Concentration method.—Defibrinated blood after filtering through gauze was centrifuged by Christophers and Fulton (1938) for concentration of parasites. The top brown layer which consisted mainly of well-developed parasites (mostly three-quarters developed) showed a very small proportion of early forms of parasites. Halwani and Sobky (1939) used this concentration method as a diagnostic measure. They used 0·5 c.c. of blood centrifuged with 10 c.c. of 1 per cent acetic acid (or formalin) solution for 10 minutes. In a comparative study with thick film preparation they have shown that while 80 per cent of their cases showed parasites in the concentration method, only 52 per cent were positive in thick film preparations.

While this method may be convenient and more accurate for laboratory studies, it is obviously time-consuming and almost impracticable for field studies in malarious regions.

Stained preparations.—It is almost unanimously agreed that stained blood smear examination is certainly the method of choice. Wilcox and Logan (1941) consider it to be the most reliable and accurate method of laboratory diagnosis so far devised. Caplan (1942) also draws attention to the importance of parasite diagnosis as a method of saving quinine. War Office Memoranda issued in 1941 (W. O. Memo., 1941) point out that of all the methods of diagnosis of malaria, blood examination is of paramount importance and in all cases thick films should also be examined. Further, it has been impressed that a single negative examination does not exclude malaria and repeated examinations may be necessary in certain cases.

While to many, a discussion on the merits of thick film preparation may seem to be quite unnecessary in view of its overwhelming advantages over thin film preparations as recognized by majority of workers on this line, there are still some who seem to prefer the thin film preparations to thick, and the difficulties of examination of thick preparations have been unduly exaggerated.

The thick film method was first evolved by Ross in 1903, almost fifty years ago (Wilcox and Logan, *loc. cit.*; Field, 1948). As pointed out by Russell *et al.* (1946) the general attitude had all along been to depend on thin smears. Simeons (1912) recorded that thick drop method was still not very popular in India and we know that in many institutions in India this method is not being given the importance it deserves. Walker (1948) holds the same view for the American institutions.

To some extent, however, this is understandable. The characteristic features of the three common species of malaria parasites in a thick film are not always present. The enlarged red blood cells with the stippling so peculiar to *P. vivax* infection is not readily discernible in completely hæmolysed specimens, nor is it easy to detect the accolé forms in *P. falciparum* infection or the band forms of *P. malariæ*. Moreover, various blood elements like platelets, leucocytes, debris of blood cells, and extraneous matters like bacteria, protozoal contamination from old distilled water (Sinton, 1922), stain deposits, dust, etc. may confuse the beginners. Given some degree of knowledge with the appearance of parasites in a thin film, a few days' practice should suffice to re-orient this knowledge and apply it to thick smear. The only deterrent is the inelegant appearance of the thick smear, otherwise its advantages are soon appreciated.

The one outstanding feature is that the parasites are concentrated in a small area and the degree of concentration should not be such as to obstruct vision. Field (1948) says that a concentration of 16 fold is satisfactory without making the smear opaque. Further, as the smear occupies much smaller space compared to a thin film, a larger amount of blood can thus be examined in much shorter time. Sinton and Banerjea (1925) have estimated that the amount of blood covered per field is increased from 10 to 50 times over that in a thin film and hence time is cut down drastically.

Again by using some of the Romanowsky stains like J.S.B. (Jaswant Singh and Bhattacharji, 1944) or Field's stain (Field, 1941), the staining time is reduced to only a few seconds.

Thus, where speed is essential and/or when large number of patients are to be examined, the thick film method saves a great deal of time and permits of the examination of many more slides in the same time than is possible by the thin film method (Christophers *et al.*, 1914 ; Russell *et al.*, *loc. cit.*). According to Green (1931) when large numbers of blood films are to be examined and the diagnosis of malaria rather than the definition of the actual parasite species becomes all important, the use of thick films becomes the method of necessity rather than choice.

In a comparative study of thick and thin film preparations for diagnosis of malaria fevers, Taylor (1917) observed that examination for 5 minutes each per slide showed 526 positive cases in thick smears but in the corresponding 526 thin films only 125 cases were found positive, examination of thin film took 30 minutes per slide. Bini (1922) observed that by adopting the thick film technique 10 per cent of more positive cases were obtained which had been judged negative by the thin film method. In a similar study, Kerim (1935) showed that while the thick film preparation showed 95·5 per cent of positive slides on an average of 38 seconds per slide, in the thin film it was only 67 per cent. Moreover, in the latter case the time required for examination was on an average 8·47 minutes per slide.

Thin film examination, however, still remains extremely valuable for the diagnosis of the species of parasite in some cases of doubt or in the study of unusual forms (Christophers *et al.*, *loc. cit.*). The same authors suggest that both thick and thin films should be taken on the same slide, though for enumeration of parasites special thick films are recommended. Barber and Komp (1929) had also recommended thin and thick smears on the same slide. For years this practice has been followed and has been found convenient, economical and a time-saving technique.

MATERIALS AND METHODS.

MATERIALS.

(*i*) *Slides.*—One of the most important pre-requisites of obtaining a satisfactory film is a clean slide. Greasy slides render good film making impossible. Slides with scratches should be discarded altogether. Used slides may be cleansed after removing cedar wood oil with xylol by boiling in soap water or keeping them in lysol solution for 24 to 48 hours. Thereafter, all traces of blood are removed from the slides with cotton or lint and then they are rinsed in warm water. Each slide is then thoroughly dried and cleaned. It is preferable to keep the slides in absolute alcohol (chemically pure) till they are required for use. Just prior to making smears, the slides should be dried first in air and then cleaned and polished with silk handkerchief or a piece of fine cloth. In field work where bulk may be a handicap, dipping in alcohol may be omitted and the clean slides should be carried in a slide box for protection against dust and flies. While handling, it is important that they are held by the edges and surface is not touched with fingers.

(*ii*) *Pricking needle.*—A triangular needle like hagedorn has been found quite useful. When not in use the needle should be kept in rectified spirit, with the eye of the needle stuck in a cork which serves as a stopper for the specimen tube in which the needle is kept.

(*iii*) *Cotton wool.*

(*iv*) *Grease pencil.*—Some prefer diamond pencil for marking. When the latter is used it is desirable that the slides are marked beforehand near one end of the slide. Some use ordinary pencil and write over the thin film itself. This procedure may be inconvenient when thin films are examined, as the continuity is broken.

(*v*) *Slide box.*

(*vi*) *Rectified spirit.*

METHODS.

Source of blood.—It is immaterial from where the blood is taken. Some prefer the pulp of the ring or little finger, or from the dorsum just below the base of the nail (Barber and Komp, *loc. cit.*). Others prefer toes when dealing with infants. Some take smears from pinna of the ear. Of necessity, source varies when dealing with birds or animals and much depends on the convenience and availability of blood easily. In monkeys, the common practice is to take blood from the ear by puncturing one of the venules. In chicks and small birds like sparrows, canaries etc., it is taken from the wing veins, while in case of fowls and pigeons from the legs and from chipped tails in respect of rats, mice and lizards.

Immediately before taking smears, it is important that the site is thoroughly cleaned with alcohol or soap water. If alcohol is used, care must be taken to ensure that it is completely dried off before using the needle.

Thick film.—It should be borne in mind that a thick film is not a thick drop as emphasized by Field (1948) who maintains that the thickness should not be more than 10 to 15 times that of a thin film (Field, 1940*b*). It is common experience

that most of the contents of a very thick film is washed away during the staining process. Moreover, even if it is properly dehaemoglobinized, very thick films cannot be properly examined due to opacity. Christophers *et al.* (*loc. cit.*) consider that it is always better to err on the thin side. However, practice soon teaches the amount of blood that should be taken and the degree of thickness required.

The usual method employed for making a thick smear is to take either a big drop or a few small drops and puddle or spread the blood either with the needle or the corner of a clean slide as advocated by Mayne (1919), Bini (*loc. cit.*), Blacklock and Southwell (*loc. cit.*), Christophers *et al.* (*loc. cit.*), Russell *et al.* (*loc. cit.*) and Hutchison and Hunter (*loc. cit.*). It is considered that the movement should be centrifugal rather than centripetal or else a thick mass will be left in the centre of the thick film.

Wilcox recommends movement of the slide in a narrow cycle, without losing contact of the drop of blood on patient's finger.

If too large a drop has been taken, excess can be removed by drawing out the drop to one corner of the slide and wiped out with a clean cloth or a wisp of cotton wool.

With regard to the size of the thick film, references are often made to the size of a finger nail or small coins like a 6-pence (English), dime (American), soldi (Italian), 2-anna or 4-anna piece (Indian) and so on. Frankly speaking it is confusing, more so, when one is not conversant with the sizes of particular foreign coins. A thick smear with a diameter of 10 mm. or slightly larger is considered to be satisfactory.

The method mentioned by Mayne (*loc. cit.*) is that drops of blood are dragged sharply on the slide which may be called a thin smear thickened. It is believed that this kind of 'drag' smear does not distort cells and parasites very much, is more uniform and ensures rapid dehaemoglobinization. Field (1948) describes the 'pipette' method and commends that it is the most versatile of all methods. It gives full control of size, shape and thickness of the film. The same worker mentions of another technique called the 'multifilm' method, suited for blood surveys when many films are required. Several thick films can be made on the same slide.

Thin film.—Preparation of thin films requires more skill than making thick ones. A small drop of blood is taken and spread with the edge of another slide kept at an angle of 30°. When the drop of blood comes in contact with the spreader, it runs along the edge of the spreading slide by capillary attraction. Spreader should be pushed forward with a steady, quick and sure movement so that the blood follows it. This gives a better control and uniformly good results. It is essential that all these directions are scrupulously observed otherwise the results would be a mass of blood clot or a clump of crushed and distorted cells, staining of which would give similar dissatisfaction as developing a photographic plate exposed two or three times. A good film is ensured with—

(*a*) clean, grease-free slides.

(*b*) smooth and uniform edge of the spreader.

(*c*) adequate amount of blood.

A good film should be evenly spread, unbroken without streaks one cell thick with continuous edges both at the top and bottom, and should terminate in finger-like process at the end.

Thick and thin films.—When thick and thin films are to be taken on the same slide, as should be the general rule, the drop for thick film should be about half an inch from one end of the slide. The drop for making a thin film should be one inch from the same end of the slide and should be spread first.

If one or both the drops are too big, the slide should be discarded and fresh drops taken on another.

Drying.—Thin films should be dried immediately, preferably by waving the slide in the air.

All seem to agree that thick film should be kept horizontally to dry. Barber and Komp (*loc. cit.*) recommend that thick films should be kept overnight as, if drying is insufficient, part of the film will come off during the staining process. Field (1940*b*) also recommended the above as one of the methods and suggested that the films could be dried for several hours. In humid atmosphere, Christophers *et al.* (*loc. cit.*) recommend exposing the films to sunlight and avoiding contamination by flies and dust. Murphy (1929) recommended warming the slide with the thick film, specially on rainy days. But all these methods are time-consuming and when speed is essential, the process can be hastened by blowing hot air over the film. Young (1938) and Wilcox and Logan (*loc. cit.*) achieved this object by using a hair dryer. This may also be conveniently used for hastily drying stained smears. Occasional practice of holding the slide near the flame of a spirit lamp or bunsen burner should be deprecated as unless care is taken the direct heat is likely to fix the film just as alcohol does. In that case it will be difficult to dehæmoglobinize the thick film.

Fixing and lysing or dehæmoglobinization.—When using Giemsa, or a water-soluble stain like J.S.B., the thin film of necessity has to be fixed in methyl alcohol. The time for dipping in alcohol varies from a few seconds to a minute or so. When dealing with both thick and thin films on the same slide, a quick dip in alcohol is preferred or else the alcohol vapour is likely to fix the thick film also. Alcohol should be commercially pure and free from moisture. As it absorbs moisture quickly from the atmosphere it should be kept in a tightly stoppered bottle or a jar.

When using stains where alcohol is the solvant like Leishman's, above procedure is unnecessary.

The thick film is not to be fixed as it will interfere with the process of dehæmoglobinization which is necessary to make the film transparent to light. Lysis, with diffusion of hæmoglobin, readily occurs during staining provided the films are not too old. The time for dehæmoglobinization depends also on the thickness of the drop and the period it has been allowed to dry. The process is complete when the film has lost all its colour and is hardly visible.

Usually the background, after staining, appears mottled and lightly coloured. Light staining produces excellent background but the parasites may appear pale. As Field recommends, a compromise between deep staining and light is necessary.

While preliminary dehæmoglobinization and fixation of thick films are considered unnecessary by Barber and Komp (*loc. cit.*), Field (1940*a*) suggests that

to avoid distortion of the leucocytes and parasites (when thick film is stained without drying for several hours thereby limiting its usefulness) a brief preliminary treatment with a basic stain consisting of methylene blue and buffer salts is helpful. The solution consists of

Methylene blue	...	0·5 gm.
Na_2HPO_4	...	3·7 gm.
KH_2PO_4	...	0·3 gm.

in 100 c.c. water.

He recommends a dip for one second before using Giemsa. Another method has also been recommended by him for hæmolysing the film with diluted stain which is isotonic with blood plasma (Field, 1941). Stains used were brilliant cresyl blue, Giemsa, mixtures of methylene blue and eosin, etc. The first of the series appeared to be most satisfactory.

For normal routine work under Indian conditions, freshly prepared thick films stained with any of the water-soluble stains without any preliminary treatment have persistently shown good results. A preliminary treatment of old films preserved by special methods like those described by Jaswant Singh *et al.* (1949) with ordinary tap water for a few minutes is sufficient for dehæmoglobinization during routine work and a little longer for very old unpreserved films.

Staining of thick and thin films.—The scope of the present paper does not permit a detailed study of stains and staining methods. As such reference to only a few important ones is made. The well-known stains used for blood films are Leishman, Giemsa and Wright. In recent years, Shute, Boye, and rapid stains like J.S.B. and Field have been introduced.

Important constituents of all are methylene blue and eosin either in combination or separately. These two stains have been used since the time of Laveran, and form the basis of all Romanowsky stains mentioned above.

Rapid stains.—Michelson and Wilcox (1940) used Wright and Giemsa for rapid staining of thick film. The stain dilution is 1 in 10 and staining time is 10 minutes. As previously mentioned, Field had used brilliant cresyl blue and also evolved an alternative stain using a mixture of methylene blue and eosin in the proportion of 0·4 gm. to 0·1 gm. in 100 c.c. of isotonic buffered water (Field, 1940*b*). The latter preparation is the well-known Field's rapid stain where two solutions are used, one containing methylene blue, azure B and buffer salts, and the other eosin and buffer salts dissolved in water (Field, 1941). The staining time of a thick film is considerably shortened by this method but this is of no value in staining thin films, and as such when dealing with thick and thin films in the same slide, this stain has not proved advantageous. On the other hand, J.S.B. stain can be used conveniently for both thick and thin films. The time for staining a thin film is a little over a minute while that for thick film is less than half a minute. For routine staining, thick and thin smears in the same slide are stained together and the procedure is the same as for staining a thin film. As a result of further modification both in the Malaria Institute of India and abroad (Manwell and Seigelson, 1948), it has been possible to achieve uniformly good results and the stained smears compare favourably with any other standard preparation. Manwell (1945) had observed that preparations stained with J.S.B. are somewhat less

resistant to fading and will stand much more exposure to light than they would ordinarily receive. The overwhelming advantage of J.S.B. over other stains is the rapidity with which a thick and thin film on the same slide can be stained. Further, by a slight change in the staining technique (unpublished) in which the smear is dipped first in solution II (eosin solution) for 3 to 5 seconds, a few dips in the washing water (buffered to pH 6·6 to 6·8) and then finally to solution I for 35 to 40 seconds (the 'reverse' process), it has been possible to limit the staining time of a thin film to about three-quarters of a minute. The parasites and the blood elements are stained equally well as with the usual procedure. In case of *P. cynomolgi* and *P. vivax*, it is the common experience by workers that stippling show up more prominently with this stain than with Giemsa. This phenomenon in respect of *P. knowlesi* has also been reported by Jaswant Singh and his co-workers (1950). By adopting the 'reverse' method, stippling is slightly more accentuated. In a comparative study of *P. berghei* stained with Giemsa or J.S.B. (Ramakrishnan and Satya Prakash, 1950), it was observed that infected corpuscles show stippling-like dots more frequently with J.S.B. than with Giemsa.

Use of J.S.B. stain for organism other than plasmodia.—Besides a number of species of plasmodia, several species of trypanosomes and *Leishmania donovani* stained with J.S.B. have been compared with Giemsa-stained smears by Manwell (*loc. cit.*) and it has been shown that the two techniques give equal differentiation. Jaswant Singh *et al.* (1950) observed satisfactory results with slides showing trypanosomes stained with J.S.B.

Trypanosomes, treponema anserini and hepatozoon have also been stained satisfactorily with this stain (Nair and Basu, 1950). In a comparative study with *Hæmoproteus columbæ* stained with J.S.B. for a little over a minute and Giemsa for 90 minutes, Ray (1949) observed equally good differentiation. He further observed excellent results when tissue smears showing toxoplasma gondii were stained with J.S.B. Sporozoites may also conveniently be stained (4 minutes) with it.

In studies in an area where malaria and filaria co-existed, Raghavan and Krishnan (1949) mention that microfilaria and plasmodia could be conveniently stained with J.S.B. in the same slide. The same observers have reported satisfactory results in routine staining of microfilariæ (staining time 4 to 5 minutes).

Diagnosis of parasites in thick and thin films: Thin films.—Detailed descriptions are available in all textbooks on the subject.

Thick films.—Diagnosis by the character of pigment: Some workers had made attempts for diagnosis of parasites by the study of the character of pigments. Roubaud (1917) used thionine or carbol violet to stain thick films. While pigment of well-developed parasites was distinguishable, he had experienced considerable difficulties in detecting pigment in earlier forms. Field (1949) has studied the characters of pigment in lysed unstained thick films and has observed differences in pigments between the three parasites. Pigment of *P. falciparum* appears early and soon concentrates and becomes a fused mass. This type of solid fusion does not occur in other species. Pigment of *P. vivax* appears like delicate rodlets which show little tendency to coalesce and the elongated granules remain discrete. Pigment of *P. malariæ* is rather round than elongated and shows a peripheral distribution. It begins to concentrate very late and to a less degree

than in the other species. As regards gametocytes no difference in the pigment in the sexes of any species could be made out.

The study of pigment in unstained thick films for differential diagnosis of parasites may be of some value but for beginners it is likely to prove an uphill task.

Identification of parasites in stained preparations : P. falciparum.—The rings in thick films usually appear as small interrupted rings, exclamation marks, 'swallow forms', 'gulls wing', 'broken ring', 'comma', 'semicolon', etc. (Field and Le Fleming, 1939 ; Field, 1948 ; Simeons, *loc. cit.*).

If the rings are small and delicate and no other forms or older stages of parasites are present, the species is likely to be *P. falciparum*. Older asexual forms are usually absent from the peripheral blood except in certain severe or moribund cases.

P. vivax.—Individually they are difficult to distinguish from those of *P. malariæ*, but if the appearance of the cytoplasm shows some irregularity, and older forms are present, it may be possible to identify the species with the help of the size of the chromatin bead which is usually large in *P. vivax*.

The cytoplasm at later stages varies in shape and size and is usually not compact. Red or magenta-coloured mass of chromatin is always present. When the parasite is fairly well grown the cytoplasm appears to be scattered. Pigment appears as yellowish granules. Suggestions of stipplings—Schüffner's dots—are occasionally found in the 'ghost' outline of the infected cells.

Schizonts are usually detected by the size of discrete and loosely concentrated pigment and the number of merozoites which are on an average 16 in number.

P. malariæ : Ring stages.—There is a single bead-like chromatin and the parasite appears as an irregular and broken ring. Later, the cytoplasm becomes irregular and shows a tendency to 'ball up' round the chromatin mass (Field, 1948) and the vacuole disappears. Thereafter, it is rounded and becomes compact unlike that of *P. vivax*. The schizonts are smaller in size than those of *P. vivax*. The number of merozoites is on an average eight and they group round the pigment. The pigment which appears as small granules and scattered in earlier stages, becomes a compact collection of distinct granules when the schizont is matured. It is usually more abundant and scattered.

Identification of gametocytes : P. falciparum.—The diagnosis of gametocytes of this species is quite easy on account of their characteristic shape. As a rule the macro-gametocytes (female) are long and slender whether at the early or mature stage. The micro-gametocytes (male) are shorter and broader. In well-stained films, the compact chromatin of macro-gametocyte may be differentiated from the diffuse type in the micro-gametocyte. The pigment in the latter is often found to be scattered from pole to pole. However, sex differentiation may not in all cases be so easy.

P. vivax.—Both the male and female gametocytes are round or oval in shape. Mature ones are larger than those of *P. malariæ*. A peripheral fringe with Schüffner's dots, if present, will confirm the diagnosis. Sometimes, however, well-developed compact trophozoites may be mistaken for half-grown macro-gametocytes but the size of the chromatin of the latter is larger and more prominent and their

characteristics should lead one to correct differentiation. The loose and diffuse character of the chromatin of micro-gametocytes and the purplish tint may make the diagnosis easy. As a rule, pigment is scattered irregularly in both male and female gametocytes.

P. malariæ.—Both the gametocytes appear round and compact. The pigment is abundant and scattered peripherally. The chromatin of female gametocytes appears more compact and larger than that of the male.

For study of unusual forms or in case of doubts regarding the species or the sex of the parasite, it would be worth while to examine the thin film.

SUMMARY.

1. The importance of blood smears as a diagnostic measure has been stressed and so also.

2. The usefulness of thick films for rapid diagnosis of malaria.

3. Routine technique of making blood smear has been discussed.

4. Mention has been made of various stains which can be used for staining of plasmodia.

5. A brief summary of the use of J.S.B. stain for organisms other than plasmodia has also been incorporated.

6. Some guidance has been given for identification of the parasites in thick films.

REFERENCES.

BARBER, M. A., and KOMP, W. H. W. (1929). *Pub. Hlth. Rep.*, **44**, pp. 2330-2341. *Abs. Trop. Dis. Bull.*, **27**, pp. 198-199.

BEAUMONT, G. E. (1948) *Medicine.* J. & A. Churchill Ltd., London, p. 733.

BINI, G. (1922) ... Policlinico. Sez. Prat., **29**, pp. 913-914.

BLACKLOCK, D. B., and SOUTHWELL, T. (1935). *A Guide to Human Parasitology.* H. K. Lewis & Co., Ltd., London, pp. 13-17 and 79.

CAPLAN, A. (1942) ... ... *Ind. Med. Gaz.*, **77**, pp. 472-475.

CHOPRA, R. N., GUPTA, J. C., and DAVID, J. C. (1927). *Ibid.*, **62**, pp. 325-327.

CHRISTOPHERS, S. R., and FULTON, J. D. (1938). *Ann. Trop. Med. Parasit.*, **32**, pp. 43-75.

CHRISTOPHERS, S. R., SINTON, J. A., and COVELL, G. (1944). *Hlth. Bull.* No. 14. *Malaria Bureau.* No. 6. Manager of Publications. Delhi.

FIELD, J. W. (1940*a*) .. *Trans. Roy. Soc. Trop. Med. Hyg.*, **33**, pp. 635-638.

Idem (1940*b*) ... *Ibid.*, **34**, pp. 195-202.

Idem (1941) ... *Ibid.*, **35**, pp. 35-42.

Idem (1948) .. *The microscopic diagnosis of human malaria. Studies from the Institute for Medical Research*, F.M.S. No. 23.

Idem (1949) *Trans. Roy. Soc. Trop. Med. Hyg.*, **43**, pp. 49-56.

FIELD, J. W., and LE FLEMING, H. (1939). *Ibid.*, **32**, pp. 467-480.

Idem (1940) *Ibid.*, **33**, pp. 507-520.

GREEN, R. (1931) .. *Gaz. Inst. Med. Res.*, F.M.S. No. 1, p. 2.

HALWANI, A., and SOBKY, M. F. (1930). *J. Egyptian Med. Assoc.*, **22**, pp. 509–516.

HARRIS, A., and REIDEL, L. M. (1948). *Amer. J. Trop. Med.*, **28**, pp. 787–795.

HORMANN, B. (1949) ... *Ztschr. f. Immunitätsf. u. Exper. Therap.*, **106**, pp. 135–141.

HUTCHISON, R., and HUNTER, D. (1949). *Clinical Methods.* Cassell & Co., Ltd., London, pp. 151–159 and 162–163.

JASWANT SINGH, and BHATTACHARJI, L. M. (1944). *Ind. Med. Gaz.*, **79**, pp. 102–104.

JASWANT SINGH, RAMAKRISHNAN, S. P., and DAVID, A. (1950). *Ind. J. Mal.*, **4**, pp. 189–192.

JASWANT SINGH, RAY, A. P., and NAIR, C. P. (1949). *Ibid.*, **3**, pp. 327–329.

Idem (1950) ... *Ibid.*, **4**, pp. 317–336.

KENT, J. F., CUTWELL, W. B., COATNEY, G. R., and COOPER, W. C. (1948). *J. Lab. Clin. Med.*, **33**, pp. 747–752.

KERIM, M. A. (1935) ... *J. Egyptian Med. Assoc.*, **18**, pp. 232–237.

LOW, C. G., and FAIRLEY, N. H. (1933). *Textbook of the Practice of Medicine.* Edited by F. W. Price, Oxford University Press, pp. 235–239.

MANWELL, R. D. (1945) ... *J. Lab. Clin. Med.*, **30**, pp. 1078–1082.

MANWELL, R. D., and FRIGELSON, P. (1948). *Ibid.*, **30**, pp. 777–782.

MAYNE, R. (1919) ... ... *Pub. Hlth. Rep. Wash.*, **34**, pp. 837–842.

MICHELSON, L., and WILCOX, A. (1940). *Ibid.*, **55**, pp. 1221–1222.

MURPHY, R. A. (1929) ... *Ind. Med. Gaz.*, **64**, pp. 557–558.

NAIR, C. P., and BASU, P. C. (1950) *Personal communication.* Delhi.

NAPIER, L. E. (1922) ... *Ind. J. Med. Res.*, **9**, pp. 830–846.

RAGHAVAN, N. G. S. (1949) ... *Ind. J. Mal.*, **3**, pp. 199–205.

RAGHAVAN, N. G. S., and KRISHNAN, K. S. (1949). *Ibid.*, **3**, pp. 39–56.

RAMAKRISHNAN, S. P., and SATYA PRAKASH (1950). *Ibid.*, **4**, pp. 361–367.

RAY, A. P. (1949) ... ... *Personal communication*, Delhi.

REIN, R. C., BURANTZ, S. C., KENT, J. F., COOPER, W. C., RUBE, D. S., and COATNEY, G. R. (1949). *Amer. J. Hyg.*, **49**, pp. 374–384.

ROUBAUD, E. (1917) ... *Bull. Soc. Path. Exot.*, **10**, pp. 702–703.

RUSSELL, P. F., WEST, L. S., and MANWELL, R. D. (1946). *Practical Malariology*, pp. 89–90. W. B. Saunders Co., Philadelphia & London.

SIMEONS, A. T. W. (1942) ... *Ind. Med. Gaz.*, **77**, pp. 725–729.

SINTON, J. A. (1922) ... *Ind. J. Med. Res.*, **10**, pp. 592–594.

SINTON, J. A., and BANERJEA, A. C. (1925). *Ibid.*, **12**, pp. 537–544.

TAYLOR, H. A. (1917) ... *J. Amer. Med. Assoc.*, **68**, pp. 771–772.

VAN ROOYEN, C. A. (1941) ... *Brit. Med. J.*, July 19, p. 87.

WALKER, A. J. (1948) ... *Amer. J. Trop. Med.*, **28**, pp. 777–785.

WAR OFFICE, LONDON (1941) ... *Memoranda on medical diseases in tropical and subtropical areas*, pp. 141–143.

WILCOX, A., and LOGAN, L. (1941) *A symposium on human malaria.* Edited by F. R. Moulton, pp. 47–62. Amer. Assoc. for advancement of science. Smithsonian Institute Building, Washington, D.C.

WORLD HEALTH ORGANIZATION EXECUTIVE BOARD (1950). *J. Nat. Mal. Soc.*, **9**, p. 191.

YORING, M. D. (1938) ... *Pub. Hlth. Rep. Wash.*, **53**, p. 1256.

Indian Journal of Malariology, **4**, 3, September 1950.

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

I. Variations in susceptibility in albino mice.

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[August 15, 1950.]

VINCKE and LIPS (1948) described *Plasmodium berghei*, a new species from tree rats in the Belgian Congo. As the first and subsequent publications of work on this parasite are in French, the highlights of the discovery are summarized here from translations of the original articles.

Consequent to finding a high sporozoite rate (7 per cent) in *A. dureni*, in a forest belt in Elizabethville in the Belgian Congo, these workers made an intensive search for the possible vertebrate host or hosts of malaria parasite in that area, and subsequent dissections of a large number of *A. dureni*, caught in nature, revealed no infection. Examination of the gut contents invariably revealed the presence of mammalian erythrocytes, and that led to an investigation of the food preferences of the mosquitoes. In a series of precipitin tests conducted against antisera of cat, dog, antelope, man and rat, consistent though delayed, positive reactions were obtained only against anti-rat serum.

Since 1947, a parasite survey of rodent blood revealed the presence of trophozoite-like forms in erythrocytes in 14 out of 93 rodents. The authors were not convinced of the nature of these parasites till they found in 1948 unquestionable plasmodia in the blood of 2 rodents (*Thamnomys surdaster*) and made subsequent studies on the morphology of the parasite.

Vincke and Lips (*loc. cit.*) were able to serially infect mice and rats by blood inoculation, and while they failed to infect rabbits and hares, Rodhain (1949) passed the infection to cotton rats successfully.

Vincke and Lips (*loc. cit.*) fed locally available anophelines without obtaining any evidence of development of parasites in them. Van den Berghe *et al.* (1950) have demonstrated the existence of the secondary tissue phase in *P. berghei*, in albino mice and rats. By sacrificing animals inoculated intraperitoneally with infected blood at various intervals during the pre-patent period, they found exo-erythrocytic schizogony in the histiocytes and monocytes of the liver and its vascular endothelium.

MATERIALS AND METHODS.

A few albino mice infected with *Plasmodium berghei* were received from London and Paris through the kind courtesy of Professors H. E. Shortt and Emile Brumpt respectively.

The stock of mice used in the present study was obtained from the Central Research Institute, Kasauli, India.

Blood was usually obtained by snipping the tip of the tail for making blood films or for the purposes of subinoculations. By gently milking the tail, it was found possible to draw as much as 0·2 c.c. of blood into 1 c.c. tuberculin syringe containing a known quantity of sterile citrate saline (2 per cent). All inoculations were made by the intraperitoneal route. Before inoculation, thorough mixing of erythrocytes in citrate saline mixture was ensured by frequently twirling the syringe between the palms of the hands.

An arbitrary scale of +1 to +7 was followed to indicate the degree of parasitaemia and the dose of inoculum. A stained blood film was examined and the number of parasites per microscopic field determined. The degree of parasitaemia was indicated as follows :—

Positive	...	One parasite in more than 10 fields.
+1	...	One parasite in 5 to 10 fields.
+2	...	One parasite in 1 to 5 fields.
+3	...	1 to 5 parasites per field.
+4	...	6 to 10 parasites per field.
+5	...	11 to 20 parasites per field.
+6	...	21 to 40 parasites per field.
+7	...	More than 40 parasites per field.

It was assumed that 0·01 c.c. of blood from a donor contained the number of parasites as indicated by an examination of a blood film and expressed in pluses as mentioned above. Appropriate dilutions of the blood were made with citrate saline to give the required dose of infection. For example, if a donor showed a parasitaemia of +6 and 0·05 c.c. of blood was drawn from it, it would contain an infection of +30. This quantity of the blood, when diluted with 9·05 c.c. of citrate saline, will contain an inoculum of +3 in each 0·1 c.c.

RESULTS.

Ninety-four albino mice (61 males and 33 females) were inoculated during the 20 serial passages in these laboratories. Dose of inoculum varied from +1 to +9. Out of the 61 males and 33 females inoculated for the first time, only 30 males

(19 per cent) and 25 females (76 per cent) became positive and the remainder were refractory to infection.

TABLE I.

Results of inoculation of different doses of infection in males.

Number of inoculation.		Dose of infection.							TOTALS.
		+1	+2	+4	+5	+6	+9	+40	
1st	Inoculated ...	36	1	8	4	9	3	...	61
	Positive ...	16	0	5	2	6	1	...	30
2nd (In mice refractory to 1st inoculation.)	Inoculated ...	7	8	2	...	2	...	3	22
	Positive ...	5	4	1	...	1	...	0	11
3rd (In mice refractory to 2nd inoculation.)	Inoculated ...	6	1	...	...	1	...	...	8
	Positive .	3	1	...	...	1	...	...	5
4th (In mice refractory to 3rd inoculation.)	Inoculated ..	2	1	2	...	..	...	...	5
	Positive ..	1	1	2	...	...	...	...	4
5th (In mice refractory to 4th inoculation.)	Inoculated ...	1	.	..	...	..	...	...	1
	Positive ...	1	..	...	...	...	...	...	1

TABLE II.

Results of inoculation of different doses of infection in females.

Number of inoculation.		+1	+2	+4	+5	+6	+9	TOTALS.
1st	Inoculated ...	9	2	10	2	7	3	33
	Positive ...	3	2	9	2	7	2	25
2nd (In mice refractory to 1st inoculation.)	Inoculated ..	1	2	2	...	3	...	8
	Positive	1	2	0	...	2	...	5
3rd (In mice refractory to 2nd inoculation.)	Inoculated .	..	...	..	...	3	...	3
	Positive ..		...	...	...	2	...	2

The animals refractory to the first inoculation after observation periods varying from 5 to 27 days were subjected to second inoculation. Of the 22 males and 8 females thus treated (Tables I and II), 11 males (50 per cent) and 5 females (63 per cent) became positive. It is noteworthy that none of the 3 males subjected to the second inoculation with a dose of an inoculum of + 40 each, took up the infection.

Of the 8 males that were refractory to the first two inoculations and subjected to the third inoculation, only 5 became positive.

The 5 males that were refractory to three inoculations were once again subjected to a fourth dose of infection, and 4 became positive.

The remaining one male was inoculated for the fifth time with positive results.

The course of infection was almost the same in all the susceptible animals. The parasitæmia progressed gradually up to +7 or more and then the animal died. A wide variation in the pre-patent as well as the period of death in the susceptible animals (Tables III and IV) was observed and may partly be due to the limitations of the method employed in estimating the dose of infection.

TABLE III.

Dose of infection.	Number of animals positive after 9 day of inoculation, in days.														Totals.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
+1	12	3	5	3	8	...	...	...	...	...	...	...	...	...	31
+2	3	2	2	1	2	...	...	...	...	...	...	...	1	...	11
+4	7	1	4	1	...	1	1	1	...	...	...	...	...	1	17
+5	2	2	...	...	...	...	...	...	...	...	...	...	...	...	4
+6	12	4	...	...	1	1	...	...	1	1	...	...	...	...	20
+9	...	2	1	...	...	...	...	...	...	...	...	...	...	...	3
Totals	36	14	12	5	11	2	[illegible]	1	1	1	...	...	1	1	86

TABLE IV.

Dose of infection.	Number of animals dead after 0 day of inoculation, in days.																		Totals.
	3	5	6	7	8	9	10	11	12	13	14	16	17	18	20	21	22	23	
+1	...	4	8	5	3	3	1	...	...	2	1	1	...	...	...	1	...	...	29
+2	...	4	3	...	...	1	1	...	...	...	...	...	...	2	...	...	...	...	11
+4	2	...	7	1	1	1	1	...	2	...	1	...	...	...	...	...	1	...	17
+5	...	...	2	...	...	...	...	...	...	...	...	...	1	...	...	...	...	1	4
+6	...	4	4	2	2	0	2	...	...	...	2	1	...	1	1	...	...	...	19
+9	...	1	...	1	...	...	...	1	...	...	...	...	...	...	...	...	...	...	3
Totals	2	13	24	9	6	5	5	1	2	2	4	2	1	3	1	1	1	1	83

DISCUSSION.

Subject to limitations of sampling and the arbitrary estimation of dosages from the observations presented above, it will be evident that certain albino mice were naturally resistant to blood inoculation of *Plasmodium berghei*. In the present series, 56 per cent of males and 24 per cent of females were refractory to the first inoculation. Vincke and Bulcke (1949) also found that some mice did not become infected on the first inoculation, but after four attempts practically no mouse remained refractory. They, however, found a much greater percentage (196 out of 199) of inoculated white mice taking the infection. The dosage they employed was not indicated. The question whether there are inherent differences in races of laboratory-bred white mice maintained in different centres is worthy of consideration.

In the present study, although the number of females and males are not the same, a higher percentage of the latter was found to be refractory to the first inoculation. That the refractoriness was not due to a small inoculum is evident from the fact that the phenomenon was observed with different doses of infection also. It would therefore seem reasonable to assume that the natural resistance is not absolute but relative, because ultimately even the most resistant became infected after the third, fourth or fifth inoculation.

The males that were susceptible when the dose of inoculum was small (from +1 to +4) did not show a uniform pre-patent period as is exhibited in 8 days old chicks when infected with a standard dose of *Plasmodium gallinaceum*. Contrary to expectations, variation was also observed when the dose of inoculum was as much as +9. The susceptible females on the other hand showed a more uniform pre-patent period as well as the day of death with inocula varying from +2 to +9, pointing to the greater utility of females for the routine maintenance of the parasite as well as experiments like screening antimalarials.

The natural infection was originally found in rats in Belgian Congo and whatever resistance the mice may exhibit must be an inherent one.

In the invertebrate host, individual refractoriness to infection in a susceptible species and race is well known. Huff (1927) showed that in the same species (*Culex pipiens*) under identical conditions, all members were not equally susceptible to infections of avian malaria. Later, Huff (1929) reported having bred susceptible mosquitoes of the species separately from the refractory ones and proved that susceptibility or refractoriness is a hereditary characteristic. It would make an interesting study to breed stocks of the susceptible and the resistant mice in pure lines after treating the infection in the former with suitable antimalarials.

The age of the recipient of infection is also an important factor and plays a part in the susceptibility or refractoriness to infection. Trager and McGhee (1950) have discussed the question of variation in resistance in adult chicks and ducks to *P. lophurae*. As all the mice used for this study had to be obtained from another laboratory, it was not possible to determine their exact age. But the majority of them were no doubt mature adults. These observations will be repeated in mice of known age when the local colonies are established.

It is well known that certain races of human beings are less susceptible to certain plasmodial infections, as for instance, the Negroes for *Plasmodium vivax* (Boyd and Stratman-Thomas, 1933). Such a resistance is considered inherent, as Negro children, born in America, known to have had no history of previous infections, also show the same refractoriness as adults. Even in susceptible races of human beings, a range of innate immunity exists in individual members to malaria (Taliaferro, 1949). Boyd (1942) described a series of 69 white patients who were refractory to the bite of one or more mosquitoes having sporozoites of *Plasmodium vivax*. Thirty of this series were exposed to a second infection (25 to a homologous strain and 5 to a heterologous strain) and all of them were successfully infected the second time. The progress of infection revealed that the immunity of the patients was due to a natural resistance rather than a deficient inoculum of sporozoites on the first occasion. The authors' findings in the white mice in relation to *Plasmodium berghei* with blood inoculations are similar.

Silenus irus monkeys exhibit a tolerance to *P. knowlesi* infections, which was interpreted by Sinton (1935) to be due to previous experience of the disease. In the present observations, infection was equally pernicious both in the susceptible animals and the ones that were refractory initially and ultimately became infected after repeated re-inoculations. Thus the word 'tolerance' will not be applicable to the refractoriness of some mice to *Plasmodium berghei*.

Mulligan *et al.* (1940) concluded that 'a high degree of natural resistance to malarial infection is dependent upon efficient, non-specific agency, the efficacy of which appears to be largely independent of the integrity of the lymphoid macrophage system. This non-specific agency is probably an inherent unsuitability of the body tissues or fluids for the development and multiplication of the parasites'. Trager and McGhee (*loc. cit.*) have demonstrated that 'the relative natural resistance to *P. lophurae* of adult chickens and certain adult ducks is at least in part dependent on the presence in their plasma of materials which in some way exert an antiplasmodial effect'. It is intended to repeat the investigation of antiplasmodial substances in the plasma of resistant mice when the local colony is established.

CONCLUSIONS AND SUMMARY.

1. A certain number of albino mice (both males and females) exhibit a natural resistance to inoculations of blood infected with *Plasmodium berghei*. This resistance has been found both when low and high doses of inocula were used. It is probable that refractoriness to infection is greater in males than in females.

2. The innate resistance is not absolute as refractory individuals become infected after repeated re-inoculations.

3. Possible relationship of the innate resistance to heredity, age and immune substances in the plasma are discussed.

ACKNOWLEDGMENTS.

The authors wish to record their grateful thanks to the Director, Central Research Institute, Kasauli, for the supply of white mice used in these observations.

They also thank Mr. R. Marathe of the All-India Radio and Dr. J. Yoeffe of the South-East Asia Regional Headquarters of the World Health Organization for kindly translating the original French publications into English.

REFERENCES.

Boyd, M. F. (1942)	*Amer. J. Trop. Med.*, **22**, p. 217.
Boyd, M. F., and Stratman Thomas, W. K. (1933).	*Amer. J. Hyg.*, **18**, p. 485.
Huff, C. G. (1927)	*Ibid.*, **7**, p. 705.
Idem (1929)	*Ann. Trop. Med. Parasit.*, **23**, p. 127.
Mulligan, H. W., Sommerville, T., and Swaminath, C. S. (1940).	*J. Mal. Inst. Ind.*, **3**, p. 563.
Rodhain, J. (1949)	*Ann. Soc. Belge de Med. Trop.*, **29**, p. 483.
Sinton, J. A. (1924)	*Ind. J. Med. Res.*, **12**, p. 341.
Idem (1935)	*Rec. Mal. Surv. Ind.*, **5**, p. 501.
Taliaferro, W. H. (1949)	*Malariology.* Edited by M. F. Boyd. Vol. II. W. B. Saunders Co., Philadelphia and London.
Trager, W., and McGhee, R. B. (1950).	*J. Exp. Med.*, **91**, p. 365.
Van den Berghe, L., Vincke, I. H., and Charlome, M. (1950).	*Ann. Soc. Belge de Med. Trop.*, **30**, p. 79.
Vincke, I. H., and Lips, M. (1948)	*Ibid.*, **28**, p. 97.
Vincke, I. H., and Van den Berghe, M. A. (1949)	*Ibid.*, **29**, pp. 525, 537 and 545.

Indian Journal of Malariology, **4**, 3, *September 1950*

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

II. Morphology, periodicity and pathogenicity in blood induced infections in mice, rats and garden squirrels.

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(October 23, 1950.)

STUDIES on the morphology of *P. berghei* reported here confirm in most respects the description of the parasite by Vincke and Lips (1948). The duration of the asexual cycle was found to be identical in five types of susceptible hosts, namely, the mice (*Mus musculus*), both the domestic and the albino varieties, rats (*R. norvegicus* white and *R. rattus*) and the garden squirrels (*Sciurus palmarum*).

MATERIALS AND METHODS.

The strain of *P. berghei* used in these studies was obtained through the courtesy of Professor Emile Brumpt, Paris. The white mice and rats were made available through the courtesy of the Director, Central Research Institute, Kasauli. The domestic mice, rats and squirrels were obtained locally.

Method of inoculation and determination of the dose and degree of infection were the same as described in part I of the studies (Ramakrishnan and Satya Prakash, 1950). The dose of infection in all cases in the present studies was plus six.

Thick and thin air-dried smears of blood were taken daily from the animals and stained after the thin smear portions had been fixed in methyl alcohol. Giemsa and J.S.B. stains were used with equally good results.

MORPHOLOGY.

The morphology of the parasites is essentially the same in all the five susceptible hosts; the differences were in the degree and course of parasitæmia.

Asexual forms.—The earliest form occurs as a small discrete dot of chromatin, about 1 micron in diameter, without any visible trace of cytoplasm. Very quickly the parasite grows into a solid, non-vacuolated body with a tiny wisp of cytoplasm. In the first 4 hours, it develops a vacuole and is seen as a ring form measuring about 2 to 3 microns in diameter usually situated eccentrically. Almost invariably the cytoplasm shows a slight thickening on the side opposite the chromatin which is prominent and almost always single and round. As a rule, it projects from the contour of the parasite giving it the classical 'signet ring' appearance. Rarely it is situated within the vacuole. More rarely it appears as two dots, both being equal in size and situated close together. Rings described as '*accolé*' and '*bird's-eye*' forms are not common, and are occasionally seen in heavy infections only. Infested cells are normal in size, shape and staining reactions and no pigment is seen at this stage.

In 4 to 8 hours, the rings become larger in size and measure about 3 to 4 microns in diameter. The cytoplasm increases in amount and may be slightly irregular in outline. Pigment is not seen and the infested red blood corpuscles are not enlarged.

In 8 to 12 hours, the parasite measures about 4 to 5 microns. The ring forms appear more solid and display an irregular outline due to a slight amœboidicity. In some cases, definite pseudopodia can be made out. The vacuole is relatively smaller and may be irregular in outline. The chromatin is larger and now appears to protrude less from the contour of the parasite and still stains a uniform brick red. No pigment is discernible but the infested red cell is slightly enlarged. Typical band forms stretching across the diameter of the red cells are seen more abundantly in the squirrels, commonly in the rats, and occasionally in the mice.

With further growth, in 12 to 16 hours, the parasite measures about 6 microns, occupying more than half of the enlarged infested red cell and occurs as a solid, rounded, oval or elongated body. The outline tends to be irregular and in the majority of forms, the vacuole disappears. The chromatin is enlarged and divided into two, each mass being irregular in shape, oval, oblong or rectangular. In most cases there seems to be a clear space or 'halo' around the chromatin. No pigment is discernible even at this stage.

Between 16 and 20 hours, the same general morphology is observed and the parasite has grown larger in size. Majority of the forms occupy almost the entire red cells which are enlarged and show a certain amount of pallor especially in heavy infections. The chromatin divides into 4 or more fragments and the 'halo' around each of them continues to be seen. Still no pigment is visible.

Between 20 and 24 hours, the parasite becomes a mature schizont and is slightly smaller than the preceding stage. In the majority of cases the infested cells remain enlarged. The number of merozoites varies from 10 to 22, usually about 12, arranged in the form of a rosette, and less frequently scattered irregularly. Each merozoite is distinct with a dense uniformly stained chromatin dot and a wisp of blue cytoplasm. In the residual mass which is often centrally situated, a small

amount of pigment can be seen for the first time in the asexual cycle of the parasite. It is usually found in a single clump and is amber-coloured. In organ smears of animals as well as in blood smears occasionally, monocytes with small amber-coloured clumps of phagocytosed pigment can be seen.

Sexual forms.—It is difficult to distinguish growing forms of gametocytes till they are mature, at which stage they appear as large-sized parasites occupying more than two-thirds of the infested cells. The outline of the parasite is usually irregular. The protoplasm of the macro-gametocyte stains blue and the chromatin which is dense and compact, is situated peripherally in a clear space almost resembling a vacuole. The margin of the chromatin mass is also irregular.

The micro-gametocyte stains poorly compared to the above and its chromatin seems a little more diffused or loosely packed and is also surrounded by a clear space resembling a vacuole. The infested cells in both cases are enlarged. In neither is any pigment discernible.

All the forms described above are illustrated in Plate XIII.

PERIODICITY OF THE SCHIZOGONY CYCLE OF *P. BERGHEI*.

In determining the periodicity of *P. berghei*, the method described by Mulligan (1935) in reference to his studies on *P. cynomolgi* and *P. knowlesi* in monkeys was followed, and arbitrary stages in which parasites can be observed and classified for purposes of differential counts were defined:

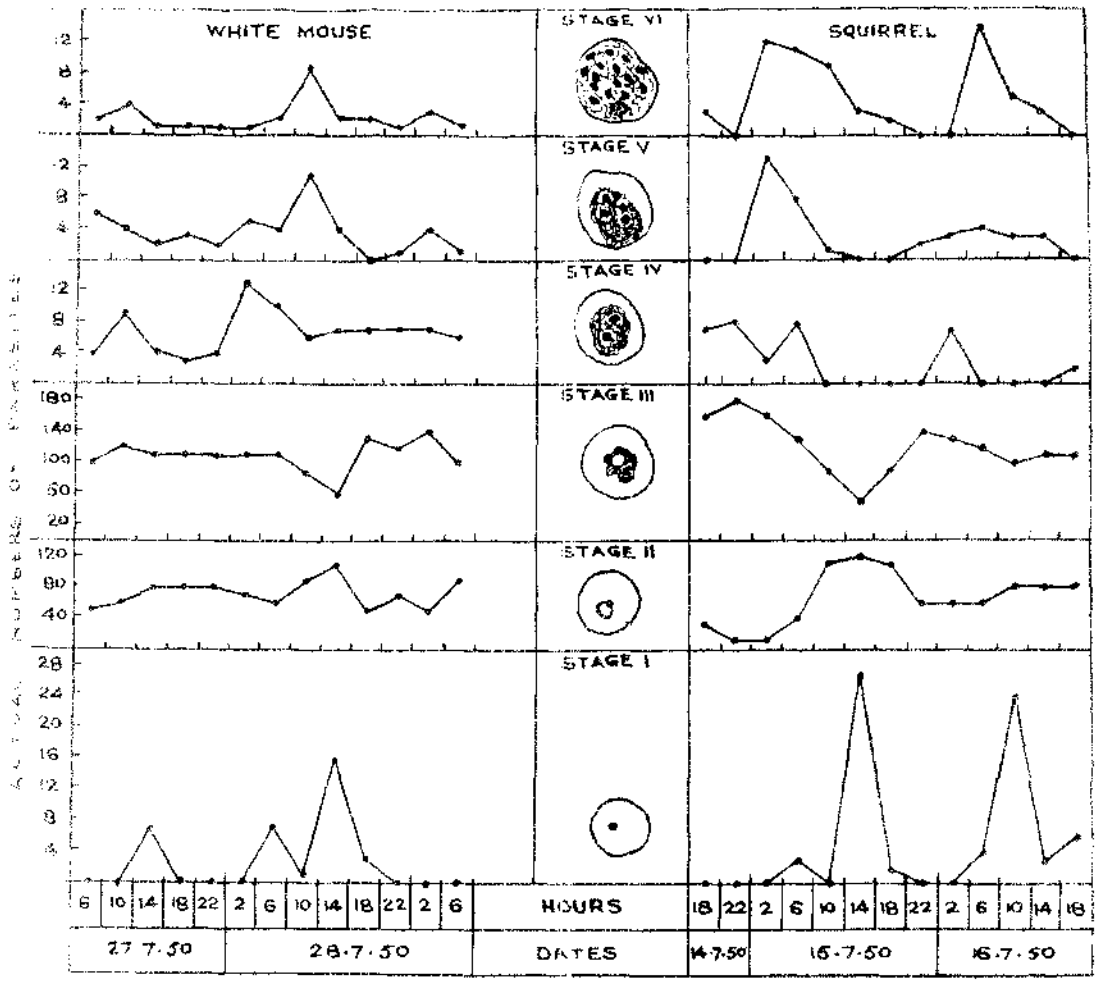
- Stage I. Early solid forms and rings; red cells unaltered.
- Stage II. Larger ring forms and young trophozoites; red cells unaltered.
- Stage III. Vacuolated trophozoite showing amœboidicity; red cell slightly enlarged.
- Stage IV. Large solid pre-schizont with two chromatin masses; red cell enlarged.
- Stage V. Developed schizont with not more than 5 chromatin masses; red cell enlarged.
- Stage VI. Mature schizonts and segmenting forms with more than 5 chromatin masses; red cell enlarged.

