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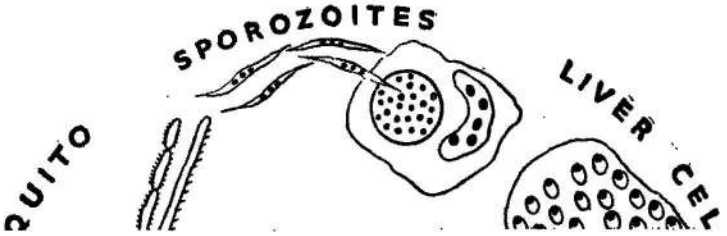

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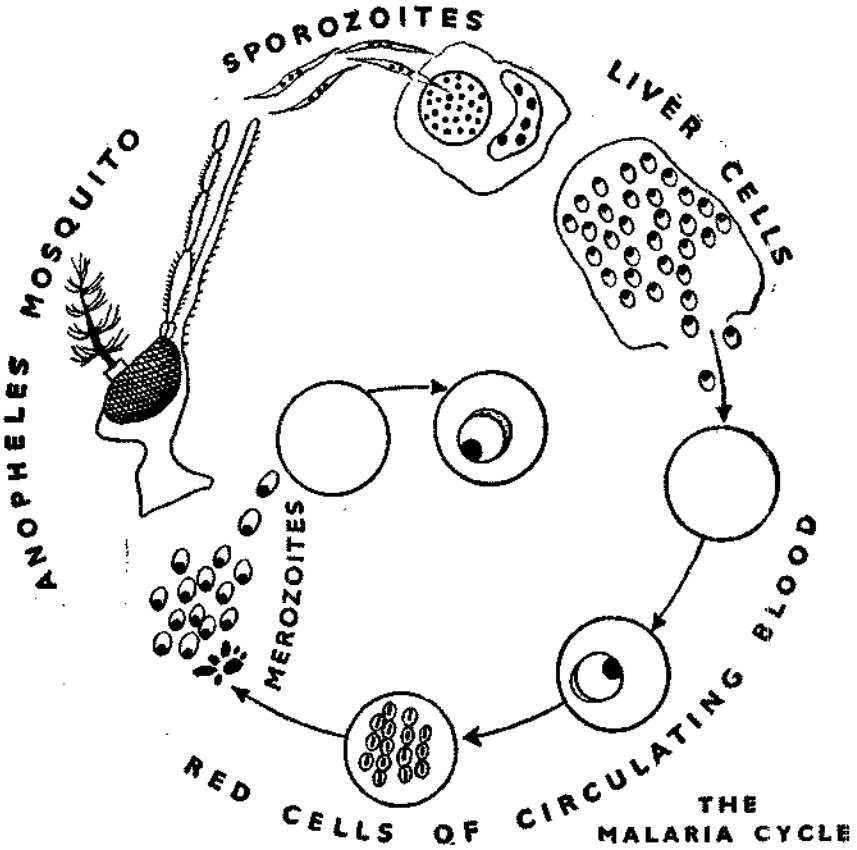

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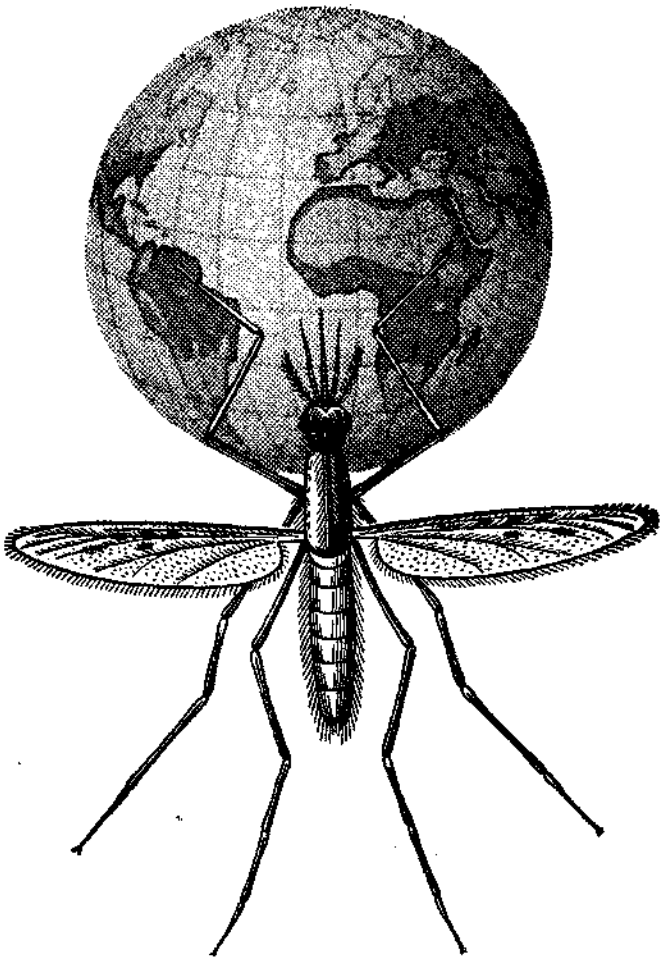