In every case, four-hourly smears were made and studied daily for a period of 6 to 7 days or till the death of the animal whichever was earlier. Random samples of 200 asexual parasites were studied in each smear for classification.

Differential parasite counts were made in 4 white mice, 3 white rats and 2 garden squirrels, and the schizogony cycle of *P. berghei* in every one of them was found to be regularly of 24 hours' duration. All growing stages, however, were found to occur more or less at all times of the day and night in the peripheral blood, presumably due to the fact that in blood induced infections, more than one brood of parasites from the donor are usually introduced. In the present studies it was invariably evident that there were at least two broods, one having its peak numbers segmenting between 10 a.m. and 2 p.m. The results of the 4-hourly differential counts in a white mouse and a squirrel are represented in Chart I. The majority of the parasites present in the peripheral blood of all the animals were at approximately the same stage of development.

CHART I.

Schizogony cycle of P. berghei *in a white mouse and a squirrel.*



PATHOGENICITY.

In general, infections of *P. berghei* are highly pathogenic to white and domestic mice, moderately so to some white and domestic rats, and least to garden squirrels. In part I of these studies (Ramakrishnan and Satya Prakash, *loc. cit.*) it was reported that some white mice showed a relative resistance to infection after the first few inoculations of infected blood. The resistance in these cases was overcome by repeated inoculations. Similar refractoriness to infection was rare in rats and squirrels. In mice, the parasitaemia was intense and continued to increase till death ensued. In some of the white rats under study, the course of the disease

was similar to that observed in the mice, while in others it tended to be subacute and chronic, then becoming latent for a period, and relapsing later. In squirrels, milder infections with latency alternating with periodical relapses were experienced. While some of the brown and white rats, and all the brown and white mice died during the acute phase of the disease on an average between 5 and 12 days of infection, the squirrels and a few of the rats lived a much longer period and infections assumed chronicity. One infected brown rat lived for 42 days after infection and the parasitæmia showed two latent periods of 10 days each. In one infected white rat, alive at the time of reporting 140 days after infection, the parasitæmia remained latent after the acute phase during four periods of varying lengths. An infected squirrel lived for 48 days after infection and showed a long period of latency for 21 days before there was a parasitæmic relapse resulting in death.

In all animals, except in mice and rats with very heavy infections, only polychromatophilic red cells were found parasitized. In heavy infections, nucleated erythrocytes were also seen and a few were found infested.

As mentioned above, the pigment, if any, is not visible in preparations stained by the Giemsa or J.S.B. methods in the growing stages of the parasite. It can be seen only in the mature schizonts and some monocytes in the blood and post-mortem smears of organs. The pigment in the mature schizonts is comparatively small in amount and is found as a single clump of amber-coloured material.

Multiple infection of the red cells was common, more so in the acute infections in the mice and some of the rats and less in the squirrels. As many as four parasites at different stages of growth were observed in one cell (Fig. 26, Plate XIII).

Especially in the mice and in the acute heavy infections of the rats, a certain number of the parasites appear extracellular in the plasma, and are invariably pre-segmentors or early gametocytes. Most of these are linear, band forms, and some measure more in length than the intracellular ones. It is possible that red cells with too many parasites are rendered fragile and disintegrate giving rise to the forms seen free in the plasma (Fig. 20, Plate XIII).

In general, the infested red cell does not show any stippling. Very rarely in mice and rats, and more often in squirrels, a coarse 'stippling' in the infested cells is seen and invariably stains bluish unlike the classical red stippling. The markings are of different sizes and do not seem to have any uniform pattern. In the mice and rats the 'stippling' is seen only in the cells containing rings, trophozoites and pre-segmentors with two chromatin masses. In the squirrels, however, in addition to the cells with the above forms of parasite, it is seen in cells with gametocytes also. 'Stippling' is encountered to a greater extent in smears stained by the J.S.B. method than with Giemsa.

Some of the infected animals exhibit certain signs of illness between 10 a.m. and 2 p.m. every day, corresponding to the maturation of schizonts and release of merozoites in the blood stream. A transient paralysis of one or more of the limbs in some, and convulsions with the fur standing erect in others, are the signs of illness which lasts for one to two hours. One white rat was observed to be definitely comatose at 10 a.m. and made a spontaneous recovery completely in about half an hour.

EXPLANATION OF PLATE XIII.

Fig. 1. Normal polychromatophilic erythrocyte of a white rat.

Figs. 2 to 8. Developmental forms of *P. berghei* in polychromatophilic erythrocytes of rats.

Figs. 9 and 10. Micro- and macro-gametocytes in polychromatophilic erythrocytes of rats.

Fig. 11. Normal erythrocyte of rat.

Figs. 12 to 17. Developmental forms of the parasite in erythrocytes of rats.

Figs. 18 and 19. Micro- and macro-gametocytes in erythrocytes of rats.

Figs. 20 and 20*a*. Extra-cellular parasites in the blood of mouse.

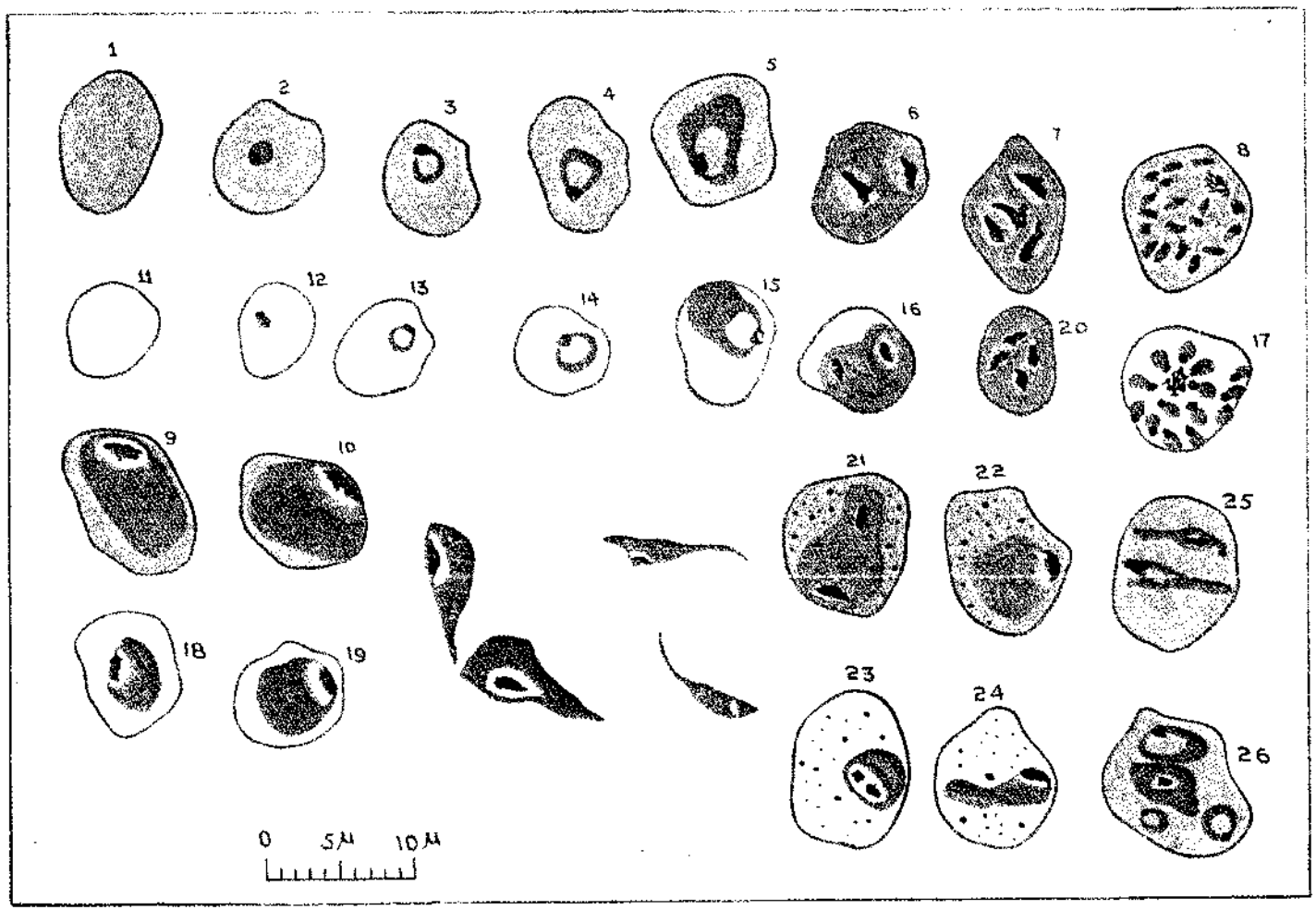
Figs. 21 and 22. 'Stippling' in infested polychromatophilic erythrocytes of a squirrel.

Figs. 23 and 24. 'Stippling' in infested erythrocytes of squirrel.

Fig. 25. Band form in polychromatophilic erythrocyte of squirrel.

Fig. 26. Multiple infection in a polychromatophilic erythrocyte of a white mouse.

Plate XIII



INSECT TRANSMISSION OF *P. BERGHEI*.

Vincke and Lips (*loc. cit.*) have reported failure to infect specimens of *A. gambiæ*, *A. coustani*, *A. maculipennis*, *A. funestus* and others with this parasite. In the course of the present studies, several hundred laboratory-bred *A. stephensi* (type) and *A. subpictus* were fed, chiefly on infected white mice, domestic and white rats, with gametocytes of varying densities in their blood. In one lot of *A. stephensi* (type) fed on an infected white mouse, five that survived 21 days, were dissected and one was found to have a few sporozoites in its salivary glands. The presence of sporozoites was confirmed by staining a part of the preparation, while the rest was aspirated into a syringe and injected intraperitoneally into a normal white mouse. No infection could be detected in the mouse during an observation period of 35 days. Further experiments on insect transmission of the parasite are in progress.

CHARACTERISTICS OF *P. BERGHEI*.

All phases of development of the asexual cycle and gametocytes of this parasite are seen in the peripheral blood and the pigment is either absent or not visible by the staining techniques used except in mature schizonts, when it is seen as a single small amber-coloured clump in the residual mass. It is also seen in monocytes both in the peripheral blood and organ smears. Further studies on pigment formation in these parasites are indicated.

The enlargement of the infested cell, preferential infestation of polychromatophilic cells, and the latency followed by relapses in some rats and squirrels, are similar to characteristics found in *P. vivax* infections of the human beings. The acute infections with high parasitæmia terminating fatally in mice may be compared to *P. falciparum* infections in man. The low pathogenicity, a larger number of "band" forms, and latency followed by relapses in squirrels may be comparable to *P. malariæ* infections of human beings.

SUMMARY.

The morphology of *P. berghei* has been studied and found to be essentially the same in five susceptible hosts, e.g. brown mice, white mice, brown rats, white rats and garden squirrels.

In all animals, the parasite preferentially invades polychromatophilic erythrocytes. In mice and some rats, the infection is acute and intense ending fatally, while in a few rats and squirrels it is more benign, and chronic with periods of latency and relapses.

The periodicity of the asexual cycle is 24 hours in all the hosts.

Insect transmission experiments revealed sporozoites in one *A. stephensi* (type). They were few in number and injection of some of them into a normal white mouse intraperitoneally did not produce infection during an observation period of 35 days.

REFERENCES.

MULLIGAN, H. W. (1935) *Arch. f. Protist.*, **84**, pp. 285-314.

RAMAKRISHNAN, S. P., and SATYA PRAKASH (1950). *Ind. J. Mal.*, **4**, pp. 361-367.

VINCKE, I., and LIPS, L. (1948) *Annals de la Societe Belge Medicine Tropicale*, **28**, p. 1.

Indian Journal of Malariology, 4, 3, September 1950.

THE IDENTITY OF THE MALARIA VECTOR, *A. LEUCOSPHYRUS*.

BY

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(September 2, 1950.)

INTRODUCTION.

A. leucosphyrus, a species widely distributed throughout the oriental region, has only comparatively recently been recognized as a malaria vector of any great importance. Earlier workers occasionally suspected it on epidemiological grounds and a few natural infections were reported, but since the published findings of Clark and Choudhury (1941) in Assam, McArthur (1947) in Borneo, and Kuitert and Hitchcock (1948) and Macan (1948) in Burma, it has become clear that this species is a dangerous, if not the principal, vector over quite large areas.

In all the above publications the vector is recorded simply as '*A. leucosphyrus*'. It is now known, however, that this name has been used in the past to include a number of different forms. The most recent review of the group (Reid, 1949) lists six forms and more recent work by Reid and the writer, as yet unpublished, makes it necessary to add a seventh. Of these, at least three have a wide distribution and are morphologically rather similar, which leaves a considerable element of doubt as to the exact identity of the incriminated vectors.

Reid (*loc. cit.*) reviewed the small amount of evidence available at the time and concluded that the vector was most probably the type form, under which designation he included *A. leucosphyrus balabacensis* Baisas. This latter form is now known to be a distinct sub-species or species and its importance as a vector has been amply demonstrated by investigations made in North Borneo during 1948-49. It is the purpose of this paper to set out the results of these investigations and to show that, for British Borneo at least, *A. leucosphyrus balabacensis* is almost certainly the only member of the group involved in malaria transmission.

Note.—Throughout this paper, the name in quotes refers to records which do not distinguish between members of the complex.

SYSTEMATIC.

The present knowledge of the *leucosphyrus* group shows it to consist of the following forms:—

1. *A. leucosphyrus leucosphyrus* Donitz, 1901.
2. *A. leucosphyrus balabacensis* Baisas, 1936.
3. *A. leucosphyrus elegans* James, 1903.
4. *A. leucosphyrus riparis* King and Baisas, 1936.
5. *A. leucosphyrus pujutensis* Colless, 1948.
6. *A. leucosphyrus hackeri* Edwards, 1921.
7. *A. cristatus* King and Baisas, 1936.

There are also other forms, notably the *hackeri*-like form of Celebes, whose exact status is not yet established.

The above list is substantially the same as that given by Reid (*loc. cit.*) although the nomenclature differs slightly since the writer prefers to recognize most of the forms as good species; *balabacensis* was included by Reid under the synonomy of the type form but, as already mentioned above, subsequent work has shown it to be a distinct form; it is here given as a sub-species since its actual status is still under investigation.

Of these forms, three have so far been found in North Borneo—*A. leucosphyrus balabacensis*, *A. pujutensis* and *A. hackeri*. The related *A. riparis* which occurs in Malaya and Mindanao as a rather common species is apparently absent from North Borneo, if not the entire island; there is no obvious reason for this surprising omission from the Borneo fauna. *A. leucosphyrus balabacensis* occurs as a common species, widely distributed throughout Borneo; *A. pujutensis* is less common though it appears to be rather widely distributed; *A. hackeri* is a very rare species of specialized breeding habits. The Borneo *balabacensis* appears to be identical with that originally described by Baisas (1936) and has been described under that name by Colless (1948).

A. LEUCOSPHYRUS BALABACENSIS AS A MALARIA VECTOR.

Over a period of several years, a large number of dissections have been carried out on Labuan Island, British North Borneo, including 1,840 specimens of *balabacensis*. The figures for 1948-49 are given in Table I and show a high natural infection rate in this form (1·6 per cent gland infections).

Material for dissection came from the Bukit Kuda, Lajau-Batu Manikar areas at the northern end of the island, comprising some five to six square miles of hilly country, mostly covered with secondary jungle or rubber, and with a high malaria endemicity; spleen rates in native children are of the order of 60 to 100 per cent. Almost all specimens were taken by hand trapping at night, the bulk being taken while attempting to feed on the collector. A very small number were also taken from outdoor resting places.

TABLE I.

Total dissections in 1948-49.

Species.	Guts examined.	Glands examined.	Total dissected.	Positives. Gut only.	Gland only.	Gut and gland.
A. leucosphyrus balabacensis	1,227	1,815	1,840	15	24	6
A. sundaicus	916	1,106	1,126	2	2	0
A. philippinensis ...	1,086	1,181	1,201	..	..	...
A. vagus	267	315	325	..	...	...
A. kochi	208	234	241	...	...	...
A. hyrcanus nigerrimus ...	90	122	127	...	...	...
A. barbirostris	58	65	66	..	...	...
A. barnari	58	66	66	..	..	..
A. separatus	17	18	18	.	..	...
A. [illegible]	13	14	14	.	..	..
A. hyrcanus C.	1	1	1	.	...	...
TOTAL ...	3,941	4,937	5,025	17	26	6

The above figures demonstrate the overwhelming importance of *balabacensis* in this area; it was the species most frequently taken and showed by far the highest sporozoite rate (1·6 per cent as compared with 0·2 per cent for *A. sundaicus* and nil for all other species). *A. pujutensis*, a rather rare species of Labuan, was never taken in adult catches.

The conclusion that *balabacensis* is the principal vector in the area is in complete agreement with all available epidemiological evidence, with the reservation that, in sea-side villages, *A. sundaicus* plays a part, the importance of which has not yet been fully determined.

SEASONAL VARIATIONS.

To illustrate the seasonal variations in malaria transmission by *balabacensis*, monthly figures for dissections of this species, and sporozoite rates, are given in Table II. These show that a variable degree of transmission may occur during most months of the year, with the possible exception of the drier months, January to March. There is no indication of any definite malaria season.

TABLE II.

Monthly dissections of A. leucosphyrus balabacensis.

Month.	1948.		1949.	
	Total dissections.	Sporozoite rate, per cent.	Total dissections.	Sporozoite rate, per cent.
Jan.	0	0	4	0
Feb.	6	0	0	0
Mar.	0	0	37	0
Apr.	0	0	102	2·0
May	19	0	123	1·6
Jun.	65	0	501	0·6
Jul.	166	5·4	152	1·3
Aug.	106	2·9	25	0
Sept.	157	3·3	63	0
Oct.	85	1·2	27	0
Nov.	55	3·6	70	0
Dec.	68	1·5	9	0
Total.	727	2·9	1,113	0·8

During the thirteen months shown in Table III, accurate trapping records were kept. The table shows the monthly average catch per man per night, the monthly sporozoite rates, and a 'transmission index' (T) derived as the product of the two former figures. This latter is designed to indicate variations in the

relative intensity of transmission, but for various reasons, probably does not give a very accurate estimate of the absolute intensity. However, multiplication by 30, as shown in the fourth column, gives some approximation to the average number of infections received per inhabitant of the area in each month.

TABLE III.

Monthly variation in malaria transmission.

Month.	Average catch man/night.	Sporozoite rate, per cent.	Transmission index (T).	T × 30.
1948				
Jul.	5·6	5·4	0·302	9·1
Aug.	2·3	2·9	0·067	2·0
Sep.	3·4	3·3	0·112	3·4
Oct.	1·9	1·2	0·023	0·7
Nov.	1·6	3·6	0·058	1·7
Dec.	1·8	1·5	0·027	0·8
1949				
Jan.	0·2	0·0	0·0	0·0
Feb.	0·0	0·0	0·0	0·0
Mar.	1·1	0·0	0·0	0·0
Apr.	3·3	2·0	0·066	2·0
May	3·1	1·6	0·049	1·5
Jun.	6·4	0·6	0·038	1·1
Jul.	5·6	1·3	0·073	2·2

It is clear from these figures that transmission is by no means steady but tends to fluctuate, probably in accordance with climatic factors. But during a large part of the year an inhabitant of the area may receive, if unprotected, something of the order of one to two infective bites per month.

PREVIOUS RECORDS.

The foregoing results settle the identity of the vector on Labuan Island. However, the earlier results of McArthur and others, from which the importance of *A. leucosphyrus* throughout Borneo has been deduced, offer no definite evidence as to the form concerned. There remains then the possibility that they refer, in

part or whole, to *A. pujutensis*. *A. hackeri* may be safely excluded by reason of its extreme rarity, and it seems most unlikely that any other form could be sufficiently abundant to be of importance and yet have escaped detection to date.

With regard to the results of McArthur (1947, and unpublished), who incriminated *A. leucosphyrus* in the Tambunan and Sandakan areas of North Borneo, there are two items of evidence :—

1. Specimens of *leucosphyrus* sent from Tambunan to the Institute for Medical Research, Kuala Lumpur, in 1941, included no *pujutensis* (Reid, *loc. cit.*).

2. Larval surveys by the writer in both the above places have shown widespread breeding of *balabacensis* and complete absence of *pujutensis*. The Tambunan survey was particularly intensive and it is impossible that any significant degree of breeding of the latter species could have been missed.

It seems then that there are no reasonable grounds for doubting that these records refer to *balabacensis*.

On several other occasions, *A. leucosphyrus* has come under suspicion in Borneo and it seems likely that in these cases also *balabacensis* was the form involved. But the evidence is so flimsy that there would appear to be little point in any detailed discussion of these records.

To sum up then, it may be stated that previous incriminations of *A. leucosphyrus* in North Borneo may be safely ascribed to *balabacensis* and that this form is most probably responsible for all *leucosphyrus*-borne malaria in the country.

RECORDS FROM OTHER COUNTRIES.

As stated in the introduction to this paper, *A. leucosphyrus* has been incriminated as a vector in Assam and Burma. The writer has seen Burmese specimens collected by T. Macan and other Burmese material kindly loaned by L. C. Knitert, and these were all *balabacensis*. This, together with the fact that no other member of the complex has been reported from those areas, points once more to *balabacensis* as the vector.

It is also of interest to note that over much of the Malay Peninsula, where *A. vagus* and *A. pujutensis* are the dominant members of the complex, *A. leucosphyrus* has for long been regarded as a harmless species and rarely, if ever, appears in adult catches. There is some reason then to doubt if these species are of any importance as vectors. There remains however the possibility that the type form, *leucosphyrus*, may be of some importance. Reid (*loc. cit.*) notes a probability that the type series of this form was captured indoors after biting, which may be of some significance, but there is at present no real evidence on this point.

SUMMARY.

1. Present published records of *A. leucosphyrus* as a vector of malaria do not distinguish between the various closely related members of the *leucosphyrus* complex.

2. Recent records from Borneo establish the presence there of three members of the complex—*A. leucosphyrus balabacensis*, *A. pujutensis* and *A. hackeri*.

3. Dissections from Labuan Island show conclusively that *balabacensis* is a dangerous vector there and it seems certain that the bulk of previous positive dissections from Borneo refer to this form.

4. Other evidence of *A. leucosphyrus* as a vector in Borneo and other countries is consistent with the view that *balabacensis* is the form referred to.

ACKNOWLEDGMENTS.

The writer wishes to acknowledge his indebtedness to his assistants, Sukarman bin A. Nasran and Sidop bin Suleiman, who carried out most of the collecting and much of the dissection upon which this paper is based; also to Mr. J. Reid of the Institute for Medical Research, Kuala Lumpur, for much valuable information on the systematics of the *leucosphyrus* complex; also to Dr. J. McArthur for helpful advice and criticism during the course of the work.

REFERENCES.

Baisas, F. E. (1936) . Notes on Philippine mosquitoes. IV. The pupal and certain adult characters of some rare species of Anopheles. *Phil. J. Sc.*, **59**, pp. 65–84.

Clark, R. H. P., and Choudhury, M. A. (1941). Observations on *A. leucosphyrus* in the Digboi Area, Upper Assam. *J. Mal. Inst. Ind.*, **4**, pp. 103–107.

Colless, D. H. (1948) . The anopheline mosquitoes of North-west Borneo. *Proc. Linn. Soc. N.S.W.*, **73**, p. 101.

Kuttert, L. C., and Hitchcock, J. D. (1948). Observations on *Anopheles leucosphyrus* at Shingbwiyang, Burma. *Proc. Ent. Soc. Wash.*, **50**, pp. 77–82.

Macan, T. T. (1948) ... Mosquitoes and malaria in the Kabaw and Kale Valleys, Burma. *Bull. Ent. Res.*, **39**, pt. 2, pp. 237–268.

McArthur, J. (1947) . The transmission of malaria in Borneo. *Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, pp. 537–558.

Reid, J. A. (1949) A preliminary account of the forms of *A. leucosphyrus* Dönitz. *Proc. Roy. Ent. Soc. Lond.*, **18**, pts. 3-4, pp. 42–53.

Indian Journal of Malariology, **4**, 3, September 1950.

ABSTRACT.

MALARIA CONTROL IN ASSAM.*

BY

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May 14, 1949.]

IN 1946, D.D.T. indoor residual spraying was started in the highly malarious areas in Assam where *A. minimus* is the chief carrier species. All human dwellings and cattlesheds were sprayed with 2·5 per cent D.D.T. suspension at the rate of 50 mg. D.D.T. per sq. ft. Epidemiological data obtained before and after the control measures is given in Tables I and II. Since the introduction of D.D.T. spraying in 1946, not only the incidence of malaria has gone down by 50 per cent but the incidence of other diseases has also shown general reduction.

The statement made by Bartram (1950) that indoor residual D.D.T. spraying is ineffective in the control of malaria in Assam, does not seem to be corroborated by the epidemiological evidence obtained during the last three years of D.D.T. spraying in Assam.

REFERENCE.

BARTRAM, D. M. (1950) Malaria control by residual insecticides. *Brit. Med. J.*, May 20, 1950, p. 1200.

* Copy of the original manuscript (9 pages with tables and 1 map) has been placed in the Library of the Malaria Institute of India, and is available on loan to workers who wish to consult it.—(*Editor.*)

TABLE 1.

Epidemiological data before and after D.D.T. indoor residual spraying.

	Before the control measures were started in 1946.			After the control measures were started			
				At the end of 1947.		At the end of 1948.	
	Spleen rate, per cent.	Parasite index, per cent.	Infant parasite index, per cent.	Spleen rate, per cent.	Infant parasite index, per cent.	Spleen rate, per cent.	Infant parasite index, per cent.
Nowgong	50·8	16·7	Not done	0·5	0·0	Not done	Not done.
Gauhati	21·6	14·8	,,	2·7	2·7	,,	,,
Mangaldai	31·5	25·7	,,	2·8	0·0	2·2	,,
North Lakhimpur	31·6	46·4	,,	21·5	20·0	8·0	,,
Haltugaon	90·0	Not done	,,	60·0	20·0	8·0	,,
Kachugaon	85·5	,,	,,	51·3	16·6	8·0	,,
Nongpoh Propa	90·6	,,	,,	34·8	16·0	8·0	,,
Lokra Charduar	15·6	,,	,,	17·0	11·1	10·0	,, (Charduar).
				9·7	3·4	2·8	,, (Lokra).
Pasighat	32·5	35·5	,,	6·0	6·5	3·7	0·0
Haflong	37·5	Not done	,,	29·3	Not done	Not done	Not done.
Bangla	80·0	,,	,,	47·3	50·0	53·5	,,
Shillong	8·7	8·69	,,	3·2	0·0	Not done	,,
Rangapahar	45·2	27·7	,,	40·47	0·0	12·6	,,
Sadiya	25·1	20·5	,,	3·4	5·5	1·5	3·0
Sukhowaghat	47·8	41·5	,,	35·5	14·2	18·02	2·6
Jorhat	9·0	Not done	,,	2·3	0·0	0·50	Not done.
Tezpur	5·1	0·9	,,	3·2	Not done	Not done	,,
Hossaingaon	57·9	35·2	26·3	51·1	61·5	,,	,,
Loshimpur	26·5	34·2	Not done	Not done	Not done	,,	,,
Tura	40·8	Not done	,,	,,	,,	25·2	,,
Kaziranga	34·4	19·1	25·7	27·2	16·3	24·2	,,

TABLE I—*concld.*

	Before the control measures were started in 1946.			After the control measures were started. At the end of 1947.		At the end of 1948.	
	Spleen rate, per cent.	Parasite index, per cent.	Infant parasite index, per cent.	Spleen rate, per cent.	Infant parasite index, per cent.	Spleen rate, per cent.	Infant parasite index, per cent.
Borlong Cinchona Plantation.	45·4	27·2	Not done	8·3	Not done	Not done	Not done.
Balek Group of Villages	33·6	Not done	..	8·6	30·5	,,	,,
Raimona ..	71·5	34·4	33·3	74·0	50·0	,,	,,
Ranikhera .	98·8	77·8	66·6	Not done	Not done	,,	,,
Halem T. E. ..	64·4	50·3	50·0	17·7	20·7	3·12	,,
Bokakhat ...	16·3	11·9	12·0	...	..	13·2	,,
Boko	74·1	66·6	..	..	.	Not done	,,
Nazira . .	32·7	15·5	13·0	.	.	13·2	,,
Nitaipukhri .	55·9	36·5	33·3	..	.	13·2	,,

TABLE II

Effect of D.D.T. indoor residual spraying in reducing the incidence of malaria and other diseases.

	Rate per thousand, before control.			Rate per thousand, after control.		
	Malaria.	Other diseases.	Kala-azar.	Malaria.	Other diseases.	Kala-azar.
Borlong Cinchona Plantation.	46·5	41·5	*Nil*	16·5	30·4	*Nil.*
Boko Group of Villages ...	230·0	374·0	5·8	163·0	309·0	3·8
Nazira	184·6	416·0	20·6	60·1	324·0	0·5
Bokakhat	163·0	260·0	*Nil*	90·6	176·3	*Nil.*
Saikhowaghat ...	206·5	620·0	*Nil*	47·0	429·0	*Nil.*
Nitaipukhri ...	140·0	354·0	10·0	20·6	92·0	*Nil.*
Rangapahar ...	93·3	275·0	*Nil*	72·1	258·3	*Nil.*
Nongpoh Proper ...	6·6	294·0	*Nil*	3·0	189·0	*Nil.*

REVIEWS.

MALARIA AND ITS CONTROL IN BOMBAY STATE.

Malaria and its Control in Bombay State. Edited and published by D. K. Viswanathan, Assistant Director of Public Health (Malariology), Bombay State, Poona-1. Price Rs. 10/-.

The book is based on the personal and intimate experience of the author in all phases of the subject dealt with in the book, which has been lucidly written. Malaria control in Bombay, which has grown very rapidly since 1942, has been dealt with in two phases, namely the activities before the advent of synthetic insecticides and thereafter.

The results achieved by the utilization of application of researches in dealing with the local problems of malaria control have been noteworthy. The bionomics of the vector species *A. fluviatilis* and its intimate relationship to the control operations forms an interesting section.

The gradual evolution of malaria control, with comparatively small beginnings and its steady growth with aspiration to extend it to the whole State, almost nearing realization, should be an example to other States in the country. The important aspects of malaria control like evaluation of results, cost, organization and administration have been dealt with due emphasis.

The difference in the methods of control between urban and rural malaria is discussed. The results in incidental prevention of plague and other insect-borne diseases are noteworthy, and are of great interest in the use of synthetic insecticides as a general public health measure.

The book is useful to workers employed differently as in teaching, field research and control.

JASWANT SINGH.

MALAWI RESEARCH CENTRE
LIBRARY
No.....
Date.....

Indian Journal of Malariology, **4**, 4, December 1950.

THE IMPORTANCE OF BORNEO ANOPHELES.

A STUDY OF THE RELATIVE IMPORTANCE OF THE DIFFERENT SPECIES OF BORNEO ANOPHELES, FROM RECORDS OF 24,000 DISSECTIONS AND OTHER EVIDENCE, BY OBSERVERS THROUGHOUT NORTH BORNEO AND LABUAN, SARAWAK, BRUNEI AND DUTCH BORNEO, 1914-49.

BY

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[September 1, 1950.]

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INTRODUCTION.

THE INCRIMINATION OF VECTORS.

The importance of a species of mosquito as a vector of malaria depends entirely on the frequency of the infective bites which it inflicts on the population.

This in turn depends on three factors: on the numbers of its adults feeding, on its 'anthropophilic index' representing its relative preference for human blood, and on its 'sporozoite rate', or the proportion which is infective to man.

In assessing the importance of a species as a vector of malaria in a locality, it is desirable to take into consideration its reputation as a vector elsewhere, its distribution and numbers in the locality, the indirect evidence of the epidemiology of malaria, its blood preferences, and finally the results of dissections.

The reputation of a mosquito in other countries is suggestive, but it may be misleading, for a species which is dangerous in one country may be harmless in another. Such evidence must be considered, but by itself cannot be taken as a guide.

The numbers of a species breeding in a locality may afford some information regarding its importance, but only in relation to other evidence for its incrimination: for a common mosquito may be harmless, and a dangerous vector may be apparently rare.

The circumstantial evidence of epidemiology may be of value in assessing the importance of a mosquito as a vector, but it has its dangers. The indirect evidence may be apparently complete, and yet—as was the case in Tambunan—it may be absolutely misleading.

The evidence of blood preferences also varies enormously according not only to the appetite of a mosquito for human blood, but according to the relative availability of animals and man, and to the method adopted for the capture of material. For this reason the anthropophilic index estimated for example from precipitin tests can only be of real value when all the circumstances are known.

The discovery of oöcysts on dissection is extremely strong evidence for a vector, for it shows that a species becomes infected, and therefore presumably can become infective: but it cannot be conclusive evidence, for it does not prove that the species actually becomes infective to man, and on the whole it leads to confusion.

The only valid evidence that a species is concerned with transmission lies in the actual finding of sporozoites in a proportion of gland dissections. This yields the direct evidence necessary for the incrimination of a vector, and is therefore the most important activity that can be carried out in assessing the importance of species.

Significant though the results of dissection are, however, even these must be considered in relation to the other evidence, particularly of the frequency with which an infective species is likely to feed on man, for a frequent biter with a low sporozoite rate may be quite as dangerous as one with a high sporozoite rate which inflicts fewer bites on the community.

In general, the most important information regarding a vector is that provided by the sporozoite rate: but even this must be subject to some interpretation by other evidence, particularly the numbers in which it actually feeds on man.

THE RECORDED DISSECTIONS OF BORNEO ANOPHELES.

It has already been stated (McArthur, 1950*b*) that 9 species or groups of Anopheles have been recorded as infected on dissection in Borneo during the first half of the twentieth century. In the present paper, the importance of each of these species or groups is considered in relation to the malaria of Borneo as a whole.

In the previous analysis, the following positive dissections were quoted, out of a total of 24,000 dissections :—

Species.	*Infected, per cent.*	*Sporozoite rate, per cent.*
A. leucosphyrus	2·8	1·5
A. umbrosus group	1·7	0·3
A. sundaicus	0·6	0·3
A. baezai	1·0	0·3
A. separatus	0·5	...
A. hyrcanus group	0·5	...
A. barbirostris	0·3	...
A. tessellatus	0·1	...
A. minimus var. *flavirostris* .	0·07	...
A. philippinensis	...	..
A. kochi	..	...
A. karwari	...	..
A. maculatus	...	..

This summary, illustrated in Chart 1, yields a fairly accurate picture of the transmission of Borneo malaria in so far as it is at present known, and the actual or suspected vectors are dealt with individually in the following studies of their importance.

I. THE IMPORTANCE OF *ANOPHELES LEUCOSPHYRUS* IN BORNEO.

A. leucosphyrus outside Borneo.

A. leucosphyrus is widely distributed across Asia from India to Celebes, particularly in the hilly jungle-covered areas and prefers shaded breeding places. Actually it is a group of mosquitoes (Reid, 1949), but the distinction between members of the group has not yet been widely applied.

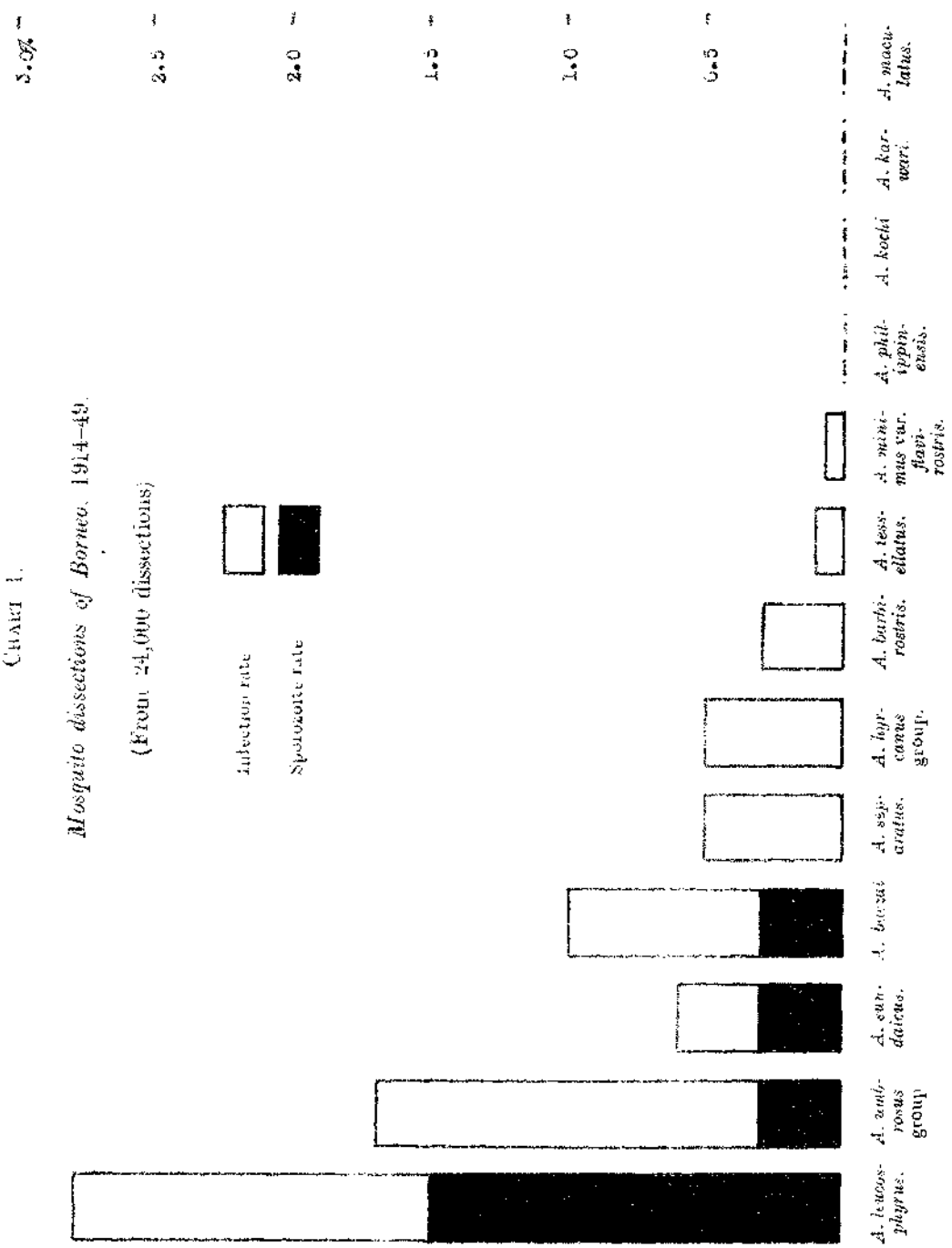
A. leucosphyrus for long was not considered of any importance in other countries, although it had been found infected. Gater (1935) in Singapore, for example, mentioning that it had been infected experimentally with benign tertian malaria, and that it had been observed naturally infected in the Dutch East Indies, stated that it is not considered, however, to be of much importance as a vector, and Christophers (1933) stated that it is not thought to play any part in malaria transmission in India.

Generally it is regarded as harmless. In Malaya, in fact, when it has been made to replace *A. maculatus*, which it does with a change of conditions, it has been

CHART 1.

Mosquito dissections of Borneo, 1914–49.

(From 24,000 dissections)



deemed to be an indication of successful malaria control, so that it has actually been encouraged than otherwise. 'Occasionally a non-vector species such as *A. leucosphyrus* invades such wells, and this alteration of the fauna is welcome as an indication of the success of the method' (Hackett *et al.*, 1938).

The fact that *A. leucosphyrus* appears to be harmless in Malaya and elsewhere, however, has given it a reputation for its innocence in other places which is perhaps not justified. It has, in fact, been shown on dissection to be a vector in several parts of the East, from Burma to Celebes, and possibly a more important vector than has hitherto been realized.

The evidence accumulated from a number of independent sources supports the belief that in spite of its elusive habits *A. leucosphyrus* is the most dangerous mosquito in Borneo.

DISTRIBUTION IN BORNEO.

A. leucosphyrus has been the most widely recognized of mosquitoes in Borneo, having been identified by practically every observer, and found in every quarter of the island. It occurs in 34 out of 42 records of identifications—more frequently than any other species—in spite of its elusiveness.

The localities indicated in Appendix Ia illustrate its wide distribution in Borneo.

A. leucosphyrus is sometimes a difficult mosquito to find, both in the larval and adult stages, on account of its peculiar breeding and feeding habits, and it is very easily overlooked, as it has been by some observers in situations where it is widespread. In spite of this, it has been recorded from every situation so far studied.

Thus, it would appear to be the most widely distributed Anopheles in Borneo, being found in every part of the island, probably wherever there is jungle shade.

EPIDEMIOLOGICAL EVIDENCE.

Long before the incrimination of *A. leucosphyrus* by dissection as a vector of malaria in Borneo, suspicion was cast upon this insect by the indirect evidence of its feeding habits.

Roper (1914) referred to *A. leucosphyrus* as being found in large numbers in coolie lines in Membakut, North Borneo, associated with many cases of malaria. Several of the species were dissected without positive result, but it was found impossible to maintain them alive for the requisite time for the digestion of a blood meal. The possibility of this insect being a vector was discussed, and Roper (*loc. cit.*) stated that 'Although from the facts recorded no definite conclusion can be drawn as to the malaria-carrying powers of this insect, I think the evidence is suggestive'.

Thirteen years later, *A. leucosphyrus* was suspected by Stookes (1927) in Miri, Sarawak. As in the case of Roper (*loc. cit.*), it was found impossible to maintain specimens alive, and no dissections were carried out; but the association of this species with a number of cases of malaria, in the virtual absence of other species, threw suspicion upon it as an efficient vector.

In the same year, *A. leucosphyrus* was suspected as a vector in Dutch Borneo, as recorded by Walch and Soesilo (1929) in Tandjong Djoeta; and later, in Boeloengan, it was noted that where malaria was severe, *A. leucosphyrus* was found, but where malaria was slight, *A. leucosphyrus* was not found.

Also in Dutch Borneo, von Kuehlewein (1929) was recorded (Goelarso, 1934) as having suggested, with all reserve, that the carriers in the region of the Upper Mahakam River, in the very centre of the island, were either *A. leucosphyrus* or *A. umbrosus*, or both.

Hutchinson (1930), Assistant Health Officer in Sarawak, made larval surveys in the vicinity of Kuching; and at Quop, ten miles from Kuching, it was thought, on indirect evidence, that *A. leucosphyrus* and *A. maculatus* were vectors, with *A. umbrosus* to a lesser degree. This was based upon the frequency in the houses of *A. leucosphyrus*, on the evil Malayan reputation of *A. maculatus*, and on the earlier suspicion thrown on *A. umbrosus* in Sarawak. In the leper camp again, the same three mosquitoes were referred to as under suspicion.

Later, Goelarso (*loc. cit.*), on the evidence of his observations in Boeloengan, and of those of Professor Walch (1932) before him in the same district, suspected *A. leucosphyrus* to be a vector some time before he was able to incriminate it by dissection in this area, although suspicion was also extended, on the evidence, to *A. maculatus*.

In Tambunan (McArthur, 1946), before the incrimination of *A. leucosphyrus* by dissection, suspicions were cast upon this insect by the indirect evidence of the peculiar distribution of the spleen rates, and of the preference of this species for human blood, in contrast to the preference of other species present for animal blood. It was found that the healthiest areas were those far from the hills, in the middle of the paddy fields; whereas the most malarious areas were the uncleared jungle ravines, where the mosquitoes breeding were *A. leucosphyrus* and *A. aitkeni*. Cleared ravines breeding *A. maculatus* to replace *A. leucosphyrus* were healthy.

Since that time, *A. leucosphyrus* has been further suspected on the east coast by Carlile (1941), who found it by far the most common species in close association with man in the areas examined, and evidence from Lahad Datu and Tawau (McArthur, 1947*c*) throws further suspicion on it in that area.

Thus, in spite of its benign reputation, this insect gave rise to suspicion on indirect evidence in several places in Borneo long before its incrimination by actual dissection, and the further evidence suggests that it will continue to be so incriminated in other areas not so far adequately studied.

BLOOD PREFERENCES.

The preference of a species of Anopheles for human or animal blood has an important influence upon its character as a vector of malaria. A species which has little appetite for human blood will have little opportunity of passing on an infection, especially considering the necessity for two blood meals to accomplish this, unless it exists in enormous numbers. On the other hand, a species which has an avidity for human blood, and which rarely feeds on animals, is likely to have many opportunities of transmitting infection, and therefore to be a dangerous vector even in small numbers.

It has been recorded on the results of precipitin tests by Walch (*loc. cit.*) that *A. leucosphyrus* feeds readily on human blood in the Netherlands Indies; and again, Ramsay, Chandra and Lamprell (1936) found *A. leucosphyrus* to have a preference for human blood in Assam and North Bengal, although no infections were found.

In Tambunan, *A. leucosphyrus* was found to have by far the greatest preference for human blood of all the local species, having an anthropophilic index of about 90 per cent, judging by the results of human and animal bait trapping. All other local species were found to have a marked preference for animal blood (McArthur, 1948).

By precipitin tests, 28 specimens of *A. leucosphyrus* taken in houses in Tambunan all contained human blood, compared with 34 specimens of other species taken in jungle, which all contained cattle blood. It was unfortunate that *A. leucosphyrus* was not taken also in jungle in order to provide a really valid comparison with the other species taken.

Thus, evidence is strong that *A. leucosphyrus* is an avid human blood feeder in Borneo, and likely to be a dangerous vector there.

INCRIMINATION BY DISSECTION IN BORNEO.

The recorded dissections of *A. leucosphyrus* to the end of 1949 have been tabulated in Appendix I*b*. In this it is shewn that during the 36 years from 1914, over 3,000 *A. leucosphyrus* have been dissected throughout Borneo. Of these, 90 have been recorded as infected, and 49 have been recorded with sporozoites.

A. leucosphyrus has been found positive on dissection by eight observers, in ten different localities throughout Borneo.

Thus, *A. leucosphyrus* has been fairly consistently incriminated as a vector of malaria in Borneo wherever adequate numbers have been dissected, with a natural infection index averaging about 2·3 per cent, rising in one series as high as 20 per cent, and with a sporozoite rate averaging 1·5 per cent.

FORMS OF *A. LEUCOSPHYRUS*.

A. leucosphyrus appears to consist not of one, but of at least six or seven forms, three of which have so far been recognized in Borneo. These have been summarized by Reid (*loc. cit.*) as follows:—

A. leucosphyrus Donitz, 1901.
A. leucosphyrus var. *elegans* James, 1903.
A. leucosphyrus var. *hackeri* Edwards, 1921.
A. leucosphyrus var. *riparis* King and Baisas, 1936.
A. leucosphyrus var. *pujutensis* Colless, 1948.
A. cristatus King and Baisas, 1936.

It seems likely that the only one of these which is of importance as a vector of malaria is the type form, with the probable exception of the Celebes var. *hackeri*.

Both the type form and var. *pujutensis* occur in Borneo, and var. *hackeri* has been recorded there, although as yet unconfirmed except in Poeloe Laoet.

The different forms are dealt with briefly as follows:—

A. leucosphyrus Donitz.—This is almost certainly the form which has been incriminated as a vector of malaria in Sumatra and Burma. It is the proved vector in Labuan, North Borneo, is shown (Colless, unpublished) to be almost certainly the form incriminated in Tambunan, and is likely to be the form which is the chief vector in Borneo.

This mosquito is distributed widely across Asia, having been identified from Bengal, Assam, Burma, the Andamans, Indo-China, Malaya, Sumatra, the Philippines and Borneo. *A. leucosphyrus* var. *balabacensis* is probably identical with it.

A. leucosphyrus var. *pujutensis*.—This form was first described from Pujut in Northern Sarawak, and it has been recorded from Brunei, North Borneo, Malaya, Dutch Borneo and Sumatra. It is probable that it has no relation to malaria transmission, and its importance may lie in its being confused with the vector.

A. leucosphyrus var. *hackeri*.—This form has been recorded from Malaya, Sumatra, Borneo and Celebes. In Borneo it appears to be rare. It has been reported with a high infection rate (30 per cent) in Celebes (Overbeck, 1941*c*), although this appears to be a distinct form.

A. leucosphyrus var. *elegans*.—This form, found in India and Ceylon, was described as *A. elegans* in 1903, but the name was abandoned when it was thought that the same mosquito has already been described under the name *A. leucosphyrus*. Reid (*loc. cit.*) however regards it as probably a distinct form, in which case the name should be revived.

A. leucosphyrus var. *riparis*, and *A. cristatus*.—These forms from the Philippines, the former found in Malaya as well, have not so far been seen in Borneo. They are probably of no importance. Var. *riparis* is one of the most common forms in Malaya, and is apparently of no importance as a vector.

Thus, although there are a number of forms of *A. leucosphyrus*, the type form appears to be the common one in Borneo, and to be that responsible for the bulk of the malaria transmission.

DISCUSSION.

A study of the records shows that *A. leucosphyrus* had been suspected on epidemiological evidence as a vector of malaria in a number of places in Borneo before it was incriminated there by actual dissection; and further evidence suggests that it will continue to be so incriminated in other areas so far not adequately studied.

A. leucosphyrus has been incriminated in Borneo in actual dissection in ten places. It has shown the highest natural infection index of any mosquito so far studied there, and has been consistently found infected whenever adequate numbers have been so studied throughout the island.

The places where this mosquito has been recorded as either suspected or incriminated as a vector, are distributed in the north, east, south, west and very centre of Borneo, so that it would appear to be a vector throughout its range.

It is concluded from the evidence collected from all over the island that *A. leucosphyrus* is the most widely distributed mosquito, the most frequently

suspected on epidemiological evidence (in spite of its elusiveness), the most avid human feeder, the most frequently incriminated by actual dissection, the most highly and the most consistently infected, and therefore the most dangerous mosquito in Borneo.

II. THE IMPORTANCE OF *ANOPHELES MACULATUS* IN BORNEO.

A. MACULATUS OUTSIDE BORNEO.

A. maculatus is a mosquito which breeds in open sunlight, generally in springs or seepages in cleared ravines associated with hilly or mountainous country. It is distributed widely across Asia, from India to Celebes, and from Java to Hong Kong.

A. maculatus is the most important vector of malaria in the interior of Malaya. For this conclusion there is the very adequate evidence of its association with both epidemic and endemic malaria, and of positive dissections.

A. MACULATUS IN BORNEO.

In Borneo, *A. maculatus* has for long been believed to be the chief vector of malaria, and there is a considerable published literature in support of this. Shircore (1937), for example, states that '*A. maculatus*, a notorious carrier in Malaya, is widely and typically distributed through the areas visited, and must be regarded as the chief vector', what is claimed to be epidemiological evidence has often been quoted, and a good deal of money has been spent for its control.

A careful study of the records, however, suggests that this belief is based almost entirely on the reputation of *A. maculatus* in Malaya. This is by no means sufficient evidence for its incrimination in Borneo, and it has often been considered dangerous on inadequate and in some cases false grounds.

A. maculatus has been accused of being a vector in Borneo for a variety of reasons: for example, because it is a vector in Malaya; because it has been found in numbers in certain localities; because it has been found near endemic malaria; because it was the only species found in the presence of epidemic malaria; because it was not found in certain healthy areas; because malaria was said to have increased after the clearance of jungle; and because it was claimed to have been found infected—in two places—on dissection by previous medical officers.

All these, if true, may be evidence; but with the exception of positive dissections, they cannot be accepted as proof; and it is of considerable importance in Borneo to determine whether the evidence is valid, and whether this mosquito is in fact the dangerous insect it has been assumed to be.

A study of the literature suggests that none of the claims made for its incrimination are valid, while studies of the insect itself, epidemiological evidence, blood preferences, and the results of dissections, lead to the conclusion that *A. maculatus* is certainly of no importance, and probably not a vector at all in Borneo.

The following is a survey of the facts available concerning the importance of *A. maculatus* in Borneo, dealing firstly with its distribution, next with evidence

that has been brought forward for its incrimination as a vector, and finally with that for its exoneration from blame.

THE DISTRIBUTION OF *A. MACULATUS* IN BORNEO.

A. maculatus was included in the first list of seven Borneo Anopheles published by Hewitt (1905) of the Sarawak museum, but later it was mentioned by Moulton (1914), of the same museum, as not having been by that time found in Sarawak itself, although present in Borneo. It was first recorded in a specific survey, by Roper (*loc. cit.*), in Membakut, North Borneo.

A. maculatus was not recorded by Swellengrebel (1921), when quoting the surveys of observers in Balikpapan Tandjong Redeb, and Moearatewe, in Dutch Borneo; neither was it recorded by Rodenwaldt (1926), in Dutch Borneo. It does not appear to have been recorded by the several writers from Dutch Borneo until the lists compiled by Swellengrebel and Rodenwaldt (1932), when it was mentioned from Ober Mahakam.

In Dutch Borneo this mosquito was later recorded by Stoker (1934) in Sarang Tioeng, Poeloe Laoet; by Goelarso (*loc. cit.*) from the Kajan and Bahau-Poedjoengan Rivers, Boeloengan; and by Kariadi (1938) in Martapoera, South-East Borneo. Records in the Eykman Institute (1938) indicate its further identification in Longnawan, Kota Baroe, and Sanking in West Borneo; and since the war it has been recorded by Heilbrunn (1948) from Mempawah, Singkawang, Ngabang, and Sanggau, all in West Borneo.

This mosquito was not found by Stookes (1927) in Miri, Sarawak, nor by Webster (1941*a*) either in Miri or other of the Oilfields Territories in Sarawak, although found by him in Jerudong, Brunei; nor has it been recorded since by the Oilfields Staff in any of their territories.

A. maculatus was stated to be absent from Kuching and certain situations in Sarawak (1930), although present in others, for example, Quop, the Leper Camp, Satang Island 'and a fair number of rural districts'. It was recorded (Sarawak, *loc. cit.*) from Bau in 1931, the Agricultural Gardens in 1932, Kg. Skrak in 1933, and Lawas in 1934.

A. maculatus was not found in Brunei until 1938, after ten years of survey, and then only in insignificant numbers in one place.

It was not found in Labuan by Scharff (1927), nor by Ou (1941), who recorded it from the neighbouring island of Pulau Da'at. It was found in Labuan for the first time by Allman (1945), and only rarely since by Borneo Malaria Research Staff.

While *A. maculatus* has not been found very frequently elsewhere in the island, it has, however, been found widely and consistently in North Borneo since the first record by Roper (*loc. cit.*), on the east coast, and particularly on the west coast and interior. It was recorded for the first time in Jesselton by Dingle (1929); in the Tenom, Keningau and Tambunan Districts by Shircore (*loc. cit.*); again in Jesselton, Tambunan and Purutan by the Malaria Research Department (McArthur, 1947*a*), and on the east coast by Carlile (*loc. cit.*).

Thus, this species would appear to be patchy in its distribution, being chiefly concentrated in North Borneo, which is the more mountainous part, but found in parts of Sarawak, Brunei and Dutch Borneo.

I. EVIDENCE FOR SUSPECTING THAT *A. MACULATUS* IS A VECTOR IN BORNEO.

A study of the evidence which has been advanced in favour of the belief that *A. maculatus* is a vector provides the following material from different parts of the island:

Evidence from Brunei.—The first reference to *A. maculatus* as a possible vector in Borneo appears to have been made in Brunei by Hoops (1921), late Director of Medical Services, Singapore. He, with his Malayan experience, rightly made strong recommendations for the preservation of jungle shade in the meantime in order to prevent any possible malaria due to *A. maculatus* breeding, until 'a careful health survey should first be made by a competent Health Officer'.

It must be noted that Hoops made no claim that *A. maculatus* was in fact a vector, but only warned against this possibility. Thus, at first, caution was properly observed, and the preservation of the *status quo* insisted upon lest false steps be taken, but only as a preliminary to the further study of the situation.

Later Hoops' recommendation was quoted (Brunei, 1929), and interpreted as meaning that *A. maculatus* had actually been incriminated, for the preservation of shade was continued and advocated. As further proof of the dangerous character of this insect it was claimed that:

> 'a similar condition prevails in Miri, the oilfields of Sarawak, where malaria was unknown prior to the clearing of the hills and ravines. Each year since, as the hills, including ravines and watercourses, have been cleared, malaria of malignant type has become commoner'.

It has not been possible to find any grounds for regarding this claim as valid. On the contrary Webster (1941*d*), on enquiry, replied as follows:

> 'Regarding the clearing of the hills and ravines in Miri as reported in the Brunei report of 1929, there is no foundation for the statement that it was followed by malaria. The reverse was the case. A very extensive clearing of the hills in the Miri area was done without reference to our department in 1929, and at that time we feared ill consequences, but nothing eventuated. In 1929, there was no malaria in the Miri area and none except imported and sporadic cases since'.

Actually *A. maculatus* was not found in Brunei until ten years later, when a very few were found breeding in a situation considered to be quite unimportant (Brunei, 1938); nor is it found in Miri to this day (Webster, 1941*a*; Johnson, 1949*a*).

Thus, although there is still the necessity for caution and for further study, there is no ground for accepting *A. maculatus* as of any importance in Brunei.

Evidence from Labuan.—In Labuan the position has been similar. A mosquito and sanitary survey of Labuan was carried out by Scharff (*loc. cit.*). *A. maculatus*

was not found breeding on the island, and the importance of keeping an open mind was emphasized. The variation in the importance of *A. maculatus* in different places was referred to, and the necessity was pointed out of determining the species of mosquitoes carrying malaria in any locality before carrying out antimalarial measures. It was mentioned that money spent on destroying the supposed breeding places of *A. maculatus* in Labuan upon the Malayan plan would have been wasted.

As a result of the recommendations made by Scharff (*loc. cit.*), however, it would appear to have been gradually assumed, without further observations, that *A. maculatus* was, in fact, a vector in the locality; and sluicing, shading, subsoiling, and other measures directed solely against *A. maculatus* were undertaken in Labuan, although this species had not as yet been recorded as found there (Ou, *loc. cit.*). Anti-*maculatus* sluices can be seen standing idle there to this day.

Again, the view was current in Labuan in 1940 that *A. maculatus* was actually incriminated as a vector of malaria by a previous medical officer, who sent specimens to Singapore for dissection. On enquiry, this was found to be without foundation. Specimens of mosquitoes had been sent to Singapore for identification, but no dissections had been carried out (Gillespie, 1940).

A. maculatus does not appear to have been found in Labuan—although found in the neighbouring island of Pulau Da'at—(Ou, *loc. cit.*) until one or two larvæ were recorded at Victoria by the Australian Forces in 1945 (Allman, *loc. cit.*).

Thus, in Labuan also, in spite of accepted beliefs, there is no evidence for the incrimination of *A. maculatus* as a vector of malaria; and its extreme rarity makes it hardly likely that it could be of any importance there, even if it were a proved vector in Borneo.

Evidence from Sarawak.—In Sarawak, *A. maculatus* has often been claimed to be a vector of malaria. Stookes (1927), for example, stated the current view that '*A. maculatus* has, I believe, been responsible for much malaria at Satang Island, about 200 miles south of Miri'.

Health reports reflect the steady growth of this belief. Hutchinson (1930), while suspecting other mosquitoes in Sarawak, stated, 'I think it probable that both *maculatus* and *leucosphyrus* are vectors at Quop'; while in the following year it was stated (Sarawak, 1931) that, in Bau, 'Malaria is endemic in the district and undoubtedly the vectors responsible for the recurrence of the infection are *A. maculatus* and *A. umbrosus*'. *A. leucosphyrus* was recorded as present.

Later, also in the Bau District, it was stated (Sarawak, 1932) that 'Malaria is endemic in this locality and undoubtedly the vector of this disease is *A. maculatus*'; and, referring to the Agricultural Gardens: '*A. maculatus*, the most dangerous vector of malaria, was found in numerous streamlets in this locality, and as a means of preventing malaria, shading the streams with thick shrubberies was recommended, so as to drive the *A. maculatus* away'.

Works directed against *A. maculatus* in Sarawak appear to have been quite extensive, and within a few years, besides larval control measures, they included condemning a Mission Station (Sarawak, 1933), the removal of the Wireless Station (Sarawak, 1934), and the abandonment of a leper colony newly built at a cost of

over fifty thousand dollars (Sarawak, 1930), on the grounds of the presence of *A. maculatus.*

Of the leper colony, for example, while its abandonment was due also to epidemic malaria and to the difficulty of access during bad weather, it was stated that 'Had there been a Health Department at the time, this expenditure might have been saved, for we have found the most virulent carrier of Malaysia —*Anopheles maculatus*—to be breeding there in numbers'. The Mission Station was advised to vacate the locality because a mosquito survey revealed several *A. maculatus* breeding places, and to eradicate these would need a great deal of money; the Wireless Station was found to be an ideal breeding ground for *A. maculatus*, control was thought to be impracticable, the area was condemned as unsuitable for human habitation, and the Wireless Station and quarters were removed.

Thus there appears to have been a progressively increasing confidence in this belief that *A. maculatus* is a dangerous vector in Sarawak, and that malaria can, therefore, be controlled there by shading and other measures found to be effective in Malaya.

An examination of the evidence, however, finds that it is not only entirely circumstantial, but of very doubtful value, for it appears to be based merely on the presence—and only in one instance on the apparently sole presence—of this mosquito, and on its reputation in Malaya. No mention was made at any time of positive dissections—which alone can be regarded as conclusive evidence—although a few negative dissections, 36 in number, are quoted, and it seems hardly likely that these would have been recorded if at any time there had been the conclusive evidence of positive dissections to offer.

The fact that the belief was held so firmly that costly measures were based upon it is no justification for regarding it as true.

Stookes' (*loc. cit.*) belief was based on the hearsay evidence that *A. maculatus* was the only species of Anopheles found during a survey of Satang Island (O'Connor, 1911). This is circumstantial evidence, but it can hardly be accepted as proof. In possibly rather similar circumstances, as one example among many, in spite of the skilled observations of Shircore (*loc. cit.*) in Tambunan, his list of mosquitoes found there included *A. maculatus*, which he believed to be the vector, but did not include *A. leucosphyrus*, which has now been found actually to be the vector. It is not unlikely that the more elusive shade-loving *A. leucosphyrus*, for example, may have been breeding and carrying malaria on Satang Island, although not found in the survey, for Satang Island is jungle-covered.

With regard to dissections, as in Labuan, the belief has been current in Sarawak (O'Connor, *loc. cit.*) that, although no records were made, dissections were made about the year 1928–30 by two medical men, with the incrimination of four species of Anopheles including *A. maculatus*.

Enquiries abroad in 1911 regarding these were unfruitful, but as one of these men published the series of 36 negative dissections already referred to, and advanced the presence of several species of Anopheles in malarious areas as evidence for their incrimination, it is hardly likely that he had the conclusive evidence of positive dissections to offer without having recorded this.

Webster (1941*b*), referring to the belief that jungle clearance had been responsible for the appearance of *A. maculatus* and malaria in Miri, denied this and writes:

> 'With regard to the actual Miri area one might say that malaria had disappeared subsequent to the clearing and drainage of the hills and drainage of the flat land lying between the hills and the sea'

and Stookes in Miri, although at first holding the current belief in *A. maculatus* as a vector as already quoted (Stookes, 1927), has since stated (Stookes, 1941) that he has never himself experienced an epidemic caused by *A. maculatus* in Borneo.

Thus it would appear that the frequently expressed belief in *A. maculatus* as an important vector in Sarawak is based upon inadequate evidence, and it has not been possible to find any direct evidence, and little indirect, that it is a vector there at all.

Evidence from North Borneo.—Although little has been published, the view expressed by Shircore (*loc. cit.*), that *A. maculatus* is the chief vector at least in parts of North Borneo, would appear to have been long accepted. That this view had been generally held was recorded also by Carlile (*loc. cit.*) on the east coast, and anti-larval measures directed specifically against *A. maculatus* by subsoiling, fascine drainage, herbage cover, and other methods advocated in Malaya, were applied.

This belief has a good deal of justification. It was supported by the early findings of the author in Tambunan, that malaria was most intense in the hills, and least at a distance from them, although several exceptions to this presented themselves. The fallacy of this was shown, when it was demonstrated that malaria did not depend upon proximity to the hills, but to the jungle with which they were almost invariably covered (McArthur, 1946).

Stookes (1941) in North Borneo stated that '*A. maculatus* and *A. sundaicus* do not seem to trouble us as in Malaya', and again, 'The facts about the absence of malaria in *A. maculatus* areas and in Jesselton confirm my impression that it cannot be an effective carrier here'. The belief in its virulence, however, was widely held by the authorities before, and by the Armed Forces during the war.

Evidence from Dutch Borneo.—*A. maculatus* does not seem to be under any great suspicion in Dutch Borneo.

Goelarso (*loc. cit.*) suggested the possibility that the vector in Boeloengan District might be either *A. leucosphyrus*, or *A. maculatus* or both, as both these species were found breeding in proximity to malaria. On dissection, however, he found that only *A. leucosphyrus* was infected, while his *A. maculatus*, and those of Stoker (1934) in Poeloe Laoet—small in numbers it is true—were negative.

Otherwise, although quite extensive observations have been carried out on malaria and its vectors in Dutch Borneo, no further information is available regarding *A. maculatus*, and it does not seem to have been considered as demanding much attention.

2. EVIDENCE THAT *A. MACULATUS* IS NOT A VECTOR IN BORNEO.

Not only is the evidence for the incrimination of *A. maculatus* as a vector of malaria in Borneo very slender, but there is on the contrary a considerable and

growing weight of evidence to show that it is not, in fact, a vector at all in certain areas at least, and possibly throughout the island.

The fact that a mosquito is dangerous in one place must not be regarded as proof that it is dangerous in another; and the fact that *A. maculatus* has proved to be the most dangerous vector in the interior of Malaya, is no evidence that it is a vector at all in Borneo. The following observations are in favour of the belief that *A. maculatus* is not responsible for the transmission of malaria in Borneo.

EPIDEMIOLOGICAL EVIDENCE FOR ITS EXONERATION.

Malaria in Borneo is by no means limited to areas where *A. maculatus* breed. On the contrary, most malarious areas are free of *A. maculatus* and at least several areas found to breed *A. maculatus* freely are healthy.

For example, *A. maculatus* was found breeding in Jesselton in considerable numbers, although the spleen rate was only 2·5 per cent; and in an area behind Jesselton it was found in even greater numbers, with the complete absence of malaria. In Menggatal, however, only a few miles away, *A. maculatus* was rare but malaria was hyperendemic.

A. maculatus is also found in Sandakan, the pre-war capital, and Stookes (1941) wrote:

> 'I do not think that a real *A. maculatus* outbreak could have occurred without some one noticing it, and if there has been no such outbreak, then *A. maculatus* is not a carrier of the usual order in Malaya, if at all. We have it in Sandakan too, and if it were a carrier of the virulence usual in Malaya, we should probably have had to abandon the town, instead of having a few occasional and rather mild cases'.

In Tambunan, North Borneo, this question was studied more closely. Here it was found (McArthur, 1946) that the distribution of the spleen and parasite rates indicated a hill or jungle breeding mosquito as the probable vector. This agreed more or less with the possibility that *A. maculatus* might be responsible.

It was found, however, that more *A. maculatus* were taken in the healthy villages than in malarious ones. Two villages in which the greatest *A. maculatus* breeding was found, were shown to be in fact the healthiest areas in the entire district, with spleen rates of only 18 and 25 per cent respectively, in contrast to the 100 per cent of many villages somewhat in similar circumstances but where *A. maculatus* was scarce or absent.

It was finally shown that the greatest intensity of malaria was not dependent on proximity to hills, but to the jungle with which the hills were usually covered. This jungle was unsuitable for the breeding of *A. maculatus*, and the malaria was finally shown to be due entirely to the shade-breeding *A. leucosphyrus*.

Thus, while the indirect evidence of epidemiology can never be wholly relied upon in assessing the importance of a species, epidemiological evidence in Borneo appears to be all in favour of the belief that *A. maculatus* has no part in malaria transmission there.

BLOOD PREFERENCES.

A. maculatus is recorded as having a high anthropophilic index in Malaya, Green (1934) finding a preference by precipitin test of 93 per cent for man; but, in Assam, Ramsay, Chandra and Lamprell (*loc. cit.*) recorded an anthropophilic index by precipitin test of only 5·4 per cent; and Walch (Overbeek and Stoker, 1937) noted that '*A. maculatus* is not particularly attracted by human beings except where there are few cattle'.

Thus, while *A. maculatus* has a great preference for human blood in Malaya, this is by no means invariable elsewhere.

In Borneo, *A. maculatus* has not been found to have much interest in human blood. In Tambunan, for example, it was rarely found attempting to feed on man. It was never found resting in houses; and using the human bait trap only one specimen was taken, on an average, every three weeks of all-night trapping, although in the presence of hyperendemic malaria (McArthur, 1948). The same was found on the coast at Menggatal, and Carlile (*loc. cit.*) described a similar experience on the east coast.

In Tambunan, *A. maculatus* was found to be much more attracted by animals than by man. Of 176 captured, 142 were taken feeding on animals, 27 were taken by natives in various ways, and only 9 taken in the human bait trap.

It was not possible in Tambunan to obtain sufficient specimens of adults for precipitin tests. The only specimen of *A. maculatus* suitable for test, captured resting in the jungle, proved to have fed on cattle, and not on man.

The indirect evidence of blood preferences is, therefore, in favour of *A. maculatus* being unimportant or harmless in Borneo.

EVIDENCE OF DISSECTIONS.

The rarity of association of *A. maculatus* with man appears to have been responsible for a difficulty in obtaining material, and for inadequate dissections of *A. maculatus*, compared with some other countries and with some species in Borneo.

No infected specimens have been found, out of 213 dissections carried out. 177 of these were made by the author in the interior and west coast of North Borneo (McArthur, 1947*a*), 36 were made by Goelarso (*loc. cit.*) and Stoker (1934) in Dutch territory and one has been recorded by Gillies (1949) in Labuan.

Thus, though the numbers remain small, such dissections as have been carried out are in favour of *A. maculatus* being at any rate unimportant.

While this series of 213 negative dissections is suggestive, it is far from conclusive. While it is in keeping with the harmlessness of *A. maculatus*, it is clear that further dissections are necessary before this evidence may be regarded as having much weight.

DISCUSSION.

A study of the facts does not reveal any convincing proof that *A. maculatus* is the chief vector, or even a vector at all, of malaria in Borneo.

Firstly, there is the epidemiological evidence of malaria-free *A. maculatus* areas, and *A. maculatus*-free malarious areas; secondly, the considerable preference

of *A. maculatus* in Borneo for animal blood; and finally—although unfortunately in still inadequate numbers—the series of negative dissections.

It is therefore concluded that *A. maculatus* is almost certainly not a vector of malaria in Borneo.

III. THE IMPORTANCE OF *ANOPHELES UMBROSUS* GROUP IN BORNEO.

MEMBERS OF THE *A. UMBROSUS* GROUP.

'*A. umbrosus*' is a shade-loving insect found particularly in jungle on coastal plains. It is distributed chiefly in Malaya, Sumatra, Java and Borneo, and is regarded as an important vector of malaria in Malaya.

'*A. umbrosus*' has, however, been found to consist not of one species, but of a group of at least 9 species of mosquitoes (Reid, in the press); and while the position is becoming clearer in Borneo, there is a good deal of uncertainty regarding the identity, for example the earlier dissections.

In Borneo it is likely that in former years *A. umbrosus* Theobald, *A. letifer* Gater, *A. sp. B* occasionally *A. baezai* Gater, and, in the larval stages, possibly also *A. separatus* Leicester and *A. brevipalpis* Roper, have been confused. Records of *A. novumbrosus* refer to true *A. umbrosus* (Colless, 1948*a*).

The relation of each of these members of the *A. umbrosus* group to malaria in Malaya is still somewhat uncertain, and it is perhaps still more so in Borneo, as most dissections, for example, have recorded only *A. umbrosus*, although many must have referred to other species.

With some exceptions, therefore, it is not possible to be certain of the identity, particularly of pre-war identifications and dissections, although it has since become possible to make an attempt to assess the importance of some of the members of the group.

For this reason it appears preferable at this stage to deal with *A. umbrosus*, *A. letifer* and *A. sp. B* together as a group, although *A. baezai* and *A. separatus* are dealt with separately.

DISTRIBUTION IN BORNEO.

The *A. umbrosus* group is widely distributed in Borneo. '*A. umbrosus*' was mentioned in the first list of Anopheles referred to by Hewitt (*loc. cit.*) as being found in Sarawak, and it has been recorded by practically every observer since.

The different members of the group have different breeding preferences, although all tend to breed under shade and around the flat coastal plain. *A. baezai* is a coastal breeder, being found in brackish water. *A. umbrosus* and *A. brevipalpis* prefer jungle on coastal plains. *A. separatus* and *A. letifer* also breed on coastal plains but tend to be under lighter secondary scrub; while in Borneo *A. sp. B* is reasonably common with *A. umbrosus* in coastal areas.

Much further work is necessary to define the distribution of the different members of the group in Borneo; but reliable identifications have been made of *A. umbrosus* in North Borneo, Sarawak and Brunei, and of *A. letifer* and *A. sp. B* in North Borneo and Brunei.

The distribution of the group as a whole—*A. umbrosus*, *A. letifer* and *A. sp. B*—according to past records is given in Appendix IIIa; but *A. separatus*, *A. baezai* and *A. brevipalpis*, being easily distinguished and not so likely to have led to confusion, are dealt with separately.

EPIDEMIOLOGICAL EVIDENCE.

'*A. umbrosus*' was regarded with suspicion in Labuan by Scharff (*loc. cit.*), although he was almost certainly dealing with *A. baezai* which at that time had not been distinguished; and its association with *A. sundaicus* during one outbreak of malaria, and with *A. kochi* in another, was noted by Stookes (1927) in Miri, Sarawak, although no dissections were carried out, and it is probable that in this instance it was suspected largely on its reputation as a vector in Malaya.

In 1929, von Kuehlewein suggested (Goelarso, *loc. cit.*), with all reserve, that in the upper reaches of the River Mahakam, Dutch Borneo, the carriers in this region were either '*A. umbrosus*' or *A. leucosphyrus*, or both; and Walch and Soesilo (*loc. cit.*) also recorded the same impression from Tandjong Djoeata, Dutch Borneo.

Goelarso (*loc. cit.*), working in the subdivision of Boeloengan, East Borneo, stated that in the Sadjau District, where malaria was found to be hyperendemic, larvæ of only *A. umbrosus* were found, and adults of only *A. leucosphyrus*. This was regarded as support for von Kuehlewein's suggestion that these two species were vectors.

Larvæ of '*A. umbrosus*' were recorded by Shircore (*loc. cit.*) from the Tenom District, North Borneo, and the species mentioned as a possible vector, although true *A. umbrosus* is unlikely from this area.

A. umbrosus has also been referred to as a possible vector by a number of other observers in Borneo—e.g., Hutchinson (*loc. cit.*), Sarawak (1931), Carlile (*loc. cit.*)—but without any definite local evidence.

There has been thus a certain amount of indirect evidence that '*A. umbrosus*' is a vector in North Borneo, Sarawak and Dutch Borneo, but suspicion would appear to have been based chiefly on its reputation in Malaya.

BLOOD PREFERENCES.

In Malaya, Green (*loc. cit.*) by precipitin tests recorded 93 per cent of '*A. umbrosus*' as having fed on human blood, and Hodgkin (1936) confirmed this preference by a comparison of catches by human and animal bait.

Walch and Sardjito (1928), working in Dutch Borneo with precipitin tests, found that all engorged females of '*A. umbrosus*' examined contained human blood. Later, Walch (*loc. cit.*) again quoted precipitin tests carried out in the Netherlands East Indies, noted that '*A. umbrosus*' was one of the species feeding readily on man and described it as the most important carrier in the interior of Western Borneo.

The evidence of blood preferences is, therefore, that '*A. umbrosus*' is a potential vector of malaria in Borneo.

INCRIMINATION BY DISSECTION.

Dissections of over 5,000 '*A. umbrosus*' in Malaya (Gater, *loc. cit.*; Hodgkin, 1937–41) yielded a gland infection of 0·35 per cent, so that it is a definite vector of malaria there.

'*A. umbrosus*' was the first mosquito recorded as being incriminated as a carrier of malaria in Borneo, when Roper (*loc. cit.*) stated that he found sporozoites in 1 out of 130 specimens of this species dissected in Membakut, North Borneo.

Roper's (*loc. cit.*) recorded incrimination of this species was confirmed by Stoker (Soesilo *et al.*, 1932) in Sanggau, Western Borneo, who found a natural infection index of 0·4 per cent.

'*A. umbrosus*' was also recorded as having been found infected 'in Soengei Kakap, Pontianak, Borneo, by Soesilo 1935 with a natural infection index of 15·2 per cent (59 species examined)' (Overbeek and Stoker, *loc. cit.*).

In 1938, 5 specimens were found infected—3 with oöcysts and 2 with sporozoites—out of 75 dissected in the Sarawak Oilfields in Bukit Sawat and Brunei under Webster (1941*a*). A further 13 from Jerudong and 1 from Matading were negative.

Since the war, positive dissections have been recorded of '*A. umbrosus*' for example by Rutledge (1947), sporozoites have been recorded in true *A. umbrosus* (Colless, 1948*c*) and *A. letifer* (Johnson, 1949*a*) and oöcysts have been found more than once in *A. sp. B*. Thus it would appear that several species are likely vectors, although further work is necessary to define their relative importance.

The dissections of the *A. umbrosus* group are tabulated in Appendix IIIb. Owing to the inadequacy of the earlier records it is impossible to say how many of the total positive dissections were gland and how many were gut infections, but 2,914 dissections of the *A. umbrosus* group have yielded 50 positives—or 1·7 per cent—of which 9 were recorded as gland infections—a sporozoite rate of 0·3 per cent—but it is possible that some others may have been gland infections also.

PRECISE IDENTIFICATION OF DISSECTIONS.

All the dissections of this group made before the war, with the exception of very few *A. separatus*, *A. baezai*, *A. brevipalpis* and '*A. novumbrosus*', were recorded as '*A. umbrosus*', so that it is impossible to tell with certainty to what they referred.

All the surviving specimens of Roper's '*A. umbrosus*' appear to be true *A. umbrosus*; and from his description of variation in his specimens, while including some *A. sp. B*, true *A. umbrosus* must have formed the bulk of his *umbrosus*-like catches (Colless, 1948*a*).

The numbers of the different species dissected have been recorded as follows :—

	Total dissected.	*Total positive.*	*Gland infections.*
'*A. umbrosus*' (Roper's, probably true *A. umbrosus*)	130	1	1
'*A. umbrosus*' (identification uncertain) ...	1,129	19	2(?)
A. umbrosus (identification reliable)	981	24	5
A. novumbrosus (presumably *A. umbrosus*) ...	12	...	...
A. letifer (identification reliable) ...	621	3	1
A. sp. B (identification reliable)	41	3	...
TOTAL	2,914	50	9(?)

SITUATION OF INFECTIONS.

'*A. umbrosus*' (probably true *A. umbrosus*) was incriminated at Membakut, North Borneo, '*A. umbrosus*' was recorded as infected, although the actual species is doubtful, at Sanggau and Soengei Kakap in Dutch Borneo, at Bukit Sawat in Brunei, and at Jesselton.

Sporozoites have been recorded in true *A. umbrosus* in the Kuala Belait-Seria area, Brunei, and gut infections in the Miri-Lutong area. Gut infections have been recorded in the Kuala Belait-Seria area in true *A. umbrosus*, *A. letifer* and *A. sp. B.*

DISCUSSION.

There is no doubt, from the records, that although more limited in distribution than *A. leucosphyrus*, and apparently exhibiting not quite as high an index of natural infection, the *A. umbrosus* group of mosquitoes must be regarded as important in relation to malaria in Borneo, in some parts of which it appears to be the chief vector.

IV. THE IMPORTANCE OF *ANOPHELES SUNDAICUS* IN BORNEO.

A. SUNDAICUS OUTSIDE BORNEO.

A. sundaicus is a coastal mosquito distributed particularly in Malaya, Sumatra and Java, but extending to the coastal areas of Orissa in India, and to Celebes.

Breeding generally in brackish pools in sunlight, this mosquito is found on the seacoast, often in cleared mangrove swamps and tidal inundations. Thus it is found often in proximity to settlements and industries, so that it may be of considerable importance if concerned in the transmission of malaria.

A. sundaicus is regarded as a dangerous mosquito throughout most of its range. Christophers (*loc. cit.*) states that 'It is one of the most effective carriers known'. It is an important vector in Malaya, Java, Sumatra, Indo-China, and in those parts of India in which it occurs.

DISTRIBUTION IN BORNEO.

A. sundaicus was first recorded in Borneo (as *A. ludlowi*) by Roper (*loc. cit.*) in Membakut, North Borneo; and subsequently by Scharff (*loc. cit.*) in Labuan, by Stookes (1927) in Miri, Sarawak, and by Dingle (*loc. cit.*) in Jesselton.

In Dutch Borneo, it was recorded by Walch and Soesilo (1929) as having been first found—as a local variation (standortsvarietat)—in Koeala Sambodja, a small port to the north of Balikpapan.

Since that time it has been fairly frequently recorded from many coastal areas, and its distribution is summarized in Appendix IV*a*.

IDENTITY OF THE BORNEO FORM.

This mosquito was originally described as a variety of the Philippine freshwater *A. ludlowi*, and is referred to in the early records as *A. ludlowi* (var. *sundaica*). It was later realized that the coastal brackish water species was quite distinct, and

the specific name *A. sundaicus* was given to the form from the Malayan Archipelago, and *A. litoralis* to the Philippine form which appears to be harmless.

Until recently there has been a good deal of uncertainty regarding the identity of the Borneo representative. A single female adult of *A. ludlowi* was taken by the author in 1941 in heavy jungle at an altitude of 2,000 feet, 25 miles from the sea, at Purutan, North Borneo; specimens from brackish water in Jesselton were identified, not as *A. sundaicus*, but as *A. litoralis* (Hodgkin, 1941); while Webster (1941*a*) identified *A. sundaicus* breeding in both fresh and brackish water in the Sarawak Oilfields. This raised the possibility of the existence of several forms of the insect in Borneo.

Colless (1948*a*), summarizing the characters which appear to be transitional between the Philippines and the East Indies forms, concludes that the Borneo form is more nearly related to *A. sundaicus*, and should be referred to as such, but that it differs significantly from the type form from the Sunda Islands, and is probably worthy of subspecific rank. This form has since been found breeding both in brackish and fresh water.

EPIDEMIOLOGICAL EVIDENCE.

Considering its possible danger to areas of economic importance, such as towns and areas under industrial development, *A. sundaicus* has been inadequately studied in Borneo, but sufficient has for long been known at least to throw strong suspicion upon it as an important vector in some areas.

Gater (*loc. cit.*) states that 'This species is regarded as one of the most important natural carriers in the coastal regions of all countries where it occurs'; but it would not be wise to conclude that it must therefore necessarily be a vector in Borneo, although it must be greatly suspected

A. sundaicus (as *A. ludlowi*) was described by Walch and Soesilo (*loc. cit.*) as 'The most dangerous of all the anopheles species found in Dutch Borneo', but this was based probably on the evidence of its virulence in Java and other parts of the Netherlands Indies, and of spleen rates of 57 and 47 per cent recorded by Dr. Zakir in proximity to its breeding places.

Scharff (*loc. cit.*) in Labuan suspected this mosquito on indirect evidence to be the malaria vector there. Only one dissection, however, and that negative, was able to be carried out during the time available.

Stookes (1927) in Miri stated that 'It occurs at times in very large numbers and is the most important carrier here'. He recorded 3 oöcysts out of 22 dissections carried out, and quoted figures showing a parallel between *A. sundaicus* breeding and epidemic malaria. This was an extremely strong evidence, although as it remained only circumstantial it was not conclusive.

Later, in the same place Webster (1941*a*) described a series of outbreaks of malaria in the Sarawak Oilfields which were considered to be due to *A. sundaicus*.

For example, evidence for the incrimination of *A. sundaicus* in Lutong was based on the fact that, from 1924 to 1933, epidemics broke out annually in spite of heavy oiling and drainage. In 1934, however, tide gates were erected, since when malaria had been under control until 1941. During this outbreak in 1941, *A. sundaicus* was found breeding in a stream which had previously been

controlled by four tide gates. An examination showed two of these to be defective, and when they were dealt with, *A. sundaicus* disappeared. No other species were found, so again it was assumed that *A. sundaicus* was the vector.

In the Tanjong Lobang area of Miri also, *A. sundaicus* was the only mosquito found breeding in spite of special search, in the vicinity of an outbreak of malaria, and it was therefore regarded as probably the vector.

Again, in the St. John's Wood area of Miri, in the vicinity of cases of malaria, *A. sundaicus* was found breeding, but in fresh water. This also, on the evidence, was considered the vector; while in the Loak-Bakan area it was found in proximity to malaria and breeding in diluted sea-water.

Further, at Bintulu, 100 miles south-east along the coast from Miri, *A. sundaicus* was again found breeding in proximity to a bungalow in which malaria had occurred, and to an epidemic which was later believed to have been malaria.

Finally, at Matading, still further along the coast, it was stated that *A. sundaicus* breeding accompanied an outbreak of malaria, and that the disappearance of both *A. sundaicus* and malaria coincided with the erection of a tide gate. This again was evidence that this mosquito was responsible.

Strahan (1947), from a very considerable experience of this mosquito in Malaya, summarizing the evidence for its incrimination in Sarawak, suggested that 'While *A. leucosphyrus* and *A. umbrosus* may play a part in the malaria of the coast, the epidemic factor would appear to be *A. sundaicus*'. He expressed Stookes' figures as a graph (Chart 2), which made these far more striking than they originally appeared to be.

Colless (1948*b*), following five weeks' survey of the Seria and Miri Oilfields, concluded that 'The epidemiological evidence against *A. sundaicus* is considered to afford satisfactory proof of the importance of this species as a vector of epidemic malaria'.

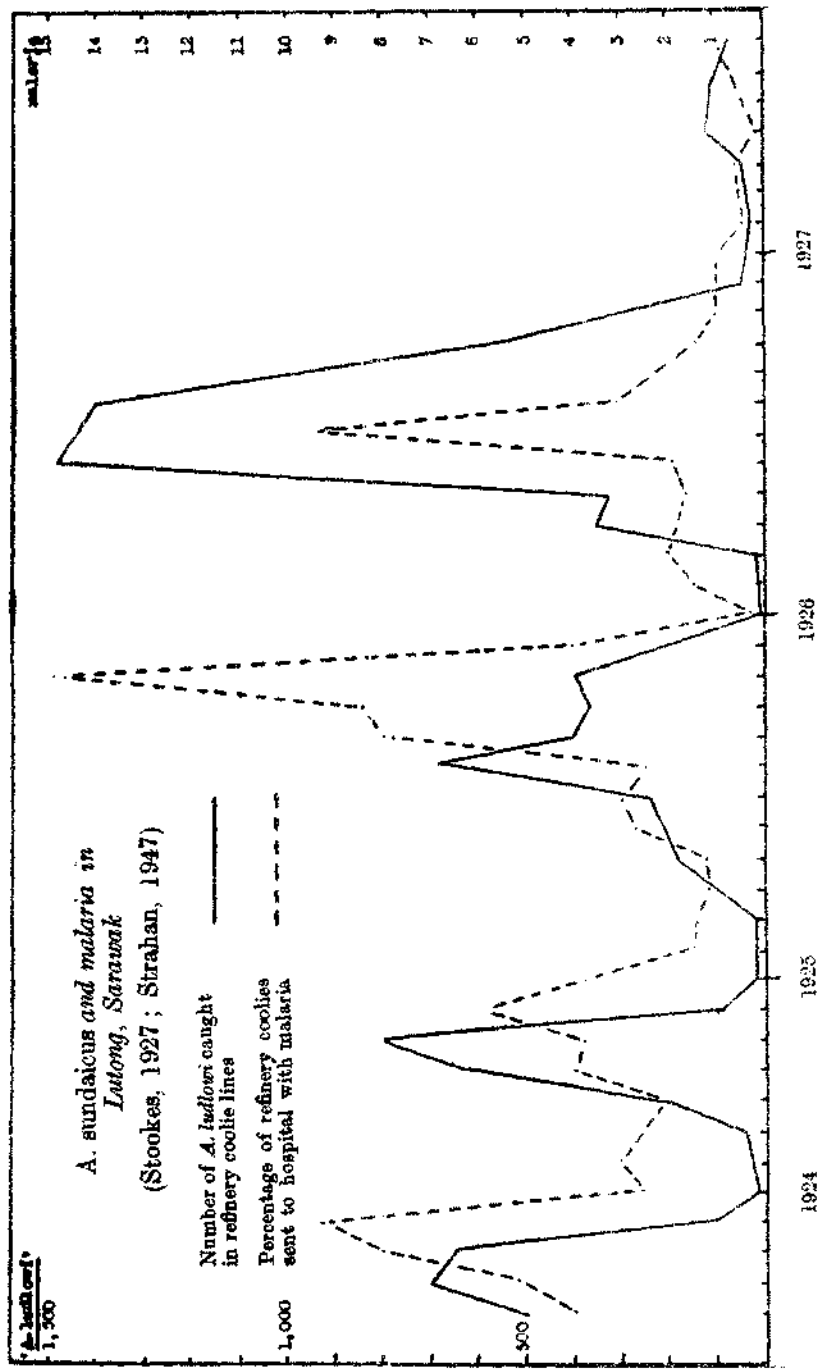
Until 1949, however, no direct evidence was forthcoming for the incrimination of *A. sundaicus* by the discovery of infected glands, although oöcysts had frequently been recorded, and there was a good deal of epidemiological evidence that it was not a vector of consequence.

For example, in 1940, *A. sundaicus* was found breeding fairly densely in a part of Jesselton, but apparently in the absence of malaria.

Again, *A. sundaicus* areas in North Borneo generally are the healthy areas —for example, the coastal parts of Labuan, Jesselton, Menggatal, Tawau and elsewhere—and when malaria had been noted in *A. sundaicus* areas in North Borneo with one exception it has always been found that there is *A. leucosphyrus* present, breeding in neighbouring jungle.

For example in Labuan, *A. sundaicus* areas on the whole are healthy, while areas free of *A. sundaicus* are often highly malarious. Areas breeding *A. sundaicus* which are also malarious are found as a rule to be subject to transmission by *A. leucosphyrus*. Only one situation has been found in Labuan in which there is hyperendemic malaria and *A. sundaicus* breeding, but with apparently very few *A. leucosphyrus*.

CHART 2.


A. sundaicus and malaria in
Lutong, Sarawak
(Stookes, 1927 ; Strahan, 1947)
Number of A. indicus caught in refinery coolie lines
Percentage of refinery coolies sent to hospital with malaria
1,000
500
1924
1925
1926
1927

Thus, epidemiological evidence has been very strong that *A. sundaicus* is a vector especially of epidemic malaria in Borneo, but there have been some notable exceptions which suggest that at least under some circumstances it may be of little or no importance, and until very recently direct evidence has been lacking.

It must also be recognized, however, that there are some peculiar aspects that require further study; for example, the occurrence of breeding in fresh water, and particularly the fact that malaria is not by any means always found in proximity to *A. sundaicus* breeding.

BLOOD PREFERENCES.

Overbeek and Stoker (*loc. cit.*) comment that *A. sundaicus* prefers feeding on human beings, even when there are cattle present; and Walch (1932) recorded an anthropophilic index of 86 per cent in *A. sundaicus* in the Netherlands Indies where there were normal numbers of cattle. It would appear to be therefore a potential vector.

EVIDENCE OF DISSECTIONS.

While the epidemiological evidence for the incrimination of *A. sundaicus* as a vector in Borneo has for long been extremely strong, it has not been felt that it was sufficient for any considerable expenditure on control measure, and its actual incrimination by the finding of infected glands on dissection was felt to be necessary.

Oöcysts had been recorded as having been found as early as 1924 by Stookes (1927), but following this for many years there was no further evidence. Sukarman (1948) recollects that one infected gut was found in 1933 by Dr. McMahon in Labuan but unrecorded. Webster (1947) recorded oöcysts in one dissection from Seria, in two from Lutong, and in three from Kuala Belait. Gillies (1948) recorded two with oöcysts in Labuan, and the Malaria Research Department (McArthur, 1949) also recorded two in Labuan.

Thus, during 25 years to the beginning of 1949, 14 infected guts had been recorded out of 1,745 dissections (0·8 per cent), but no infected glands had ever been found.

In spite therefore of the reputation of this insect in other countries, of its apparent association with malaria in many parts of Borneo, and of infected guts on dissection, the direct evidence of infected glands was lacking; and the possibility was bound to be considered that this mosquito might after all be of no importance. In support of this was the evidence of areas free of *A. sundaicus* with very high spleen rates, but *A. sundaicus* areas with very low spleen rates.

Finally, however, in 1949, sporozoites were found on dissection almost simultaneously in all three states of British Borneo, firstly in Sarawak by the Oilfields Medical Staff in June, and in the following month again by the Oilfields Staff in Brunei, and by the Malaria Research Department in Labuan.

Thus, at the end of 1949 out of 4,485 dissections of *A. sundaicus*, there were recorded 17 infected guts, and 12 infected glands, representing an infection rate of 0·6 per cent and a sporozoite rate of 0·3 per cent (Appendix IV*b*).

V. THE IMPORTANCE OF *ANOPHELES BAEZAI* IN BORNEO.

A. BAEZAI OUTSIDE BORNEO.

A. baezai is a member of the *A. umbrosus* group. It is now readily distinguishable from the rest, but before 1933 it was confused with *A. umbrosus*, so that there are no earlier records of it.

A. baezai is a coastal mosquito breeding in stagnant brackish water under *nipah* or other shade. It has been recorded from Malaya, Indo-China, the Philippines, Celebes, Bali and Java.

BLOOD PREFERENCES.

A. baezai has been under some suspicion as a vector. In Malaya it has a certain interest in human blood (Hodgkin, 1939-40), and has shown a 0·9 per cent gland infection (17 with sporozoites) out of 1,891 dissections in Malaya (Hodgkin, 1936-41), although doubt was expressed whether these were in fact human infections.

EVIDENCE OF DISSECTIONS.

In 1949, positive dissections were recorded in Borneo by the Sarawak Oilfields Medical Staff, 1 with sporozoites and 5 with oöcysts in the Miri-Lutong area (Johnson, 1949*c*), and 1 with sporozoites in Kuala Lumut (Johnson, 1949*d*), making a total of 2 gland infections out of 653 dissections. Thus it appears that *A. baezai* may have to be taken into account in the malaria situation in Borneo, and shaded mangrove and *nipah* regarded as a potential danger.

VI. THE IMPORTANCE OF *ANOPHELES SEPARATUS* IN BORNEO.

A. SEPARATUS OUTSIDE BORNEO.

A. separatus is classified as a member of the *A. umbrosus* group, although it is readily distinguished from the rest. It breeds in grassy open swamps on coastal plains, and is distributed near coastal regions in Malaya, Sumatra, Borneo and elsewhere.

A. separatus has been under some suspicion on account of its interest in human blood. Gater (*loc. cit.*), commenting on the absence of infections in the few dissections carried out, comments that 'There appears to be no evidence that it is concerned in transmission, though it certainly feeds on man'.

Hodgkin (1935-41) found one gland infection (0·14 per cent)—apparently the only one recorded—out of a total of 717 dissected in Malaya, and it is generally considered to be unimportant.

DISTRIBUTION IN BORNEO.

A. separatus has been frequently recorded on the coastal plains of North Borneo and Labuan, Sarawak, Brunei and Dutch Borneo. Its fairly wide distribution is summarized in Appendix VI*a*.

BLOOD PREFERENCES.

In Borneo, *A. separatus* has been observed to have an interest in human blood, although not nearly so great as that of the known vectors *A. leucosphyrus* and *A. umbrosus*. In Jesselton in 1942, it was the only Anopheles found in houses, and Allman (*loc. cit.*) noted that it had a fair preference for human blood in North Borneo. For these reasons it has been regarded as possibly having some small place as a vector in Borneo, although it has been seen in numbers in malaria-free areas.

EVIDENCE OF DISSECTIONS.

In July 1949, 13 specimens of *A. separatus* were recorded by the Sarawak Oilfields Medical Staff as having gut infections out of 934 dissections, although no gland infections were found in the Miri-Lutong area (Johnson, 1949*c*).

While *A. separatus* has been recorded with oöcysts, however, none have ever been recorded with sporozoites in Borneo out of a total of 3,583 dissections.

VII. THE IMPORTANCE OF *ANOPHELES HYRCANUS* GROUP IN BORNEO.

DISTRIBUTION.

The *A. hyrcanus* group of mosquitoes is distributed widely across Asia from India to Celebes. It breeds in swamps and paddy fields, and is regarded in many places as having considerable importance as a vector of malaria.

MEMBERS OF THE *A. HYRCANUS* GROUP.

The classification of the *A. hyrcanus* group is at present in some confusion. *A. hyrcanus* has generally been referred to in two varieties—*A. hyrcanus* var. *sinensis* and *A. hyrcanus* var. *nigerrimus*—both of which are recorded from Borneo; but recent work has shown that each of these divisions may include a number of distinct forms. Others have been defined, including three Philippine sub-species, as well as *A. hyrcanus X* of Venhuis (1940), and the sp. nr. *sinensis* of Colless (1948*a*), now known as 'Coastal Form'.

In Malaya, Crawford (1938) established the presence of five distinct forms, and since then yet others have been found there. Altogether some nine forms have been distinguished, but their precise identification remains to be defined.

A. HYRCANUS GROUP OUTSIDE BORNEO.

Outside Borneo the three forms, *A. hyrcanus* var. *sinensis*, *A. hyrcanus* var. *nigerrimus* and *A. hyrcanus X*, have all been recorded as infected. *A. hyrcanus* var. *sinensis* has been recorded as a vector in China (Feng, 1932) and Formosa (Anazawa, 1931). Both *A. hyrcanus* var. *sinensis* and *A. hyrcanus* var. *nigerrimus* have been recorded as vectors in Malaya (Hodgkin, 1933), Indo-China (Gaschen, 1936) and particularly in Sumatra (Soesilo *et al.*, 1933; Walch and Walch-Sorgdrager, 1921). In Java, *A. hyrcanus* var. *nigerrimus* has been recorded as a vector by Soesilo *et al.* (1933) and by Overbeek and Stoker (*loc. cit.*); but in both Java and

Celebes, evidence has been quoted that *A. hyrcanus X* is a vector (Covell, 1944; Stoker, 1949).

Thus, even if the former classification is not valid, *A. hyrcanus* appears to be definitely a vector in several of its forms, and in a number of countries in the Far East.

DISTRIBUTION IN BORNEO.

A. hyrcanus in one form or another has been recorded fairly widely in Borneo. The recorded distribution of the different forms is given in Appendix VII*a*, but both *A. hyrcanus* var. *sinensis* and *A. hyrcanus* var. *nigerrimus* have been recorded from all parts—North Borneo and Labuan, Brunei, Sarawak and Dutch Borneo—while *A. hyrcanus X* has been recorded from several places in Western Dutch Borneo. Records of *A. hyrcanus* var. *sinensis* from North Borneo and Sarawak refer almost certainly to the 'Coastal Form'.

BLOOD PREFERENCES.

A. hyrcanus var. *nigerrimus* has not been considered to have any particular preference for human blood, and Ramsay, Chandra and Lamprell (*loc. cit.*) recorded an anthropophilic index of only 3·8 per cent; but there are several records of *A. hyrcanus* var. *sinensis* having a considerable appetite for man, for example, Walch (*loc. cit.*) recorded an anthropophilic index of 83 per cent in the Netherlands Indies.

In Labuan, Colless (1948*a*), however, concluded from a comparison of the results of human and animal bait trapping that *A. hyrcanus* var. *nigerrimus* had a decided preference for human blood.

EVIDENCE OF DISSECTIONS.

There is no particular evidence, either from the epidemiology or from blood preferences, that *A. hyrcanus* in any form is a vector of importance in Borneo, but dissection records yield some information.

1,557 dissections of the *A. hyrcanus* group have been recorded in Borneo—as summarized in Appendix VII*b*—most of these without more precise identification.

Of these, 4 '*A. hyrcanus*' were recorded as 'positive' in Tanah Grogot, Dutch Borneo, by Stoker (Overbeek and Stoker, *loc. cit.*) and 2 '*A. hyrcanus*' were recorded with oöcysts in Labuan by Gillies (1948).

Unfortunately, the original records of the dissections in Dutch Borneo appear to have been destroyed, and there was some confusion in the identification, one reference recording it as '*A. hyrcanus*' (Overbeek and Stoker, *loc. cit.*), another as '*A. hyrcanus* var. *sinensis*' (Stoker, 1931), and a third as '*A. hyrcanus* var. *nigerrimus*' (Overbeek, 1941*a*).

There is no record of sporozoites ever having been found in any of the forms of *A. hyrcanus* in Borneo, and the evidence that it is a vector of importance is not strong.

If *A. hyrcanus* is a vector in Borneo, there is no evidence, direct or indirect, regarding which form may be responsible; and much further work is necessary in order to classify the forms and to define their distribution and possible relation to malaria.

VIII. THE IMPORTANCE OF *ANOPHELES BARBIROSTRIS* IN BORNEO.

A. BARBIROSTRIS OUTSIDE BORNEO.

A. barbirostris is one of the most widely distributed mosquitoes in Asia, its range extending throughout nearly the whole of India and Ceylon in the west, and as far as Celebes in the east. It breeds in pools, swamps, ricefields and a variety of other situations, so that it may be a very common and widespread insect.

A. barbirostris is not regarded as having a great interest in human blood, or as a vector of importance. Gater (*loc. cit.*) states that 'It is not generally considered to play an important part in transmission'. It has however been found infected on a number of occasions in different parts of the world, for example, in Malaya by Hodgkin (1935–40), Sumatra by Doorenbos (1925), and Celebes by Machsoes (1939).

DISTRIBUTION IN BORNEO.

A. barbirostris is one of the most common and widely distributed Anopheles in Borneo.

EPIDEMIOLOGICAL EVIDENCE.

In Borneo, *A. barbirostris* has not been under any great suspicion. In Tambunan, areas of greatest *A. barbirostris* breeding, for example the ricefields, were healthiest; while the most malarious areas were those of least *A. barbirostris* breeding.

There are several forms of *A. barbirostris*, some of which may be vectors, but so far nothing is known of their relation to the Borneo *A. barbirostris*.

BLOOD PREFERENCES.

In Tambunan, *A. barbirostris* was the most common mosquito found in larval surveys, forming more than 50 per cent of all Anopheles found breeding, yet it formed only 6 per cent of captures in the human bait trap. It cannot therefore have any great appetite for human blood.

EVIDENCE OF DISSECTIONS.

One specimen of *A. barbirostris* was found with a gut infection in Jesselton, however, by Colless (1948*b*), another gut infection was recorded by Rutledge (*loc. cit.*) in the same place, and yet another in the Miri-Lutong area by Johnson (1949*c*), so that it cannot be altogether ignored.

CONCLUSION.

All things considered, however, with 1,002 dissections, 3 gut infections of *A. barbirostris* can hardly be considered significant, and this mosquito can probably be regarded as harmless under ordinary circumstances.

IX. THE IMPORTANCE OF *ANOPHELES TESSELLATUS* IN BORNEO.

A. TESSELLATUS OUTSIDE BORNEO.

A. tessellatus breeds in pools, swamps, buffalo wallows and other situations. It is not considered to be a vector of any importance although it has been recorded as naturally infected in several places—China, Indo-China, Formosa and elsewhere.