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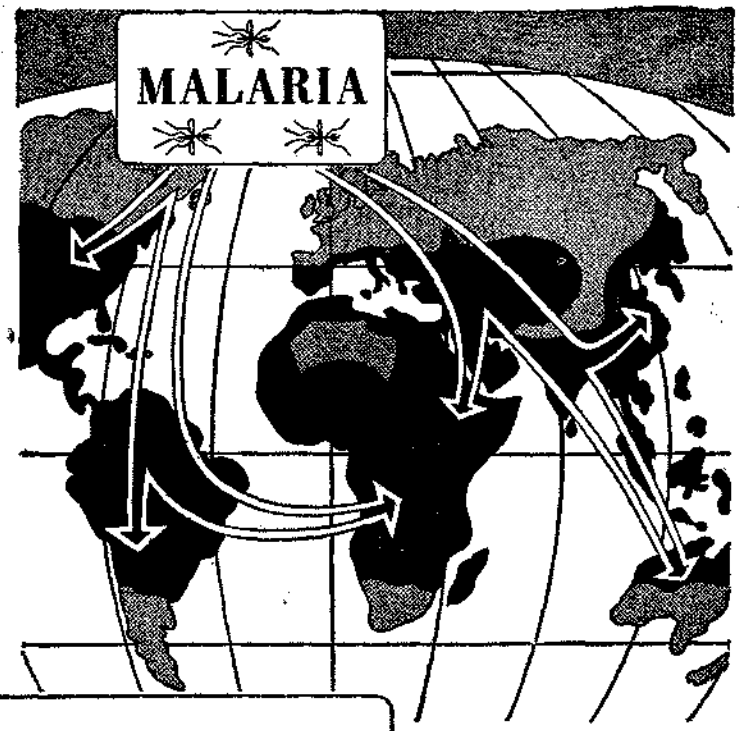

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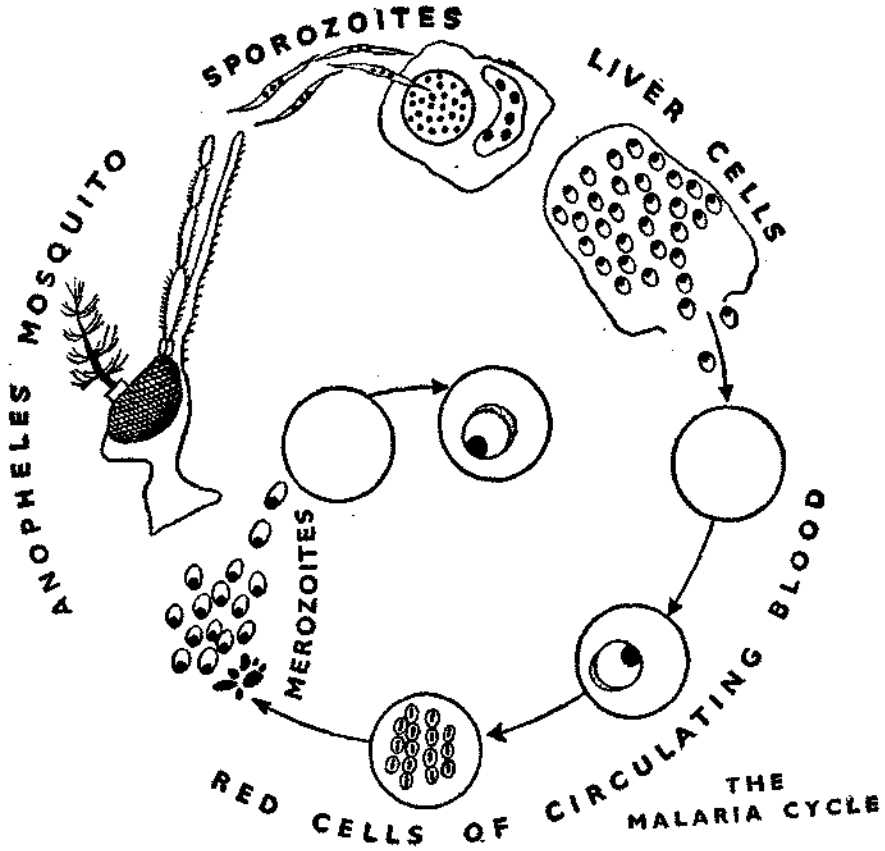

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MALARIA IN CEYLON.

Part I.

THE CONTROL AND PREVENTION OF EPIDEMIC MALARIA BY THE RESIDUAL SPRAYING OF HOUSES WITH D.D.T.