DISTRIBUTION IN BORNEO.

A. tessellatus is fairly widely distributed in Borneo, and is reasonably commonly recorded as a result of surveys.

BLOOD PREFERENCES.

This mosquito is rather variable in its blood preferences in other countries, but it has never been taken in the human bait trap in Tambunan, and there is no evidence that it has any marked preference for human blood in Borneo, or that it has ever been responsible for malaria transmission there.

EVIDENCE OF DISSECTIONS.

Out of exactly 1,000 dissections carried out throughout Borneo, 2 have yielded gut infections (0·2 per cent). Infected glands have never been found.

In the absence of any other evidence, these two positive gut dissections cannot be considered significant, and there is no reason to suppose that this species takes any part in the malaria transmission in Borneo.

X. THE IMPORTANCE OF *ANOPHELES MINIMUS* VAR. *FLAVIROSTRIS* IN BORNEO.

A. MINIMUS OUTSIDE BORNEO.

A. minimus is a mosquito which is widely distributed in Asia, and regarded as an important vector of malaria wherever it occurs.

A. minimus has been recorded from all the countries surrounding Borneo—Java and Alor to the south, Sumatra and the Malayan Peninsula to the west, Siam, Cochin-China, Annam, Tongkin, China and Formosa to the north, and Celebes and the Moluccas to the east. *A. minimus* var. *flavirostris* has been recorded in the Philippines to the north, Bali to the south, and—as considered below—in Pocloc Laoet, Dutch Borneo.

A. minimus is regarded as the chief vector of malaria in parts of India, Assam, Burma, Indo-China, Formosa and elsewhere; and *A. minimus* var. *flavirostris* is regarded as the chief vector in the Philippines.

DISTRIBUTION IN BORNEO.

Although *A. minimus*—or *A. minimus* var. *flavirostris*—has been recorded from all the countries around Borneo, it has never, curiously, been recorded from the mainland of Borneo itself.

EVIDENCE OF DISSECTIONS.

It is recorded, however (Overbeck, 1941*b*), that in 1934, 2,770 *A. minimus* var. *flavirostris* were dissected, with 2 positives, in three villages in Poeloe Laoet, an island off the south-east of Dutch Borneo.

This series of dissections was referred to as indicating that this mosquito was the most important vector of malaria at that place (De voornaamste malariaoverbrenger) by Soesilo *et al.* (1935).

Neither *A. minimus* nor *A. minimus* var. *flavirostris*, however, have been recorded before or since in Poeloe Laoet, nor have they been recorded anywhere on the mainland of the island of Borneo. It is not known by whom the dissections were carried out, nor how reliable the identifications might have been. If in fact it does exist in Poeloe Laoet, its distribution must be very limited.

Further, the very small number of positive dissections—and whether sporozoites or oöcysts is not stated—that are recorded regarding this mosquito in Poeloe Laoet, is not in keeping with its being a dangerous vector, and might well be the result of accidental infection of a harmless species in an area of intense malaria. The fact that *A. leucosphyrus* had already been incriminated in Poeloe Laoet with an infection index of 6·3 per cent (Stoker, 1934) would appear to be sufficient to explain the malaria incidence and to throw doubts upon the importance of the 0·07 per cent index of *A. minimus* var. *flavirostris* there.

It is therefore concluded that, while *A. minimus* var. *flavirostris* has been claimed to be the chief vector of malaria in Poeloe Laoet, this is in some doubt; and if it is present on the mainland of Borneo at all—which appears to be improbable—it is unlikely to be of any importance.

XI. THE NON-VECTORS OF BORNEO.

Of the Anopheles dissected in adequate numbers—that is, of 500 or more—only two have given sufficient evidence, both indirectly from a study of epidemiology, and directly from dissections, that they have normally no place whatever in the transmission of Borneo malaria.

Of these two, *A. philippinensis*, a paddy field breeder, is an important vector in parts of India, although in other parts it appears to be harmless. The other, *A. kochi*, a pool breeder, although regarded as harmless in India, is considered to be of some importance in Sumatra and elsewhere in the Netherlands Indies.

During the Tambunan research in North Borneo it was found that where malaria was most intense, these mosquitoes were scarce, but that where they abounded, malaria was slight or absent.

Finally, throughout Borneo, *A. philippinensis* has yielded 1,880 and *A. kochi* over 875 negative dissections. Thus it appears safe to conclude that these two very common and widespread Anopheles are normally harmless.

Of the remaining Anopheles of Borneo, insufficient evidence is available to permit an assessment of their precise importance, but there is no strong epidemiological evidence that any species other than those considered has any relation to Borneo malaria.

CONCLUSIONS.

It is concluded, from these studies of the possible vectors of Borneo malaria, that:

1. Of the many species of Anopheles which have been identified throughout Borneo, nine have been recorded as having been found infected with malaria, and four as having yielded sporozoites on dissection.
2. *A. leucosphyrus* is the chief vector of malaria throughout the greater part of Borneo.
3. *A. maculatus*, widely held in past years to be the chief vector, is of little and probably no importance.
4. The *A. umbrosus* group is of considerable importance in some areas, but is neither so virulent nor so widely distributed as *A. leucosphyrus*.
5. *A. sundaicus* may be of some importance, possibly more in occasional epidemics than in the widespread endemic malaria.
6. There are several other possible vectors—*A. baezai*, *A. separatus*, the *A. hyrcanus* group and perhaps others—the importance of which is probably limited but which has not so far been well defined.
7. Only two species of Anopheles—*A. philippinensis* and *A. kochi*—have given fair evidence, though necessarily negative, from a study of epidemiology and of dissections, that they have normally no place in the transmission of Borneo malaria.
8. While the picture of Borneo malaria transmission is becoming more clear, much remains to be done to assess the distribution, the precise identity, and the relative importance of some of the Borneo vectors, and to define their habits, and consequently the means for their control.

REFERENCES.

ALLMAN, S. L. (1945) ... Personal Communication, Aug. 6, 1945, Labuan, regarding work of the *Aust. Mob. Ento. Sec., 1945.*

ANAZAWA, K. (1931) ... *Taiwan Igakkai Zasshi.*, **30**, pp. 1027–1049, Tohoku.

BROOKE (1930) ... ... *Sarawak Ann. Med. Rep., 1930*, Kuching.

BRUNEI (1929–38) ... ... *Gov'. Annual Medical Reports.*

CARLILE, J. P. (1941) Personal Communication, Oct. 31, 1941, Sandakan.

CHRISTOPHERS, S. R. (1933) ... *Fauna of Brit. India, Diptera*, **4**, pp. 182 and 285. Taylor and Francis, London.

CLAPHAM, G. (1948) ... Personal Communication, June 24, 1948, Kuching.

COLLESS, D. H. (1947) ... *Malaria Research Dept. Entomologist's Rep.*, Dec. 1947, Sandakan.

Idem (1948*a*) ... *Proc. Linnean Soc.*, New South Wales, **73**, Parts 3-4, pp. 71–119.

COLLESS, D. H. (1948*b*)	Personal Communication, June 16, 1948, Labuan.
Idem (1948*c*)	*Report of a Malaria Survey in the Seria and Miri Oilfields*, May to June 1948, Labuan.
Idem (1948*d*)	*Report of a Malaria Survey in the Miri-Lutong Area, Sarawak, November to December 1948*, Labuan.
Idem (1949)	*Malaria Research Dept. Entomologist's 'Summary of Entomological Research during 1948'*, Labuan.
Idem (1950)	*Ind. J. Mal.*, **4**, pp. 377–383.
COVELL, G. (1941)	*J. Mal. Inst. Ind.*, **5**, pp. 399–434.
CRAWFORD, R. (1938)	*Some anopheline pupae of Malaya*, Government Printing Office, Singapore.
DINGLE, P. A. (1929)	*An anopheline mosquito survey of Jesselton*. Rep. to N. B. Govt., 1929, Sandakan.
DOORENBOS, W. B. (1925)	M. D. Thesis, Univ. of Leyden.
ENGLISH, J. C. (1945)	Malaria in the Borneo Operations, May 1–Sept. 1. Australian Military Forces.
EYKMAN INSTITUTE (1938)	From maps in the Institute recording surveys in Dutch Borneo, 1930–1938 (McArthur, 1938).
FENG, L. C. (1932)	*Geog. Trop. Med. Hyg.*, **35**, pp. 98–202.
GASCHEN, H. (1936)	*Bull. Soc. Path. Exot.*, **29**, pp. 1093–1095.
GATER, B. A. R. (1935)	*Gels to the identification of anopheline imagines in Malaya, Singapore.*
GILLESPIE, F. (1949)	Personal Communication, May 9, 1949.
GILLIES, D. (1948)	*Annual Medical Report, 1947.*
Idem (1949)	*Ibid., 1948.*
Idem (1950)	*Ibid., 1949.*
GOELARSO (1934)	*Geneesk. Tidjschr. Ned.-Ind.*, **74**, pp. 1345–1352, Batavia.
GREEN, R. (1934)	*Rep. Inst. Med. Res., F.M.S., 1933*, pp. 75–109.
HACKETT, L. W., RUSSELL, P. F., SCHAREF, J. W., and SENIOR WHITE, R. (1938).	*Bull. Hlth. Orgn. League of Nations*, **7**, Extract No. 36, p. 1038.
HEILBRUNN, A. (1948)	Personal Communication, Apr. 30, 1948, Sintang.
HEIM, F. (1948)	*Report on a second visit to Ulu Kinabatangan*, Sandakan.
HEWITT, J. (1905)	*Report of Sarawak Museum*, pp. 7–8, Kuching.
HODGKIN, E. P. (1933)	*Bull. Inst. Med. Res., F.M.S.*, No. 1.
Idem (1935–41)	*Ann. Reps. Inst. Med. Res. Malaya*, Kuala Lumpur.
HOOPS, A. L. (1921)	Report on visit to Brunei.
HUTCHINSON, W. J. (1930)	Sarawak Health Dept. Annual Report, Appendix XIX, Kuching.
JOHNSON, A. W. (1949*a*)	Personal Communication, Jan 25, 1949, Kuala Belait.
Idem (1949*b*)	*Ibid.*, Mar. 8, 1949, Kuala Belait.
Idem (1949*c*)	*Ibid.*, Jul. 14, 1949, Kuala Belait.

JOHNSON, A. W. (1949*d*)	Personal Communication, Aug. 18, 1949, Kuala Belait.
Idem (1950)	*Ibid.*, Jan. 3, 1950, Kuala Belait.
KARIADI (1938)	*Geneesk. Tidjschr. Ned.-Ind.*, **74**, No. 21.
KUEHLEWEIN, VON (1929)	*Meded. D. V. G.*, *1930*, No. 1.
McARTHUR, J. (1938)	*A Study of Malaria in the Far East*, North Borneo Govt.
Idem (1939)	*Report on a Malaria Survey of Jesselton*. Report to North Borneo Govt., Jesselton, 1939.
Idem (1940-1)	*Monthly Reports of Malaria Research Dept.*, Tambunan.
Idem (1944)	Unpublished records made during Japanese occupation, Kuching.
Idem (1946)	*Malaria Transmission in Borneo*, *Lancet*, July 27, 1946, p. 117, London.
Idem (1947*a*)	'The Transmission of Malaria in Borneo.' *Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, 5, pp. 537–558.
Idem (1947*b*)	*Monthly Report of Malaria Research Headquarters*, July 1947, Sandakan.
Idem (1947*c*)	*A Malaria Survey of East Coast of North Borneo*. Report to North Borneo Govt., Sandakan.
Idem (1948)	*Malaria in Borneo. Report on the Work of the Malaria Research Dept., North Borneo, 1939–42*. Cyclostyled by Colonial Office, London.
Idem (1949)	*Annual Report on the Work of Malaria Research Dept., 1948*, Labuan.
Idem (1950*a*)	*Annual Report on the Work of Malaria Research Dept., 1949*, Labuan.
Idem (1950*b*)	Malaria and its Vectors in Borneo. *Ind. J. Med.*, **4**, pp. 1–90.
MACHSOES, M. (1939)	*Geneesk. Tidjschr. Ned.-Ind.*, **79**, pp. 2500–15.
MOULTON, J. C. (1914)	*13th Report of the Sarawak Museum*. Govt. Printing Office, Kuching.
NORTH BORNEO (1929)	*Report to N. B. Govt. on larval surveys of Sandakan, Keningau and Tambunan*, by Anti-Malarial Field Assistant, Sandakan.
Idem (1931)	*Report to N. B. Govt. on larval surveys of Tawau*, by Anti-Malarial Field Assistant, Sandakan.
O'CONNOR, M. P. (1941)	Personal Communication, July 2, 1941, Kuching.
OU, KOK BOU (1941)	*Ibid.*, Apr. 5, 1941, Labuan.
OVERBEEK, J. G. (1941*a*)	*Ibid.*, Aug. 16, 1941, Batavia.
Idem (1941*b*)	*Ibid.*, Aug. 30, 1941, Batavia.
Idem (1941*c*)	Personal Communication destroyed during the war.
OVERBEEK, J. G., and STOKER, W. J. (1937).	*Malaria in the N.E.I. and its control*, Batavia.
RAMSAY, G. C., CHANDRA, S. N., and LAMPRELL, B. A. (1936).	*Rec. Mal. Surv. Ind.*, **6**, pp. 49–52.

REID, J. A. (1949) ...	*Proc. Roy. Ent. Soc. Lond.*, **18**, pp. 42–53.
RODENWALDT, E. (1926)	*Geneesk. Tidjschr. Ned.-Ind.*, **66**, pp. 787–799, Batavia.
ROPER, R. (1914) ...	*Bull. of Ent. Research*, **5**, Pt. 2, pp. 137–147.
RUTLEDGE, N. H. (1947)	Personal Communication. Nov. 10, 1947, Jesselton.
SARAWAK (1930–35)	*Govt. Annual Medical Reports*, Kuching.
SCHARFF, J. W. (1927)	*Malayan Med. J.*, **2**, pp. 88–94.
SHIRCORE, J. O. (1937)	*Report on native health, British North Borneo*, Govt. Press.
SOESILO *et al.* (1932)	*Geneed. Dienst. Volksgezondh. Ned.-Ind.*, **18**, pp. 102–111. Batavia.
Idem (1933) ..	*Ibid.*, **22**, pp. 99–120.
Idem (1935) .	*Afdeeling Malariabestrijding. Ned.-Ind.*, **24**, 4, pp. 386–402, Batavia.
STOKER, W. J. (1931)	*Geneesk. Tidjschr. Ned.-Ind.*, **71**, 14, pp. 1200–1209, Batavia.
Idem (1934) .	*Ibid.*, **74**, 21, pp. 1342–1344.
Idem (1949)	Personal Communication, Feb. 8, 1949.
STOOKES, V. A. (1924) .	Notes on the Control of Malaria in Sarawak. *Trans. 5th Congr. F.E.A.T.M.*, pp. 38–45.
Idem (1927) .	*Trans. 7th Cong. F.E.A.T.M.*, held in India, **3**, pp. 103–115.
Idem (1941)	Personal Communication, May 24, 1941.
STRAHAN, J. H. (1947) .	*Med. J. Malaya*, **2**, 2, pp. 83–92.
SUKARMAN bin A. NASRAN (1948)	Personal Communication. June 12, 1948, Labuan.
SWELLENGREBEL, N. H. (1921)	*De Anophelinen van Nederlandsch Oost-Indie*, Amsterdam, 2nd Edn., 1921.
SWELLENGREBEL, N. H., and RODENWALDT, E. (1932).	*Die Anopheles von Niederlandisch-Ostindien*, Jena.
VENHUIS, W. G. (1940) ..	*Geneesk. Tidjschr. Ned.-Ind.*, **80**, pp. 27–43.
WALCH, E. W. (1932) ...	*Ibid.*, **72**, pp. 682–709.
WALCH, E. W., and SARDJITO, M. (1928).	*Ibid.*, **68**, pp. 247–268.
WALCH, E. W., and SOESILO, R. (1929).	*Geneed. Dienst. Volksgezondh. Ned.-Ind. Foreign Edn.*, **18**, pp. 193–207, Batavia.
WALCH, E. W., and WALCH-SORODRAGER, B. (1921).	*Med. Barg. Ned.-Ind.*, **1**, pp. 2–47.
WALLACE, E. B. (1949) ...	*Report of a tour in the Lower Rejang.* Sarawak Govt., Kuching.
WEBSTER, F. L. (1941*a*) ...	*Report on an outbreak of malaria associated with* A. sundaicus *showing differing breeding habits.* Personal Communication. Oct. 27, 1941, Miri.
Idem (1941*b*) .	Personal Communication, June 30, 1941, Miri.
Idem (1941*c*) ..	*Ibid.*, Aug. 12, 1941, Miri.
Idem (1941*d*) ...	*Ibid.*, Oct. 5, 1941, Miri.
Idem (1941*e*) .	*Ibid.*, Oct. 27, 1941, Miri.
Idem (1947) .	*Ibid.*, Oct. 12, 1947, Serin.

APPENDIX Ia.

Recorded distribution of A. leucosphyrus *in Borneo.*

Roper (1914)
Membakut, N.B.

Swellengrebel (1921)
Balikpapan, D.B., Tandjong Redeb and Moeara Tewe.

Stookes (1924)
Lutong, Sarawak.

Stookes (1927)
Miri, Sarawak.

Dingle (1929)
Jesselton, N.B., Sandakan, N.B.

von Kuehlewein (1929)
Upper Mahakam River.

Watch and Seesile (1929)
Tandjong Palas, Boeloengan.

Hutchinson (1930)
Kuching District, Sarawak.

Stoker (1931)
Sanggau, D.B.

Swellengrebel and Rodenwaldt (1932)
Tandjong Redeb, D.B., Tandjong Selor, D.B., Tandjong Palas, D.B., Tandjong Djoetar, D.B., Sanggau, D.B., Balikpapan, D.B., Samarinda, D.B., Tenggarong, D.B., Moeara Tewe, D.B., Ober Mahakam, D.B., Sekejam, D.B., Tajan, D.B., Melian, D.B., Tarakan, D.B., Sekedau, D.B., Nangkatajap, D.B.

Stoker (1934)
Poeloe Laoet, D.B.

Soelarso (1934)
Tandjong Palas, D.B., Tandjong Selor, D.B., Sadjau River, D.B., Binai River, D.B., Kajan River, D.B., Boeloengan River, D.B., Bahau-Poedjoengan River, D.B., Kg. Moeara Pangean, D.B.

Shircore (1937)
Tenom, N.B., Kemabong, N.B., Melalap, N.B., Keningau, N.B., Purutan, N.B.

Kariadi (1938)
Martapoera, S.E.B.

Eykman Institute (1938)
Mampawah, D.B., Bankajang, Tajan, Sanggau, Melian, Sekedau, Nangka Tajap, Boven Loop Mahakam, Kota Baroe, Setagen, Balikpapan, Tenggarong, Samarinda, Longnawan-Tarakan, Pontianak, Sambas.

Brunei (1932)
Brunei, Kuala Belait, Brunei, Tutong Road Area, Brunei.

Sarawak (1931-5)
Kuching, Kuching, Simanggan Road, Bau, Ulu Bau, Ulu Sadong, Miri.

Ou (1941)
Labuan.

Webster (1941a)
Miri, Sarawak, Jerudong, Brunei.

McArthur (1939)
Jesselton, N.B., Tambunan, N.B.

McArthur (1940)
Apin Apin, N.B., Purutan, N.B.

Carlile (1941)
Sandakan Area, N.B., Tawau Area, N.B.

English (1945)
Balikpapan, Tarakan, Labuan, Brunei.

Allman (1945)
Brunei, Seria, Limbang, Lutong, Labuan, Beaufort.

Rutledge (1947)
Jesselton.

Heilbrunn (1948)
Sintang, Sanggau, Ketapang, Ngabang, Bengkjang, Janggal, Sambas, Singkawang, Mempawah.

McArthur (1947b)
Sandakan.

McArthur (1947c)
Lahad Datu.

Heim (1948)
Kinabatangan River.

Colless (1948a)
Brunei, Labuan, Jesselton, Seria (*A. leucosphyrus pujutensis*), Pujut (*A. leucosphyrus pujutensis*), Beaufort (*A. leucosphyrus pujutensis*).

APPENDIX Ia—concld.

Clapham (1948)
Kuching.

Gillies (1948)
Labuan.

Johnson (1949*b*)
Miri (*pujutensis*).

Colless (1949)
Labuan (*A. leucosphyrus leucosphyrus*), Labuan (*A. leucosphyrus pujutensis*).

APPENDIX IIa.

Recorded distribution of A. maculatus *in Borneo.*

Hewitt (1905)
? (Borneo, not Sarawak).

Roper (1914)
Membakut, N.B.

Dingle (1929)
Jesselton, N.B.

North Borneo (1929)
Sandakan, Keningau, Tambunan.

Sarawak (1930)
Quop, Kuching, Sarawak, Leper Camp, Satang Island.

Sarawak (1931)
Bau.

Sarawak (1932)
Agricultural Gdns., Kuching.

Sarawak (1933)
Kg. Skrak, Sarawak.

Sarawak (1934)
Lawas, Sarawak.

Swellengrebel and Rodenwaldt (1932)
Ober Mahakam, D.B.

Stoker (1934)
Poeloe Laoet.

Goelarso (1934)
Bahau-Poedjoengan River, Boeloengan, Kajan River.

Kariadi (1936)
Martapoeara.

Shircore (1937)
Tenom District:
Tenom, Melalap, Kemabong, Pahl, Tendaran.
Keningau District:
Mentailan, Agudun, Dangalud, Kadulakang, Keningau, Masak, Tuarid.
Tambunan District:
Tambunan, Bahru, Pomttodon, Nambaian, Dar, Mogong, Papar, Tuntuhu, Karanan, Lintun, Timbau, Mansangoh, Kerokot, Tendagong, Bundu Tuhan, Matan, Maraku, Latud, Kota Belud.

Brunei (1938)
Brunei ('outside sanitary board area').

Eykman Institute (1938)
Boven Loop Kajan, D.B., Boven Loop Mahakam, D.B., Longnawan, D.B., Kota Baroe, D.B., Sacking.

Webster (1941)
Jerudong, Brunei.

Ou (1941)
Pulau Da'at, N.B.

Carlile (1941)
East Coast.

Stookes (1941)
Sandakan.

McArthur (1946)
Jesselton, Tambunan Plain, Sunsurun, N.B., Purutan, N.B.

Allman (1945)
Labuan.

Colless (1948*a*)
Labuan.

Heilbrunn (1948)
Mempawah, D.B., Singkawan, D.B., Ngbang, D.B., Sanggau, D.B.

Gillies (1949)
Labuan.

APPENDIX IIIa.

Recorded distribution of the A. umbrosus *group in Borneo.*

Recorded as '*A. umbrosus*' (IDENTIFICATION UNCERTAIN).

Eykman Institute (1938)
Teloek Bajoer, Bandjermasin, Tanah Grogot, Balikpapan, Koboe, Tini.

Webster (1941*a*)
Jerudong, Brunei, Bukit Sawat, Brunei, Matading, Sarawak, Miri, Sarawak.

Carlile (1941)
Sandakan Area, N.B., Tawau, N.B.

Allman (1945)
Kuala Belait.

Webster (1947)
Kuala Belait, Seria, Lutong.

Rutledge (1947)
Jesselton.

Heilbrunn (1948)
Singkawang, Sanggau, Benkanjang, Ngabang, Menpawah, Pontianak, Sekadau, Ketapang, Sedjiran, Poetoessibau, Sintang, Nanga Pinoh.

Wallace (1949)
Kuala Matu, Sarawak.

Recorded as '*A. novumbrosus*' (PRESUMABLY *A. umbrosus*).

Sarawak (1931)
Miri.

Swellengrebel and Rodenwaldt (1932)
Sekejam.

Brunei (1938)
Brunei.

Webster (1941*a*)
Miri, Lutong, Bukit Sawat, Brunei.

Allman (1945)
Kuala Belait, Seria.

English (1945)
Balikpapan.

Heilbrunn (1948)
Sintang.

Recorded as '*A. leiifer*'.

Colless (1948*c*)
Seria, Kuala Belait Road.

Johnson (1949*b*)
Kuala Belait, Seria, Miri.

Colless (1949)
Labuan.

Recorded as *A.* sp. *B.*

Reid (in the press)
Membakut.

Colless (1948*a*)
Kuala Belait.

Colless (1948*c*)
Seria, Kuala Belait Road.

Johnson (1949*a*)
Kuala Belait, Seria.

Johnson (1949*c*)
Kuala Lumut.

Recorded as '*A. umbrosus*' (IDENTIFICATION RELIABLE).

Roper (1914) (Reid, in the press)
Membakut, N.B.

Colless (1948*a*)
Kuala Belait.

Colless (1948*c*)
Seria, Kuala Belait Road, Seria.

Colless (1948*d*)
Kg. Loak, Miri, Pangkalan Gdns., Lutong.

Johnson (1949*b*)
Kuala Belait, Seria, Miri, Lutong.

Recorded as '*A. umbrosus*' (IDENTIFICATION UNCERTAIN).

Swellengrebel (1921)
Balikpapan, D.B.

Stookes (1927)
Miri, Sarawak.

Scharff (1927)
Labuan.

Dingle (1929)
Jesselton, N.B.

North Borneo (1929)
Sandakan, N.B.

APPENDIX IIIa—concld.

North Borneo (1931)
Tawaii.

Sarawak (1931)
Kuching.

von Kuehlewein (1929)
Ober Mahakam, D.B.

Walch and Soesilo (1929)
Tg. Djoeata, D.B.

Hutchinson (1930)
Quop (Kuching Area). Sarawak.

Brooke (1930)
Satang Island.

Stoker (1931)
Sanggau, D.B.

Swellengrebel and Rodenwaidt (1932)
Sanggau, Simpang, D.B. (? Sintang), Nangkatajap, Balikpapan, Koeala Sambodja, Sekadau, Tg. Redeb, Tenggarong, Tarakan, Ober Mahakam, Tini, Tg. Selor, Sakajam.

Brunei (1932)
Brunei.

Sarawak (1932)
Bau, Sarawak, Miri, Sarawak.

Goelarso (1934)
Sadjau, D.B.

Overbeek and Stoker (1937)
Sungei Kakap, D.B.

Shircore (1937)
Tenom, N.B.

Kariadi (1938)
Martapoerta, D.B.

Eykman Institute (1938)
Mampawah, Benkajang, Kakap, Pontianak, Tajan, Sanggau, Meliau, Sekadau, Nangkatajap, Sintang, Longnawan, Boven Loop Mahakam, Boven Loop Kajan, Semblimbingan, Sambodja, Tenggarong, Samarinda, Tg. Redeb, Sadjau, Tarakan.

APPENDIX IVa.

Recorded distribution of A. sundaicus *in Borneo.*

Roper (1914)
Membakut (*A. ludlowi*).

Stookes (1927)
Miri (*A. ludlowi*).

Scharff (1927)
Labuan (*A. ludlowi*).

Dingle (1929)
Jesselton (*A. ludlowi*).

North Borneo (1929)
Sandakan.

North Borneo (1931)
Tawain.

Walch and Soesilo (1929)
Koeala Sambodja (a local variation).

Sarawak (1931)
Kuching (*A. ludlowi*).

Sarawak (1933)
Santubong.

Sarawak (1934)
Miri.

Brunei (1938)
Kuala Belait.

Webster (1941)
Miri, Matading.

Ou (1941)
Labuan.

Carlile (1941)
East Coast.

McArthur (1939)
Jesselton (*A. litoralis*).

Webster (1941*a*)
Miri, Lutong, T. Lobang, Loak-Bakan Area, Bintulu, Matading.

English (1945)
Balikpapan.

APPENDIX IVa—concld.

Rutledge (1947)
Jesselton.

Allman (1945)
Kuala Belait-Seria, Labuan, Papar.

Colless (1948*a*)
Kuala Belait, Seria, Baram River, Labuan.

Heilbrunn (1948)
P. Lemockoetan.

Heim (1948)
Kinabatangan River.

Gillies (1948)
Labuan.

Webster (1947)
Seria, Kuala Belait, Lutong.

Strahan (1947)
Pujut.

Johnson (1949*b*)
Kuala Belait, Miri, Seria, Lutong, Kg. Gana, Kuala Lumut, Kg. Lumut, Kg. Loak, Sungei Krokop.

APPENDIX Va.

Recorded distribution of A. baezai *in Borneo.*

Brunei (1938)
Brunei.

Brunei (1935)
Kuala Belait.

Sarawak (1935)
Kuching, Miri.

Webster (1941)
Lutong, Miri, Matading, Sarawak.

Ou (1941)
Labuan.

Clapham (1948)
Kuching.

English (1945)
Balikpapan.

Allman (1945)
Brunei, Kuala Belait-Seria, Lutong, Labuan, Kimanis, Papar.

Gillies (1948)
Labuan.

Webster (1947)
Seria, Kuala Belait, Lutong.

Colless (1947)
Sandakan.

Johnson (1949*b*)
Kuala Belait, Seria, Miri, Lutong.

Colless (1948*a*)
Kuala Belait, Brunei, Lutong, Labuan, Kimanis.

Wallace (1949)
Sarekei, Kg. Sai.

APPENDIX VIa.

Recorded distribution of A. separatus *in Borneo.*

Roper (1914)
Membakut.

Moulton (1914)
Sarawak.

Stookes (1927)
Miri.

Scharff (1927)
Labuan.

Sarawak (1931)
Kuching, Miri.

Sarawak (1934)
Bau.

APPENDIX VIa—concld.

Swellengrebel and Rodenwaldt (1932)
Amoentai.

Brunei (1935)
Brunei, Kuala Belait.

Webster (1941*b*)
Jerudong, Lutong, Bukit sawat and Brunei, Matading, Miri.

Ou (1941)
Labuan.

Carlile (1941)
East Coast.

McArthur (1942)
Jesselton.

English (1945)
Balikpapan.

Rutledge (1947)
Jesselton.

Allman (1945)
Brooketon, Brunei, Kuala Belait-Seria, Labuan.

Webster (1947)
Seria, Kuala Belait, Lutong.

Colless (1948*a*)
Brooketon, Brunei, Lambang, Kuala Belait, Labuan, Jesselton, Papar.

Heilbrunn (1948)
Singkawang, Nagabang, Pontianak.

Gillies (1948)
Labuan.

Johnson (1949*b*)
Seria, Kuala Belait, Miri-Lutong.

Colless (1948*c*)
Kuala Belait-Seria.

Colless (1948*d*)
Miri-Lutong.

Wallace (1949)
Selalang, Sarawak, Binatang Sarawak, Sarikei Sarawak, Kuala Matu, Sarawak Matu, Sarawak, Kg. Tien, Sarawak.

APPENDIX VIIa.

Recorded distribution of the A. hyrcanus *group in Borneo.*

Recorded as *A. hyrcanus*.

Swellengrebel and Rodenwaldt (1932)
Sanggau, Singkawang, Barabai, Kandangan, Bandjermasin, Tajan, Tenggarong, Tandjong Redeb, Sangkoelirang, Balikpapan, Tarakan, Obere Mahakam, Nangatajap.

Brunei (1935)
Brunei, Kuala Belait.

Allman (1945)
Brooketon, Kuala Belait-Seria, Seria.

Webster (1947)
Kuala Belait, Lutong, Miri, Kuala Belait-Seria.

Johnson (1949*a–d*)
Miri-Lutong, Selalang, Lower Rajang.

Wallace (1949)
Binatang, Sacakei, Kuala Matu, Sungei Sok.

Gillies (1949)
Labuan.

Recorded as '*A. hyrcanus sinensis*'.

Swellengrebel (1921)
Tandjong Redeb.

Stookes (1924)
Lutong, Miri, Pujut.

Scharff (1927)
Labuan.

Dingle (1929)
Jesselton.

North Borneo (1929)
Sandakan.

Sarawak (1931)
Kuching.

Sarawak (1932)
Bau.

APPENDIX VIIa—concld.

Sarawak (1933)
Sibu.

Sarawak (1934)
S. Tengah Estate, 38th–40th Mile.

Stoker (1934)
Pueloe Laoet.

Kariadi (1938)
Martapoera.

Webster (1941*b*)
Lutong, Miri, Matading.

Clapham (1948)
Kuching.

Allman (1945)
Kuala Belait-Seria, Labuan.

Heilbrunn (1948)
Pemangkat, Singkawang, Mempawah, Ngabang.

Gillies (1948)
Labuan.

Johnson (1949*b*)
Kuala Belait, Miri, Seria, Lutong.

Recorded as '*A. hyrcanus nigerrimus*'.

Clapham (1948)
Kuching.

English (1945)
Balikpapan.

Allman (1945)
Labuan.

Colless (1948*a*)
Brooketon, Labuan, Papar, Jesselton.

Gillies (1948)
Labuan.

Johnson (1949*b*)
Kuala Belait, Miri, Seria, Lutong.

Recorded as '*A. hyrcanus* N.'.

Heilbrunn (1948)
Singkawang, Mempawah, Sintang, Nanga Pinoh.

Recorded as '*A. hyrcanus* Coastal Form' (Subsp. *nr. sinensis*).

Colless (1948*a*)
Kuala Belait.

Colless (1948*c*)
Seria.

Colless (1948*d*)
Kg. Loak, Kg. Senadin, Pankalan Gdns., Lutong.

Colless (1949)
Labuan.

Recorded as '*A. hyrcanus* C'.

Colless (1949*a*)
Labuan.

APPENDIX VIIIa.

Recorded distribution of A. barbirostris *in Borneo.*

Hewitt (1905)
Sarawak.

Roper (1914)
Membakut.

Stookes (1924)
Miri, Pujut.

Swellengrebel (1921)
Tg. Redeb.

Scharff (1927)
Labuan.

Dingle (1929)
Jesselton.

Swellengrebel and Rodenwaldt (1932)
Tg. Redeb, Sanggan, Malian, Samarinda, Tenggarong, Boeloengan, Bandjermasin, Rantau Pandjang.

Goularse (1934)
Tg. Selor, Tg. Palas, Seljan Kajan River, Bahau-Poedjoengan River.

Kariadi (1938)
Martapoera.

Brunei (1929)
Brunei Town.

Brunei (1936)
Kuala Belait.

APPENDIX VIIIa—concld.

Sarawak (1931)
Kuching, Kuching-Simanggang Road.

Sarawak (1932)
Bau, S. Tengah Estate.

Sarawak (1933)
Sibu.

Shircore (1937)
Tenom District, Malalap.

Webster (1941)
Jerudong, Lutong, Miri, Bukit Sawat, Brunei, Matading.

Ou (1941)
Labuan.

North Borneo (1929)
Sandakan, Keningau, Tambunan.

North Borneo (1931)
Tawaii.

Carlile (1941)
East Coast.

McArthur (1941)
Jesselton, Tambunan, Apin Apin, Menggatal.

Clapham (1948)
Kuching.

English (1945)
Balikpapan.

Rutledge (1947)
Jesselton.

Allman (1945)
Brooketon, Brunei, Lambang, Kilanis, Lutong, Labuan, Beaufort, Kimanis, Papar.

Colless (1948*a*)
Miri, Jesselton, Brooketon, Brunei, Limbang, Kilanis, Lutong, Beaufort, Papar.

Heilbrunn (1948)
Bonkajang, Mempawah, Ngabang, Sanggau, Ketapang, Sintang.

Gillies (1948)
Labuan.

Webster (1947)
Seria, Kuala Belait, Lutong.

Johnson (1949*a*)
Kuala Belait, Miri, Lutong.

Colless (1948*b*)
Labuan.

Colless (1949*a*)
Kg. Senadin.

APPENDIX IXa.

Recorded distribution of A. tessellatus *in Borneo.*

Swellengrebel (1921)
Balikpapan (*punctulatus*).

Swellengrebel (1921)
Tg. Redeb (*punctulatus*).

Dingle (1929)
Jesselton.

North Borneo (1929)
Sandakan.

Stoker (1931)
Sanggau.

Swellengrebel and Rodenwaldt (1932)
Sanggau, Singkawang, Kendangan, Samarinda, Tenggarong, Boeloengan, Mocara Tewe, Tandjong Redeb.

Goelarso (1934)
Tg. Selor, Tg. Palas, Sadjau, Kajan (*punctulatus tessellatus*).

Kariadi (1938)
Martapoera.

Webster (1941)
Jerudong, Bukit Sawat and Brunei, Lutong, Miri.

Ou (1941)
Labuan.

Brunei (1929–38)
Brunei Town, Kuala Belait.

Sarawak (1931–35)
Kuching, Bau.

*APPENDIX IXa—*concld.

Shircore (1937)
Miri, Tomani.

Carlile (1941)
East Coast.

McArthur
Tambunan.

Clapham (1948)
Kuching.

Allman (1945)
Kuala Belait-Seria, Labuan.

English (1945)
Balikpapan

Colless (1948*a*)
Kuala Belait, Labuan, Jesselton.

Heilbrunn (1948)
Sanggau (*A. punctulatus tessellatus*), Singkawang, Benkajang, Mempawah, Pontianak, Ngabang.

Webster (1947)
Seria, Kuala Belait, Lutong.

Johnson (1949*b*)
Kuala Belait, Miri, Seria.

Colless (1948*c*)
Seria-Kuala Belait Road.

Colless (1948*d*)
Kg. Senadin, Pangkalan Gdns.

APPENDIX Xa.

Recorded distribution of A. minimus flavirostris *in Borneo.*

Overbeek (1941*b*)
Kg. Semblimbingan, Kg. Tengah, Kg. Semboedoean.

APPENDIX Ib.

Recorded dissections of A. leucosphyrus *in Borneo.*

Reference and place.	Number dissected.	G+.	Gl+.	G+Gl+.	Total positive.
Roper (1914)					
Membakut	17	...	...	...	..
Sarawak (1931)					
Sarawak	4	...	...	...	...
Soesilo and others (1932)					
Sanggau	1	...	...	...	...
Stoker (1934)					
Sarang Tioeng	57	6	...	...	6
Stoker (1934)					
Pedamban	53	1	...	...	1

G+ = gut infected. Gl+ = salivary glands infected.

APPENDIX Ib—contd.

Reference and place.	Number dissected.	G+	Gl+	G+Gl+	Total positive.
Goolarse (1934)					
Sadjau River	5	...	...	...	...
Buai River	1	...	...	...	...
Kg. Moeara Pangean	13	...	...	...	1
McArthur (1940)					
Tambunan	41	...	...	...	...
Purutan	6	...	...	...	...
Apin Apin	34	...	...	...	...
Jesselton	2	...	...	...	...
McArthur (1941)					
Tambunan	639	7	13	...	19
Apin Apin	11	...	2	...	2
Purutan	13	...	1	...	1
Apin Apin	15	1	2	...	3
Webster (1941)					
Jerutong	28	...	...	...	...
Rutledge (1947)					
Jesselton	173	8	1	...	9
McArthur (1947*b*)					
Sandakan	12	1	...	...	1
Clapham (1948)					
Kuching	100+	1	...	...	1
Colless (1948*b*)					
Brunei	1	...	...	...	...
Labuan	6	...	...	...	...

G+ = gut infected. Gl+ = salivary glands infected.

APPENDIX Ib—concld.

Reference and place.	Number dissected.	G+	Gl+	G+ Gl+	Total positive.
Sukarman (1948)					
Labuan	6	...	...	...	...
Gillies (1948)					
Labuan	6	...	...	..	.
Gillies (1949)					
Labuan	133	1	...	..	1
McArthur (1949)					
Labuan	727	9	16	5	30
Gillies (1950)					
Labuan	21	..	...	..	.
McArthur (1950*a*)					
Labuan	1,109	6	8	1	15
TOTAL	3,235	41	43	6	90

G+ = gut infected. Gl+ = salivary glands infected.

APPENDIX IIb.

Recorded dissections of A. maculatus *in Borneo.*

Reference and place.	Total dissections.
Stoker (1934)	
Kg. Sarang Tioong	11
McArthur (1947*a*)	
Pambuan, 1940	28
Purutan ,,	1
Apin Apin ,,	2
Jesselton ,,	1
McArthur (1947*a*)—*concld.*	
Tambunan, 1941	138
Apin Apin ,,	1
Purutan ,,	3
Gillies (1950)	
Labuan	1
TOTAL	186

APPENDIX IIIb.

Recorded dissections of the A. umbrosus *group in Borneo.*

Reference and place.	Number dissected.	G+.	Gl+.	G+Gl+.	Total positive.	Per cent positive.
Recorded as *A. umbrosus* (probably true *A. umbrosus*).						
Roper (1914)						
Membakut, North Borneo	130	...	1		1	...
Recorded as *A. umbrosus* (identification uncertain).						
Sarawak (1932)						
Ban, Sarawak	...	...	...	...	...	...
Suesito and others (1932)						
Sunggau	889	2	1	1	3	0·4
Overbeek and Stoker (1937)						
Soengei Kakap ...	59	1	1	1	9	15·2
Webster (1941*b*)						
Bukit Sawai, Brunei	75	3	2	...	3	4·0
Jerudong, Brunei	13	...	...	...	...	...
Maradong, Sarawak ...	4	...	...			...
Rutledge (1947)						
Jesselton	50	4	...	...	4	...
Webster (1947)						
Seria, Brunei	206	...	...	...	...	...
Kuala Belait, Brunei	8	...	...	...	...	...
Miriong, Sarawak	16	...	...	...	...	...
Wallace (1949)						
Kuala Matu	7	...	...	...	...	...
Recorded as *A. umbrosus* (identification reliable).						
Colless (1948*b*)						
Kuala Belait	3	...	...	...	...	...
Colless (1948*c*)						
Seria-Kuala Belait Rd. ...	131	6	1	1	8	...
Seria	11	...	...	...	...	...

G+ = gut infected. Gl+ = salivary glands infected.

APPENDIX IIIb—concld.

Reference and place.	Number dissected.	G+.	Gl+.	7+Gl+.	Total positive.	Per cent positive.
Recorded as *A. umbrosus* (identification reliable)—*concld.*						
Colless (1948*d*)						
Kg. Loak, Miri	8	...	...	...	...	...
Pangkalan Gdns., Lutong	2		...	...	...	...
Johnson (1949*a*)						
Kuala Belait-Seria	209	8	3	...	11	...
Miri and Lutong	5		...	...	...	...
Johnson (1950)						
Miri-Lutong	167	5	...	...	5	...
Kuala Belait-Seria	145	...	...	...		...
Recorded as *A. novumbrosus* (presumably *A. umbrosus*).						
Webster (1941*b*)						
Bukit Sawat and Brunei	7	...	...	...	...	...
Colless (1948*b*)						
Kuala Belait	5	...	...	...		
Recorded as *A. latifer*.						
Colless (1948*c*)						
Seria-Kuala Belait Rd.	1	...	...	...		...
Johnson (1949*a*)						
Kuala Belait-Seria	27	...	1	...	1	...
Johnson (1950)						
Miri-Lutong	4	...	...			
Kuala Belait-Seria	183	2	...	...	2	
Recorded as *A. sp. B.*						
Colless (1948*c*)						
Seria-Kuala Belait Rd.	4	1	...	...	1	...
Johnson (1949*a*)						
Kuala Belait-Seria	11	...	...	...		
Johnson (1950)						
Kuala Belait-Seria	26	2	...	...	2	...
TOTAL *A. umbrosus* group ...	2,914	31(1)	8(1)	1	50	...

G+ = gut infected. Gl+ = salivary glands infected.

APPENDIX IVb.

Recorded dissections of A. sundaicus *in Borneo.*

Reference and place.	Number dissected.	G+.	Gl+.	Total positive.
Recorded as *A. indiana.*				
Stookes (1927)				
Miri	22	3	.	3
Scharff (1927)				
Labuan	1	...	..	...
Recorded as *A. litoralis.*				
McArthur (1947)				
Jesselton	30	...		.
Menggatal	1	..		.
Recorded as *A. sundaicus.*				
Webster (1941*b*)				
Matading	261		.	..
Miri	52	.	.	...
Rutledge (1947)				
Jesselton	50	.		.
Webster (1947)				
Seria	5	1	.	1
Kuala Belait	31	3		3
Lutong	25	2	.	2
Sukarman (1948)				
Labuan	23	1	.	...

G+ = gut infected. Gl+ = salivary glands infected.

APPENDIX 1/b—concld.

Reference and place.	Number dissected.	G+.	Gl+.	Total positive.
Recorded as *A. sundaicus*—*concld.*				
Colless (1948*b*)				
Kuala Belait ...	29	...	...	...
Jesselton	1	...	...	...
Gillies (1948)				
Labuan ...	14—	2	...	2
Gillies (1949)				
Labuan	430	...	...	.
Johnson (1949*a*)				
Kuala Belait-Seria .	5	...	...	...
Miri and Lutong ..	235	...	...	..
McArthur (1949)				
Labuan	414	2	...	2
Colless (1949)				
Miri-Lutong .	1	...	...	...
Johnson (1950)				
Miri and Lutong .	1,597	2	3	5
Kuala Belait-Seria ..	520	1	7(13?)	8(14?)
Gillies (1950)				
Labuan	6	...	...	...
McArthur (1950*a*)				
Labuan	712	...	2	2
TOTAL .	4,485	17	12(18?)	29(35?)

G+ = gut infected. Gl+ = salivary glands infected.

APPENDIX Vb.

Recorded dissections of A. barzai *in Borneo.*

Reference and place.	Number dissected.	G+	Gl+	Total positive.
Webster (1941*b*) Matading, Sarawak	3	...	...	...
Webster (1947) Sera, Brunei ...	237	...	...	...
Webster (1947) Kuala Belait, Brunei	139	...	...	...
Webster (1947) Lutong, Sarawak	19	...	...	...
Sukarman (1948) Labuan ...	9	...	...	...
Clapham (1948) Kuching, Sarawak ...	1	...	...	...
Gillies (1948) Labuan ...	3	...	...	...
Gillies (1949) Labuan	3	...	...	...
Johnson (1949*a*) Kuala Belait-Seria	17	...	...	...
Miri-Lutong	1	...	...	...
Johnson (1950) Miri-Lutong ...	130	5	1	6
Colless (1948*d*) Pangkalan Gdns., Lutong	48	...	...	...
Johnson (1950) Kuala Belait-Seria ...	47	...	1	1
Gillies (1950) Labuan ...	23	...	...	...
McArthur (1950*a*) Labuan	14	...	...	...
TOTAL	693	5	2	7

G+ = gut infected. Gl+ = salivary glands infected.

APPENDIX VIb.

Recorded dissections of A. separatus in Borneo.

Reference and place.	Number dissected.	G+
Webster (1941*b*)		
Bukit Sawat and Brunei	[illegible]	[illegible]
Rutledge (1947)		
Jesselton	50	[illegible]
Webster (1947)		
Seria	8	[illegible]
Kuala Belait	16	[illegible]
Lutong	4	...
Sukarman (1948)		
Labuan	7	...
Colless (1948*b*)		
Brooketon	102	
Kuala Belait	124	...
Jesselton	24	...
Gillies (1948)		
Labuan	7	...
Colless (1948*c*)		
Seria, Kuala Belait Bai.	9	...
Colless (1948*d*)		
Kg. Senadin	15	...
Pangkalan Gdus., Lutong	22	...
McArthur (1949)		
Labuan	8	...
Wallace (1949)		
Lower Rajang	28	...
Johnson (1949*a*)		
Kuala Belait-Seria	125	...
Miri-Lutong	29	...
Gillies (1950)		
Labuan	3	...
McArthur (1950*a*)		
Labuan	9	...
Johnson (1950)		
Miri-Lutong	2,704	16
Kuala Belait-Seria	279	1
TOTAL	3,583	17

G+ = gut infected.

APPENDIX VIIb.

Recorded dissections of the A. hyrcanus *group in Borneo.*

Reference and place.	Number dissected.	G+.	Total positive.
Recorded as '*A. hyrcanus*'.			
Overbeek (1941*a*)			
Tanah Grogot	186	...	4
Webster (1947)			
Seria	8	...	...
Kuala Belait	61	...	...
Lutong	14		...
Miri	1	...	...
Colless (1948*b*)			
Kuala Belait	9	...	...
Gilles (1948)			
Labuan	66	2	2
Johnson (1949*a*)			
Kuala Belait-Seria	144	...	...
Miri and Lutong	90	...	...
Recorded as '*A. hyrcanus sinensis*' or '*A. sinensis*'.			
Stoker (1934)			
Poebo Lamet	32		...
Webster (1941*b*)			
Matoading	4	...	...
Gilles (1949)			
Labuan	4		
Wallace (1949)			
Kuala Matu	1	...	
Johnson (1950)			
Miri and Lutong	233	...	...
Kuala Belait-Seria	344	2	2
Recorded as '*A. hyrcanus nigerrimus*'.			
Ailman (1945)			
Labuan	5	...	...
Recorded as '*A. hyrcanus nigerrimus*'—*concld.*			
Colless (1948*b*)			
Labuan	68	...	...
McArthur (1949)			
Labuan	26	...	...
Wallace (1949)			
Kuala Matu	18	...	...
Sungei Sok	4	...	...
Gilles (1950)			
Labuan	18	...	...
McArthur (1950*a*)			
Labuan	100	...	...
Johnson (1950)			
Miri and Lutong	1	...	...
Recorded as '*A. hyrcanus* both *sinensis* and *nigerrimus*'.			
Clapham (1948)			
Kuching	100	...	...
Recorded as '*A. hyrcanus*' (Classified).			
Colless (1948*a*)			
Seria	2	...	...
Colless (1948*b*)			
Kg. Loak	1	...	...
Kg. Samadin	36	...	...
Pangkalan Gdns.	4	...	...
McArthur (1950*a*)			
Labuan	1	...	...
TOTAL *A. hyrcanus* group.	1,557	4	8

G+ = gut infected.

APPENDIX VIIIb.

Recorded dissections of A. barbirostris *in Borneo.*

Reference and place.	Number dissected.	G+.
Roper (1914)		
Membakut	3	...
Stookes (1927)		
Miri	3+	...
Sarawak (1932)		
Bau	[illegible]	...
Webster (1941)		
Bukit Sawat, Brunei ...	1	...
Jerudong	36	...
Allman (1945)		
Labuan	4	...
McArthur (1947)		
Pambunan, 1940 ...	26	...
Apin Apin	6	...
Jesselton	1	...
Menggatal	2	...
Pambunan, 1941 ...	483	...
Apin Apin, 1941	3	...
Webster (1947)		
Seria	7	...
Kuala Belait	5	...
Lutong	1	...
Sukarman (1948)		
Labuan	15	...
Clapham (1948)		
Kuching	50+	...
Colless (1948*b*)		
Labuan	4	...
Colless (1948*b*)		
Jesselton	4	1
Gillies (1948)		
Labuan	11	...
Gillies (1949)		
Labuan	14	...
Johnson (1949*a*)		
Miri and Lutong ...	19	...
McArthur (1949)		
Labuan	33	...
Colless (1948*d*)		
Kg. Senadin	4	...
Johnson (1950)		
Miri	157	1
McArthur (1950*a*)		
Labuan	30	...
Rutledge (1947)		
Jesselton	80	1
TOTAL ...	1,002	3

G+ = gut infected.

APPENDIX IXb.

Recorded dissections of A. tessellatus *in Borneo.*

Reference and place.	Number dissected.	G+.
Soesilo and others (1932)		
Sanggau	10	...
Webster (1941)		
Bukit Sawat, Brunei ..	1	...
Allman (1945)		
Labuan . ..	1	...
McArthur (1947)		
Tambunan	12	...
Webster (1947)		
Seria	2	...
Kuala Belait	9	...
Lutong .. .	1	..
Sukarman (1948)		
Labuan	10	...
Giapham (1948)		
Kuching	[illegible]	...
Colless (1948*b*)		
Kuala Belait	10	..
Colless (1948*c*)		
Seria, Kuala Belait Rd. ...	2	...
Colless (1948*d*)		
Kg. Senadin ...	14	...
Pangkalan Gdns. ...	5	...
Gillies (1948)		
Labuan	20	...
Gillies (1949)		
Labuan	8	...
Johnson (1949*a*)		
Kuala Belait-Seria ...	41	...
Miri, Lutong ...	7	...
McArthur (1949)		
Labuan	119	...
McArthur (1950*a*)		
Labuan	191	...
Johnson (1950)		
Miri and Lutong ...	450	1
Johnson (1950)		
Kuala Belait-Seria ...	87	...
TOTAL ...	1,000	1

G+ = gut infected.

APPENDIX Xb.

Recorded dissections of A. minimus flavirostris *in Borneo.*

Reference and place.	Number dissected.	Total positive.
Overbeek (1941*b*)		
Kg. Semblimbingan ..	2,137	1
Kg. Tengah	401	1
Kg. Semboelocan ...	232	...
TOTAL ...	2,770	2

APPENDIX XIb.

Recorded dissections of A. philippinensis *in Borneo.*

Reference and place.	Number dissected.
Allman (1945)	
Labuan	14
McArthur (1947)	
Tambunan, 1940	231
Apin Apin, 1940	2
Jesselton	1
Menggatal	58
Tambunan, 1941	274
Apin Apin, 1941	1
Rutledge (1947)	
Jesselton	25
Colless (1948*b*)	
Labuan	35
Gillies (1949)	
Labuan	33
McArthur (1949)	
Labuan	787
Gillies (1950)	
Labuan	64
McArthur (1950*a*)	
Labuan	361
TOTAL	1,880

APPENDIX XIIb.

Recorded dissections of A. kochi *in Borneo.*

Reference and place.	Number dissected.
Roper (1914)	
Membakut	8
Stookes (1927)	
Miri	+
Goelarso (1934)	
Tandjong Selor	13
Tandjong Palas	8
Webster (1941)	
Jerudong	10

+ = some dissections.

APPENDIX XIIb—concld.

Reference and place.	Number dissected.
Allman (1945)	
Labuan	2
McArthur (1947)	
Tambunan, 1940	237
Apin Apin	6
Menggatal	8
Tambunan, 1941	290
Sukarman (1948)	
Labuan	20
Clapham (1948)	
Kuching	?
Colless (1948*b*)	
Labuan	2
Gillies (1949)	
Labuan	22
McArthur (1949)	
Labuan	184
Johnson (1950)	
Miri and Lutong	11
McArthur (1950*a*)	
Labuan	54
TOTAL	875

? = some dissections.

Indian Journal of Malariology, 4, 4, December 1950.

VEGETABLE AND ESSENTIAL OILS AS MOSQUITO LARVICIDES: A REPORT OF SOME PRELIMINARY INVESTIGATIONS.

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[September 2, 1950.]

THIS investigation into the possibility of developing larvicides out of vegetable oils was prompted by the scarcity of crude oil and kerosene oil during the Second World War.

The compositions generally used for killing mosquito larvæ contain crude oil, kerosene oil and a toxic agent. A small proportion of vegetable oil—particularly castor oil—as well as surface active agents are also added to improve the spreading power of the mixture.

Although a considerable amount of work has been done to define the characteristics of an oil or oil mixture which would prove efficient as a larvicide, little work has been done to investigate if any of the indigenous vegetable oils can be used as the major constituent of such a formulation. Wats and Bharucha (1938) have studied the larvicidal properties of several indigenous vegetable oils when used in mixtures varying from 1 per cent to 'near saturation' with kerosene oil and found that the results were disappointing except in the case of cashew nut shell oil (Anacardium) when used in a mixture with 96 per cent of kerosene oil. Such a mixture would obviously be inapplicable under conditions of scarcity of kerosene oil.

THE MODE OF ACTION OF OILS ON MOSQUITO LARVÆ.

When oils were first suggested for use as larvicides, it was believed that they owe their action to the formation on the surface of the water of an inert film which prevents access of the larvæ to air. Freeborn and Atsatt (1918), Hacker (1925) and Murray (1936) showed that the action of oil is really many-sided and demonstrated that the oil actually penetrates the tracheal tubes of the larvæ.

The main factors that determine the suitability of an oil or oil mixture for use as a larvicide are that it should be (1) sufficiently light so as to float on the water, (2) capable of spreading rapidly and forming a permanent film, and (3) sufficiently fluid to be able to penetrate the tracheal tubes of the larvæ. Preferably the oil mixture should contain either naturally present or added materials

which are toxic to the larvæ. Incidentally, the added ingredients should be such that they would not volatilize readily.

The methods employed in the investigation of an oil or oil mixture should, therefore, determine the (1) specific gravity, (2) viscosity, (3) spreading and film-forming properties, and (4) toxicity. In this preliminary study, the simpler methods described by David (1942) were employed. The viscosity was expressed as a ratio of the time taken by the oil to flow through two fixed points in a vertically clamped 50 c.c. pipette to the time taken by a 50 : 50 mixture of glycerine and water taken as 100. The spreading pressure was gauged by competitive spreading against castor oil. The toxicity was evaluated as follows: A thoroughly cleaned porcelain dish of 30 cm. diameter was filled with water and a 3 oz. wide-mouthed glass bottle, whose bottom had been cut off, was placed in the centre. 0·5 c.c. of the oil under test was added to the water surface and allowed to form a film. Ten larvæ (Culex) of a uniform size were introduced into the wide-mouthed bottle which was then gently lifted up so that the larvæ were forced out into the water covered by the film of oil. After a period of 30 minutes, the larvæ were picked out by means of a pipette without bringing them in contact with the surface film of oil and were transferred to a beaker containing water from its natural habitat. A few shreds of algæ were added to the water in the beaker. Observations for signs of recovery at the end of 24 hours were recorded.

EXPERIMENTAL.

The oils from the following were investigated:

Arachis hypogæa (groundnut).
Bassia longifolia (iluppai).
Brassica juncea (mustard).
Melia azadirachta (neem).
Sesamum indicum (gingelly).
Calophyllum inophyllum (pinnakottai).
Pongamia glabra (pungam).
Anacardium occidentale (cashew nut shell).

With the exception of cashew nut shell oil, the other oils spread very rapidly and may be arranged in the following order of decreasing spreading power: pinnakottai, groundnut, kerosene, iluppai, crude oil, and cashew nut shell oil.

From the relative viscosities as compared to that of 50 per cent glycerine taken as 100, it will be seen that all the vegetable oils are too viscous according to the standard prescribed by David (*loc. cit.*) (Table I).

TABLE I.

	Relative viscosity.
Kerosene oil	84·8
Crude oil	94·4
Groundnut oil	319·2
Pungam oil	321·5
Neem oil	360·5
Pinnakottai oil	435·9
Castor oil	2,854·0

Penetration of the tracheal tubes of the larvæ was studied by the method described by Hacker (*loc. cit.*) and it was found that none of the vegetable oils was taken in. Emulsification of the oil with soap solution or admixture of the oil with 'surface tension reducers' did not make any difference.

Toxicity tests showed that the larvæ were quite undisturbed in water with a surface film of the vegetable oils, whereas in the control experiments with kerosene oil or malariol, the mortality was appreciable. The use of vegetable oils mixed with 'tricresol' up to a concentration of 3 per cent and the addition of surface tension reducers did not produce any kill amongst the larvæ.

Attention was, therefore, diverted to two members of the essential oil group, viz. pine oil and lemon grass oil. Both these oils are known as mosquito repellents and skin irritants. De and Subrahmanyan (1929) and Bose *et al.* (1949) have studied the possibility of employing emulsions of these oils as antiseptics.

These oils spread easily on the water surface and easily penetrate the larval trachea. When used at 0·5 c.c. for an area of 30 cm., both these oils effected 100 per cent mortality among the larvæ as against only 60 per cent caused by the same amount of malariol. Smaller volumes were therefore tried and it was found that 0·1 c.c. pine oil was as effective as 0·5 c.c. malariol. Since this small quantity of oil was unlikely to form a satisfactory film, it was considered desirable to study the behaviour of mixtures of the essential and vegetable oil or of emulsions of such mixtures with soap. Toxicity experiments were conducted with 0·5 c.c. of a mixture (1 : 4) of essential oil and vegetable oil or with 1·0 c.c. of an emulsion (1 : 4 : 5) of essential oil, vegetable oil and soap solution respectively, so that in all cases the effective concentration of the essential oil was 0·1 c.c. for an area of 30 cm. diameter.

TABLE II.

Used as	Essential oil.	For area of 30 cm. diameter.		Number of experiments.	Mortality of larvæ (average), per cent.	
		Total volume, c.c.	Effective volume of essential oil, c.c.		Experimental.	Malariol B at 0·5 c.c. /same area.
Without admixture	Lemon grass oil	0·5	0·5	4	100·0	62·6
Do.	Pine oil	0·5	0·5	4	100·0	62·6
Do.	Do.	0·1	0·1	14	69·3	61·0
Mixed with veg. oil (1 : 4)	Do.	0·5	0·1	19	35·0	59·0
Emulsion (1 : 4 : 5)	Do.	1·0	0·1	17	59·5	58·0

The results set forth in Table II show that the emulsion of the pine oil compares favourably with malariol B. A few field experiments were, therefore, conducted with emulsions of both pine oil and lemon grass oil. These emulsions were applied at doses sufficient to give effective concentrations of about 0·5 c.c. of the essential oil per square foot of surface area. Alternate cess pools were treated with 2 c.c. of malariol B. The number of larvæ and pupæ at the beginning of the experiment and at the end of 24 hours after application of the oil was computed by introducing a sampling pan 10–15 times at various parts of the cess pool.

The results set forth in Table III show that emulsions equivalent to 0·5 c.c. of the two essential oils were as effective as 2 c.c. of malariol per square foot area.

TABLE III.

Emulsion of oil used.	Total volume c.c./sq. ft.	Effective volume of essential oil, c.c./sq. ft.	Average mortality of larvæ.		Average mortality of pupæ.	
			Experimental.	Malariol.	Experimental.	Malariol.
Pine	5·0	0·5	48	50	40	30
	6·0	0·6	80	54	10	26
	6·0	0·6	78	50	10	30
Lemon grass	5·0	0·5	85	50	60	32
	5·0	0·5	80	60	40	30
	10·0	1·0	94	33	62	25

PHENOL LARVICIDES.

Knowles *et al.* (1941) have studied mixtures of sulfonated oils and cresols as larvicides and claim that they are specially useful in heavily polluted waters, stagnant pools and in relatively inaccessible places. Since sulfonated castor oil ('Turkey red oil') is a locally available product, it was considered desirable to conduct a few exploratory tests with a mixture of commercial cresol and this material. The toxicity was slightly less than that of malariol B as seen from the results in Table IV. But when pine oil was employed in place of tricresol, the toxicity showed an improvement.

TABLE IV.

Mixture used 0·5 c.c. per surface 30 cm. diameter.	Number of experiments.	Average mortality of larvæ.	
		Experimental mixture.	Malariol B.
Turkey red oil + 3 per cent cresol	12	48	60
Turkey red oil + 3 per cent pine oil	14	71	62

REDUCTION OF SURFACE TENSION.

Larvæ keep afloat because the spiracle and—to a lesser extent—the palmate hairs resist wetting by water. Reduction of the surface tension of the water would be expected to prevent the larvæ from keeping afloat. Russell and Rao (1941) found that anopheline larvæ cannot keep afloat if the surface tension is reduced below 27 dynes per square centimetre and that the requirements of culicine larvæ are not much different.

In this preliminary investigation no determinations of surface tension were undertaken but a few experiments were conducted to explore the possibility of killing larvæ by the addition of soap solution to the water. To a number of beakers containing the water from the natural habitat of the larvæ, a strong solution of soap was added to give concentrations ranging from 0·05 to 0·25 per cent of the water and thoroughly mixed up. The larvæ were then introduced and kept under observation. In another series of experiments the larvæ were first introduced into the water and then the soap solution was added. It was observed that the larvæ (Culex) could keep afloat with difficulty in a 0·125 per cent solution but were unaffected at lower concentrations. At a level of 0·25 per cent soap all the larvæ were drowned and killed.

The use of soap or other surface tension reducers suffers from the limitation that it can be used only for shallow collections of water since the quantity required is determined by the total volume of the water and not—as in the case of oil—on the surface exposed. Nevertheless, this measure also appears to deserve further study especially in the direction of investigating the possibility of using soaps prepared from cheap materials such as fish oil and the possibility of incorporating pine oil or lemon grass oil in the soap solution.

DISCUSSION.

It must be emphasized that this work is of a preliminary and exploratory character and that more work under controlled conditions is necessary before definite conclusions are drawn. Nevertheless, it can be concluded from this study that:

(1) Vegetable oils are unable to penetrate the tracheal tubes of the larvæ or to effect mortality among larvæ when used in their natural state or with admixture of cresol or after emulsification with soap and hence cannot form the major constituent of a larvicidal mixture. They are, however, able to spread rapidly and may, therefore, find a useful place as adjuncts in larvicidal mixtures.

(2) On the other hand, the essential oils proved very satisfactory as judged by the toxicity tests in the laboratory. Emulsions of these oils with vegetable oils and soap appeared to be superior to malariol B as judged by a few field trials on Culex larvæ.

A difficulty encountered in the course of this work was that the toxicities of different samples of the same essential oil were not consistent. Recent work of Bose *et al.* (1950) has also shown the wide variations in the antiseptic properties of commercial samples of lemon grass oil. These workers have shown that the antiseptic property of an emulsion of lemon grass oil depends on the (1) citral

content of the oil, (2) the efficiency of emulsification, and (3) the type and proportion of the emulsifying agent used. They have also studied the methods of preventing the decline (which is due to the auto-oxidation of the citral) in the antiseptic power of the oil.

The promising results indicated in this study would make it worth while to further investigate the subject particularly in the light of the information made available by Bose *et al.* (1950).

The experiments with Turkey red oil admixed with pine or lemon grass oil are too few to warrant any definite conclusions but they suggest the advisability of pursuing this line of investigation.

This work could not be pursued because of the author's transfer and it is hoped that the publication of the results of this preliminary study will stimulate further work in this field.

SUMMARY.

It has been shown that films formed with common vegetable oils alone or in admixture with tricresol or with tricresol and soap are unable to kill mosquito larvæ. On the other hand, oils of pine and lemon grass are very effective when used either by themselves or in the form of emulsion, and thus appear worthy of a closer study in the light of the recent work on factors affecting their antiseptic properties.

ACKNOWLEDGMENT.

The author wishes to place on record his thanks to Dr. C. G. Pandit for his keen interest in this work.

REFERENCES.

BOSE, S. M., BHIMA RAO, C. N., and SUBRAHMANYAN, V. (1949). *J. Sci. Ind. Res.*, **8**, B, pp. 157–160.

Idem (1950) *Ibid.*, **9**, B, pp. 37–42.

DAVID, W. A. L. (1942) *Bull. Ent. Res.*, **33**, pp. 195–203.

DE, P. K., and SUBRAHMANYAN, V. (1929). *Ind. J. Med. Res.*, **17**, pp. 1153–1159.

FREEBORN, S. B., and ATSATT, R. F. (1918). *J. Econ. Ent.*, **11**, pp. 299–308.

HACKER, H. P. (1925) ... *Malaria Bureau Reports, Fed. Malay States*, **3**, pp. 1–62.

KNOWLES, F. L., PARKER, W. V., and JOHNSON, H. A. (1941). *Pub. Health Reports*, Washington, **56**, pp. 1637–1641.

MURRAY, D. R. P. (1936) ... *Bull. Ent. Ind.*, **4**, pp. 263–296.

RUSSELL, P. F., and RAO, T. R. (1941). *J. Mal. Inst. Ind.*, **4**, pp. 263–296.

WATS, R. C., and BHARUCHA, K. H. (1938). *Ibid.*, **1**, pp. 217–219.

Indian Journal of Malariology, 4, 4, December 1950.

ACTIVITY OF BROMO ANALOGUE OF PALUDRINE (BROMOGUANIDE)* AGAINST AVIAN AND SIMIAN MALARIAS.

BY

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[October 10, 1950.]

DURING the course of systematic work on the synthesis of new antimalarials, Bami and Guha (1949) studied a number of new substituted biguanide derivatives which included certain isomers and analogues of paludrine. Of these, a number of representative compounds having different types of chemical structures have been screened against avian and simian malarias with varying results (Jaswant Singh *et al.*, 1949 ; Bami *et al.*, 1949). These studies revealed that the para-bromo-analogue of paludrine termed as bromoguanide was an active substance. The encouraging results obtained with this compound have already been indicated by Jaswant Singh (1949) and the present paper embodies the details and further observations.

CHEMICAL NAME, FORMULA AND PROPERTIES.

Chemical name.—

N^1-p-bromophenyl-N^5-isopropyl biguanide (hydrochloride salt).

Formula.—

NH-C-NH-C-NH-CH $(CH_3)_2$ HCl NH^{II} NH^{II}

* The quantities of bromoguanide hydrochloride sufficient for these trials were specially prepared by Dr. H. L. Bami in the Chemical Laboratories of the Malaria Institute of India, Delhi, in continuation of his previous work at the Indian Institute of Science, Bangalore, as a Research Fellow of the Indian Council of Medical Research.

Properties.—Bromoguanide is similar to proguanil (paludrine) with the difference that the chlorine atom at the paraposition of the phenyl ring has been replaced by bromine. The hydrochloride salt of bromoguanide which is a white crystalline powder (melting point 245°C.) has been used in these studies. It is sparingly soluble in water and has a base content of nearly 89·2 per cent.

METHODS AND MATERIAL.

The therapeutic trials were carried out against blood-induced infections of *P. gallinaceum* in fowls and against *P. knowlesi*, *P. cynomolgi* and *P. inui* infections in *Silenus rhesus* monkeys of northern India. The method of testing the effects of antimalarials in monkeys is according to the standardized technique already discussed by Jaswant Singh *et al.* (1950). The drug in watery suspension was administered orally using Ryle's stomach tube. The technique followed in the case of birds varied in certain details. The weight of the birds varied from 1 to 2·5 kg. and the standard inoculum for producing intravenous infections in fowls was 30 million parasites per kg. body weight. Parasite densities in donors were determined against total R.B.C. counts per cubic millimetre and also in terms of percentage of infected red blood corpuscles.

The dosages employed varied in general from 0·5 to 50 mg. base per kg. body weight in two divided doses a day (10 a.m. and 5 p.m.) and continued for 4 days in the case of fowls and 7 days in monkeys.

Side by side with the chemotherapeutic studies, the acute toxic effect of the drug in varying doses administered orally was also studied in fowls, and monkeys of an average weight of 2·5 kg. and 4·5 kg. respectively (38 fowls and 47 monkeys). In these experiments, the drug was well diluted and given in all cases on empty stomach. The amount administered was in terms of the base of the drug per kg. body weight of the animals. Further details regarding the test are similar to those mentioned in connection with the acute toxicity test with paludrine lactate (Jaswant Singh *et al.*, 1950).

RESULTS OF THERAPEUTIC TRIALS.

Trials with P. gallinaceum.—The tests were carried out in 15 fowls using dosages varying from 1 to 50 mg. per kg. body weight and the results are given in Table I.

Dosages ranging from 5 to 50 mg. base were effective in controlling the initial infection whereas 1 mg. dosage proved ineffective. Out of two birds treated with 1 mg. dosage regime, one died of heavy infection during treatment and in the other, the infection passed on to a chronic stage. In the higher dosage, parasites were cleared from the peripheral blood within an average of 89 hours (range 60 to 122 hours) from the commencement of treatment.

Asexual parasites disappeared within an average of 66 hours (range 60 to 84 hours). In all birds, repeated short-term relapses occurred within 6 to 13 days after the initial cure. No acute toxic effect was noticed in any of the dosages employed.

Trials with P. knowlesi.—The results obtained in the treatment with different dosages against *P. knowlesi* in the monkeys are given in Table II.

TABLE I.

Therapeutic tests with bromoguanide against P. gallinaceum.

Daily dosage mg. base per kg. body weight.	Parasite clearance (hours).		Relapses.		Remarks.
	All stages.	Asexual stages.	Number.	Interval between the cessation of drug administration and first relapse (days).	
50	60	60	Frequent	11	
50	60	60	...	...	Died 3 days after treatment.
35	108	72	Frequent	7	
30	84	60	,,	9	
30	72	60	,,	11	
30	122	72	,,	13	
20	84	72	,,	7	
20	108	84	,,	7	
15	80	60	...	...	Died on 2nd day after treatment due to unknown cause.
10	84	72	Frequent	8	
10	84	72	,,	8	
5	108	60	,,	6	
5	108	60	...	...	Died on the 6th day after treatment.
1	Died due to heavy infection.			...	Inactive.
1	Initial infection became chronic.			...	Inactive.

TABLE II.

Therapeutic trials with bromoguanide against P. knowlesi.

Daily dosage mg. base per kg. body weight.	Parasite clearance (hours).		Relapses.		Total amount of drug administered (in terms of mg. base per kg. body wt.) before death.	Remarks.
	All stages.	Asexual stages.	Number.	Interval between the cessation of drug administration and the first relapse (days).		
50	..	..	...	...	50	Died during treatment.
50	.	..	...	...	50	Do.
40	...	...	...	...	120	Do.
40	216	168	1	18	...	..
30	..	...	...	...	90	Died during treatment.
20	204	168	1	10	...	..
20	204	192	1	10	...	...
10	120	108	Frequent	10	...	..
10	132	120	Do.	7	...	...
10	144	132	Do.	14	...	...
5	120	96	1	7	...	...
5	168	122	...	...	...	No relapse during an observation period of 32 days.
5	..	Parasites continued to circulate in peripheral blood stream.			...	Inactive.
5	...	Do.			...	Do.

Dosages varying from 5 to 10 mg. per kg. body weight were found to be effective in controlling the blood-induced infection. The time taken for clearing the parasites from the circulation was on an average 164 hours (range 120 to 216 hours). Asexual parasites disappeared from the peripheral blood much earlier than the sexual parasites, the time taken for the former being 96 to 192 hours (range) and 138 hours (average). One or two relapses were observed in all except in one monkey but these were of very short duration (1 to 2 days) and the first relapse occurred 7–18 days after the initial disappearance of parasites. Four out of five monkeys treated with 30 to 50 mg./kg. base dosages died after receiving 90 to 120 mg./kg., due, in all probability, to the toxic effect.

Trials with P. cynomolgi.—Sixteen monkeys were put on treatment against *P. cynomolgi* in different dosage regimes ranging from 0·5 to 50 mg. base and the findings are shown in Table III.

TABLE III.

Therapeutic trials with bromoguanide against P. cynomolgi.

Daily dosage mg. base per kg. body weight.	Parasite clearance (hours).		Relapses.	
	All stages.	Asexual stages.	Number.	Interval between the cessation of drug administration and the first relapse (days).
50	72	60	1	9
50	72	60	2	12
10	168	108	2	10
10	144	120	2	10
30	132	84	2	2
30	132	84	4	2
20	132	84	Nil	...
20	144	96	Nil	...
10	132	84	1	2
10	132	84	5	2
5	72	60	Frequent	5
5	90	84	,,	13
5	144	108	,,	7
5	138	96	,,	7
1	168	108	,,	9
0·5	Initial infection passed on to chronic state.			

It will be observed that while 0·5 mg. dosage proved ineffective, dosages ranging from 1 to 50 mg. modified the infections in all cases. However, with 50 mg. the parasite clearance was more rapid than with the other dosage regimes although the same was observed in respect of one out of 3 animals which received only 5 mg./kg. Parasites disappeared from peripheral blood in all effective dosage regimes within an average of 125 hours (range 72 to 168 hours). Effect on asexual

parasites was noticed within an average of 88 hours (range 60 to 120 hours). Thirteen out of 15 monkeys employed, relapsed within a period of 2 to 12 days after the cessation of treatment. In 40 and 50 mg. dosage regimes, only transient parasitæmia was observed, whereas in dosages of 5 and 1 mg. frequent relapses occurred. An interesting feature is that monkeys on 10 and 30 mg. dosages relapsed 1 to 5 times, but with 20 mg. dosage no relapses were observed during the observation period of 90 days. No toxic effects of the drug were noticed in any of the monkeys.

Treatment with P. inui.—Ten monkeys were placed on treatment against *P. inui* using dosages varying from 1 mg. to 50 mg. base. Details of the results are shown in Table IV.

TABLE IV.

Therapeutic treatment with bromoguanide against P. inui.

Daily dosage mg. base per kg. body weight.	Parasite clearance (hours).		Relapses.		Total amount of drug administered (in terms of mg. base per kg. body weight) before death.	Remarks.
	All stages.	Asexual stages.	Number.	Interval between the cessation of drug administration and the first relapse (days).		
50	...	...	...	...	200	Died during treatment.
50	132	120	...	...	...	No relapse during an observation period of 42 days.
20	144	96	3	5	...	
20	144	120	3	5	...	
10	120	108	1	20	...	
10	120	120	3	6	...	
5	156	120	Frequent	7	...	
5	165	132	Do.	5	...	
1	156	108	Do.	5	...	
1	168	132	Do.	8	...	

All the dosage regimes employed were found to be effective. The time taken for the complete disappearance of parasites varied from 120 to 168 hours with an average of 145 hours (all regimes combined). Higher dosages ranging from 10 to 50 mg. cleared parasites from the peripheral blood within an average of 132 hours whereas the lower ones took 162 hours for the same. Asexual parasites disappeared

within an average of 118 hours. Relapses were recorded within 5 to 20 days in all except in the one that was treated with 50 mg. dose. In dosages ranging from 10 to 40 mg., relapses were few (1 to 3) whereas in lesser dosages they were frequent. Fifty mg. dosage appeared toxic to one of the two monkeys employed and it died after receiving 200 mg./kg. of the drug.

RESULTS OF ACUTE TOXICITY TESTS.

Acute toxicity tests in fowls.—Thirty-six fowls were used for this test. The maximum dose, which did not cause any mortality in any of the birds tried, was 150 mg. The minimum dosage to give 50 per cent mortality was 250 mg. and for 100 per cent mortality 600 mg./kg. base. The details of the findings are given in Table V.

TABLE V.

Acute toxicity tests with bromoguanide in fowls.

Single dosage mg./kg. base.	Number of fowls.	Number died.	Interval in hours between death and drug administration.	Lethal dose.
150	4	0	.	L.D. 0
200	6	2	18	L.D. 33
250	4	2	46 to 90	L.D. 50
300	3	2	53	L.D. 66
550	4	3	3½ to 128	L.D. 75
600	4	4	¼ to 48	L.D. 100
650–1,000	11	11	¼ to 58	L.D. 100

In the above doses, fowls died within 15 minutes to 128 hours after the administration of the drug. The symptoms observed in such cases were restlessness, ataxia and excessive salivation followed by spasmodic contraction and relaxation of the body with apparent stoppage of breathing. On post-mortem examination, intestines were found bloated and there was congestion of the lungs.

Acute toxicity tests in monkeys.—Acute toxicity of bromoguanide was determined in 47 monkeys. The details of the results are shown in Table VI.

TABLE VI.

Acute toxicity tests with bromoguanide in monkeys.

Single dosage mg./kg. base.	Number of monkeys used.	Number died.	Interval in hours between death and drug administration.	Lethal dose.
45	14	0	...	L.D. 0
50	10	5	20 minutes to 12 hours	L.D. 50
60	10	6	5 to 45 minutes	...
75	10	9	5 to 10 minutes	...
100	3	3	Immediate to 5 minutes	L.D. 100

It was found that the maximum tolerated dose was 45 mg. base per kg. body weight. The minimum dosages for L.D. 50 and L.D. 100 were 50 mg. and 100 mg. respectively. Death in lethal doses occurred in almost all cases either immediately or within 45 minutes after the drug administration. Only in a few cases this was delayed up to 12 hours.

Toxic manifestations consisted of immediate flushing of the face followed by extreme paleness, vomiting, violent restlessness, ataxia, copious salivation and rapid breathing followed by coma and death. On post-mortem examination acute congestion of all internal organs was noticed. Frothy exudate was observed in trachea, bronchi, broncheoles and alveoli. Stomach and intestines were also bloated.

DISCUSSION.

The lowest dose of bromoguanide that effected complete clearance of parasites (temporarily) from the peripheral blood was found to be 5 mg./kg. base daily for 4 to 7 days in *P. gallinaceum* and *P. knowlesi* infections, whereas similar results were obtained with 1 mg./kg. base in *P. cynomolgi* and *P. inui* infections. The clearance of all stages of the parasites from peripheral blood was achieved within an average period of 89 to 164 hours in the infections produced by all the different species of plasmodia. Judged from the time taken for the disappearance of parasites from peripheral blood, the comparative efficacy of the drug was in the following order of magnitude: *P. gallinaceum*, *P. cynomolgi*, *P. inui* and *P. knowlesi*. The gametocytes persisted for an average period of 23 to 27 hours after disappearance of asexual forms of *P. gallinaceum*, *P. knowlesi* and *P. inui*, and for 37 hours in the case of *P. cynomolgi*. No special advantage was noticed in treating *P. gallinaceum*, *P. knowlesi*, and *P. cynomolgi* infections with higher dosages for the control of initial infection, but in *P. inui* infection slightly enhanced results were obtained with the daily dosages of 10 mg. and above. This absence of correlation between the dosage and activity of drug cannot be explained fully. According to Wiselogle (1946) the absence of correlation between the higher dosage of the drug and its activity may be due to the toxic nature of the drug or

its poor absorption from the alimentary tract when administered orally. If it is presumed that bromoguanide is a drug of higher toxic nature, it fails to reveal the ascending order of activity in all dosages less than maximal effective dosage, a factor which is again applicable to toxic drug (Wiselogle, *loc. cit.*). Regarding the absorption of the drug from the alimentary tract as a factor for the absence of correlation between the higher dosage of the drug and its activity, further investigations are necessary.

Regarding the prevention of relapses, all dosage regimes proved invariably ineffective.

It has been suggested that chlorophenyl group present in paludrine is responsible for the plasmodicidal action, and the alkyl biguanide chain only helps to admit the drug into the site of action (Spinks, 1947). Similarly in mepacrine and pamaquin, the parasiticidal action is supposed to be confined to the acridine and quinoline ring systems present in the respective drugs (Spinks, 1946). With a view to finding out the interchangeability of the acridine and quinoline rings of mepacrine and pamaquin respectively with chlorophenyl group of proguanil, various analogues have been prepared. These have been achieved by replacing the chlorophenyl group of paludrine by suitable acridine and 8-quinoline ring systems, but these compounds unfortunately have not shown any antimalarial activity (May *et al.*, 1947; Gupta, 1950).

The activity of paludrine seems therefore to be a resultant of specific and collective action of all its chemical components. Any replacement with active chemical groups from other well-known antimalarials, disturbs the original mechanism of action and results in the formation of inactive analogues. However, in view of the findings that bromoguanide is an effective antimalarial drug (Jaswant Singh *et al.*, 1949), it is assumed that if replacement involves only change of chlorine atom with bromine without disturbing the basic paludrine structure, the antimalarial activity is retained.

During the course of survey of literature by the authors, they came across a reference to Dr. D. G. Davey's unpublished work by Ainley and his co-workers (1949) which reads as under :—

'.....since the assay of N^1-p bromophenyl N^5 isopropyl-biguanide had disclosed that it possessed somewhat higher antimalarial activity than paludrine.....'.

In the authors' trials in fowls and monkeys both with paludrine and bromoguanide under identical conditions (unpublished), it has been found that though the latter compares somewhat favourably with the former, it is not superior to paludrine, especially with regard to the effect against *P. knowlesi*. As against an average period of 122 hours that was required for the control of initial infection with paludrine, the corresponding period with bromoguanide was 164 hours. Sometimes in higher doses (daily dose of 10 mg. and above) paludrine acts somewhat quicker and clears parasites within 101 hours. A similar correlation has not been generally applicable with bromoguanide. Regarding the time required for the disappearance of asexual parasites, as against an average of 116 hours with paludrine, the corresponding period with bromoguanide is 138 hours. Considering the effect of bromoguanide in preventing relapses, it appears to be less potent than paludrine. Bromoguanide treatment results in more frequent relapses in *P. gallinaceum* infections than in the

case of paludrine. In *P. knowlesi* infection, against a figure of 46 per cent relapses with paludrine, it is 87·5 per cent with bromoguanide. With 30 mg. dosages (daily) and below, more frequent relapses occur with bromoguanide than with paludrine in *P. cynomolgi* infection. Likewise in *P. inui* infection for a relapse rate of 30 per cent with paludrine, the corresponding figure for bromoguanide is 90 per cent. Similarly in the toxicity tests it has been found that paludrine is less toxic than bromoguanide. L.D. 0 in monkeys is 55 mg./kg. of paludrine whereas with bromoguanide it is 45 mg./kg. Fifty mg. single dose of bromoguanide caused 50 per cent mortality in monkeys. This leaves only a very small margin of safety to the extent of 5 mg. between the L.D. 0 and L.D. 50. This is at variance with the results obtained with paludrine where the margin between L.D. 0 and L.D. 50 is observed to be 105 mg. base per kg. body weight. These differences in the toxic doses between the two drugs are perhaps due to the fact that chlorine in paludrine is a normal physiological constituent of living cells and that bromine in the bromoguanide is altogether a foreign element and the breakdown product or products of bromoguanide in the body are more lethal as compared to paludrine. To be definite, a knowledge of the metabolic changes, breakdown products, distribution and tissue concentration of bromoguanide in the body system is strongly indicated.

SUMMARY.

Para-bromo-analogue of paludrine termed as bromoguanide was studied in detail regarding :—

(1) Its therapeutic effect against *P. gallinaceum* in fowls ; and *P. knowlesi*, *P. cynomolgi* and *P. inui* infections in monkeys, and

(2) Its acute toxic effects on fowls and monkeys.

Effective doses ranged from 5 to 50 mg. base per kg. body weight a day in two divided doses for 4 days in fowls infected with *P. gallinaceum* and for 7 days in monkeys infected with *P. knowlesi*. While the maximum effective dose applied equally to *P. cynomolgi* and *P. inui* also, the minimum effective dose in them was as low as 1 mg. base per kg. body weight. In the above doses, parasites cleared from the peripheral blood of the animals infected with *P. gallinaceum*, *P. knowlesi*, *P. cynomolgi* and *P. inui* within an average period of 78, 164, 128 and 145 hours respectively from the commencement of treatment.

Relapses were common following the drug administration in all species of plasmodial infections.

Toxic effects were observed in some monkeys treated with 30 to 50 mg./kg. base a day for 7 days. The maximum single dose which caused no mortality was found to be 45 mg./kg. in monkeys, and 150 mg./kg. in fowls. The respective doses for effecting 50 and 100 per cent mortality were 250 and 600 mg. per kg. body weight in fowls, and 50 and 100 mg. in monkeys.

REFERENCES.

AINLEY, A. D., CURD, F. H. S., *J. Chem. Soc.*, Jan., p. 98.
and ROSE, F. L. (1949).
BAMI, H. L., and GUHA, P. C. *J. Ind. Inst. Sci.*, **31a**, part 1, p. 1.
(1949).

BAMI, H. L., *et al.* (1949)	..	*Curr. Sci.*, **18**, p. 50.
GUPTA, P. R. (1950)	...	Personal communication.
JASWANT SINGH (1949)	...	*Ind. J. Mal.*, **3**, p. 413.
JASWANT SINGH, NAIR, C. P., BASU, P. C., and RAY, A. P. (1950).		*Ibid.*, **4**, p. 337.
JASWANT SINGH, RAY, A. P., NAIR, C. P., and BASU, P. C. (1949).		*Ibid.*, **3**, p. 405.
MAY, E. L., *et al.* (1947)	...	*J. Org. Chem.*, **12**, p. 869.
SPINKS, A. (1946) ...	...	*Ann. Trop. Med. Parasit.*, **40**, p. 153.
Idem (1947) ...	...	*Ibid.*, **41**, p. 30.
WISELOGLE, F. Y. (1946)	...	*Survey of Antimalarial Drugs*, 1941–1945. Edwards Bros., Ann. Arbor., Michigan, U.S.A.

Indian Journal of Malariology, **4**, 4, December 1950.

LEUCOCYTE COUNT IN NORMAL *SILENUS RHESUS* AND *SINICUS* MONKEYS.

BY

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(*October 10, 1950.*)

EXPERIMENTAL studies on human malaria generally involve estimation of the total R.B.C. and W.B.C. counts, and calculation of the numerical densities of malaria parasites. In field conditions, for R.B.C's., a count of 5 million per c.mm. of blood is usually taken as a standard; and for W.B.C's., a count of 7,500 for a similar quantity of the blood.

Observations made and difficulties experienced in setting up a similar standard of leucocyte counts in *rhesus* and *silenus* monkeys have been presented in this paper.

METHODS AND MATERIAL.

Normal healthy monkeys, two *rhesus* and three *sinicus* of about the same weight, were used for these investigations. The animals were caught gently without exciting them too much and blood for leucocyte counts was taken from the ears. The W.B.C. counts of *rhesus* monkeys were taken thrice daily for 9 days. The first daily count was done before the forenoon feed, i.e. between 9.30 and 10.00 a.m., second count between 1.00 and 2.00 p.m., i.e. between the forenoon and afternoon feeds, and the third count just after the afternoon feed, i.e. between 4.30 and 5.00 p.m. In the case of the *sinicus* monkeys, the estimation was done at 2.00 p.m. only daily but the period of observation lasted from 33 to 66 days in the different animals.

RESULTS.

Results of the leucocyte counts are given in Table I in respect of the *rhesus* monkeys, and in Table II in the case of *sinicus* monkeys.

TABLE I.

Leucocyte count of rhesus *monkeys.*

Day of count.	Leucocyte count of *rhesus* monkey No. 1. (Number per c.mm.)			Leucocyte count of *rhesus* monkey No. 2. (Number per c.mm.)		
	10.00 a.m.	1.00 p.m.	5.00 p.m.	10.00 a.m.	1.00 p.m.	5.00 p.m.
1st	20,600	25,600	15,400	11,200	9,600	8,800
2nd	15,000	18,000	21,600	9,400	10,600	10,000
3rd	22,200	18,800	19,400	10,400	11,000	12,000
4th	22,000	11,400	24,000	7,800	8,000	16,400
5th	18,100	17,000	18,000	7,800	10,400	7,000
6th ...	9,000	9,400	9,800	5,200	4,400	5,400
7th	9,400	9,800	7,000	4,800	5,200	4,600
8th	12,000	8,000	15,000	4,000	5,000	13,000
9th ...	7,000	6,200	9,000	9,600	10,200	8,400
Average count at different hours of the day.	15,033	13,800	15,466	7,800	8,266	9,733
Mean for the period		14,766			8,600	

TABLE II.

Leucocyte count of sinicus *monkeys.*

Monkey numbers.	Period of count (days).	Leucocyte count.		
		Minimum.	Maximum.	Average.
1	66	5,200	48,000	13,586
2	66	2,400	17,000	8,873
3	33	4,500	13,200	7,088

The mean leucocyte count for the whole period of observation shows highly divergent figures in the two *rhesus* monkeys. In one it was 14,766 and in the second 8,600. A marked fluctuation is observed in the counts taken on different days and at different hours of the day in both the monkeys. Broadly speaking, variation ranged between 6,200 to 25,600 W.B.Cs. per c.mm. in one, and 4,000 to 16,400 in the other. The average figure of the counts taken at any fixed hour of the day during the entire period of observation was however fairly constant for a particular monkey, the counts being 15,033, 13,800 and 15,466 in one; and 7,800, 8,266 and 9,733 in the second one for the periods 10.00 a.m., 1.00 p.m., and 5.00 p.m. respectively.

In the *sinicus* monkeys also, a wide fluctuation in the daily leucocyte count was observed. In one, it varied from 5,200 to 48,000, in the second 2,400 to 17,000 and in the third 4,500 to 13,200. The corresponding average count for the three monkeys was 13,586, 8,873 and 7,088 respectively.

DISCUSSION.

A study of the works of Wilh and Stuart; Anderson and Neill; Billimoria, Krumbhar and Musser; Lucas and Prizer; Taylor; Hecktoen and Eggers; Fox; Hall; Shukers and Purdy (as quoted by Kalra, 1947) indicates that the total count in *rhesus* monkeys fluctuates from a minimum of 5,000 to a maximum of 82,600 with averages ranging from 11,080 to 26,500. From simultaneous total and differential leucocyte counts Kalra (*loc. cit.*) observed that the daily fluctuation in these animals was mainly due to changes in neutrophil proportion and that the lymphocytes maintain a fairly constant level.

In the present studies, the daily fluctuation of the leucocytes in *rhesus* monkeys recorded by other workers is corroborated, and furthermore counts taken at different hours of the day fluctuated widely. Another noteworthy point is that the counts taken at a particular time though showed wide fluctuation when compared to the counts made at the same time on different days, the average figures for the different fixed times for a particular monkey were more or less constant for the whole period of 9 days. This seems to indicate that the high or low leucocyte counts obtained at different periods of the same day have no relationship to the feeding, i.e. to the state of empty or full stomach. The results obtained also show that a fairly constant variation in the level of total leucocytes is maintained in a particular animal and only the changes beyond this minimum and maximum figures, provided these have been previously estimated, can be considered as pathological. It again indicates that there is no fundamental change in the total leucocyte pattern of the *rhesus* and *sinicus* monkeys and the general conclusion drawn from the findings in *rhesus* monkeys appears to be applicable to *sinicus* monkeys also.

SUMMARY AND CONCLUSION.

Total leucocyte count estimation was made thrice a day for 9 days in *rhesus* monkeys and once a day for 33 to 66 days in 3 *sinicus* monkeys.

A very wide daily fluctuation in white blood corpuscles was observed in both the species of monkeys. In addition, a similar change was observed in the counts taken at different periods of the day in *rhesus* monkeys. But the average count

taken at any fixed time during the period of observation remained fairly constant for the particular monkey.

There was no particular relationship between the food and fluctuation in white cells in these monkeys.

There was no fundamental change in the leucocyte pattern of *rhesus* and *sinicus* monkeys.

No average leucocyte standard can be laid down in *rhesus* and *sinicus* monkeys for the speedy calculation of rough parasite densities in a given volume of blood by the method of simultaneous parasite and white cell counts from the thick and thin blood smears. Therefore, counting of the parasites against the R.B.Cs. and calculation of the parasite density based upon average R.B.C. count is to be preferred.

REFERENCE.

KALRA, S. L. (1947) . . Leucocytes in normal *rhesus* monkeys. *Ind. Med. Gaz.*, **82**, p. 383.

Indian Journal of Malariology, **4**, 4, December 1950.

SEASONAL PREVALENCE OF MALARIA IN SOUTHERN FORMOSA.

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[November 27, 1950.]

GREAT discoveries in medical science often seem to make us disregard previously gained knowledge and to stifle further research. This was the case with knowledge and research on some aspects of nutrition in diabetics after the discovery of insulin, and with the bacterial infections after the discovery of the antibiotics.

The recent advent of potent and fairly cheap insecticides and antimalarial drugs seems to have bred a contempt in some people for the time-honoured approach to malaria problems—the careful collection of data as a basis for control measures. It is true that potent new drugs and insecticides bring malaria control, almost anywhere in the world, into the realm of practical possibility. But one sometimes hears statements that detailed malaria studies are no longer needed, that malaria control to-day requires only the application of rule-of-thumb techniques, and money. Since money for health work in general and malaria work in particular always has been, and still is hard to obtain, any information that will lower the cost of malaria control should of course be sought.

This awareness of the need for sensible and economical approaches to the control of local malaria problems underlay the programme of malaria work in Formosa which was established in 1917 when our first field station was located in the town of Ch'ao Chow, in southern Formosa. The following report on the seasonal prevalence of malaria in the general vicinity of Ch'ao Chow is the result of studies which began in March 1948. The observations reported seem to apply to most of the plains region of southern Formosa and to supply information necessary for proper planning of a programme of D.D.T. residual spraying of homes for malaria control.

STUDY CONDITIONS.

The people of southern Formosa live in compact population units. Isolated houses in suburban or rural areas are rarely seen. The smallest villages usually have a population of more than 200 persons, and the average size is probably more than 500 persons. In general, housing is good in comparison with housing throughout the Orient. Farm animals are stabled in the villages.

The bulk of the population is rural and the villages are surrounded by fields tilled by the villagers. These fields contain either rice paddies or irrigated row crops such as sugarcane. The latter crop has increasingly replaced rice crops during the past three years. Of forty-odd sugar factories, nearly 40 were wholly or partly destroyed by bombing in 1944 and 1945 but their rehabilitation has created a demand for cane that is being met.

The sheets of water in the paddies and pools of water of various sorts serve as breeding places for *A. hyrcanus* var. *sinensis*. The marginal portions of streams and irrigation channels offer places for *A. minimus* to propagate. These are the only mosquitoes proved to be vectors of malaria in the Ch'ao Chow area. Of some eight other anophelines in the area, *maculatus*, *tessellatus*, *annularis* and *sundaicus* (*hudsonii*) were thought by Japanese malaria workers to be vectors, but their prevalence is low or of short duration or they have habits which prevent their being important vectors.

The point to be emphasized is that the human population of southern Formosa for the most part is intimately associated with the anopheline population, particularly with anophelines known to transmit malaria. The people may have some protection from anophelines with zoophilic traits through the use of animal barriers unconsciously raised by the stabling of buffalo near the homes. Their houses offer no protection against mosquitoes and the effective use of bed nets, widely promoted by the Japanese government, is not very prevalent.

Primary education for five years was compulsory in Formosa under the Japanese government and resulted in a high level of literacy. Larger villages have primary schools and consolidated primary and secondary schools are found in most towns. Under the Japanese government there was considerable regimentation of the general population and health programmes were administered under the general supervision of the police. All of these factors—literacy, habitual obedience to directives by authority, concentration of population into discrete units, and numerous local schools—are helpful in malaria studies and control work.

METHODS.

The observations on malaria prevalence reported here were obtained by three methods: examination of infants and children up to 25 months old, examination of primary school children, and study of Japanese records.

Infant malaria.—As Balfour (1935) pointed out 15 years ago, the infection rate of infants is probably the most sensitive and accurate comparative measurement of new malaria infections. These data are not easy to obtain, for mothers the world over take a dim view of having their babies contribute blood to science. Two girls, middle-school graduates, were trained to make thick and thin blood films and to keep simple records. Since March 1948, these girls have covered 13 villages

in the Ch'ao Chow area on bicycles, collecting data on malaria prevalence in infants and small children. Children come into this study population at birth and remain in it until they are 25 months old, or until they demonstrate malaria parasitism. Blood films are taken from each child at intervals of about 30 days. Whenever the children are ill, mothers are encouraged to bring them into the malaria centre at Ch'ao Chow. This helps to find malaria infections which otherwise might be treated for malaria at home between visits by the technicians, so that the blood film would be negative at a regular visit. Even so, the data collected probably underestimate the prevalence of malaria in this infant and child population. Some malaria cases were not identified because of home treatment or for other reasons; others were identified during a later relapse or at the time of a second infection. The monthly average number of examinations for the 25 months ending in March 1950 was 580; the maximum number in one month was 1,154.

School surveys.—During 1949 the children of two primary schools were examined at monthly intervals. One of these schools was at Tso Tien, a large village in Ch'ao Chow District but outside the township. The area about this village was exposed to a higher level of anophelism than the average for Ch'ao Chow Township. The other school was at Chu Tien, a small town about 10 km. from Ch'ao Chow Town and just outside the district. This town is in an area where row crops predominate over rice crops; there is less anophelism than in Ch'ao Chow District. No data on seasonal prevalence from primary schools in Ch'ao Chow Township are analysed in this report, because malaria control operations had begun to affect malaria rates in these schools by the summer of 1948.

Japanese records.—Records of the malaria treatment centre for Ch'ao Chow District for the years 1937 to 1944 escaped destruction when the town was bombed in 1945. These records are on a daily basis and give the number of blood films examined and the number found positive for malaria parasites. There is no correction for persons who have completed treatment with quinine, nor for other variables. In addition to these factors which make for underestimation of malaria prevalence, there was the inefficiency of the technician. Parasite indices were computed from these data on a monthly basis for the 12 years covered by the record. While this index underestimates prevalence by at least 50 per cent for any given period, the large numbers involved make for statistical stability of the rates.

Data on anophelism.—Since January 1947, anopheline mosquitoes have been collected at weekly intervals from animal stable collecting stations throughout Ch'ao Chow District. These collections have been supplemented by night collections from buffalo and daily collections from artificial diurnal resting places, underground shelters. Anopheline density reported here has been computed from routine stable collections in terms of numbers collected per man-hour of work.

Results.

Anophelism.—Data on anophelism are given for 1949 only, and for only three species. Figure 1, which gives mean values for collections from seven stations in Ch'ao Chow Township, shows the relationship between the cultivation of rice and the prevalence of *A. hyrcanus* var. *sinensis*. The decline in *sinensis* propagation after its peaks of prevalence in March-April and September-October, is due principally to progressive dewatering of ricefields. The midsummer decline

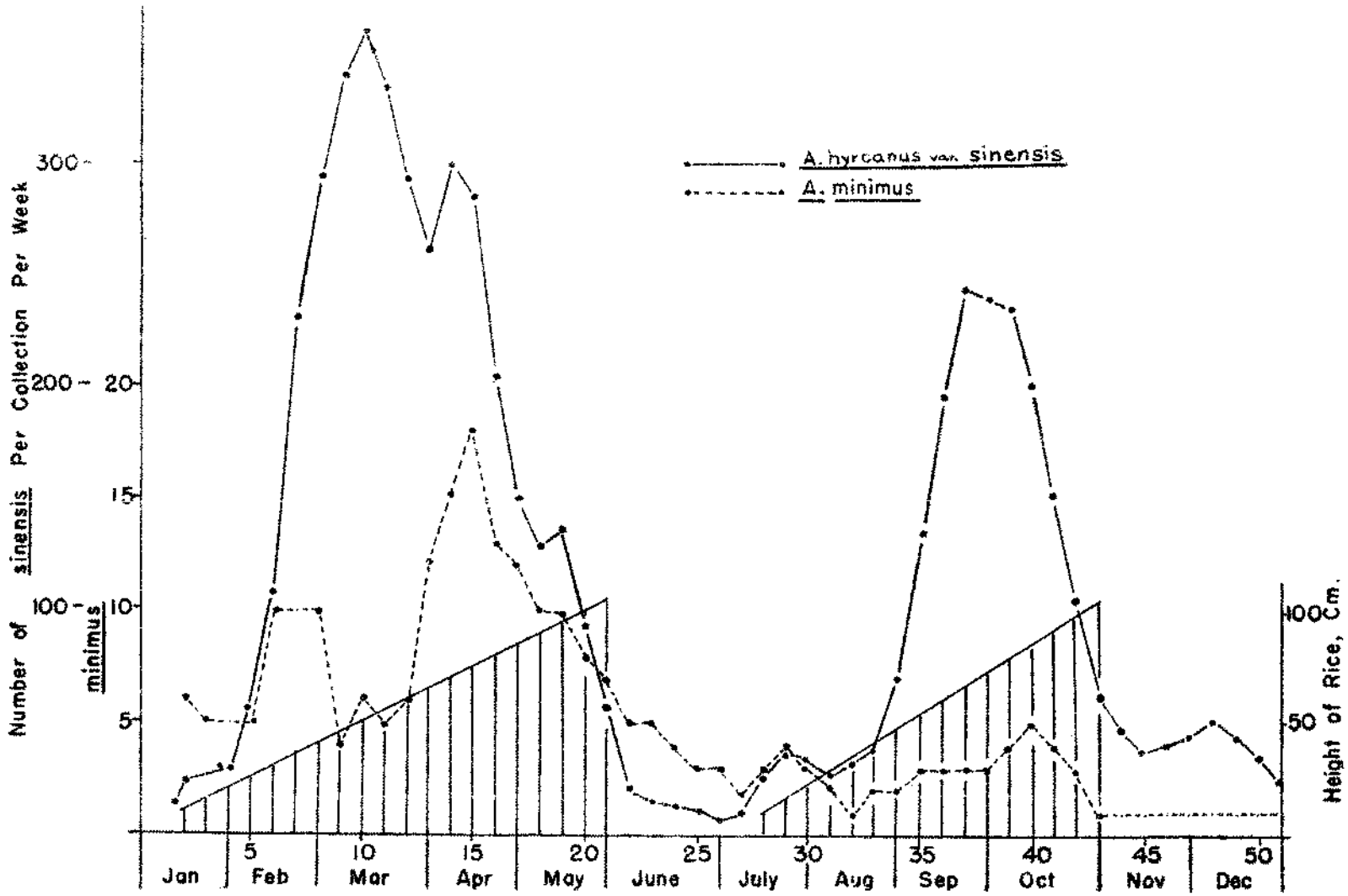

FIG. 1.—Three weeks moving mean of density values for *A. hyrcanus* var. *sinensis* and *A. minimus*, from seven collecting stations in Ch'ao Chow Township, 1949. The approximate heights of the rice crops are based upon measurements made in 1948.

in *minimus* prevalence and its lower densities in late summer and autumn, as compared with spring, are due to flushing of streams and irrigation channels during the rainy season from May to October. The largest collection of *sinensis*, 428 per man-hour, during the spring peak of prevalence was in March (11th week); and during the autumn peak, 427 per man-hour, it was in September (37th week). The largest number of *minimus* collected during the year, 45 per man-hour, was in April (15th week). The rate curves for these mosquitoes during 1949 are quite similar to those for 1947 and 1948 and are believed to be typical of areas in southern Formosa where rice is grown fairly extensively. Annual variations in the *sinensis* density rates are usually quantitative rather than qualitative.

Figure 2 records rates for the same mosquitoes and for *A. sundaicus* from seven stations in Ch'ao Chow District but outside the township. Rice is grown somewhat more extensively in this area than in the township; therefore, while the rate curve for *sinensis* is qualitatively quite similar to that in Figure 1, the values are higher. Peak density was also reached during the 11th week, with average collections of 1,424 mosquitoes per man-hour. The peak autumn density was 644 mosquitoes per man-hour in the 39th week. Peak *minimus* prevalence was 10 mosquitoes per man-hour in the 14th week, and this mosquito was less abundant during the year than for the township area.

A curve for *sundaicus* density is given in Figure 2. This curve is unusual in that it shows a midsummer peak of prevalence. In most years everywhere and in 1949 in the township, there is no midsummer increase in the density of *ludlowii*. The late winter peak of prevalence shown in Figure 2 is typical; this mosquito is found throughout the year, but its density is low after the 1st of May, usually about the same or lower than for *minimus*. This is because optimum conditions for *sundaicus* propagation occur in the late winter dry season: its larvæ are found typically in small pools with sandy or stone bottoms, without vegetation and exposed to direct sunlight, along the margins of streams. These breeding places are destroyed during the rainy season.

While *sundaicus* appears to be zoophilic, it will bite man. It is not known what part it plays as vector in southern Formosa. Anazawa (1931) reported an infection rate of 0·6 per cent for *sundaicus*; the authors did not find an infected specimen in three years among 3,591 dissections.

No data are shown for the density of *A. tessellatus* and *A. annularis*. These mosquitoes are zoophilic and 'wild'; they are not often taken from collecting stations and what is known of their density has been obtained through night catches from buffalo bait. Peak density of these mosquitoes is reached in the autumn (Chang, Watson and Chow, 1950) and corresponds qualitatively to the curve for autumnal *sinensis* prevalence. Only a few of these mosquitoes were dissected and no infected specimen was found. Anazawa (*loc. cit.*) reports rates of 0·2 per cent for *annularis* and 1·9 per cent for *tessellatus*. In the opinion of the authors these rates over-emphasize considerably the possible importance of these mosquitoes.

Mosquito dissections.—During 1949, 27,934 *A. hyrcanus* var. *sinensis* were dissected. No oöcysts were found, but four specimens were positive for sporozoites: an infection rate of 0·015 per cent. Three infected specimens were collected in February, the other in March. All of the infected specimens and 96 per cent of mosquitoes dissected were collected from animal shelters. Of 2,424 dissections

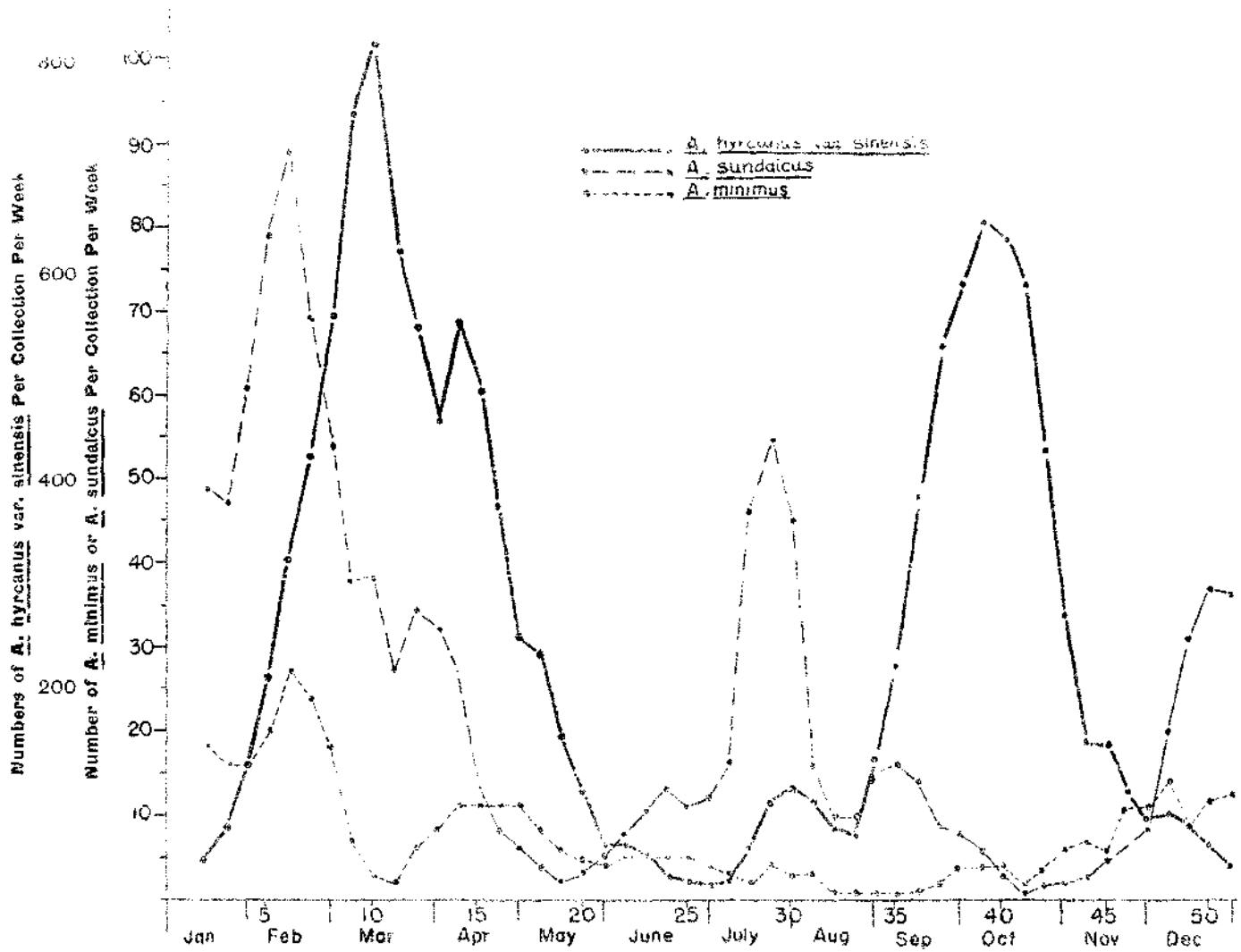

FIG. 2.—Three weeks moving mean of density values for three anopheline mosquitoes collected in Chiao Chow District, 1940.

of seven other anophelines in 1949, none was positive; *minimus* contributed 363 specimens to this group. However, an infected *minimus* was found in 1948.

NEW MALARIA INFECTIONS OF INFANTS AND YOUNG CHILDREN.

Seasonal prevalence.—It has been assumed that all malaria infections found in the monthly surveys of infants and children less than 25 months old were primary infections. In common practice infant infection rates are usually computed with data from infants less than 13 months old, but such rates are most often based upon one or more surveys in a year, less often upon monthly surveys of the same population. The broader age base of the data is justified by the fact that infants were observed at monthly intervals from birth; thus the chance of an infection escaping attention was rather small.

The seasonal prevalence of these infections is shown in Figure 3. The figure is for a composite year, compiled from data collected from March 1948 through March 1950, in villages in Ch'ao Chow Township without a malaria control programme. The case rates are calculated in terms of 1,000 person-days' exposure to the hazard of becoming infected with malaria. No allowance was made for the estimated

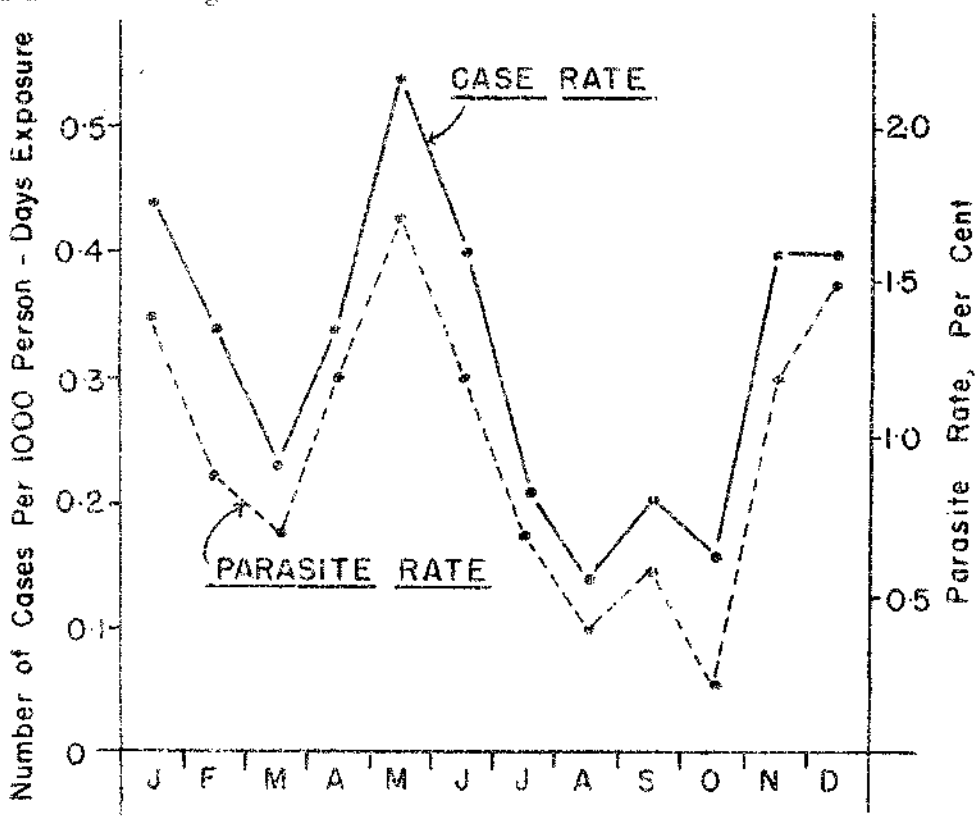

FIG. 3.—New malaria infections of infants and young children of Ch'ao Chow Township. The rates are computed for a composite year from data collected from March 1948 through March 1950.

date of infection; that is, the period of exposure for an individual was terminated on the day his blood film was found to contain malaria parasites. This makes for an error in the rates, particularly with respect to *P. malariæ* infections. A correction for this error and computation of rates at biweekly intervals might give a curve that anticipates the one in the figure, but the number of cases is so small that the value of further refinement of the data seems questionable for the purposes of this report. There is, of course, close agreement between the case rate curve and the curve of parasite rates.

Peak prevalence of new infections is seen to fall in May, with a secondary peak of prevalence in December. There are thus two waves of increasing prevalence, in late spring and in late autumn; and two waves of decreasing prevalence, during early summer and late winter.

Species parasitism.—Data on species parasitism are shown in Figure 4 in the form of numbers of cases of the three infections by months. The 93 cases shown were found throughout Ch'ao Chow Township; that is, from villages where control work was and was not being done. It is to be noted that *vivax* infections occurred in every month, *falciparum* infections in all months save one (July) and *malariæ*

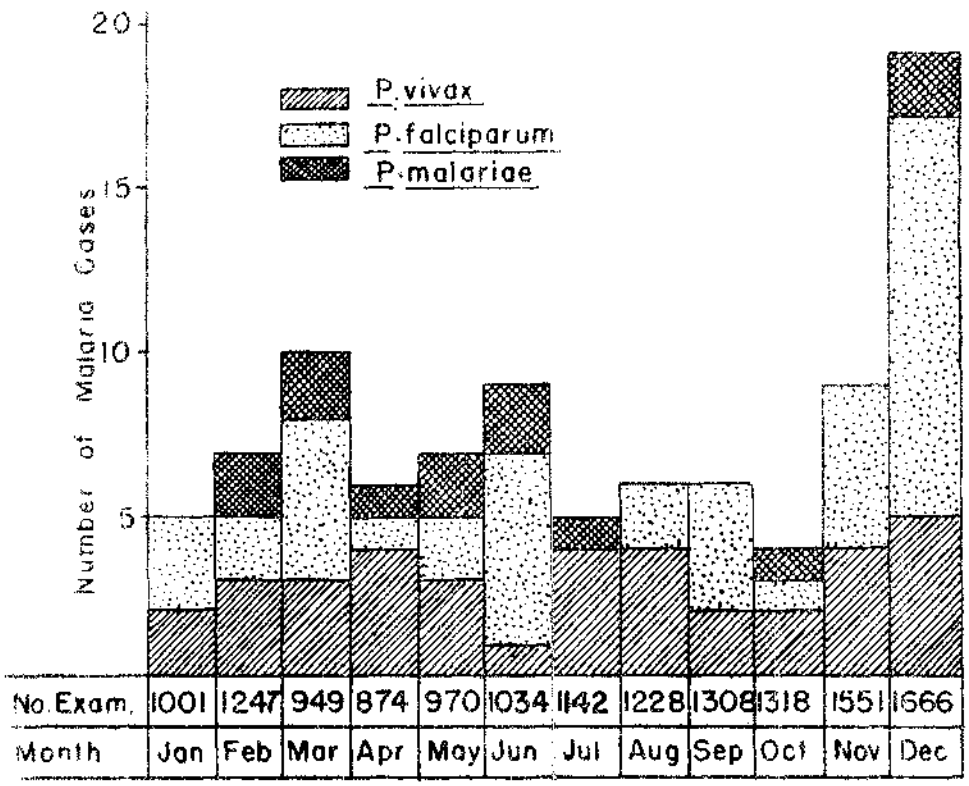

FIG. 4.—Species parasitism in 93 malaria infections of infants and young children, March 1948 to March 1950, Ch'ao Chow Township.

infections in eight months. For the composite year the cases may be divided as follows: *P. vivax*, 37 (40 per cent); *P. falciparum*, 43 (46 per cent); and *P. malariæ*, 13 (14 per cent).

MALARIA INFECTIONS OF PRIMARY SCHOOL CHILDREN.

Seasonal prevalence.—Figure 5 gives parasite rates by months for the two schools surveyed in 1949 and for the composite year for persons of all ages, compiled from the Japanese records, which cover all of Ch'ao Chow District.

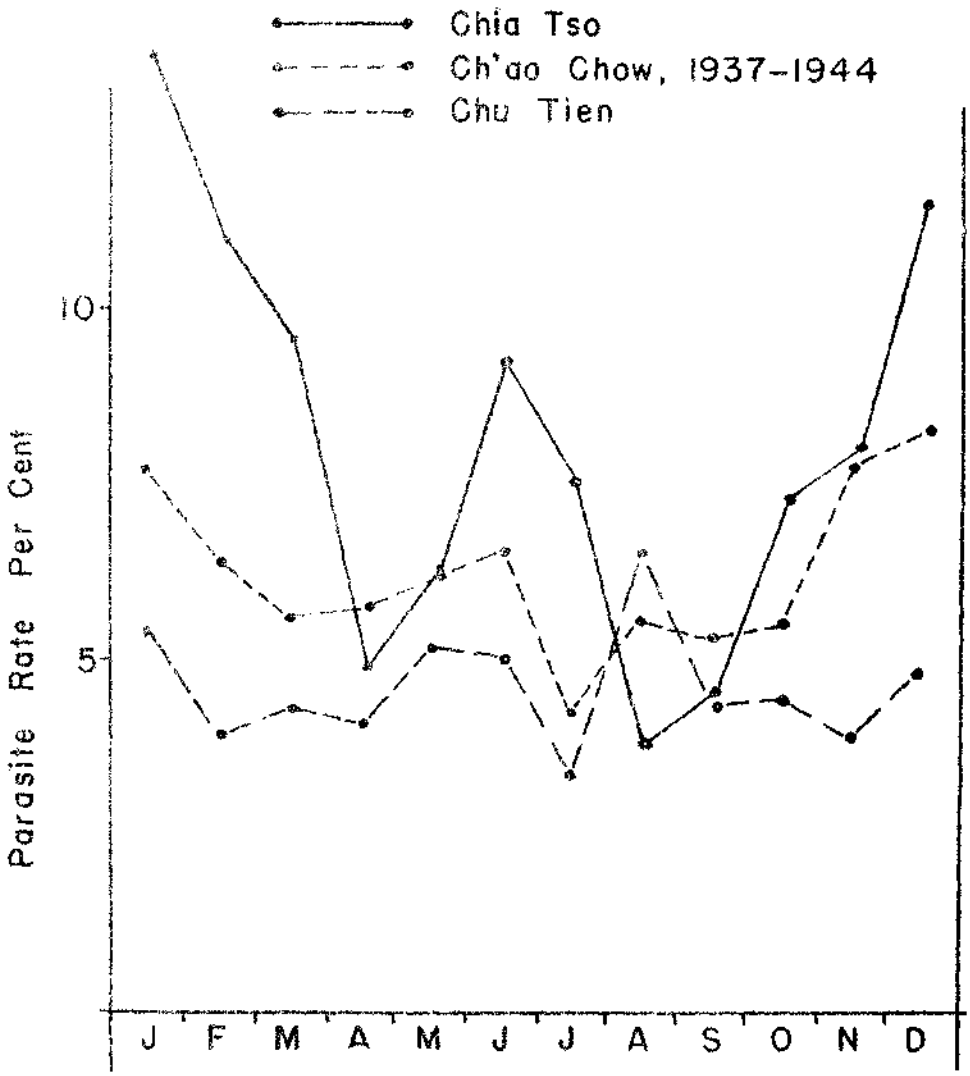

FIG. 5.—Prevalence of malaria among primary school children, 1949, and for the general population of Ch'ao Chow District, 1937–1944.

The mean number of examinations per month at Chia Tso primary school was 559, the range 184 to 627. The lower figure was for August, the summer vacation, and was one of only two months when the examinations fell under 500. The mean computed without the August examinations was 602. The mean parasite rate for the entire year was 7·6 per cent. The parasite rate curve for Chia Tso school children is qualitatively similar to the infant rate curves in Figure 3: there are two peaks of prevalence, in January and in June, that is, one month later than the peaks for new infections of infants.

At Chu Tien primary school the mean number of examinations per month was 906, the range 547 to 1,085. Again, the lower figure was for the August vacation, the only month when less than 900 examinations were made. The mean without the August examinations was 939. The mean parasite rate for the year was 4·5 per cent. The parasite rate curve for Chu Tien school conforms more closely to that for Ch'ao Chow District than to the rate curve for Chia Tso school. The two former curves exhibit a May-June increase in prevalence less sharp than this part of the Chia Tso curve; both curves also demonstrate a transient upward trend in August which is not exhibited by the Chia Tso curve. The August rate for Chu Tien is 6·58 per cent, the highest of the year. There is an autumn-winter wave of prevalence in the Chu Tien curve, but it is not marked; indeed, there is no great deviation from the mean annual rate. The high point of this wave is 5·38 per cent in January.

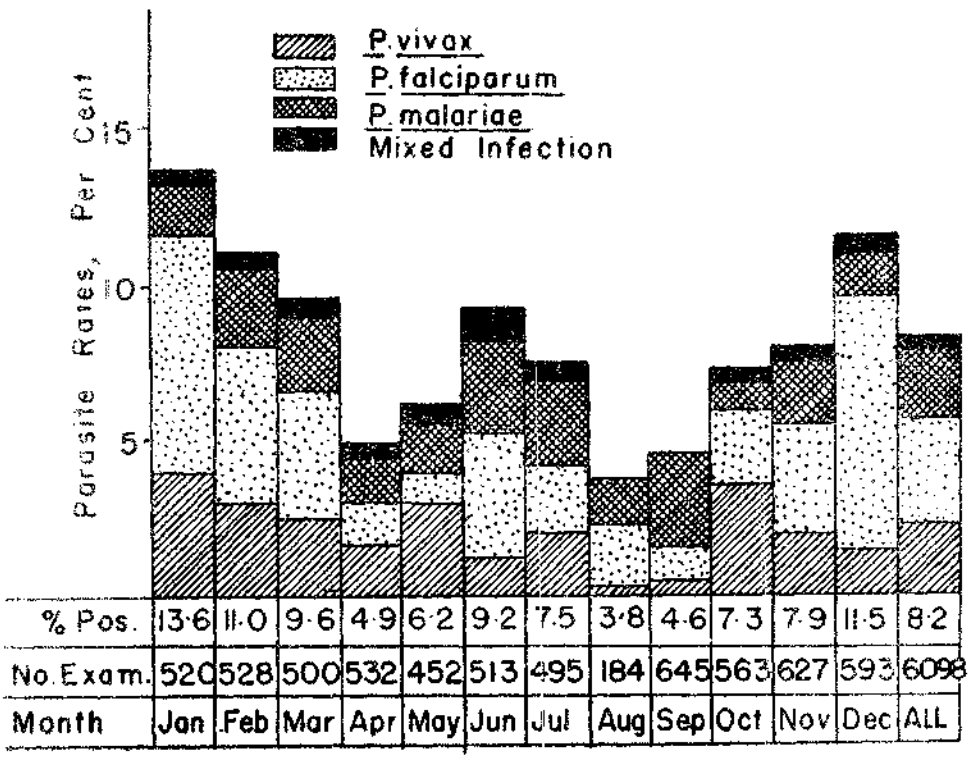

FIG. 6.—Species parasitism among the children of Chia Tso primary school, 1943.

Species parasitism.—Figure 6 shows that infections with *P. falciparum* predominated slightly over *vivax* and *malariæ* infections (which were about the same), on a yearly basis; also *falciparum* infections were predominant in seven months. Infections with *P. vivax* were predominant in two months, May and October; and malariæ infections in two months, July and September. The three species contributed almost equally to the infections found in April, with perhaps a slight preponderance in *vivax* infections. A substantial proportion of the children exhibited mixed infections in every month except August and September, when none was found.

Figure 7 gives data on species parasitism for the pupils of Chu Tien school. On a yearly basis, *P. falciparum* was most commonly found, comprising 197 (39 per cent) of the 499 infections found. *Malariæ* infections were a close second (176 or 35 per cent), while *vivax* infections numbered 113 (22 per cent). Infections with *vivax* predominated in only one month, *falciparum* was definitely predominant in five months, *malariæ* in three months, while in three months there was no significant difference between the prevalence of *malariæ* and *falciparum* infections. The larger proportion of *malariæ* infections at the expense of *vivax* infections is the principal difference noted when these data are compared with those in Figure 6.

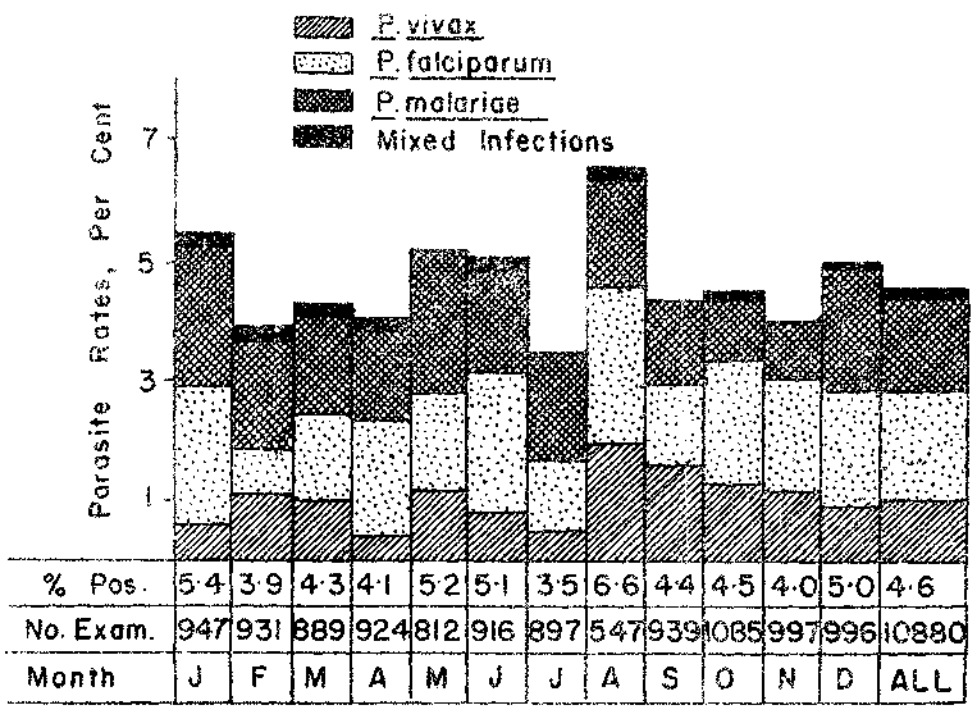

FIG. 7.—Species parasitism among the children of Chu Tien primary school, 1942.

When the data in Figures 6 and 7 are examined with respect to seasonal prevalence of all infections, it is noted that *falciparum* infections are predominant during the months of relatively high prevalence. This is particularly noticeable in Figure 6.

Data on species parasitism are not available from the Japanese records for Ch'ao Chow District.

DISCUSSION.

A notion of malaria prevalence in specific locations within Ch'ao Chow District may be obtained from the data from village surveys in Table I. At the time of each survey, malaria control work had not been carried out in these villages. An idea of changes in general malaria prevalence in the district during 1947–1949 can be had from the data for Ch'ao Chow primary school included in this table.

From these data it is evident that at the beginning of 1947 Ch'ao Chow District was a hyperendemic area or the remains of one. It is known as a matter of fact that the district had suffered severely from malaria during 1945 and 1946, when normal treatment of malaria cases had almost stopped for lack of drugs. Relief agencies brought in supplies of quinacrine hydrochloride in 1946 but there had not been much distribution of this drug when our work started.

In the data in Table I for Ch'ao Chow school there is evidence of a decreased rate of malaria transmission in 1948 and 1949. Our malaria control programme was in progress in some of the villages in the district, and since 1947 there had been progressively less planting of rice and more planting of cane. Yet the unprotected villages at the end of 1949 still suffered severely from malaria.

The data on infant malaria indicate that the highest rate of malaria transmission was during the period from about the first of April to the middle of May. The second highest rate of transmission was from about the middle of October to the middle of December. These periods of increased transmission rate are in part responsible for the vernal and autumnal waves of malaria prevalence. But new cases of malaria have been recorded for every month of the year, a certain indication that infected anophelines are a part of the epidemiological population throughout the entire year. This may mean that there is sufficient propagation of vectors with sufficiently equable climatic conditions to permit malaria transmission to proceed at a reduced rate in midwinter and in midsummer. Very low levels of *sinensis* and *minimus* anophelism would thus be sufficient to effect a low rate of malaria transmission. A more likely explanation is that a sufficient number of infected anophelines survive from the periods of high density and high transmission rates to effect transmission in the inter-wave periods. Both factors may prevail.

The May peak of new cases falls about two months after the peak of anophelism in the spring. But *sinensis* anophelism was maintained consistently at levels above 200 mosquitoes per man-hour per collection from about the middle of February until the end of April. Likewise, *minimus* anophelism reached its annual peak density in April. Therefore, the May peak of new cases may be said to fall about 30 days after peak *minimus* anophelism and after *sinensis* anophelism had been maintained for six weeks at a high level.

The autumn peak of new infections falls in late November or early December, say, about the 1st of December. The autumn peak of *sinensis* anophelism was reached in September and levels above 200 per man-hour's collection occur from the middle of September until the middle of October. The autumn peak of *minimus* production is about the middle of October. Therefore, the autumn wave of new cases falls about eight weeks after highest *sinensis* production and six weeks after highest *minimus* production. The spring wave of anophelism from these two mosquitoes is longer and higher than the autumn wave; so also the spring incidence of new malaria cases is higher than the autumn level.

It seems therefore that there is a sufficiently high degree of correlation between *sinensis* and *minimus* anophelism and the occurrence of new malaria cases to warrant the conclusion that these mosquitoes are intimately concerned with malaria transmission in the Ch'ao Chow area of southern Formosa. Of the two mosquitoes, *sinensis* is believed to be more important because of its much higher seasonal densities. However, it is possible that *sundaicus*, *annularis* and *tessellatus* play some part as vectors.

The July-August increase in *sundaicus* noted in Figure 2 would furnish a convenient explanation of the temporary increase in new cases in September; but this *sundaicus* peak is unusual and in any case could not have affected the incidence of malaria in the infant population surveyed. Moreover, the composite year for the period 1937 to 1944 shows a temporary increase of cases in August. The authors are inclined to view this late summer increase of new cases as due to *sinensis* density levels of about 50 mosquitoes per man-hour's collection which often occur in mid-July. This density level may be the critical level, above which transmission proceeds at an increased rate, below which transmission falls off rapidly. If *sundaicus* is a vector, its importance is during the late winter when it acts as an assistant to *sinensis*. Likewise with *annularis* and *tessellatus*, whose peak densities occur in October and early November (Chang, Watson and Chow, *loc. cit.*) and which are hard to find at other times; during the autumn they may be vectors of secondary importance to *sinensis*.

The highest prevalence of malaria among the children of Chia Tso school came during June-July and December-January, approximately one month after the peak of new infections in infants. It is believed that this is due to the contribution made by relapsing infections to the total infections found. A similar finding is noted in the curve for Ch'ao Chow District, 1937 to 1944.

The curve for children of Chu Tien school does not conform to this pattern in one important respect; the greatest prevalence during the year was found in August. It is not known why this should be, and the differences between this rate for August and those for July and September are significant statistically. A possible explanation is that the school was having a holiday when these examinations were made; consequently the children had to be sought in their homes. Children ill with malaria were more likely to be found at home than well children; therefore this rate may be weighed in favour of parasitism. There is a tendency towards an increase in malaria prevalence during August as shown by the curve of prevalence for 1937 to 1944; this may be due to relapses of infections acquired during the previous autumn. A third possibility is unusual prevalence of vectors. However, this can be ruled out in so far as collection from catching stations is concerned. Figure 8 indicates that the prevalence of both *A. sinensis*

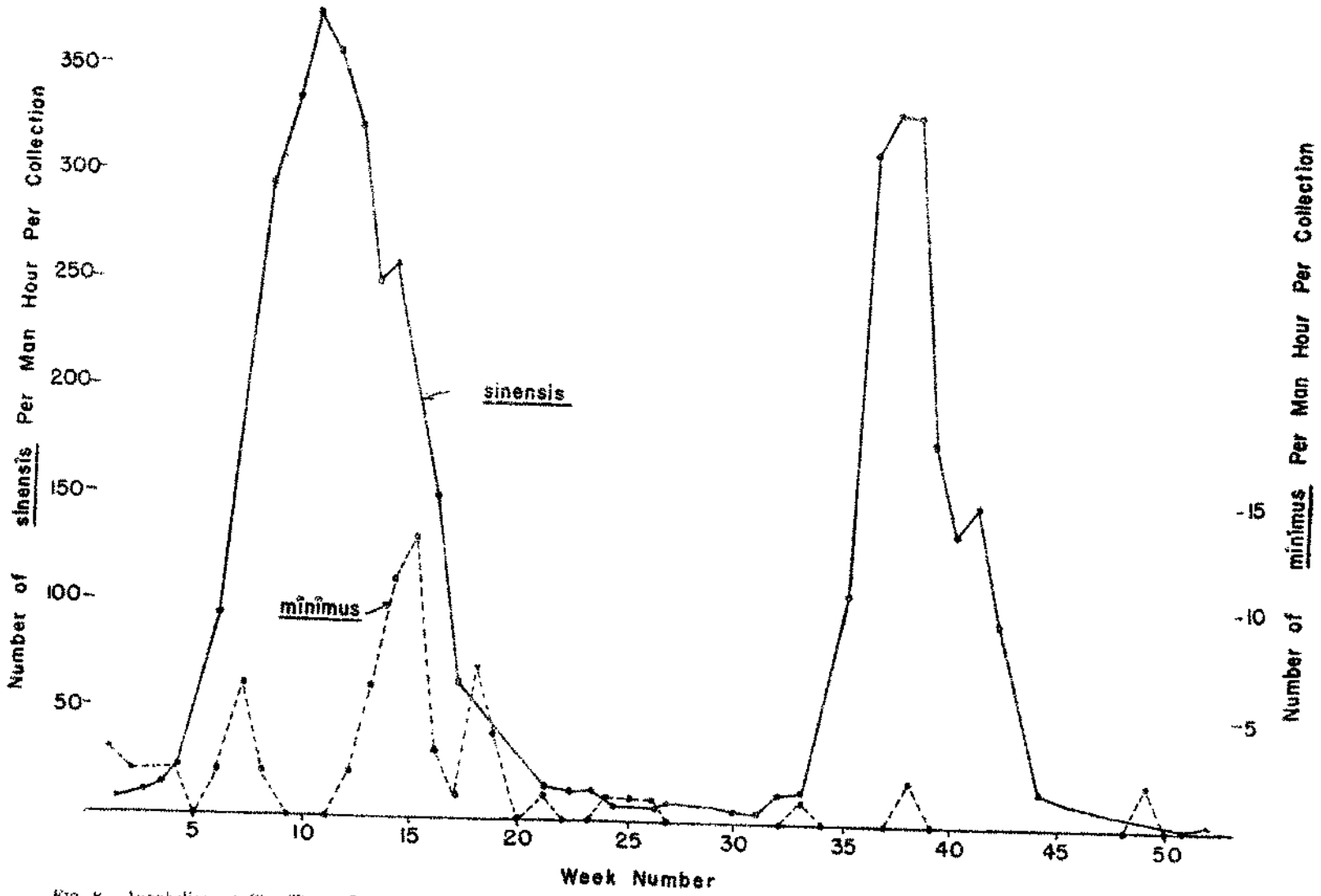

FIG. 8.—Anophelism at Chu Tien. The curve for *sinensis* is a three weeks moving mean of collections from three catching stations. The values for *A. minimus* are actual numbers of mosquitoes collected.

and *A. minimus* was less for Chu Tien than for Ch'ao Chow Township and Ch'ao Chow District (Figures 1 and 2). There was also a lower density at Chu Tien than elsewhere for *A. sundaicus*, *A. annularis* and *A. tessellatus*.

The practical implications of this study seem obvious in relation to a malaria control programme based upon residual spraying of premises with insecticides. Assuming an effective period of six months for the spray and that the critical level of *minimus* is 10, and of *sinensis* 50, mosquitoes per man-hour's collection, then the first spraying during 1949 should have been done about the 1st of February for Ch'ao Chow Township and about three weeks earlier for the area outside the township. The second application of spray should have been made about the middle of August and the middle of July respectively for these two areas.

One of the great advantages of residual insecticides is that they can be used within fairly wide limits of dosage and time between applications, and still be effective. Such studies of local anophelines make for greater efficiency and greater economy of control operations.

SUMMARY.

In the vicinity of Ch'ao Chow, a town in southern Formosa, records of new malaria infections of infants and children less than 25 months of age were collected from March 1948 to March 1950. Monthly surveys of two primary schools were made during 1949, and Japanese records of malaria prevalence in the district for the years 1937 to 1944 were examined.

Study of these records indicates that new malaria infections occur in every month of the year. It is likely that transmission of the three common species of malaria parasites occurs in every month. There are two periods during the year when new malaria infections are most prevalent; April to June and November to January, with the greatest incidence of cases falling in May and December. These periods antedate the peak incidence of all malaria infections by about a month.

There is a high degree of positive correlation between the occurrence of these waves of prevalence and the propagation of *A. hyrcanus* var. *sinensis* and *A. minimus*, proven vectors of malaria at Ch'ao Chow. These are considered to be the important vectors of malaria in the area, but *A. sundaicus* may transmit malaria during the late winter and *A. annularis* and *A. tessellatus* may be vectors during the autumn.

The need for precise knowledge of local vectors is emphasized as a preliminary to the efficient and economical use of residual insecticides for malaria control.

REFERENCES.

ANAZAWA, K. (1931) ... Observations on natural malaria-infection of the various Formosan anophelines, with reference to the critical value of each species from the malaria epidemiological point of view. *J. Med. Assoc.*, Formosa, **30**, pp. 1027–1049.

BALFOUR, M. C. (1935) ... Malaria studies in Greece; measurements of malaria, 1930–1933. *Amer. J. Trop. Med.*, **15**, pp. 301–330.

CHANG, T. L., WATSON, R. B., and CHOW, C. Y. (1950). Notes on the seasonal prevalence of anopheline mosquitoes in southern Formosa. *Ind. J. Mal.*, **4**, pp. 281–293.

TABLE I.

Summaries of records of malaria surveys of primary schools and villages in Ch'ao Chow Township, 1947–1949.

Year.	Month.	Place.	Number examined.	Rates, per cent.		Survey population.
				Spleen.	Parasite.	
1947	January ...	Ch'ao Chow ...	1,291	65·5	37·2	School.
	May ...	San Hsing ...	388	71·6	18·2	Village.
	June ...	P'eng Cheng ...	569	57·5	16·0	Village.
	September ...	Ssu Ling ..	320	62·2	13·8	School.
	September ...	Ch'ao Chow ...	1,455	72·5	16·5	School.
1948	February ...	Kuang Ch'un ...	263	70·9	11·4	Village.
	March ...	Wu Kuai ...	373	53·5	15·5	Village.
	April ...	Lan-tze Ting ...	213	78·9	27·2	Village.
	April ...	Ssu Ling ...	1,130	53·4	13·0	Village.
	May ...	Lu Liao ...	387	49·8	14·7	Village.
	July ...	Wan Luan ...	565	41·9	11·0	Village.
	November ...	Ch'ao Chow ...	1,613	50·4	8·7	School.
1949	January ...	Kuang Ch'un ...	67	78·0	6·0	Village.
	February ...	Lu Liao ...	239	44·9	6·7	Village.
	February ...	Ch'ao Chow ...	1,635	43·0	5·1	School.
	May ...	Lu Liao ...	323	43·3	16·1	Village.
	May ...	Kuang Ch'un ...	248	59·3	11·0	Village.
	May ...	Ch'ao Chow ...	1,612	50·6	3·7	School.
	October ...	Lu Liao ...	368	41·0	13·0	Village.

Indian Journal of Malariology, 4, 4, December 1950.

FIELD EXPERIMENTS TO DETERMINE THE RELATIVE EFFICACY IN MALARIA CONTROL OF DIFFERENT DOSAGE REGIMENS OF DICHLORO-DIPHENYL-TRICHLOROETHANE (D.D.T.) AS JUDGED BY MOSQUITO DENSITIES, SPLEEN RATES, PARASITE RATES AND CHEMICAL ESTIMATION OF THE RESIDUAL DEPOSITS OF D.D.T. AT VARYING INTERVALS AFTER EACH APPLICATION AS AN INDOOR SPRAY.

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(September 9, 1950.)

INTRODUCTION.

D.D.T. has been successfully employed as an indoor residual spray for the control of malaria in several parts of the world. References are too numerous to be cited in this paper. Swellengrebel and Stack (1949) have, however, drawn attention to the inefficacy of this method for the control of malaria in New Guinea Island in the Malay Archipelago where the vector species is *A. punctulatus*, which not only rests but also feeds outdoors and hence no insecticidal application to

residential houses has had any value whatever in the control of malaria. Kennedy (1947) has drawn attention to the manifestation of a 'repellent' effect on exposure of *A. atroparvus* to short and therefore sublethal contact with D.D.T.-sprayed surfaces. Muirhead-Thomson (1947) has further reported on the failure of D.D.T. to bring about effective reduction in *A. gambiae* population and in their natural infection rates though the numbers caught in houses may show a great reduction. Gabaldon (1949) has referred to the inefficacy of D.D.T. in the control of malaria transmitted by *A. bellator* and *A. cruzi* in Brazil, quoting a private communication from Pinoti. On the other hand, as stated earlier, D.D.T. has been successfully used against most anopheline vectors in both the hemispheres. In Europe, the United States of America, Venezuela and other places, the dosage of D.D.T. employed in malaria control centres around 200 mg./sq. ft. A single application has been adequate in most places while in certain other places it has been repeated twice, thrice, or even four times in a year, but never earlier than once in 3 months. In India, on the other hand, Viswanathan and Ramachandra Rao (1947, 1948, 1949) and several others have employed a dosage of 56 mg./sq. ft. and repeated it about three times during the malaria season at intervals of 6 weeks to 2 months. There are several districts in India where the transmission of malaria is confined to about 4 months in a year. While the expenditure on insecticides, solvents, emulsifiers, etc., would remain the same, whether a single massive dosage is applied once during the year or it is split up into two or three applications at suitable intervals, the expenditure on labour and supervision would naturally be of a very much higher order in the second case. In order to gather some data on the relative merits of a single large dosage and multiple smaller dosages, an experiment was carried out in Ahmedabad District during the year 1949, the results of which are recorded here.

BRIEF EPIDEMIOLOGY OF MALARIA IN THE AREA OF EXPERIMENT.

In this district, not a single village is free from malarial endemicity, the spleen rates exceeding 10 per cent in every village. The average spleen rate is 46·1 per cent for the whole district and the average parasite rate is 11·2 per cent. Both *falciparum* and *vivax* are prevalent in about equal proportions. The vector is *A. culicifacies*, with a natural infection rate of 0·54 per cent (4 out of 737, all showing sporozoites). The season of transmission as judged from the dispensary figures is from September to December and hence control measures would need to be taken up from July-August to the end of November.

PLAN OF EXPERIMENT.

In three out of 10 talukas in the district (Ghogo, Modasa and Prantij*), D.D.T. was applied in a single dosage of 168 mg./sq. ft. in the month of August 1949. No further application was made during the year. The total population of sprayed

* Of these, Modasa and Prantij have since been taken out of the district and hence collection of data was discontinued.

villages is 117,559. In three other talukas, viz. Dholka, Danduka and Ranpur, during the month of August, a dosage of 112 mg./sq. ft. was applied and from October 1 to 15 a second application of 56 mg./sq. ft. was made. The population of the sprayed villages in this group is 203,920. In the remaining four talukas of the district, namely, Viramgam, Sanand, Daskroi and City Mahal, three rounds of application of D.D.T. in a dosage of 56 mg./sq. ft. were applied from August 1 to November 15, at intervals of 6 weeks. In this group the population receiving the benefit of the spray is 331,813. Stirrup pumps (double acting) with nozzles delivering 600 c.c. per minute in a cone-shaped spray were used in all cases. No accurate measurements were made either of sprayed surfaces or quantities of spray fluid applied. But, as in the general experience of the organization, total quantities sprayed each day by different units remained almost the same, the number of houses also was almost equal.

In each of the above 3 groups of areas, a number of villages whose total population is equal to one-eighth of the population of the group as a whole, was left, for purposes of comparison, without any application of D.D.T.

DATA COLLECTED.

Spleen rates and parasite rates among children between 2 and 10 years of age were compiled in the district when a malaria survey was carried out in 1947. At the end of the experiment and the season of malaria transmission in 1949, similar data were collected for sprayed and unsprayed villages in each group. Infant parasite rates were also compiled. As regards anopheline collections, the data collected by the insect collectors attached to the malaria control scheme in the district are not reliable since almost all of them were employed for the first time during that year and hence were not adequately trained. Use has therefore been made only of such data as were collected either by the senior author or his medical assistant during the course of their tours, with the help of specially trained insect collectors attached to the headquarters organization in Poona. In conformity with the general practice of this organization, timed collections were made and the densities recorded on a man-hour basis.

RESULTS.

Mosquito densities.—Table I shows the densities of female *culicifacies* in sprayed and unsprayed villages in each of the 3 experimental groups.

Discussion.—During September the density of female *culicifacies* in unsprayed villages ranged between 13·0 and 25·8 per man-hour. In the first group of villages the density was the highest (25·8) during the 2nd week of September. In the other two groups the maximum densities (17·8 and 14·7) are practically the same. After spraying a single massive dose of 168 mg./sq. ft., the densities rose from 4·0 per man-hour in the 1st week to 6·5 in the 2nd, 8·2 in the 4th and 23·1 in the 6th week. The rise till the end of the 4th week is slight and is consistent with the possibility of their being principally primiparous adults, since if they were older

TABLE I

Densities of A. culicifacies (females) in sprayed and unsprayed villages.

A. After application of a single dose of 168 mg. sq. ft.

Date of collection.	1st week of September.		2nd week of September.						1st week of December.		2nd week of December.	
Interval after spray.	1 week.		2 weeks.		4 weeks.		6 weeks.		16 weeks.		18 weeks.	
	Sp.	Unsp.	Sp.	Unsp	Sp	Unsp.	Sp.	Unsp.	Sp.	Unsp.	Sp.	Unsp
Actuals	12	81	39	155	49	155	149	155	11	14	[illegible]	[illegible]
Per man-hour ...	4·0	20·2	6·5	25·8	8·2	25·8	23·1	25·8	1·8	4·7	[illegible]	[illegible]

B-1. After application of 112 mg. sq. ft.

B-2. After 2nd application of 56 mg./sq. ft. following an application of 112 mg. sq. ft. 6 weeks earlier.

Date of collection.	1st week of September.		2nd week of September.				3rd week of September.		2nd week of September.		1st week of December.		2nd week of December.	
Interval after spray.	1 week.		2 weeks.		4 weeks.		6 weeks.		1 week.		11 weeks.		12 weeks.	
	Sp.	Unsp.	Sp.	Unsp.	Sp.	Unsp.	Sp.	Unsp.	Sp.	Unsp.	Sp.	Unsp.	Sp.	Unsp
Actuals	26	78	31	89	46	89	90	96	9	96	4	40	2	8
Per man-hour ..	3·2	13·0	6·2	17·8	9·2	17·8	15·0	16·0	1·5	16·0	1·0	2·5	0·5	2·0

	C-1. After application of 56 mg. sq. ft.						C-2. After 3rd application of 56 mg./sq. ft. following two earlier similar applications at intervals of 6 weeks.					
Date of collection.	2nd week of September.						1st week of December					
Interval after spray.	2 weeks.		3 weeks.		4 weeks.		1 week.		2 weeks.		4 weeks	
	Sp.	Unsp.	Sp.	Unsp.	Sp.	Unsp.	Sp.	Unsp.	Sp.	Unsp.	Sp.	Unsp.
Actuals	10	44	11	44	28	44	0	7	0	7	0	7
Per man-hour ...	2·5	14·7	5·5	14·7	9·3	14·7	0	1·2	0	1·2	0	1·2

Sp. = sprayed. Unsp. = unsprayed.

specimens, they would have laid multiple batches of eggs and there would have resulted a larger increase in adult densities in houses especially at a period most favourable for mosquito propagation. The steep rise, however, from the 4th to the 6th week (from 8·2 to 23·1) and the density in sprayed villages reaching nearly as high as in unsprayed villages (23·1 and 25·8 respectively) furnish strong evidence of the survival of multiparous females on the one hand and the loss of residual insecticidal effect of the sprayed surfaces on the other hand. Viswanathan and Ramachandra Rao (1948, 1949) have shown that with persistence of insecticidal effect there would be waves in the density of human or animal house-haunting anophelines and with a loss of such effect there would be a steep rise reaching the densities of unsprayed villages. There is not much difference between the 2nd and 3rd group. In the 2nd group (dosage 112 mg./sq. ft.) the densities in sprayed and unsprayed villages are 9·2 and 17·8 respectively at the end of the 4th week. In the 3rd (56 mg./sq. ft.), similar figures are 9·3 and 14·7. Purely by accident the densities in the unsprayed villages are highest in those situated close to 1st group of villages. While, if, despite this difference, the massive application resulted in low densities, the experiment would clinch its superiority, the present data show that, in areas with high mosquito densities, massive application of D.D.T. loses its residual effect by about the end of 6 weeks. In the 2nd group too, at the end of 6 weeks the densities in sprayed and unsprayed villages are nearly the same (15·0 and 16·0, respectively). No data are available regarding densities at the end of 6 weeks in the 3rd group of sprayed villages. But there is no reason to doubt that the insecticidal effect will almost be completely lost. On the whole, therefore, larger dosages than 56 mg./sq. ft. have good results only in the earlier weeks but, by the end of 6 weeks, their effects appear to be nearly fully lost.

Table II gives the spleen rates and parasite rates among children 2 to 9 years of age and infant malaria (parasite) rates in each of the 3 groups separately for sprayed and unsprayed villages in December 1947 and December 1949. The data presented in Table II show that in each of the groups, spleen rates, parasite rates and infant malaria rates have undergone a distinct reduction at the end of the season of transmission in 1949 as compared with 1947. It may be stated here that in 1948 the entire district experienced a severe draught and special famine works were undertaken by the Government. As malaria in this district is principally dependent on rainfall, 1948 was an unusually healthy year. Due to interruption in active transmission during that year even in unsprayed villages, data for 1949 show some reduction in 1949 as compared with 1947 (*vide* spleen rates in Groups 1 and 3). The percentage reduction in spleen rates in sprayed villages has been the highest in Group 3 (63 per cent), nearly as high in Group 2 (60 per cent) and least in Group 1 (45 per cent). Parasite rates show very little reduction except in Group 3, but Group 2 again shows a larger reduction than Group 1. Infant malaria rates are least in Group 3 with the largest reduction (from 11·1 to 2·5 per cent). In Group 2, this rate is 5 per cent as compared with 16·7 per cent in the unsprayed villages during the same period and 13·3 per cent in the same villages at the end of 1947. Thus individually and collectively all data seem to betoken the greatest reduction in the prevalence of malaria during the 1949 season in Group 3 villages and the least reduction in Group 1.

TABLE II.

Spleen and parasite rates.

	December 1947.		December 1949.		December 1947.		December 1949.		December 1947.		December 1949.	
	Sprayed villages.	Unsprayed villages.	Sprayed villages.	Unsprayed villages.	Sprayed villages.	Unsprayed villages.	Sprayed villages.	Unsprayed villages.	Sprayed villages.	Unsprayed villages.	Sprayed villages.	Unsprayed villages.
	Group 1.				**Group 2.**				**Group 3.**			
Number of children examined.	378	77	363	66	1,033	372	1,314	220	2,710	183	2,383	187
Spleen rate, per cent ...	44·4	40·3	24·5	33·3	56·0	59·7	22·4	55·5	43·1	43·8	15·9	39·0
Number of children examined.	115	99	161	45	271	74	451	191	362	46	880	57
Parasite rate, per cent ...	10·4	10·0	9·3	11·1	11·5	9·5	8·2	8·9	10·3	8·7	4·7	12·3
Number of infants examined.	25	16	24	15	30	35	20	48	45	Not examined.	118	18
Infant malaria (parasite) rate, per cent.	20·0	18·8	8·3	13·3	13·3	14·3	5·0	16·7	11·1	...	2·5	11·1

DISPENSARY DATA.

Table III shows the number of malaria cases treated as out-patients (based on clinical but not on microscopical diagnosis) in various dispensaries in each group every year from 1944 to 1949. The number treated in the 3rd and 4th quarters of each year is also separately shown.

TABLE III

Malaria cases (out-patients) treated in dispensaries.

	1944.	1945.	1946.	1947.	1948.	1949.
Group 1.						
3rd quarter	290	706	224	128	45	27
4th ,,	198	295	564	427	24	149
Annual	581	1,089	526	672	196	228
Group 2.						
3rd quarter	4,435	2,538	2,707	2,018	1,383	1,490
4th ,,	5,013	2,981	3,792	3,266	1,614	2,193
Annual	13,824	8,157	8,966	8,137	5,693	5,794
Group 3.						
3rd quarter	2,351	4,956	2,017	1,743	1,229	1,361
4th ,,	2,884	3,038	2,924	1,823	1,707	1,506
Annual	7,206	7,603	7,028	6,462	5,733	4,316

As stated earlier, due to unprecedented draught in 1948, there was very little malaria transmission. Excluding that year's figures from consideration, one would notice a substantial reduction in malaria cases in all groups in 1949 as compared with the years prior to 1948. Too much reliance cannot be placed on dispensary data except to show trends over a period of time. It is therefore premature to draw any valid conclusions.

CHEMICAL ANALYSIS OF D.D.T. RESIDUAL DEPOSITS.

The arrangements for the chemical analysis of scrapings from D.D.T.-sprayed surfaces got under way only in November 1949, and hence the shortest interval between the application of D.D.T. in a single massive dosage of 168 mg./sq. ft. and the date of collection of the scrapings from sprayed surfaces was about 17 weeks. In the case of areas in which two applications of D.D.T. were made, the 1st with 112 mg./sq. ft. and the 2nd with 56 mg./sq. ft., the shortest interval after the 2nd application was 10 weeks. The longest interval between the date of 2nd application in these areas and date of collection of scrapings for analysis was 20 weeks. In the case of areas where three rounds of D.D.T. were applied in a dosage of 56 mg./sq. ft., the shortest interval was 1 week and the longest 14 weeks after the 3rd application. In the beginning it was not intended as a part of the experiment to resort to chemical estimation of residual deposits of D.D.T. in sprayed surfaces. But when the services of a research assistant were later made available, his services were utilized on this behalf. By that time, however, a considerable period of time had passed since the application of the single heavy dosage of D.D.T. and it is unfortunate, but unavoidable, that results of analysis of scrapings in earlier weeks are not available. Even after the above arrangements were completed, it took some further period to obtain Alessandrini's (1948) colorimetric scales. Pending their arrival, in the earlier analysis, the quantitative determination of D.D.T. (technical) was made from the hydrolysable chlorine content of the scrapings estimated by the method of Gunther (1945), modified by Laclair (1946). For the purpose of this estimation, scrapings were collected from an area about 6 × 6 inches and of thickness varying from 1 to 3 mm. depending on the ease with which the surface could be scraped and on the porosity of the surface. In the case of estimation of D.D.T. by Alessandrini's method, scrapings were collected from an area 5 cm. square and through a thickness varying between 1 and 3 mm. as stated above. In view of the fact that the amount of cowdung present on the walls caused a certain amount of discoloration and, in view also of the fact that the separating funnel tended to be clogged, Alessandrini's method was modified to the extent of extracting D.D.T. from the scraped sample with sulphuric ether, the solvent which she herself has suggested. The solvent was later allowed to evaporate and the residue proceeded with as described in her method.

CORRELATION BETWEEN THE ESTIMATE OF D.D.T. BY HYDROLYSABLE CHLORINE AND ALESSANDRINI'S METHODS.

In Thana District where every village has been sprayed with D.D.T. as a malaria control measure thrice during 1949, the first two rounds with about 60 mg./sq. ft. and the third round with 40 mg./sq. ft., estimation of D.D.T. deposit on sprayed surfaces was made by both methods at intervals of 4, 6, 8 and 10 weeks respectively after the third application. The smaller dosage in the third round is due to the need to restrict the total annual consumption of D.D.T. to the sanctioned quantity of 20 tons. The frequency of application in this district is about 2 months. Samples of scrapings were taken from 3 villages in each of the above 4 groups and in each village from 3 houses and in each house from

3 different portions of the walls. Thus a total of 108 samples is available. Among these as many as 24 gave 0 values by either method and are therefore excluded. The remaining 84 pairs of values are furnished below in the form of a correlation table.

TABLE IV.

Correlation table between observed values by hydrolysable chlorine method and Alessandrini's test (D.D.T. in mg./sq. ft.).

Alessandrini's test	Hydrolysable chlorine method. 0–	3–	6–	9–	12–	15–	18–	21–	24–	27–29·99	TOTAL
0	2	...	...	...	...	...	...	...	...	..	2
3	...	3	20	...	...	...	...	...	...	...	23
6	...	...	7	18	1	2	...	...	...	..	28
9	...	...	...	...	...	...	...	...	...	...	...
12	..	...	...	1	1	5	...	12	...	2	21
15	..	...	...	...	...	...	...	...	...	..	...
18	...	...	...	...	...	...	...	...	...	..	...
21	...	...	...	...	...	...	...	3	...	7	10
TOTAL ..	2	3	27	19	2	7	...	15	...	9	84

There is a high and significant degree of correlation between the values obtained by the two methods, the correlation coefficient being $0{\cdot}927 \pm 0{\cdot}016$. The regression between the values obtained by the two methods is given by the equation $\overline{X} = 0{\cdot}3 + 0{\cdot}94\ Y$. For our purpose it is enough to assume that the values given by Alessandrini's test is approximately seven-tenths of the value given by the hydrolysable chlorine method. It is only this latter correction factor that is utilized later in the limited cases where the value obtained by one method has been converted into its equivalent by the other method to facilitate comparisons with other values obtained by the latter method.

ESTIMATION OF RESIDUAL DEPOSITS OF D.D.T. ON SPRAYED SURFACES AT VARYING INTERVALS OF TIME AFTER APPLICATION IN DIFFERENT DOSAGES.

Table V shows the quantities of D.D.T. in mg./sq. ft. remaining on the sprayed surfaces after an application of 168 mg./sq. ft. 17 and 18 weeks earlier.

TABLE V.

D.D.T. (technical) in mg./sq. ft. estimated by hydrolysable chlorine method.

Serial number.	17 weeks after spray.	18 weeks after spray.
1	0	0
2	0	0
3	0	0
4	3·52	1·76
5	3·52	1·76
6	1·76	0
7	0	0
8	0	0
9	0	0
Mean	0·98 ± 0·51	0·39 ± 0·27

Each column represents the results of analysis of 9 samples taken from 3 houses in a village and in each house from 3 different portions of sprayed walls. It will be seen that from the figures given above there is practically no residual deposit of D.D.T. 17 weeks after an application of 168 mg./sq. ft. In view of the above negative finding there is no need to convert the D.D.T. determined by hydrolysable chlorine method into its equivalent by Alessandrini's method. For the reasons stated earlier it has not been possible to determine residual deposits at any earlier period.

Table VI shows the quantities of D.D.T. in mg./sq. ft. remaining on sprayed surfaces after a 2nd application of 56 mg./sq. ft. following an application of 112 mg./sq. ft. 6 weeks earlier.

TABLE VI.

D.D.T. (technical) in mg./sq. ft. estimated by hydrolysable method (A) or Alessandrini's method (B) at stated weeks after 2nd application of 56 mg. following 112 mg. applied 6 weeks earlier.

Serial number.	Method A.			Method B.	
	10 weeks.	12 weeks.	14 weeks.	18 weeks.	20 weeks.
1	0·0	10·64	0·0	0·0	3·6
2	0·0	14·20	1·76	0·0	0·0
3	0·0	14·20	0·0	0·0	0·0
4	14·20	10·64	10·65	3·6	0·0
5	14·20	10·64	10·65	3·6	0·0
6	17·75	3·52	0·0	0·0	0·0
7	10·65	14·20	14·20	0·0	0·0
8	14·20	0·0	10·65	3·6	3·6
9	14·20	0·0	7·10	0·0	0·0
Mean	9·47 ± 2·5	8·67 ± 1·33	6·11 ± 1·9	1·2 ± 0·6	0·8 ± 0·52

At the end of 18 weeks after an application of 56 mg./sq. ft. following 112 mg./sq. ft. applied 6 weeks earlier still, there is practically no residual deposit. 10, 12 and 14 weeks after the 2nd application, the residual deposits were, say, about 10 mg., 8 mg., and 6 mg. (very roughly) as determined by hydrolysable chlorine method or 7 mg., 5·6 mg., and 4 mg. respectively by Alessandrini's method.

Table VII furnishes the results of estimation of residual D.D.T. 1, 2, 4, 6, 8, 10, 12 and 14 weeks respectively after the third round of application of 56 mg./sq. ft. following two earlier applications of a similar dosage at intervals of approximately 6 weeks.

TABLE VII.

D.D.T. (technical) in mg./sq. ft. estimated by hydrolysable chlorine method (A) or Alessandrini's method (B) at stated weeks after 3rd application of 56 mg. following two earlier applications of 56 mg. at 6 weekly intervals.

Serial number.	Method A.				Method B.			
	1 week.	2 weeks.	4 weeks.	6 weeks.	8 weeks.	10 weeks.	12 weeks.	14 weeks.
1	42·6	56·8	32·0	24·0	3·6	3·6	3·6	0·0
2	71·0	56·8	32·0	21·3	7·2	3·6	3·6	0·0
3	42·6	49·7	32·0	17·8	7·2	0·0	3·6	0·0
4	78·1	63·9	7·1	21·3	14·4	7·2	0·0	3·6
5	71·0	17·8	7·1	21·3	14·4	7·2	0·0	0·0
6	63·9	17·8	17·8	21·3	21·6	3·6	0·0	3·6
7	78·1	63·9	32·0	0·0	0·0	0·0	3·6	7·2
8	71·0	49·7	35·5	7·1	3·6	3·6	7·2	0·0
9	63·9	56·8	28·4	7·1	3·6	7·2	3·6	0·0
Mean ..	64·7 ± 4·5	48·4 ± 6·0	24·9 ± 3·7	15·8 ± 2·9	...	...	...	...
Equivalent by method A.	45	34	17	11	8·4 ± 2·2	4·0 ± 0·9	2·8 ± 0·8	1·6 ± 1·0

Thus, after a period of 1, 2, 4, 6, 8, 10, 12 and 14 weeks respectively after the 3rd application of 56 mg./sq. ft. following two similar earlier applications at intervals of 6 weeks, the D.D.T. residual deposits were found to be 45, 34, 17, 11, 8·4, 4·0, 2·8 and 1·6 mg./sq. ft. These data are compared in Table VIII with the results of scrapings at varying intervals after a single application of 56 mg./sq. ft. in Thana District.

TABLE VIII.

Analysis of scrapings from surfaces sprayed with 56 mg./sq. ft. of D.D.T. (technical) at various intervals after the spray (Thana District).

Serial number.	Village.	Date of spraying.	Date of scraping.	Period in weeks.	D.D.T. (TECHNICAL) IN MG./SQ. FT. ESTIMATED BY ALESSANDRINI'S METHOD.										
					1	2	3	4	5	6	7	8	9	Mean.	Standard deviation of mean.
1	Shirvana	May 21, 1950	May 21, 1950	0	72·0	36·0	36·0	72·0	144·0	72·0	72·0	36·0	36·0	64	11·7
2	Nahur	June 27, 1950	July 6, 1950	1	36·0	72·0	21·6	7·2	3·6	28·8	36·0	72·0	144·0	45·2	14·6
3	Kamba	Do.	July 11, 1950	2	21·6	14·4	7·2	36·0	36·0	28·8	21·6	14·4	36·0	28·4	3·3
4	Vadap	Do.	Do.	2	28·8	28·8	72·0	14·4	14·4	36·0	36·0	36·0	28·8		
5	Nandkar	June 19, 1950	July 17, 1950	4	21·6	21·6	14·4	3·6	28·8	28·8	14·4	21·6	21·6	19·6	2·6
6	Duggad	June 6, 1950	Do.	6	14·4	3·6	3·6	14·4	3·6	14·4	3·6	28·8	14·4	9·8	1·8
7	Ogde	June 5, 1950	Do.	6	21·6	14·4	7·2	7·2	0·0	0·0	3·6	7·2	14·4		
8	Saivan	May 2, 1950	July 16, 1950	8	7·2	3·6	0·0	3·6	14·4	7·2	14·4	3·6	0·0	6·0	1·8
9	Mohop	May 8, 1950	July 19, 1950	10	7·2	0·0	0·0	7·2	7·2	3·6	7·2	3·6	3·6	4·4	1·0

Table IX shows the mean D.D.T. residual deposits at various weeks after a single application of 56 mg./sq. ft. in Thana District compared with similar data in Ahmedabad District after three such applications.

TABLE IX.

Mean residual D.D.T. deposit after one and three applications respectively of 56 mg./sq. ft.

Interval in weeks.	Single application.						3 applications.					
	1	2	4	6	8	10	1	2	4	6	8	10
D.D.T (mean) in mg./sq. ft.	15·2	28·4	19·6	9·8	6·0	4·4	46·5	34·6	17·9	11·3	8·4	4·0
Standard deviation of the mean.	14·6	3·3	2·6	1·8	1·8	1·0	3·2	4·3	2·7	2·1	2·2	0·9

It will be found from Table IX that there is no significant difference in the mean D.D.T. residual deposit in any of the weeks after a single or three applications in a dosage of 56 mg./sq. ft. In conformity with the authors' previous experience, densities of *culicifacies* reach critical threshold by the end of 6 weeks after an application of 56 mg./sq. ft. On that basis a residual deposit of about 10 mg./sq. ft. seems to be the minimum necessary for effective insecticidal action. There appears to be a greater rate of loss per week in the first 2 weeks (about 30 per cent). In later weeks there is an average loss of about 20 per cent of D.D.T. per week. On this basis, with a single large dosage of 168 mg./sq. ft. there will be about 10 mg./sq. ft. on 12th-13th week. Yet in the present studies, even at the end of 6 weeks, mosquito densities steeply rose. Apparently there is either a larger rate of loss after massive applications or a larger quantitative loss at some period after the application of such a dosage, which in the absence of data of analysis of residual D.D.T. in earlier weeks after massive dosage, is not possible to determine.

DISCUSSION.

There are some limitations to a critical appraisal of the data presented in this paper. Firstly, in the absence of data regarding residual D.D.T. deposits in the earlier weeks after larger dosages of application, mosquito densities cannot strictly be correlated to determined quantities of residual deposits. Secondly, mosquito densities are recorded in certain villages but residual deposits determined in certain other villages. Data regarding both in the same villages will offer a more valid correlation. Thirdly, the various other indices of malariometry are compiled in a certain number of villages in which residual deposits were not determined. This study, therefore, lacks the precision attached to compilation of all indices including determination of residual deposits in a few specified villages. On the

other hand it has one merit from the practical point of view. In large scale malaria control projects, it is not possible to determine exact epidemiological features in each village. Only random samples can be utilized both for purposes of survey—and determination of malarial endemicity—and for appraisal of results after institution of control measures. Judged from this point of view the present data are worthy of consideration so long as general conclusions are not prematurely drawn on the results of this single study in one area in one year. There are two other disturbing factors. The first is that in the talukas of Modasa and Prantij, where mosquito data were not collected due to their later removal from the district, casual spleen census reveals at least as much reduction in spleen rates and parasite rates and malaria cases treated in the dispensaries as in the 3rd group. Secondly, it has now come to light that in the areas where a single large dose was applied the normal mosquito densities (as judged by data for comparison villages) are very much higher. Hence the results are not strictly comparable. By and large however the studies herein reported show that a single large dose of D.D.T (168 mg./sq. ft.) cannot be reckoned as adequate for control of malaria in all areas. It may be enough in some areas but it may be quite inadequate in others. Pending further studies on the problem—which are in progress—it may be stated that in the absence of precise knowledge of local epidemiology of each village it is prudent to resort to three applications as hitherto in progress in the State of Bombay. This has the added merit that collateral effects are secured and trained staff are employed over a longer period. Again organizationally with the extensive malaria control programmes undertaken by the Government of Bombay it will be extremely difficult to find the staff to apply one large dose of D.D.T. simultaneously in several talukas of each district. Again the practice of renovation of walls and thatched roof entirely nullifies a single application whereas with multiple applications there will be some benefits secured. All these organizational advantages are not strictly relevant to the determination purely from the scientific point of view whether under Indian conditions a single large dose will be as fruitful as multiple doses. The limited answer to that is that so far it has not been proved to be so in all cases. Until such proof is forthcoming the present practice should and will continue as much from the scientific as from the pragmatic point of view.

SUMMARY AND CONCLUSIONS.

(1) The results of comparative trials with a single application of 168 mg./sq. ft. of D.D.T.; two applications, the first in a dosage of 112 mg., and the second, 6 weeks later, in a dosage of 56 mg.; and three applications, at six weekly intervals, of 56 mg./sq. ft. carried out in Ahmedabad District, Bombay State, are presented.

(2) *A. culicifacies* is the local vector with a natural infection rate of 0·54 per cent. The season of transmission is from July-August to November. The average spleen rate for the whole district is 46·1 per cent.

(3) At the end of 6 weeks in either of the 2 groups where larger dosage was applied, densities of female *A. culicifacies* are found to reach the same level as in neighbouring unsprayed villages. Data are not available for the 3rd group at this period but elsewhere in the State previous studies have shown that after 56 mg./sq. ft. dosage densities come up to the level of comparison villages at the end of 6 weeks.

(4) Spleen rates, parasite rates and infant malaria rates show greater reduction in the experimental area after three rounds of applications of 56 mg./sq. ft. of D.D.T. than after fewer applications of larger dosage.

(5) Analysis of residual deposits of D.D.T. shows that 17 weeks after a dosage of 168 mg./sq. ft., there is no residue whatever. Data for earlier periods are not available. At the end of 6 weeks after a dosage of 56 mg./sq. ft., the residual deposit is only 10 mg./sq. ft. This is presumably the minimum residual deposit necessary for effective reduction of vector density below critical levels.

(6) After a single application of 56 mg./sq. ft., there appears to be a loss of 20 per cent of D.D.T. every week. Although 6 weeks after an application of 168 mg./sq. ft. there should on this basis still be a residual deposit of about 48 mg./sq. ft., present studies show a loss of insecticidal action at that period. The rate of loss after larger applications appears therefore to be of a different order.

(7) The present studies, on account of several limitations, are not conclusive. But they do not furnish any evidence of the superiority of a single large dosage of 168 mg./sq. ft. of D.D.T. in malaria control over three applications of 56 mg./sq. ft. at 6 weekly intervals.

ACKNOWLEDGMENT.

The authors wish to thank Dr. K. Venkataraman and Dr. G. P. Kane, Department of Chemical Technology, University of Bombay, in whose laboratories the chemical analysis reported in this paper was carried out by the junior author.

REFERENCES.

ALESSANDRINI, M. R. (1948) . *Ved. Ann. di Chem.*, app. 38.
FISHER, R. A. (1934) . *Statistical Methods for Research Workers*. Fifth edition. Oliver and Boyd, London.
GARSDON, A. (1949) . *Malariology*. Edited by Mark F. Boyd, p. 1413. W. B. Saunders Co., Philadelphia and London.
GUNTHER, F. A. (1945) *Indust. Eng. Chem.* (Anal. 2nd), **17**, p. 149.
KENNEDY, J. S. (1947) .. *Bull. Ent. Res.*, **37**, pp. 593–607.
LACLAIR, J. B. (1946) ... *Indust. Eng. Chem.* (Anal. 2nd), **18**, p. 763.
MUIRHEAD-THOMSON, R. C. (1947) *Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, p. 511.
SWELLENGREBEL, N. H., and STACK, T. (1949). *Ann. Soc. Belge de Med. Trop.*, **29**, 2, pp. 207–212.
VISWANATHAN, D. K., and RAMACHANDRA RAO, T. (1947). *Ind. J. Mal.*, **1**, pp. 503–542.
Idem (1948) *Ibid.*, **2**, pp. 157–210.
Idem (1949) *Ibid.*, **3**, pp. 269–312.
VISWANATHAN, D. K., RAMACHANDRA RAO, T., and JUNEJA, M. R. (1949). *Ibid.*, **3**, pp. 57–67.

Indian Journal of Malariology, **4**, 4, December 1950.

FURTHER NOTES ON THE USE OF BENZENE HEXACHLORIDE AS A RESIDUAL INSECTICIDE COMPARED WITH DICHLORO-DIPHENYL-TRICHLOROETHANE.

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[December 31, 1950.]

INTRODUCTION.

VISWANATHAN *et al.* (1949) recorded results of a preliminary field trial with benzene hexachloride and dichloro-diphenyl-trichloroethane in their residual insecticidal action against anopheline mosquitoes and effects on malaria transmission. The area selected for the experiment lies in Mokhada Taluka, Thana District, Bombay State, about 80 miles north of Bombay City. In the preliminary trials carried out in 1948, 2 groups of villages in this taluka were treated with benzene hexachloride (B.H.C.) and dichloro-diphenyl-trichloroethane (D.D.T.) respectively, and a third group was studied for purposes of comparison. During those trials there were certain limitations, such as lack of adequate data regarding the vector species, their densities and their vectorial capacity. Further, when the first round of spraying was commenced malaria transmission had already occurred for some weeks. It was therefore decided that further work should be conducted during the next malaria season. This paper records the results of the further experiments thus carried out.

VILLAGES SELECTED FOR THE EXPERIMENT.

The villages selected for treatment with B.H.C. and D.D.T. and for comparison were as follows :—

Comparison villages.		Benzene hexachloride villages.		D.D.T. villages.	
Village.	Population.	Village.	Population.	Village.	Population.
1. Chas	692	1. Vashala ...	1,018	1. Karegaon ...	715
2. Jondale B ...	679	2. Satundi ...	687	2. Gonghar ...	759
3. Morsande ...	924	3. Mokhada ...	3,157	3. Suryamal ...	579
		4. Poshera ...	1,283		
		5. Khodala ...	962		

ANOPHELINE FAUNA.

In addition to the 16 different species of Anopheles recorded in the preliminary note, viz. *annularis, barbirostris, culicifacies, fluviatilis, jamesi, jeyporiensis, maculatus, moghulensis, pallidus, philippinensis, splendidus, subpictus, tessellatus, theobaldi, vagus* and *varuna*, 5 more species were recorded during the further experiment, viz. *aconitus, hyrcanus* var. *nigerrimus, karwari, stephensi* and *turkhudi*. As in the preliminary trial during the present experiment also, no natural infections were met with amongst anophelines. Altogether 64 *fluviatilis* and 195 *culicifacies* were dissected during the second year as compared to 150 and 226 respectively in the former year, all with negative results, but from the authors' experience elsewhere in the district and in neighbouring districts, there would seem no doubt that both *culicifacies* and *fluviatilis* should be deemed as the vector species in this locality, the former probably being far more important on account of its much larger numerical prevalence.

DETAILS OF EXPERIMENT.

B.H.C., supplied for purposes of the experiment by the Imperial Chemical Industries (India) Limited, in the form of a wettable powder labelled by them as "Gammexane P.520" and said to contain 50 per cent B.H.C. or about 6·5 per cent *gamma* isomer, was sprayed in a dosage of 12 oz. of wettable powder suspended in one gallon of water and applied over 2,000 sq. ft. (11 mg. of *gamma* isomer per sq. ft.). D.D.T. was applied in the form of a 5 per cent emulsion made with soap

from a solution of D.D.T. (Technical) in "aromex", at a dosage of 56 mg. per sq. ft. The first round of spray with benzene hexachloride was applied between July 19 and 21, and the second round between October 25 and 27, 1949. The first round of D.D.T. was sprayed in all the three villages on July 22, and the second round was not applied in one village (Suryanal), while in two others it was applied on October 28 and November 21, 1949 (Karegaon and Gonghar respectively).

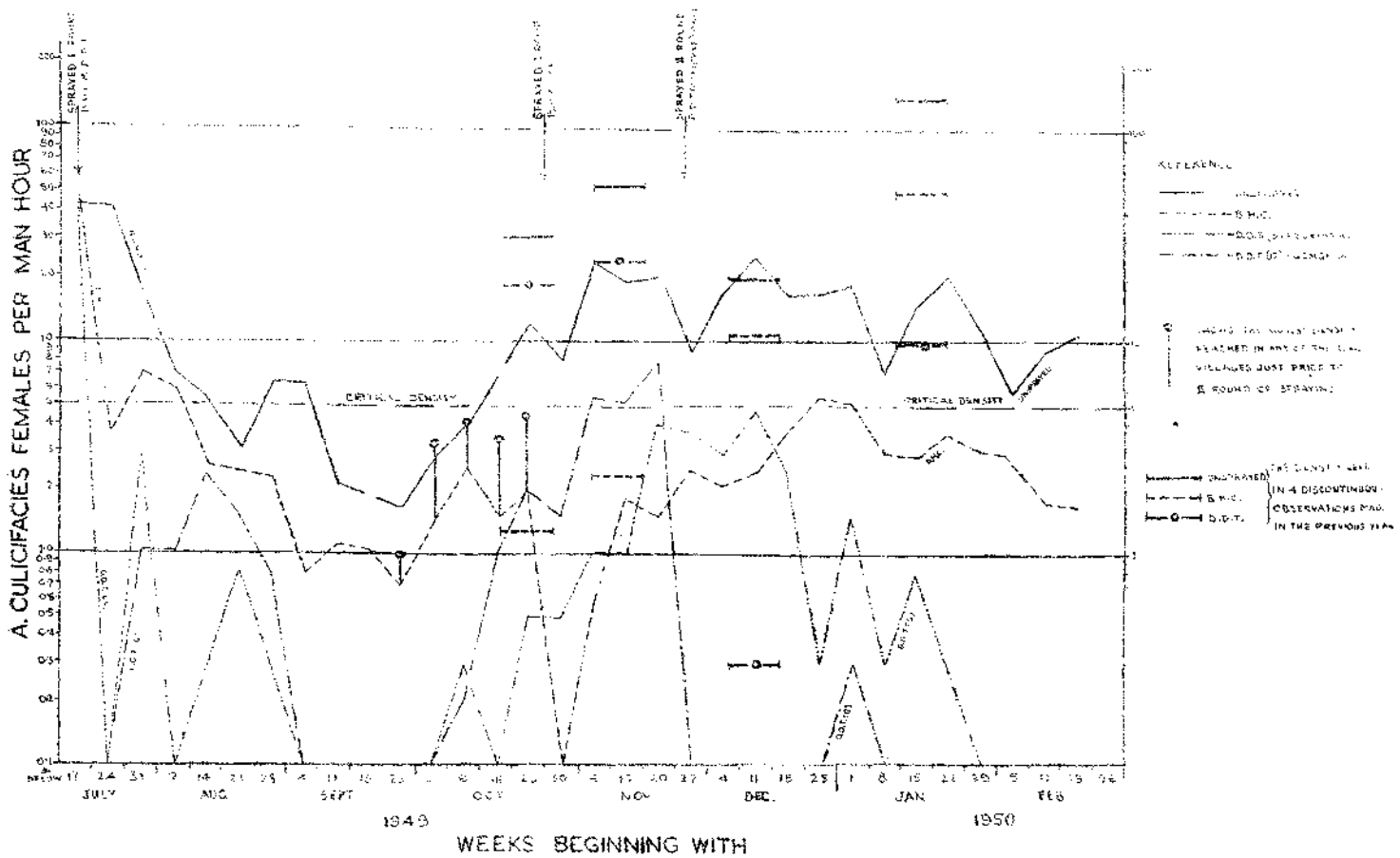
Stirrup pumps with oil-resisting hose, brass lance and a nozzle with an orifice 3/64 inch in diameter, delivering 600 c.c. per minute in a cone-shaped spray were used. All structures including human dwellings, mixed dwellings and cattlesheds were sprayed in both the groups of villages.

ENTOMOLOGICAL DATA.

Entomological data were collected in the sprayed villages as well as in the comparison group from the middle of July 1949 till the end of February 1950. Mosquitoes were collected for 1 man-hours once a week in each of the villages between 07.00 and 12.00 hours. The bulk of the collections were made in mixed dwellings (housing both men and animals) though human dwellings and cattlesheds were also searched. The nature of the dwellings altered often during the course of these investigations and what was a pure human dwelling sometimes became a mixed dwelling and *vice versa*. Therefore, while, in Table I, a summary of all anophelines collected by species and resting places throughout the period of the experiment is presented, for the sake of completeness and for study of the relative proportions of the several species, data with respect to mixed dwellings only are utilized for purposes of critical study. In Tables II, III and IV, data for mixed dwellings are presented for each group, viz. comparison villages, B.H.C. villages and D.D.T. villages respectively, showing only the numbers of *culicifacies* and *fluviatilis* and all anophelines collected, together with the time spent and the per man-hour figure for each week. The densities of *culicifacies* females are also shown in Chart I. In Table IV, the figures for Suryanal, Gonghar and Karegaon, all sprayed with D.D.T., are separately shown as the first village was sprayed only once, the second was sprayed twice and the third one also twice but on the second occasion due to a mistake. On this account mosquito densities for this village have not been shown in Chart I. Data regarding mosquito densities determined at four different periods during the previous season are also shown in the chart.

Comparison villages (Table II).—The densities of mosquitoes in the comparison villages had already reached high points in the third week of July when these observations were started. *Culicifacies* females had an average density of 11·1 per man-hour and formed the bulk of the specimens collected. From then onwards till the end of September, there was a gradual but steady decline in the densities of all Anopheles including *culicifacies*. They rose again gradually until the peak was reached in the months of November and December with a slight decline again till the end of February when the observations were suspended. The considerable fall in densities of *culicifacies* and of all anophelines in August and September can be associated with the high rainfall occurring during these months in this area, which obviously was responsible for flushing away the larvæ from the breeding places. The densities of *culicifacies* females remained below 5 per

CHART


A. CULICIFACIES FEMALES PER MAN HOUR
CRITICAL DENSITY
CRITICAL DENSITY
JULY
AUG.
SEPT
OCT
NOV
DEC.
JAN
FEB
1949
1950
WEEKS BEGINNING WITH

man-hour, the assumed critical density for the species, for nearly 6 weeks during September and October.

B.H.C. villages (Table III). At the commencement of experiment, the densities of *culicifacies* and all anophelines were very high in all the 5 villages selected for spraying with B.H.C., and *culicifacies* again formed the bulk of anophelines collected. The average density of *culicifacies* in the five villages was 46·1 per man-hour, well above the assumed critical density of 5 per man-hour. All the villages were sprayed between July 19 and 21, resulting in immediate fall in densities. In the week immediately after spraying, the density of *culicifacies* was reduced to 3·1 per man-hour. In individual villages, it was 5·8 per man-hour in Mokhada, 0·3 in Vashala, 2·8 in Saturli, 2·0 in Poshera and 5·5 in Khodala. From then onwards, in conformity with the general fall in the density of anophelines in the whole experimental area, as shown in the comparison villages, the densities in the B.H.C. villages also underwent a gradual decline and stayed low till the 1st week of October. The lowest density recorded was 0·7 in the last week of September.

By the middle of October, *culicifacies* densities showed an increase. It had been decided that the second round of spraying should be carried out when the *culicifacies* densities reached or approximated to the critical density and such a tendency was manifest for more than 2 weeks not necessarily in the same village. In the weeks beginning with October 9, 16, 16, 23 and 23 the densities had already risen to 4·3 in Mokhada, 2·5 in Vashala, 3·5 in Poshera, 4·5 in Saturli and 3·5 in Khodala respectively. Thus from October 9 to October 23, there was a tendency for the *culicifacies* density to come up to the level of the assumed critical density though it was still below it. Another factor influencing the decision regarding the date of the second round of spraying was the reported increase in malaria cases both by the Medical Officer of Mokhada and the people themselves during the third week of October. The question for decision was whether one should wait for the actual increase of *culicifacies* to more than 5 per man-hour or to anticipate that level being reached, and commence the second round of spray. It was decided in favour of the latter course especially because the people of the district were conscious of malaria control being successfully established with D.D.T. elsewhere and were somewhat critical of the experiment in progress with another insecticide. Hence taking all factors into consideration it was decided to carry out the second round of spray with B.H.C. on October 25, 26 and 27. Yet another factor that was taken into consideration was the general liability for malaria prevalence in the month of November in this area and the need to anticipate such prevalence. It may well be that in a month most liable to malaria prevalence the critical density is even less than the average assumed level throughout the season. On the whole one had to balance between the needs of a strictly scientific experiment and the effects of its probable failure on the health of the people, especially in a field experiment of this kind. In the event of delaying the second round, the density would have undoubtedly gone up above the assumed critical level. The densities after the second round of spray remained more or less uniform in all the B.H.C. villages. Though during this period of observation they were extremely high in the unsprayed villages, the densities in the B.H.C.-sprayed villages remained below the critical level and at a far lower level than in the comparison villages. They exceeded the critical limit only towards the end of December for a couple of weeks and showed a decline thereafter.

D.D.T. villages (Table IV).—In the villages to be sprayed with D.D.T. also, *culicifacies* densities were very high in the middle of July (with per man-hour figures of 40·0 in Suryamal, 35·5 in Gonda and 43·0 in Karegaon). After the first round of spraying, the reduction in D.D.T. villages was far more marked than in the B.H.C. villages. During the middle of October when the rapid rise in the densities in B.H.C. villages necessitated the second round of spraying, the densities in D.D.T. villages remained low and in fact for five weeks continuously *culicifacies* was totally absent in the catches made in all the three villages. But by the first week of November the density of *culicifacies* had risen to above the critical density in Gonghar and remained high in the following two weeks. A second round of spraying was therefore carried out on November 26, two weeks after the rise was noticed. The density in Suryamal remained very low throughout the period except for a few weeks in November and December when it began to show tendency to reach almost the critical density but soon declined again. In Karegaon also the densities were low and negligible throughout the period but owing to a misinterpretation of instructions to the staff engaged in the general spraying work in the district, it was sprayed for the second time on October 28. In both Gonghar and Karegaon the *culicifacies* density has been practically negligible after the second round of spraying: while during the same period the B.H.C. villages, and to a more marked extent the unsprayed villages, were showing increased *culicifacies* prevalence.

Night mosquito collections.—In order to study the variations in mosquito densities during day and night respectively, collections were made one night every week in one B.H.C. village (Mokhada), one D.D.T. village (Suryamal) and one comparison village (Gonda). These collections were made between 20.00 and 21.00 hours in six mixed dwellings in each village, spending 10 minutes in each mixed dwelling. These data are being separately reported in connection with a comparative study of Anopheles densities during mornings, afternoons and nights (Viswanathan, Ramachandra Rao, Halgeri and Karandikar, 1950). It can however be mentioned here that the night densities were normally higher than in the morning. While both in the unsprayed village and in the D.D.T. village the night collections were nearly twice as high as the morning ones, in the B.H.C. village the night collections were about 40 per cent more than in the morning.

SPLEEN AND PARASITE RATES.

Spleen and parasite rates compiled twice during the season of 1949-50, amongst children between two and ten years of age in all the sprayed and comparison villages, are summarized in Table V. Similar data for 1948-49 have been published already (Table IV of Viswanathan, Ramachandra Rao and Juneja, 1949). In the comparison villages, the total spleen rates of all the three villages were 75·2 per cent in September 1948, 82·8 per cent in January 1949, 74·3 per cent in July 1949 and 88·5 per cent in January 1950. These figures show a small but significant rise in January over those recorded in July and September. In the B.H.C. villages these figures are 50·3 per cent in September 1948, 44·4 per cent in January 1949, 40·0 per cent in July 1949 and 32 per cent in January 1950. These show a small but significant and gradual reduction. In the D.D.T. villages the spleen rates were 42·1 per cent in September 1948, 34·4 per cent in January 1949, 24·9 per cent in July 1949 and

22·0 per cent in January 1950. The reduction is of a greater order in the D.D.T. villages.

As regards parasite rates, in the comparison villages they were 15 per cent in September 1948, 29·9 per cent in January 1949, 34·1 per cent in July 1949 and 36·7 per cent in January 1950. While in 1949 the January figures are significantly in excess of the previous September figures, during the season of 1949 the parasite rates have shown no significant variation between July 1949, at the beginning of the season, and January 1950, at its end. In the B.H.C. villages, the figures were 5·7 per cent in September 1948, 17·3 per cent in January 1949, 10·2 per cent in July 1949 and 12·1 per cent in January 1950. While in the season of 1948, the B.H.C. villages showed a significant increase in parasite rates at the end of the transmission season, the increase is not significant during the transmission season in 1949. In the case of the D.D.T. villages similar figures were 6·7 per cent in September 1948, 12·9 per cent in January 1949, 13·1 per cent in July 1949 and 1·8 per cent in January 1950. Thus in 1948 there was a small but significant increase in the parasite rates in the D.D.T. villages but in 1949 there was a marked and significant reduction.

INFANT PARASITE RATES.

Table VI shows the infant parasite rates in the three groups of villages in July 1949 and January 1950. In the unsprayed villages, the infant parasite rate was 25·0 per cent in July 1949 and 32·1 per cent in January 1950. The increase, however, is not significant. The infant parasite rate in January 1949 in the unsprayed villages was 50 per cent, showing a much higher order of transmission during that season, a fact also borne out by the higher mosquito densities recorded.

In the B.H.C. villages, the infant parasite rate was 6·2 per cent in July 1949 and 5·2 per cent in January 1950. The reduction, however, is not significant. Similar figures in the previous year were 2·4 per cent in September and 9·5 per cent in the following January. The infant parasite rates at the end of the present year were significantly lower than at the end of the transmission season in the previous year. In the D.D.T. villages, the infant parasite rate was 7·1 per cent in July 1949 and 3·7 per cent in January 1950. Here, too, the reduction is not statistically significant. During the past year the infant parasite rate was nil in September 1948 and 4 per cent in January 1949. The infant parasite rate in the D.D.T. group has thus been of the same order at the end of the transmission season during both the years but it has been the lowest amongst the three groups of villages under study.

DISPENSARY FIGURES.

Table VII shows the number of malaria cases treated in the out-patient department of Mokhada dispensary in each month during the three years 1947, 1948 and 1949. It must be emphasized that these figures relate entirely to out-patient diagnosis and are not based upon microscopic examination of blood. No reliance can therefore be placed upon these figures qualitatively. They can only be deemed to give an idea of the general trend of malaria. In 1949, there has been a little more

than 40 per cent reduction in the incidence of malaria as compared to 1947, and a little less than 40 per cent reduction as compared to 1948. It will however be observed that during the month of December 1949 there has been a rise.

DISCUSSION.

The objectives of these studies were :—

1. To compare the relative merits of D.D.T. and B.H.C. as residual insecticides in malaria control; and
2. To determine the frequency of application and number of rounds of spray required for successful establishment of malaria control in Thana District.

In order to arrive at a valid conclusion as regards these two objectives, all data including mosquito findings, spleen rates, childhood parasite rates, infant parasite rates and dispensary figures must be assessed cumulatively rather than individually.

Data presented in Table I show that throughout the period of experiment, the total number of anophelines (females only) collected were 18,279 in the comparison villages in a total period of 659 hours 35 minutes. The per man-hour total anopheline density is therefore 28. The *culicifacies* density (total = 8033) in these villages is 12 per man-hour. In the B.H.C. villages similar figures were: Total female anophelines 10,227 and total female *culicifacies* 3,340, and the densities 9 and 3 per man-hour respectively. In the D.D.T. villages total female anophelines were 1,436 and female *culicifacies* were 687 and the densities 2 and 1 respectively. These gross figures by themselves show the marked superiority of D.D.T. over B.H.C. as a residual insecticide, provided, however, there was no true repellent mechanism involved or again the absence of mosquitoes from sprayed structures as judged by manual catch may be taken to truly reflect mosquito reduction or interception or both, and is not an evidence of their staying away from normal resting places due to an exciting effect on the part of the insecticide, which, however, does not prevent their entry at night for purposes of feeding with consequent transmission of malaria.

A reference to Tables II, III and IV and Chart I would show that, in the absence of any true repellency or excito-repellent mechanism, D.D.T. is superior to B.H.C. in its residual insecticidal action throughout the period of experiment. Not only is there a much more significant reduction in Anopheles densities immediately after spraying with D.D.T. than with B.H.C. but the mosquitoes reappear after a much more prolonged period.

While Kennedy (1947), Thompson (1950) and Davidson (1950) have referred to the phenomenon of an 'excito-repellent' mechanism following the application of D.D.T., and the latter two on its relative absence after spraying with B.H.C., in the case of Indian anophelines excepting Bertram (1950) working with *minimus*, no evidence has so far been presented in support of such a phenomenon occurring in the wake of a D.D.T. spray. Even with *minimus* other workers in Assam, notably Hay Arthur and Gilroy (private discussions) have reported effective mosquito and malaria reduction in *minimus* areas with the use of D.D.T. The authors' own

observations, which are separately under report, do not indicate any such phenomenon after the application of either D.D.T. or B.H.C. Mosquito collections made indoors at any part of the day or night seem to be subject to nearly the same degree of variation irrespective of the fact whether the structures are sprayed with one or other insecticide or are unsprayed. The authors are therefore of the opinion that the usual morning collections can be relied upon to assess the density of mosquito prevalence after the application of an insecticide and to determine, therefrom, the duration of the residual action.

As regards the second objective, the present experiment shows that, inasmuch as mosquito densities reach a very high level even earlier than July, the best period for the first round of spray in Thana District is perhaps in May-June. In August and September on account of the high rainfall, there is a natural drop in the numerical prevalence of the vector species. As regards the second round of spray, it is necessary to complete it before the end of October and if the area to be included is large and the staff employed small, it may be necessary to commence the second round even in September, so that the entire area may be completed before the end of October. The fact that in the case of Suryamal Village, a single application of D.D.T. has been effective during the year 1949, should not lull one into a sense of security that a single application would be adequate to bring about malaria control. Mosquito densities and malaria transmission must inevitably differ from village to village and no organization can ever establish all the details of epidemiology of malaria in every village that is under its care. The fact that in Gonghar Village the mosquito density reached well over the critical point in November, points to the need for a second round of spray with D.D.T. as well as with B.H.C.

The next question is whether a third round of spray is necessary or not. The mosquito densities recorded in January (Chart 1) show a very high level even at that period of the year. The high prevalence of malaria in December, January and February shown by the dispensary figures also points to the need for a third round of spray to cover that period. In the case of B.H.C., a third round of spray commencing from November 1 to be completed by the middle of December would definitely appear to be necessary. If this was done, perhaps the tendency for a rise in malaria in December, as shown by the dispensary figures at Mokhada in December 1949, may be controlled. Whether a similar third round of application would be necessary in the case of D.D.T., cannot be stated with confidence. On the whole, however, based on the experience with this insecticide in several other parts of the State, the authors are inclined to recommend a third round of spray with D.D.T. as well. Therefore, under conditions prevailing in the experimental area—Thana District as a whole for that matter—three rounds of spray are recommended, whether with B.H.C. or D.D.T., the first in May-June, the second in September-October and the third in November-December. The dosages recommended are as applied in the present experiment, namely 11 mg. of *gamma* isomer of B.H.C. and 56 mg. of D.D.T. per square foot.

Thompson (1950) has shown the superiority of B.H.C. over D.D.T. with respect to *gambiae*. His dosages were, however, much higher than recorded in this experiment. Gammexane was applied at the rate of 24 mg. *gamma* isomer per sq. ft., but D.D.T. also was applied in his experiments at the rate of 400 mg./sq. ft. Several American and European workers have adopted a single application of a high dose

of 200 mg. D.D.T. per sq. ft. for malaria control. In this country, higher dosages of residual insecticides are not generally in vogue. The authors themselves have repeatedly stressed the need for a larger number of spraying rounds with smaller dosages than a single application with a large dosage. Whether a larger dosage of B.H.C. would give better results compared with the dosage of D.D.T. as generally adopted in this country, namely 56 mg./sq. ft., is not of much practical importance inasmuch as cost would alone determine choice of the insecticides in malaria control. At current price levels the dosages that have been applied in the present experiment would make B.H.C. comparable to D.D.T. in so far as costs are concerned, though the resultant efficacy is of a lower order. If the price of B.H.C. is capable of further reduction, while there is no such prospect in the case of D.D.T., then economic considerations might influence the use of B.H.C. in preference to D.D.T. even though it may be less effective.

Another factor for consideration is the difference in the formulation of the two insecticides used. B.H.C. was applied as a wettable powder while D.D.T. in the form of an emulsion. Viswanathan and Rao (1947, 1948, 1949) have referred to the superiority of D.D.T. when applied in the form of an emulsion over its application as to wettable powder. Whether B.H.C. when applied as a wettable powder will equal the efficacy of D.D.T. when applied also as a wettable powder, or even be superior to it, the authors' experiments provide no data to assess.

SUMMARY AND CONCLUSIONS.

1. Results of continued field trials to test the comparative efficacy of D.D.T. and B.H.C. for malaria control in Thana District, Bombay State, are presented.

2. B.H.C. was applied in 5 villages as a wettable powder in a dosage of 11 mg. of *gamma* isomer per sq. ft. and D.D.T. in 3 villages as an emulsion at 56 mg./sq. ft. In the dosages and formulations applied, in an area where *culicifacies* and possibly *fluviatilis* are vectors, both insecticides are capable of establishing malaria control. The degree of control established was of a higher order with D.D.T. than with B.H.C.

3. In Thana District three applications of either insecticides are considered necessary in a year, the first in May-June, the second in September-October and the third in November-December. The third application of D.D.T. may not be necessary in some villages but as a working rule, three applications are recommended for the whole of the district.

4. D.D.T. would appear to be the better insecticide insofar as most Indian anophelines are concerned but B.H.C. can also be used successfully in malaria control if supplies of D.D.T. are either scarce or too costly.

ACKNOWLEDGMENTS.

The authors feel greatly obliged to Messrs. Imperial Chemical Industries (India) Ltd., Bombay, for a further free supply of Gammexane P.520 for these trials.

REFERENCES.

BERTRAM, D. M. (1950) ..	*Ann. Trop. Med. Parasit.*, **44**, pp. 242–254.
DAVIDSON, G. (1950) ...	*Ibid.*, pp. 1–26.
KENNEDY, J. S. (1947) .	*Bull. Ent. Res.*, **37**, pp. 595–607.
THOMPSON, R. C. MUIRHEAD (1950).	*Trans. Roy. Soc. Trop. Med. Hyg.*, **43**, pp. 401–412.
VISWANATHAN, D. K., and RAMACHANDRA RAO, T. (1947).	*Ind. J. Mal.*, **1**, pp. 503–542.
Idem (1948) ..	*Ibid.*, **2**, pp. 157–210.
Idem (1949) ...	*Ibid.*, **3**, pp. 269–312.
VISWANATHAN, D. K., RAMACHANDRA RAO, T., HALGERI, A. V., and KARANDIKAR, V. S. (1950).	*Ibid.*, **4**, pp. 533–547.
VISWANATHAN, D. K., RAMACHANDRA RAO, T., and JUNEJA, M. R. (1949).	*Ibid.*, **3**, pp. 57–67.

TABLE

Mokhada Petha—Summary of Anopheles adults

	Comparison villages.							
	Human dwelling.		Mixed dwelling.		Cattleshed.		Total.	
Time: Hrs.—Mts.	147—0		457—20		55—15		659—35	
	♂	♀	♂	♀	♂	♀	♂	♀
annulus	...		0	7	0	1	0	8
annularis	0	2	0	10	...		0	12
barbirostris	..		0	11	0	3	0	14
culicifacies	88	975	383	6,021	70	1,037	541	8,033
fluviatilis	4	132	0	201	0	29	4	362
fuscus	0	9	0	6	0	3	0	18
jamesi	1	24	0	157	0	33	1	214
aegyptiacus	20	742	75	4,337	7	816	102	5,895
karwari	0	3	0	13	0	3	0	19
maculatus	0	7	0	22	0	5	0	34
minimus	0	69	0	318	0	105	0	492
pallidus	2	194	20	1,092	14	279	36	1,565
philippinensis	0	6	0	27	0	8	0	41
splendidus	0	1	0	14	0	4	0	19
stephensi	0	2	0	15	...		0	17
subpictus	33	151	113	562	44	245	190	958
theobaldi	0	23	0	107	0	34	0	164
vagillatus	0	16	1	127	0	22	1	165
varshani	0	1	0	26	0	4	0	31
vagus	0	29	2	154	0	33	2	216
varani	...		0	2	...		0	2
TOTAL	148	2,386	594	13,229	135	2,664	877	18,279

♂ = male. ♀ = female.

1

collected by species—July 1949 to February 1950.

B.H.C. villages.								D.D.T. villages.							
Human dwelling.		Mixed dwelling.		Cattleshed.		Total.		Human dwelling.		Mixed dwelling.		Cattleshed.		Total.	
292—0		584—0		292—0		1,168—0		183—0		366—0		183—0		732—0	
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
..		0	9	0	3	0	12	...		...		...		...	
0	4	0	9	0	3	0	16	...		0	2	...		0	2
0	4	0	13	0	4	0	21	...		...		0	2	0	2
18	344	108	1,906	82	1,090	208	3,340	11	45	19	420	9	222	39	687
0	12	1	89	0	42	1	143	0	1	0	11	0	6	0	21
...		...		0	1	0	1	..		0	4	0	1	0	5
0	14	0	69	0	48	0	131	0	1	0	37	0	9	0	47
12	381	55	2,319	51	1,255	118	3,955	2	24	4	249	3	95	9	368
0	3	0	21	1	4	1	28	...		0	4	...		0	4
0	5	0	49	0	12	0	66	...		.		0	1	0	1
0	34	0	264	1	134	1	132	..		0	17	0	6	0	23
0	78	6	462	3	255	9	795	0	7	0	45	0	16	0	68
0	5	0	16	0	9	0	30	...		0	5	...		0	5
		0	11	0	3	0	14	...		0	2	..		0	2
		0	9	0	7	0	16	...		0	2	0	1	0	3
7	95	34	413	30	270	71	778	0	3	6	72	3	25	9	100
0	10	0	79	0	46	0	135	0	4	0	27	0	7	0	38
0	10	0	82	0	26	0	118	0	7	0	16	0	12	0	35
.		0	2	0	5	0	7	0	1	0	3	...		0	4
1	12	0	120	1	49	2	181	...		0	15	1	4	1	19
1	1	0	5	0	2	0	8	...		..		0	2	0	2
38	1,012	204	5,917	169	3,268	411	10,227	13	93	29	934	16	409	58	1,436

♂ = male. ♀ = female.

TABLE II.

Anopheline densities in three unsprayed villages—mixed dwellings only.

Week beginning with	Total man-hours spent.	Number collected.						Average density per man-hour for females only.		
		Cul.		Flu.		All.		Cul.	Flu.	All.
		♂	♀	♂	♀	♂	♀			
1949										
July										
17	17·0	118	698	0	2	161	1,003	41·1	0·1	59·0
24	8·5	16	357	0	4	29	520	42·0	0·5	61·2
31	20·0	42	332	1	4	55	722	16·6	0·2	36·1
August										
7	14·5	13	108	0	6	23	312	7·4	0·4	21·5
14	11·0	20	60	0	0	32	170	5·4	..	15·4
21	11·0	1	35	0	2	12	91	3·2	0·2	8·3
28	11·0	5	71	0	0	18	192	6·4	0·5	17·4
September										
4	14·5	7	71	0	2	11	193	6·3	0·1	13·3
11	17·0	0	37	0	0	4	135	2·2	..	25·0
18	...									...
25	14·0	0	23	0	16	2	256	1·7	1·1	18·3
October										
2	17·0	0	19	0	14	6	255	2·9	0·9	15·0
9	17·0	0	68	0	14	11	539	4·0	0·8	31·7
16	...									...
23	17·0	3	205	0	21	28	1,185	12·1	1·2	69·7
30	17·0	3	137	0	24	13	624	8·1	1·4	36·7

♂ = male. ♀ = female.

Cul. = *culicifacies*. Flu. = *funestus*. All. = all anophelines.

TABLE II—*concld.*

Week beginning with	Total man-hours spent.	Number collected.						Average density per man-hour for females only.		
		Cul.		Flu.		All.		Cul.	Flu.	All.
		♂	♀	♂	♀	♂	♀			
1949—*concld.*										
November										
6	17·0	12	400	0	16	15	987	23·5	0·9	58·1
13	14·0	17	269	0	5	23	470	19·2	0·3	33·6
20	17·0	13	369	0	3	14	576	21·7	0·2	33·9
27	14·0	0	125	0	8	1	319	8·9	0·6	22·8
December										
4	11·0	4	186	0	6	5	279	16·9	0·5	25·4
11	17·0	6	521	0	5	9	795	30·6	0·3	46·8
18	17·0	5	279	0	5	5	425	16·4	0·3	25·0
25	14·5	4	246	0	3	4	352	17·0	0·2	24·3
1950										
January										
1	17·0	10	215	0	2	10	342	12·6	0·1	20·1
8	11·0	3	79	0	4	3	149	7·2	0·4	13·5
15	17·0	15	254	0	6	15	344	14·9	0·4	20·2
22	6·0	11	123	0	1	12	144	20·5	0·2	24·0
29	17·0	5	366	0	2	6	276	21·4	0·1	16·2
February										
5	17·0	3	93	0	1	5	132	5·5	0·1	7·8
12	11·0	5	103	0	1	3	119	9·4	0·1	10.8
19	17·0	11	180	0	2	11	229	10·5	0·1	13·5

♂ = male. ♀ = female.

Cul. = *culicifacies*. Flu. = *fluviatilis*. All. = all anophelines.

TABLE III.

Anopheline densities in five B.H.C. villages—mixed dwellings.

Week beginning with	Time spent, man-hours.	Number collected.						Average densities per man-hour for females only.		
		Cul.		Flu.		All.		Cul.	Flu	All.
		♂	♀	♂	♀	♂	♀			
1949										
July										
17 ...	10	40	461	0	7	45	647	46·1	0·7	64·7
24* ..	18	8	56	0	1	11	112	3·1	0·1	6·2
31 ..	20	9	127	0	0	8	168	6·4	0·0	8·4
August										
7 ..	20	5	118	0	2	7	225	5·9	0·1	11·3
14 ..	18	2	48	0	10	3	133	2·7	0·6	7·4
21 ..	20	7	49	0	1	14	194	2·5	0·1	9·7
28 ..	18	2	43	0	0	13	152	2·4	0·0	8·4
September										
4 .	18	0	15	0	0	1	105	0·8	0·0	5·8
11 ..	20	0	23	0	0	6	128	1·2	0·0	6·4
18 ..	20	0	21	0	1	3	256	1·1	0·1	12·8
25 ...	18	0	14	0	6	4	192	0·8	0·3	10·7
October										
2 ..	20	0	28	0	6	1	370	1·4	0·3	18·5
9 ..	18	0	51	0	11	12	474	2·8	0·6	26·3
16 ...	18	0	33	0	4	6	471	1·8	0·2	26·2
23 ...	16	1	30	0	5	15	390	1·9	0·3	24·4
30 ...	18	0	1	0	0	2	35	0·1	0·0	1·9

* First round sprayed: July 19, 20 and 21.
Second round sprayed: October 25, 26 and 27.

♂ = male. ♀ = female.
Cul. = *culicifacies*. Flu. = *fluviatilis*. All. = all anophelines.

TABLE III—*concld.*

Week beginning with	Time spent, man-hours.	Number collected.						Average densities per man-hour for females only.		
		Cul.		Flu.		All.		Cul.	Flu.	All.
		♂	♀	♂	♀	♂	♀			
1949—*concld.*										
November										
6	20	0	10	0	3	9	129	0·5	0·2	6·5
13	16	0	29	0	2	15	127	1·8	0·1	7·9
20	20	1	31	0	4	12	177	1·6	0·2	8·9
27	18	1	45	0	3	3	191	2·5	0·2	10·6
December										
4	16	2	34	0	16	3	151	2·1	1·0	9·4
11	20	1	48	0	4	2	140	2·4	0·2	7·0
18	20	7	75	0	6	7	158	3·8	0·3	7·9
25	20	5	110	0	5	7	177	5·5	0·3	8·9
1950										
January										
1	16	8	84	0	1	8	123	5·3	0·1	7·7
8	20	4	61	0	4	5	127	3·1	0·2	6·4
15	20	6	58	0	5	7	144	2·9	0·3	7·2
22	8	2	30	0	0	2	46	3·8	0·0	5·8
29	18	4	59	0	0	4	115	3·3	0·0	6·4
February										
5	20	3	61	0	0	3	97	3·1	0·0	4·9
12	8	1	15	0	0	0	30	1·9	0·0	3·8
19	20	3	34	0	0	3	52	1·7	0·0	2·6
26	...	...		...		...		...	...	...

♂ = male. ♀ = female.

Cul. = *culicifacies*. Flu. = *fluviatilis*. All. = all anophelines.

TABLE IV.

Anopheline densities—D.D.T. villages—mixed dwellings only.

Week beginning with	Time spent in collection, man-hours.	Number collected.						Density per man-hour.			Remarks.
		Cul.		Flu.		All.		Cul.	Flu.	All.	
		A. Suryamal.									
1949		♂	♀	♂	♀	♂	♀				
July											
17 ...	2	4	80	0	0	4	109	40·0	0·0	54·5	
24 ...	4	0	0	0	0	0	0	0·0	0·0	0·0	Sprayed on Jul. 22, 1949.
31 ...	4	1	11	0	0	1	13	2·8	0·0	3·8	
August											
7 ...	4	0	0	0	0	0	1	0·0	0·0	0·3	
14 ...	4	0	1	0	0	0	2	0·3	0·0	0·5	
21 ...	4	0	3	0	0	0	5	0·8	0·0	1·3	
28 ...	4	0	1	0	0	0	3	0·3	0·0	1·3	
September											
4 ...	4	0	0	0	0	0	0	0·0	0·0	0·0	
11 ...	4	0	0	0	0	0	1	0·0	0·0	0·3	
18 ...	4	0	0	0	0	0	2	0·0	0·0	0·5	
25 ...	2	0	0	0	0	0	2	0·0	0·0	1·0	
October											
2 ...	4	0	0	0	0	0	5	0·0	0·0	1·3	
9 ...	4	0	1	0	0	0	7	0·3	0·0	1·8	
16 ...	4	0	0	0	0	0	7	0·0	0·0	1·8	
23 ...	4	0	2	0	0	0	22	0·5	0·0	5·5	
30 ...	4	0	2	0	3	0	29	0·5	0·8	7·3	

♂ = male. ♀ = female.

Cul. = *culicifacies*. Flu. = *fluviatilis*. All. = all anophelines.

TABLE IV—*contd.*

Week beginning with	Time spent in collection, man-hours.	Number collected.						Density per man-hour.			Remarks.
		Cul.		Flu.		All.		Cul.	Flu.	All.	
					A. Suryamai—concld.						
1949—*concld.*		♂	♀	♂	♀	♂	♀				
November											
6 ...	4	1	4	0	2	2	36	1·0	0·5	9·0	
13 ...	2	0	2	0	0	0	12	1·0	0·0	6·0	
20 ..	4	0	16	0	2	0	45	4·0	0·5	11·3	
27 ..	4	0	15	0	3	0	43	3·8	0·8	10·8	
December											
4 ...	4	0	11	0	0	0	38	2·8	0·0	9·5	
11 ...	4	0	19	0	0	0	37	4·8	0·0	9·3	
18 ..	4	0	10	0	0	0	34	2·5	0·0	8·5	
25 ..	4	0	1	0	1	0	14	0·3	0·3	3·5	
1950											
January											
1 ...	4	0	6	0	0	0	10	1·5	0·0	2·5	
8 ...	4	0	1	0	1	0	11	0·3	0·3	2·8	
15 ...	4	1	3	0	0	1	6	0·8	0·0	1·5	
22 ..	4	0	1	0	0	0	7	0·3	0·0	1·8	
29 ...	4	0	0	0	0	0	1	0·0	0·0	0·3	
February											
5 ...	4	0	0	0	0	0	0	0·0	0·0	0·0	
12 ...	4	0	0	0	0	0	0	0·0	0·0	0·0	
19 ..	4	0	0	0	0	0	0	0·0	0·0	0·0	

♂ = male. ♀ = female.

Cul. = *culicifacies*. Flu. = *fluviatilis*. All = all anophelines.

TABLE IV—*contd.*

Week beginning with	Time spent in collection, man-hours.	Number collected.						Density per man-hour.			Remarks.
		Cul.		Flu.		All.		Cul.	Flu.	All.	
		♂	♀	♂	♀	♂	♀				
		B. Gonghar.									
1949											
July											
17 ..	2	1	71	0	0	1	86	35·5	0·0	43·0	Sprayed on Jul. 22, 1949
24 .	4	0	0	0	0	0	3	0·0	0·0	0·0	
31 .	2	0	2	0	0	0	3	0·0	0·0	0·0	
August											
7 ...	4	0	4	0	0	0	7	1·0	0·0	1·8	
14 ..	4	0	9	0	1	0	17	2·3	0·3	4·3	
21 ..	4	0	5	0	0	0	7	0·8	0·0	1·0	
28 ...	4	0	3	0	0	0	7	0·8	0·0	1·8	
September											
4 ...	4	0	0	0	0	0	3	0·0	0·0	0·8	
October											
9 ..	6	0	1	0	0	0	15	0·2	0·0	2·5	
16 ..	2	0	2	0	0	0	11	1·0	0·0	5·5	
23 ...	2	0	4	0	0	2	27	2·0	0·0	13·5	
30 ...	2	0	3	0	1	1	19	1·5	0·5	9·5	

♂ = male. ♀ = female.

Cul. = *culicifacies.* Flu. = *fluviatilis.* All. = all anophelines.

TABLE IV—*contd.*

Week beginning with	Time spent in collection, man-hours.	Number collected.						Density per man-hour.			Remarks.
		Cul.		Flu.		All.		Cul.	Flu.	All.	
		♂	♀	♂	♀	♂	♀				
		B. Gomghar—concld.									
1949—*concld.*											
November											
6 ...	2	0	11	0	2	0	32	5·5	1·0	16·0	
13 ...	4	0	21	0	2	1	39	5·3	0·5	9·8	
20 ...	4	3	32	0	4	5	55	8·0	1·0	13·3	Sprayed on Nov. 21, 1949.
27 ...	4	0	0	0	0	0	0	0·0	0·0	0·0	
December											
4 ...	4	0	0	0	0	0	0	0·0	0·0	0·0	
11 ...	2	1	0	0	0	1	0	0·0	0·0	0·0	
18 ...	4	0	0	0	0	0	3	0·0	0·0	0·8	
25 ...	4	0	0	0	0	0	0	0·0	0·0	0·0	
1950											
January											
1 ...	4	0	1	0	0	0	2	0·3	0·0	0·5	
8 ...	4	0	0	0	0	0	1	0·0	0·0	0·3	
15 ...	4	0	0	0	0	0	2	0·0	0·0	0·5	
22 ...	4	0	0	0	0	0	0	0·0	0·0	0·0	
29 ...	4	0	0	0	0	0	0	0·0	0·0	0·0	
February											
5 ...	4	0	0	0	0	0	0	0·0	0·0	0·0	
12 ...	4	0	0	0	0	0	0	0·0	0·0	0·0	
19 ...	4	0	0	0	0	0	0	0·0	0·0	0·0	

♂ = male. ♀ = female.

Cul. = *culicifacies*. Flu. = *fluviatilis*. All. = all anophelines.

TABLE IV—*contd.*

Week beginning with	Time spent in collection, man-hours.	Number collected.						Density per man-hour.			Remarks.
		Cul.		Flu.		All.		Cul.	Flu.	All.	
				C. Karegoon.							
1949		♂	♀	♂	♀	♂	♀				
July											
17 ...	2	8	86	0	0	8	116	43·0	0·0	58·0	Sprayed on Jul. 22, 1949.
24 ...	4	0	2	0	0	0	12	0·5	0·0	3·0	
31 ...	2	1	3	0	0	1	4	1·5	0·0	2·5	
August											
7 ...	4	0	2	0	0	0	2	0·5	0·5	0·5	
14 ...	4	0	3	0	0	0	9	0·8	0·0	2·3	
21 ...	4	0	1	0	0	0	8	0·3	0·0	2·0	
28 ...	2	0	2	0	0	0	9	1·0	0·0	4·5	
September											
4 ...	4	0	4	0	0	0	5	1·0	0·0	1·3	
11 ...	4	0	1	0	0	0	2	0·0	0·0	0·5	
18 ...	4	0	1	0	0	0	7	0·3	0·0	1·8	
25 ...	4	0	0	0	0	0	8	0·0	0·0	2·0	
October											
2 ...	4	0	1	0	0	0	8	0·3	0·0	2·0	
9 ...	4	0	2	0	0	0	11	0·5	0·0	2·8	
16 ...	4	0	0	0	0	3	15	0·0	0·0	3·8	
23 ...	2	0	2	0	0	0	12	1·0	0·0	6·0	Sprayed on Oct. 28, 1949.
30 ...	2	0	0	0	0	0	0	0·0	0·0	0·0	

♂ = male. ♀ = female.

Cul. = *culicifacies*. Flu. = *fluviatilis*. All. = all anophelines.

TABLE IV—*concld.*

Week beginning with	Time spent in collection, man-hours.	Number collected.						Density per man-hour.			Remarks.
		Cul.		Flu.		All.		Cul.	Flu.	All.	
		C. Karegaon—*concld.*									
1949—*concld.*		♂	♀	♂	♀	♂	♀				
November											
6 ...	2	0	0	0	0	0	1	0·0	0·0	2·0	
13 .	2	0	0	0	0	0	6	0·0	0·0	3·0	
20 ...	4	0	1	0	0	0	4	0·3	0·0	1·0	
27 ...	4	0	1	0	0	0	9	0·3	0·0	2·3	
December											
4 .	4	0	1	0	0	0	7	0·3	0·0	1·8	
11 .	4	0	3	0	0	0	5	0·8	0·0	1·3	
18 .	4	0	1	0	0	0	4	0·3	0·0	1·0	
25 .	4	0	0	0	0	0	3	0·0	0·0	0·8	
1950											
January											
1 ...	4	0	1	0	0	0	2	0·3	0·0	0·5	
8 ..	4	0	0	0	0	0	0	0·0	0·0	0·0	
15 .	4	0	1	0	0	0	2	0·3	0·0	0·5	
22 ...	2	0	1	0	0	0	1	0·5	0·0	0·5	
29 ..	2	0	0	0	0	0	0	0·0	0·0	0·0	
February											
5 .	4	0	0	0	0	0	0	0·0	0·0	0·0	
12 ..	2	0	0	0	0	0	0	0·0	0·0	0·0	
19 ...	4	0	0	0	0	0	0	0·0	0·0	0·0	

♂ = male. ♀ = female.

Cul. = *culicifacies*. Flu. = *fluviatilis*. All. = all anophelines.

TABLE V

Mokhada Petha—Spleen and parasite rates, 1949-50.

Group.	Village.	Date of examination.	Spleen. Number examined.	Spleen. Number positive.	Spleen. Spleen rate, per cent.	Blood. Number examined.	Blood. Number positive.	Blood. Parasite rate, per cent.	Parasite species. V.	Parasite species. F.	Parasite species. M.
Unsprayed	Gonda	Jul. 1949 ...	32	21	65·6	32	11	34·4	8	2	1
		Jan. 1950 ...	51	49	96·1	40	14	35·0	...	10	4
	Morhande	Jul. 1949 ...	51	36	70·6	40	15*	37·5	8	8	0
		Jan. 1950 ...	55	44	80·0	40	13	32·5	1	9	3
	Chas	Jul. 1949 ...	30	27	90·0	29	5	17·2	2	3	0
		Jan. 1950 ...	42	38	90·4	40	17*	42·5	4	12	2
Total ...		Jul. 1949 ...	113	84	74·3	91	31	34·1	18	13	1
		Jan. 1950 ...	148	131	88·5	120	44	36·7	5	31	9
B.H.C.	Mokhada	Jul. 1949 ...	50	21	42·0	39	2	5·1	1	1	0
		Jan. 1950 ...	81	21	25·9	41	2	4·9	1	1	0
	Poshera	Jul. 1949 ...	55	34	61·8	47	7	14·9	3	4	...
		Jan. 1950 ...	89	46	51·6	40	10	25·0	0	9	1
	Khodala	Jul. 1949 ...	48	17	35·4	40	2	5·0	2	0	0
		Jan. 1950 ...	83	22	26·5	40	2	5·0	0	2	0

	Saturli	Jul. 1949	...	32	8	25·0	30	3	10·0	2	1	0
		Jan. 1950	...	38	11	28·9	27	3	11·1	0	3	0
	Vashala	Jul. 1949	...	40	10	25·0	20	4	20·0	3	1	0
		Jan. 1950	...	26	4	15·3	26	4	15·4	0	2	2
Total		Jul. 1949	...	225	90	40·0	176	18	10·2	11	7	0
		Jan. 1950	...	317	104	32·8	174	21	12·1	1	17	3
D.D.T.	Suryamal	Jul. 1949	...	31	8	25·8	30	1	3·3	1	0	0
		Jan. 1950	...	32	4	12·5	32	1	3·1	0	1	0
	Gomghar	Jul. 1949	...	31	13	41·9	30	6	20·0	6	0	0
		Jan. 1950	...	32	7	21·8	32	0	0·0	0	0	0
	Karegaon	Jul. 1949	...	44	16	36·4	39	6	15·4	4	2	0
		Jan. 1950	...	45	14	31·1	40	4	10·0	0	2	2
Total		Jul. 1949	...	106	37	34·9	99	13	13·1	11	2	0
		Jan. 1950	...	109	25	22·9	104	5	4·8	0	3	2

V. = *vivax*. F. = *falciparum*. M. = *malaria*. * One mixed infection.

TABLE VI

Mokhada Petha—Infant parasite rates, 1949-50

Group.	Village.	July 1949.						January 1950.					
		Number examined.	Number positive.	Infant parasite rate, per cent.	Species.			Number examined.	Number positive.	Infant parasite rate, per cent.	Species.		
					V.	F.	M.				V.	F.	M.
Unsprayed	Gonda B. ...	8	3	...	3	0	0	9	2	22·2	1	1	0
	Morhande...	15	3	...	2	1	0	15	2	13·3	1	0	1
	Chas ...	9	2	...	2	0	0	13	8	61·5	2	5	1
	Total ...	32	8	25·0	7	1	0	37	12	32·4	4	6	1
B.H.C.	Mokhada ...	19	0	...	...	...	...	31	0	0·0	...	...	...
	Poshera ...	29	1	...	1	0	0	23	2	8·7	0	2	0
	Khodala ...	16	0	...	0	0	0	22	1	4·5	0	1	0
	Saturli ...	13	1	...	1	0	0	10	1	10·0	0	1	0
	Vashala ...	13	3	...	3	0	0	11	1	9·1	0	1	0
	Total ...	81	5	6·2	5	0	0	97	5	5·2	0	5	0
D.D.T.	Suryamal ...	6	0	...	...	...	...	7	...	...	...	...	...
	Gomghar ...	6	0	...	...	...	...	7	...	...	...	...	...
	Karegaon ...	16	2	...	2	0	0	7	1	14·3	0	0	1
	Total ...	28	2	7·1	2	0	0	21	1	3·7	0	0	1

V. = *vivax*. F. = *falciparum*. M. = *malariæ*.

Note.—The infant parasite rates in 1948-49 were as follows :—

	September 1948.	January 1949.
Unsprayed ...	7·4	50·0
B.H.C. ...	2·4	9·5
D.D.T. ...	0·0	4·0

TABLE VII.

Mokhada dispensary—Malaria figures, by months in 1947, 1948 *and* 1949.

Month.	Number of malaria cases treated.		
	1947.	**1948.**	**1949.**
Jan. ...	144	197	48
Feb. ...	90	146	77
Mar. ...	88	141	62
Apr.	48	75	43
May	56	54	43
Jun.	36	38	33
Jul.	48	49	37
Aug. ...	32	71	59
Sep. ...	103	118	50
Oct. ...	112	146	90
Nov. ...	250	90	83
Dec. ...	287	30	106
TOTAL ...	1,294	1,155	731

Indian Journal of Malariology, **4**, 4, December 1950.

OBSERVATIONS ON ANOPHELES DENSITIES IN INDOOR SHELTERS DURING THE FORENOON, AFTERNOON AND NIGHT.

BY

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[December 31, 1950.]

INTRODUCTION.

THE density of vector species of Anopheles plays an important part in the establishment of a host-parasite relationship under different levels of malarial endemicity. Epidemic malaria, especially of the type that occurs in the Punjab and Sind, is associated with an increase in the density of the vector as well as its longevity and degree of association with man. In the past, when malaria control was established mainly by antilarval methods, densities of the vector species used to be systematically compiled as a check on the maintenance of successful malaria control on the assumption that such densities are a measure of the total population of the vector species. Any sudden increase in such densities betokened the creation of a hitherto undiscovered breeding ground or a breeding ground previously determined as only potential being converted into an actual breeding place either on account of recent rain or on account of irrigation. A measure of Anopheles densities in day-time indoor shelters would naturally have practical value only with respect to such species as resort to indoor resting places during the day in large proportions.

In recent years, malaria control in rural areas has been established with the application of any of the new residual insecticides. Successful malaria control may be achieved with this method under widely varying relationship between the density of larval breeding and adult anophelines, for, theoretically at all events, it will be possible to establish complete malaria control almost entirely through the phenomenon of interception, in which, without actual reduction in the density of the vector species as judged by larval figures, the longevity of the adult mosquito is effectively reduced to below the period required for the extrinsic incubation of human Plasmodia. On this account it is sometimes postulated that densities which would normally be considered as high in the absence of any malaria control may be ignored after the application of a residual insecticide, inasmuch as the Anopheles thus found are destined to die ere long.

While at the end of the malaria season, resort would naturally be had to the various other malariometric indices such as spleen rates, parasite rates, infant parasite rates, malaria morbidity rates, etc., it would naturally be necessary to have a more effective and immediate check on the progress of malaria control by a proper understanding of the behaviour of the vector species. Density as such has been a very important characteristic in such behaviour but, for the reasons stated above, there seems to be some uncertainty regarding the time of day at which mosquitoes should be collected in order that the best assessment may be made of the prevailing density of the vector species which in due course may build up a critical density and longevity adequate for the maintenance of malaria transmission. It is sometimes held that mosquitoes collected during the early morning from day-time resting places may not have had adequate contact with the sprayed surface, but if left undisturbed they would continue to have the contact and eventually die. Hence if collections are made during the afternoon by which time there would have been a greater degree of contact, such data would perhaps throw a better light on the density of the surviving Anopheles and therefore on the degree of effectiveness of the insecticide.

Yet another complicating circumstance arises out of the observations made by Thomson (1950), Bertram (1950), and Wharton and Reid (1950) that short and, therefore, sublethal contact of certain Anopheles like *gambiæ* and *minimus* with a sprayed surface excites them and makes them fly away from such shelters without resting therein as they would otherwise have done. Such an action, however, is not inconsistent with the Anopheles having a feed during which process they may either get infected or be infective. They, therefore, argue that an almost entire absence of Anopheles during the day time in indoor resting places may be consistent with continued malaria transmission. Under such conditions, therefore, recording of densities of the vector species from indoor shelters during the day time is of no value whatever in determining the efficacy of malaria control.

For the reasons stated above, it would be necessary to establish in each area for each vector species the normal correlation between morning and afternoon densities of Anopheles in indoor shelters on the one hand in sprayed and unsprayed areas; and on the other hand, the relation between morning densities in indoor shelters and the densities of Anopheles which enter at night, obviously for the purpose of feeding, should likewise be determined in sprayed and unsprayed areas.

In this paper, field data collected in the malaria season of 1949-50 which throw some light on these aspects are presented. The observations were made in three villages in Thana District with spleen rates of over 50 per cent. situated in forest-clad foothills in which malaria is transmitted principally by *culicifacies* and perhaps to a lesser extent by *fluviatilis* and in four villages in the Deccan Plateau in Poona District irrigated by the Nira Canals. In the latter area, malaria is of moderate endemicity and is transmitted by *culicifacies*, *fluviatilis* and *stephensi* var. *mysorensis*, all of which, however, have an extremely low vectorial capacity. The natural infection rate is in the first or sometimes even in the second decimal place. Collections of Anopheles were made in these observations by trained mosquito collectors manually with the aid of suction tubes and flashlights from dwellings housing both men and cattle under the same roof.

CORRELATION BETWEEN FORENOON AND AFTERNOON DENSITIES.

These studies were made in four villages in Poona District, two of which (Ambirna and Hol) were unsprayed, while the other two (Pimpli and Mekhli) were sprayed with D.D.T. A trained insect collector would arrive at one of these villages on a fixed day of the week and search under supervision six predetermined catching stations, spending 30 minutes in each between 11.00 and 15.00 hours. The next morning he would search six other similar stations in the same village between 7.00 and 11.00 hours. For obvious reasons the same catching stations could not be searched both in the morning and in the afternoon. Nor was it considered that alternating the period of collection in each catching station from week to week would be suitable, since in the authors' opinion a span of a fortnight is too great an interval to show the normal variations effectively. As only mixed dwellings were included in the catching stations they may be taken as fairly typical of similar dwellings in the entire village.

The combined total of female *culicifacies*, *fluviatilis* and *stephensi* var. *mysorensis* collected in 3 man-hours per week, as well as the per man-hour figure are presented in Table I, at weekly intervals for 32 weeks between July 1949 and February 1950. It will be found from this table that, in general, the afternoon collections were smaller than those made in the mornings. The mean morning density for the entire season in the two unsprayed villages was 22·0 and 14·3 per man-hour, with an average of 18·1. The mean of the afternoon densities was 17·1 and 8·7 respectively, with an average of 12·1.

The data presented in Table I are also shown in the Chart in the form of a scatter diagram of the paired observations. The morning and afternoon densities have a high and significant positive correlation with a coefficient equal to $+0{\cdot}812 \pm 0{\cdot}05$. The relationship between the two densities may be expressed by the following regression equation :

Mean afternoon density $= 0{\cdot}6455 \times$ morning density $+ 1{\cdot}3$.

Similarly, in the D.D.T.-sprayed villages too, there is significant positive correlation between morning and afternoon densities. The morning density for the entire season in the two sprayed villages was 4·5 and 8·2, with an average of 6·4 and the afternoon density 3·1 and 5·8 respectively, with an average of 4·8. The

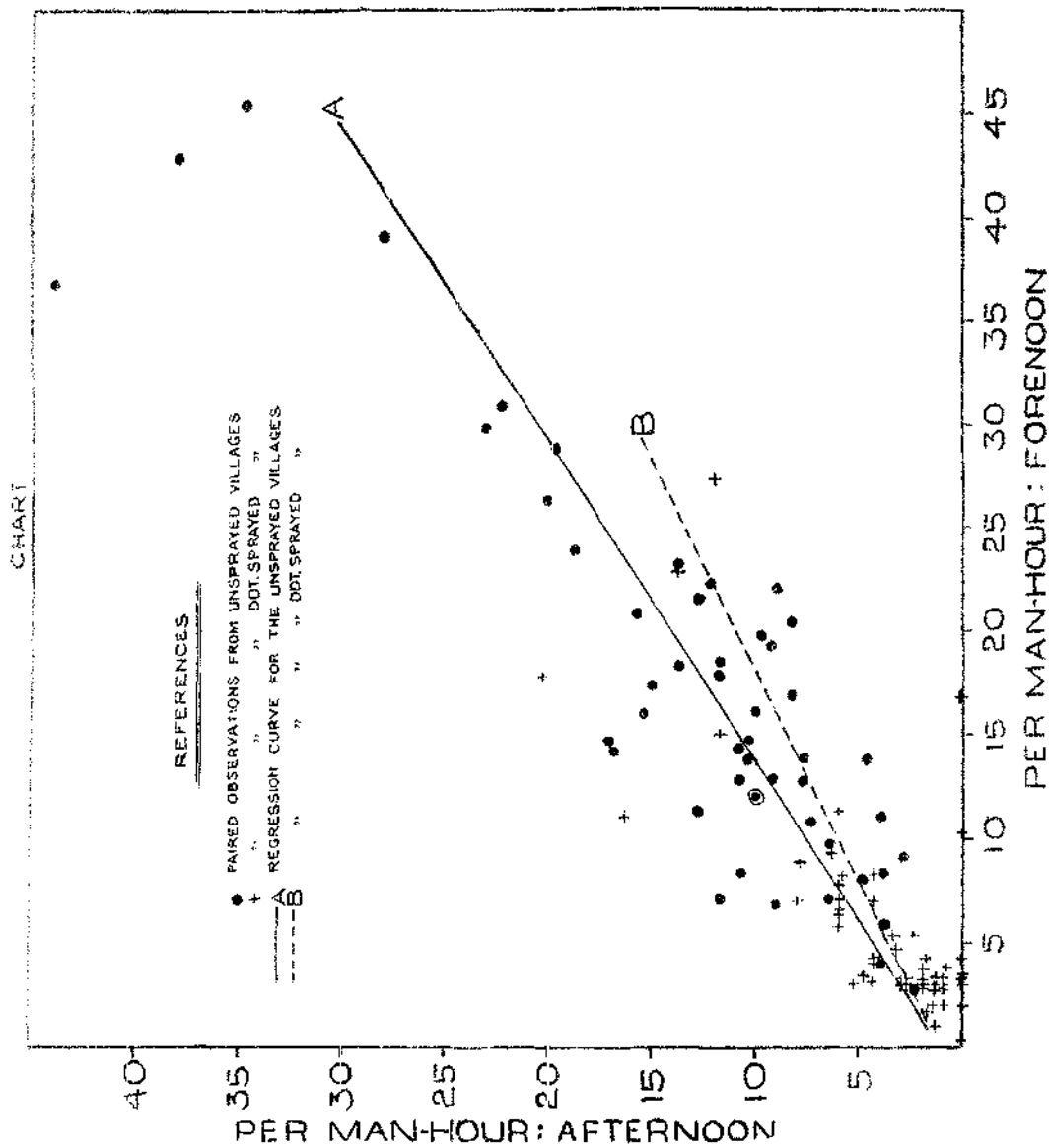
CHART I
REFERENCES
PAIRED OBSERVATIONS FROM UNSPRAYED VILLAGES
" " " DDT. SPRAYED "
REGRESSION CURVE FOR THE UNSPRAYED VILLAGES
" " " DDT. SPRAYED "
A
B
PER MAN-HOUR: AFTERNOON
PER MAN-HOUR: FORENOON
5
10
15
20
25
30
35
40
45

correlation coefficient in this case is $+ 0{\cdot}65 \pm 0{\cdot}08$. The regression equation is as below:

Mean afternoon density $= 0{\cdot}488 \times$ morning density $+ 1{\cdot}27$.

It would at first sight seem that the slope of the line showing the relation between afternoon and morning densities in sprayed areas is slightly less steep than in unsprayed areas, but applying the test of significance (Fisher, 1934), it is found that the difference between the Z values of the two coefficients is less than twice its standard deviation. Hence there is no evidence to show that the two samples in the sprayed and the unsprayed villages are not drawn from equally correlated populations.

If, however, the slightly smaller density during the afternoon as compared with morning density in the sprayed areas is real, if not demonstrable, it is consistent with the view that some of the anophelines which are found in indoor shelters during the morning inevitably undergo a further degree of mortality. On the whole, however, the data presented do not warrant the view that the afternoon collections are a definite improvement upon those made in the morning in assessing the density of surviving anophelines after an insecticidal application

COMPARISON OF MORNING AND NIGHT COLLECTIONS.

These observations were made in 6 villages (3 villages in each area). In the Nira Canals Zone, one unsprayed village and two D.D.T.-sprayed villages were selected and in Thana District one unsprayed village, one D.D.T.-sprayed village and one B.H.C.-sprayed village. The morning collections were made on the same lines as described earlier. Night collections were made once each week in each village between 20 and 21 hours in six mixed dwellings in each village, spending 10 minutes in each dwelling. These data are presented in Tables II and III. In the Nira Canals Zone the combined totals of the females of the three vector species are included and in Thana District only female *culicifacies*.

On the whole, despite the comparative difficulty of collecting anophelines during the night when they are particularly active, night densities have been significantly higher than those in the morning, both in sprayed and in unsprayed villages in both areas. In the D.D.T.-sprayed villages, the night density is, as in the unsprayed villages, double that of the morning. In the B.H.C.-sprayed village, the night density was a little over 40 per cent more than in the morning.

DISCUSSION.

Data presented in this paper show that with *culicifacies*, *fluviatilis* and *stephensi* var. *mysorensis* in the areas under study, mosquito collections made in the mornings as reported hitherto provide a fairly reliable basis for assessing the persistence of the residual action of the insecticide used. On the basis of such morning densities, the frequency of application of the insecticide was determined earlier. The data now presented do not show any evidence that such determination is likely to be erroneous.

Likewise, in the technical papers published by some of the authors (Viswanathan and Ramachandra Rao, 1947, 1948 and 1949), low Anopheles densities as determined during the forenoon in indoor resting places were furnished

as evidence of the efficacy of the insecticide. The present studies seem to show that in the various parts of Bombay State where malaria control is in progress with the application of D.D.T. as an indoor residual spray, the absence or the paucity of the vector species in early morning collections does not betoken any excito-repellent effect which has been reported by other workers in the case of certain anophelines like *gambiae* and *minimus*. In the solitary B.H.C. village included in the series, the night densities were only about 40 per cent higher than the morning densities, while in the case of all the D.D.T.-sprayed villages as well as in unsprayed villages, night densities are nearly twice as high as the morning densities. While this may be consistent with the observations made by Thomson (*loc. cit.*) and others that there is a far lesser degree of excito-repellent phenomenon with B.H.C. than with D.D.T., it would not be safe to draw conclusions from these small-scale studies. On the whole, however, the data presented in this paper do not warrant the view that this phenomenon occurs in any very large degree, either with B.H.C. or with D.D.T., and that therefore day-time densities from indoor shelters give a fair estimate of the surviving mosquito population which is likely to have a bearing on malaria transmission. We may, in this connection, also refer to yet another observation that we have made, that while the insecticide is persistent in its action there are oscillations in the morning densities, with the crest of the waves, however, well below the critical density. When there is waning of the insecticidal action succeeding waves increase in their amplitude and the critical density is soon reached or exceeded. This is the period when the authors usually determine that the second round of application of the insecticide is necessary.

SUMMARY.

Observations on the densities of the adults of *culicifacies*, *fluviatilis* and *stephensi* var. *mysorensis* were made between 7.00 and 11.00 hours and between 11.00 and 15.00 hours in two unsprayed villages and two villages sprayed with D.D.T. in the Nira Canals Zone of Poona District. The afternoon collections were found to be on the whole smaller, by an average of about 30 per cent. than the morning collections. Positive and significant correlation was found between the forenoon and afternoon densities in both groups of villages and there was no evidence that the two samples were not drawn from equally correlated populations.

Observations were also made at weekly intervals in 6 villages (3 in the Nira Canals Zone and 3 in Thana District) on the densities of the females of the vector species during day time (between 7.00 and 11.00 hours) and at night (between 20.00 and 21.00 hours). Three of the villages were sprayed with D.D.T., one with B.H.C. and 2 were unsprayed. Night densities were nearly twice as high as in the mornings in the unsprayed villages and those sprayed with D.D.T., and only about 40 per cent higher than in the mornings in the B.H.C. village. There was no evidence to warrant the view that with the species studied the phenomenon of excito-repellency occurred to any large degree.

It was concluded that there would seem to be no need to resort to afternoon collections for the sole purpose of assessing the effect of the residual insecticide on day-time densities of Anopheles adults and that the day-time densities in indoor shelters give a fair estimate of the surviving mosquito population which is likely to have a bearing on malaria transmission.

REFERENCES.

BERRYMAN, D. M. (1950) ..	*Ann. Trop. Med. & Parasit.*, **44**, pp. 242–254.
FISHER, R. A. (1934) ..	*Statistical Methods for Research Workers*. Oliver and Boyd, Edinburgh and London.
THOMSON, R. C. MUIRHEAD (1950)	*Trans. Roy. Soc. Trop. Med. Hyg.*, **43**, pp. 401–412.
VISWANATHAN, D. K., and RAMACHANDRA RAO, T. (1947).	*Ind. J. Mal.*, **1**, pp. 503–542.
Idem (1948) ...	*Ibid.*, **2**, pp. 157–210.
Idem (1949) ...	*Ibid.*, **3**, pp. 269–312.
WHARTON, R. H., and REID, J. A. (1950).	*Nature*, Jan. 7, 1950, **165**, pp. 28–29.

TABLE 1.

Morning and afternoon collections of anophelines in unsprayed and D.D.T.-sprayed villages in Nira Canals Zone, Poona District, July 1949 to February 1950.

Week beginning with		July 17.	July 24.	July 31.	August 7.	August 14.	August 21.	August 28.	September 4.	September 11.	September 18.	September 25.
Unsprayed, Athurna.												
Morning	(a)	79	49	89	52	61	51	...	86	136	110	117
	(b)	26·3	16·3	29·7	17·3	20·3	17·0	...	28·7	45·3	36·7	39·0
Afternoon	(a)	...	46	69	45	...	41	109	59	104	132	84
	(b)	...	15·3	23·0	15·0	...	13·7	36·3	19·7	34·7	44·0	28·0
Unsprayed, Hol.												
Morning	(a)	43	41	44	55	78	145	104	53	21	22	24
	(b)	14·3	13·7	14·7	18·3	26·0	48·3	34·7	17·7	7·0	7·3	8·0
Afternoon	(a)	...	24	31	35	...	49	38	35	19	36	14
	(b)	...	8·0	10·3	11·7	...	16·3	12·7	11·7	6·3	12·0	4·7
D.D.T-sprayed, Mekhli.												
Morning	(a)	32	19	...	6	13	...	10	13	9	7	...
	(b)	10·7	6·3	0	2·0	4·3	...	3·3	4·3	3·0	2·3	...
Afternoon	(a)	33	16	0	0	1	4	13	5	9	...	...
	(b)	11·0	5·3	0	0	0·3	1·3	4·3	1·7	3·0	...	...
D.D.T-sprayed, Pimpli.												
Morning	(a)	108	0	0	50	34	...	9	67	53	33	...
	(b)	36·0	0	0	16·7	11·3	...	3·0	22·3	17·7	11·0	...
Afternoon	(a)	77	0	0	1	18	19	16	41	61	49	26
	(b)	25·7	0	0	0·3	6·0	6·3	5·3	13·7	20·3	16·3	8·7

(a) Actual total number of females of *culicifacies*, *fluviatilis* and *stephensi* var. *mysorensis* collected in 3 man-hours, each week.

(b) Per man-hour figures.

Note.—Mekhli sprayed on July 30, 1949.

Pimpli sprayed on July 26 and October 24, 1949.

TABLE I—*contd.*

Week beginning with		October 2.	October 9.	October 16.	October 23.	October 30.	November 6.	November 13.	November 20.	November 27.	December 4.	December 11.
Unsprayed, Athurna.												
Morning	(a) ...	64	128	92	71	62	76	33	38	55	41	36
	(b) ...	21·3	42·7	30·7	23·7	20·7	25·3	11·0	12·7	18·3	13·7	12·0
Afternoon	(a) ...	38	114	67	55	47	60	12	28	41	31	30
	(b) ...	12·7	38·0	22·3	18·3	15·7	20·0	4·0	9·3	13·7	10·3	10·0
Unsprayed, Hol.												
Morning	(a) ...	13	32	43	68	25	61	44	29	66	38	58
	(b) ...	4·3	10·7	14·3	22·7	8·3	20·3	14·7	9·7	22·0	12·7	19·3
Afternoon	(a) ...	12	22	32	41	32	25	52	19	27	30	28
	(b) ...	4·0	7·3	10·7	13·7	10·7	8·3	17·3	6·3	9·0	10·0	9·3
D.D.T.-sprayed, Mekhli.												
Morning	(a) ...	6	5	...	8	13	31	21	10	21	16	26
	(b) ...	2·0	1·7	...	2·7	4·3	10·3	7·0	3·3	7·0	5·3	8·7
Afternoon	(a) ...	4	5	...	4	13	...	13	14	24	7	23
	(b) ...	1·3	1·7	...	1·3	4·3	0	4·3	4·7	8·0	2·3	7·7
D.D.T.-sprayed, Pimpli.												
Morning	(a) ...	82	45	...	10	0	1	9	10	3	9	25
	(b) ...	27·3	15·0	...	3·3	0	0·3	3·0	3·3	1·0	3·0	8·3
Afternoon	(a) ...	36	35	...	0	0	0	6	4	4	6	13
	(b) ...	12·0	11·7	...	0	0	0	2·0	1·3	1·3	2·0	4·3

(a) Actual total number of females of *culicifacies*, *fluviatilis* and *stephensi* var *mysorensis* collected in 3 man-hours, each week.

(b) Per man-hour figures.

Note.—Mekhli sprayed on July 30, 1949.

Pimpli sprayed on July 26 and October 24, 1949.

TABLE 1—*concld.*

Week beginning with		December 18.	December 25.	January 1.	January 8.	January 15.	January 22.	January 29.	February 5.	February 12.	February 19.	Means.
Unsprayed, Atburna.												
Morning	(a)	43	73	67	48	44	59	67	34	38	36	...
	(b)	14·3	24·3	22·3	16·0	14·7	19·7	22·3	11·3	12·7	12·0	22·0
Afternoon	(a)	51	...	...	30	25	29	38	38	23	...	...
	(b)	17·0	...	...	10·0	8·3	9·7	12·7	12·7	7·7	...	17·1
Unsprayed, Hol.												
Morning	(a)	20	24	24	41	27	36	27	25	17	8	...
	(b)	6·7	8·0	8·0	13·7	9·0	12·0	9·0	8·3	5·7	2·7	14·3
Afternoon	(a)	27	...	...	14	...	30	8	11	11	7	...
	(b)	9·0	...	...	4·7	...	10·0	2·7	3·7	3·7	2·3	8·7
D.D.T.-sprayed, Mekhli.												
Morning	(a)	12	21	9	16	6	11	8	11	10	6	...
	(b)	4·0	7·0	3·0	5·3	2·0	3·7	2·7	3·7	3·3	2·0	4·5
Afternoon	(a)	13	20	6	9	8	...	3	6	8	3	...
	(b)	4·3	6·7	2·0	3·0	2·7	...	1·0	2·0	2·7	1·0	3·1
D.D.T.-sprayed, Pimpli.												
Morning	(a)	28	17	19	23	11	9	8	10	10	10	...
	(b)	9·3	5·7	6·3	7·7	3·7	3·0	2·7	3·3	3·3	6·3	8·2
Afternoon	(a)	19	18	18	18	2	...	3	0	3	18	...
	(b)	6·3	6·0	6·0	6·0	0·7	...	1·0	0	1·0	6·0	5·8

(a) Actual total number of females of *culicifacies*, *fluviatilis* and *stephensi* var. *mysorensis* collected in 3 man-hours, each week.

(b) Per man-hour figures.

NOTE.—Mekhli sprayed on July 30, 1949.

Pimpli sprayed on July 26 and October 24, 1949.

TABLE 11

Morning and night collections of anophelines in unsprayed and D.D.T.-sprayed villages in Nira Canals Zone, Poona District, August 1949 to February 1950.

Week beginning with		August 14.	August 21.	August 28.	September 4.	September 11.	September 18.	September 25.	October 2.	October 9.
Unsprayed, Athurna.										
Morning	(a)	...	31	...	86	136	116	117	64	128
	(b)	...	17·0	...	28·7	45·3	36·7	39·0	21·3	42·7
Night	(a)	..	36	68	50	...	75	76	47	86
	(b)	...	36·0	68·0	50·0	...	75·0	76·0	47·0	86·0
D.D.T.-sprayed, Mekhli.										
Morning	(a)	13	...	10	13	9	7	...	6	5
	(b)	4·3	..	3·3	4·3	3·0	2·3	...	2·0	1·7
Night	(a)	0	..	13	10	...	.	...	2	3
	(b)	0	...	13·0	10·0	...	.	...	2·0	3·0
D.D.T.-sprayed, Pimpli.										
Morning	(a)	34	...	9	67	53	33	...	82	45
	(b)	11·3	...	3·0	22·3	17·7	11·0	...	27·3	15·0
Night	(a)	32	...	...	...	...	51	18	23	33
	(b)	10·7	...	...	...	...	51·0	18·0	23·0	33·0

(a) Actual total number of females of *culicifacies*, *fluviatilis* and *stephensi* var. *mysorensis* collected in 3 man-hours.

(b) Per man-hour figures.

Note.—Mekhli sprayed on July 30, 1949.

Pimpli sprayed on July 26 and October 24, 1949.

TABLE II—*contd.*

Week beginning with		October 16.	October 23.	October 30.	November 6.	November 13.	November 20.	November 27.	December 4.	December 11.	December 18.
Unsprayed, Athurna.											
Morning	(a)	92	71	62	76	33	38	55	41	36	43
	(b)	30·7	23·7	20·7	25·3	11·0	12·7	18·3	13·7	12·0	14·3
Night	(a)	42	79	47	41	9	17	48	20	21	39
	(b)	42·0	79·0	47·0	41·0	9·0	17·0	48·0	20·0	21·0	39·0
D.D.T.-sprayed, Mekhli.											
Morning	(a)	...	8	13	31	21	10	21	16	26	12
	(b)	...	2·7	4·3	10·3	7·0	3·3	7·0	5·3	8·7	4·0
Night	(a)	...	3	8	7	18	10	12	16	17	12
	(b)	...	3·0	8·0	7·0	18·0	10·0	12·0	16·0	17·0	12·0
D.D.T.-sprayed, Pimpli.											
Morning	(a)	...	10	0	1	9	10	3	9	25	28
	(b)	...	3·3	0	0·3	3·0	3·3	1·0	3·0	8·3	9·3
Night	(a)	45	...	0	0	3	10	8	14	13	15
	(b)	45·0	...	0	0	3·0	10·0	8·0	14·0	13·0	15·0

(a) Actual total number of females of *culicifacies*, *fluviatilis* and *stephensi* var. *mysorensis* collected in 3 man-hours.

(b) Per man-hour figures.

Note.—Mekhli sprayed on July 30, 1949.

Pimpli sprayed on July 26 and October 24, 1949.

TABLE II—*contd.*

Week beginning with		December 25.	January 1.	January 8.	January 15.	January 22.	January 29.	February 5.	February 12.	February 19.	Means.
Unsprayed, Athurna.											
Morning	(a)	73	67	48	44	...	59	67	34	38	...
	(b)	24·7	22·3	16·0	14·7	...	19·7	22·3	11·3	12·7	21·1
Night	(a)	...	...	19	40	...	12	12	26	19	...
	(b)	...	...	19·0	40·0	...	12·0	12·0	26·0	19·0	39·1
D.D.T.-sprayed, Mekhli.											
Morning	(a)	21	9	16	6	11	8	11	10	6	...
	(b)	7·0	3·0	5·3	2·0	3·7	2·7	3·7	3·3	2·0	4·4
Night	(a)	4	2	4	0	...	2·2	2·0	3·0	1·0	...
	(b)	1·0	2·0	4·0	0	...	2·0	2·0	3·0	1·0	6·8
D.D.T.-sprayed, Pimpli.											
Morning	(a)	17	19	23	11	9	8	10	10	19	...
	(b)	5·7	6·3	7·7	3·7	3·0	2·7	3·3	3·3	6·3	6·8
Night	(a)	...	...	...	...	10	...	13	...	10	...
	(b)	...	...	...	...	10·0	...	13·0	...	10·0	14·0

(a) Actual total number of females of *cuticifacies*, *flavitidis* and *stephensi* var. *mysorensis* collected in 3 man-hours.

(b) Per man-hour figures.

Note.—Mekhli sprayed on July 30, 1949.

Pimpli sprayed on July 26 and October 24, 1949.

TABLE III.

Morning and night collections of anophelines in unsprayed and sprayed villages in Mokhada Petha, Thana District, September 1949 to February 1950.

Week beginning with		September 18.	September 25.	October 2.	October 9.	October 16.	October 23.	October 30.	November 6.	November 13.	November 20.	November 27.	December 4.
Unsprayed, Gonda.													
Morning	(a)	...	9	20	21	...	31	17	9	33	10	13	15
	(b)	...	1·8	4·0	4·2	...	6·2	3·4	1·8	6·6	2·0	2·6	3·0
Night	(a)	...	10	13	12	...	9	10	5	24	15	13	18
	(b)	...	5·0	6·5	6·0	...	4·5	5·0	2·5	12·0	7·5	6·5	9·0
B.H.C.-sprayed, Mokhada.													
Morning	(a)	2	4	13	17	6	0	0	3	12	10	6	15
	(b)	0·5	1·0	3·3	4·3	1·5	0	0	0·8	3·0	2·5	1·5	3·8
Night	(a)	3	1	8	4	5	0	2	4	12	15	5	20
	(b)	1·5	1·0	4·0	2·0	2·5	0	1·0	2·0	6·0	7·5	5·0	10·0
D.D.T.-sprayed, Suryamal.													
Morning	(a)	0	0	0	1	0	2	2	4	2	16	15	11
	(b)	...	0	0	0·3	0	0·5	0·5	1·0	1·0	4·0	3·8	2·8
Night	(a)	...	0	0	0	0	0	4	4	2	5	5	12
	(b)	...	0	0	0	0	0	2·0	2·0	2·0	2·5	2·5	6·0

(a) Actual number of females of *culicifacies* collected.

(b) Per man-hour figures.

Note.—Mokhada sprayed on July 19 and October 25, 1949.

Suryamal sprayed on July 22, 1949.

TABLE III—*concld.*

Week beginning with		December 11.	December 18.	December 25.	January 1.	January 8.	January 15.	January 22.	January 29.	February 5.	February 12.	February 19.	Means.
Unsprayed, Gonda.													
Morning ...	(a) ...	13	7	6	23	9	18	...	19	10	3	3	...
	(b) ...	2·6	1·4	1·2	4·6	1·8	3·6	...	3·8	2·0	0·6	0·6	3·0
Night ...	(a) ...	6	5	12	23	12	17	...	12	20	2	5	...
	(b) ...	3·0	2·5	6·0	11·5	6·0	8·5	...	6·0	10·0	1·0	2·5	6·1
B.H.C.-sprayed, Mokhada.													
Morning ...	(a) ...	8	15	37	28	14	26	...	19	26	10	5	...
	(b) ...	2·0	3·8	9·3	7·0	3·5	6·5	...	4·6	6·5	2·5	1·3	3·2
Night ...	(a) ...	19	12	0	15	18	20	...	13	20	5	4	...
	(b) ...	9·5	6·0	0	7·5	9·0	10·0	...	6·5	10·0	2·5	2·0	4·6
D.D.T.-sprayed, Suryamal.													
Morning ...	(a) ...	19	10	1	6	1	3	1	0	0	0	0	...
	(b) ...	4·8	2·5	0·3	1·5	0·3	0·8	0·3	0	0	0	0	1·1
Night ...	(a) ...	13	10	8	18	9	5	5	2	2	0	0	...
	(b) ...	6·5	5·0	4·0	9·0	4·5	2·5	2·5	1·0	1·0	0	0	2·4

(a) Actual number of females of *culicifacies* collected.

(b) Per man-hour figures.

Note.—Mokhada sprayed on July 19 and October 25, 1949.

Suryamal sprayed on July 22, 1949.

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