BY

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[October 3, 1950.]

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INTRODUCTION.

Although malaria has probably existed in Ceylon for centuries there is no written reference on it. The earliest record that such a pestilence did exist is in Plancius' map of 1592 (*see* reproduction in Pieris, 1913), where it is stated that the 'Kingdom of Jala (was) deserted and depopulated for 300 years by reason of unhealthiness'. Jala or Yala, as it is known today, is in the south-east of the island in an area of what must have been hyperendemic malaria.

According to Nicholls (1921), the areas in the North Central Province, known as Anuradhapura and Polonnaruwa, could never have supported such a vast population nor could its ancient civilization have reached its zenith had malaria been in existence then. The decline of this civilization from the beginning of the 13th century, Nicholls attributes to the importation of malaria from India and 'possibly also the anopheline mosquitoes, the conveyors of the disease'. This was written before *Anopheles culicifacies* was found to be the sole vector of malaria in Ceylon. But it is rather difficult to believe today that this mosquito, which breeds heavily in these areas at certain times of the year and is considered to be primarily a mosquito of the drier areas of Ceylon, was not present in ancient times. However, Nicholls does make a significant observation that the Tamil invaders, who made many attempts to occupy these areas, eventually drifted to the less fertile lands in the north of the island.

The meagre references to malaria in Portuguese, Dutch and early British times are summarized by Briercliffe (1935). These leave no room for doubt that malaria was a serious problem throughout the island which exacted a heavy toll of life, both amongst the settlers and the inhabitants.

There are no satisfactory mortality figures for the 19th century, as the registration of deaths was optional prior to 1897. A Civil Medical Department was created in 1858 and its earliest available administration report is for 1867, when a reference is made to an unusual prevalence of fever in the Eastern Province and amongst labourers working on bridges over the Deduru Oya. From 1877 onwards, frequent references are made in these reports to outbreaks of epidemic fever. Many of these refer to areas which in recent times have been regarded as being hyperendemic and in which annual seasonal fever rises have been the rule. But it is also apparent that periodical epidemics have also been experienced in the main river basins of the western and south-western parts of the island.

In this century, epidemics have occurred in 1906, 1911, 1914, 1919(?), 1923, 1928-29, 1934-35, 1939-40, 1943 and 1945-46. Gill (1935) has pointed out that these epidemics tended to occur approximately at intervals of five years. In 1919, the very high death rate was due to pandemic influenza which caused approximately 67,000 deaths. But there is evidence to show that it was associated with epidemic malaria in the months of January and February, but the actual death rate due to malaria cannot be ascertained now. The epidemics of 1943 and 1945-46 were more or less confined to the upper catchment of the Deduru Oya and the Maha Oya river basins. However, by now malaria control measures had been highly organized in all areas liable to epidemics and their effect must have been considerable in localizing the epidemics.

In the normal course of events an outbreak of malaria in the upper catchment of the Deduru Oya and the Maha Oya basins in 1948 was probable, as this was a year of severe and widespread drought. In actual fact, 1948 was a very healthy year. It is proposed in the final section of this paper to discuss in some detail why such an event did not take place although conditions were so favourable.

In this paper when reference is made to the adults of *A. culicifacies* it is to the female mosquito, unless otherwise stated.

RELIEF, PHYSICAL FEATURES AND CLIMATE.

Physiography.—The island of Ceylon which lies between 5°55′ and 9°50′ north latitude and 79°42′ and 81°53′ east longitude has an area of 25,332 square miles. Its greatest length is 270 miles and its greatest breadth 140 miles.

According to Wadia (1941) the earth's crust in Ceylon is composed of extremely ancient crystalline and metamorphic rocks, and has since the dawn of geological time remained an area that has not been submerged below the sea nor been elevated through the processes of mountain-building. Almost the chief feature in the physiography of the island is its terraced structure in three well-marked terraces, or peneplains. These terraces have arisen as a series of uplifts from the ocean-bed, separated by vast intervals of time and have throughout been subjected to denudation by the elements. Of the two major uplifts that have occurred, the last must have taken place during the Pliocene or even later.

The first peneplain or the coastal plain is about 20,000 square miles in extent, occupying most of the northern half of the island. The second peneplain, which is over 1,500 feet above sea-level, is about 4,000 square miles in extent; while, the third peneplain which is over 5,000 feet above sea-level is limited to an area of about 1,000 square miles. Each of those peneplains rises rather abruptly proceeding inland. The coastal plain rises about 500 feet around the foot of the steep ascent to the second plain which in turn rises over 2,000 feet to reach the third.

Ceylon is well provided with rivers, which radiate from the hills out to sea in all directions (Map 1). Streams, too, are innumerable in all parts of the island. This abundant network of rivers and streams is attributed by Cook (1931) to two main causes, i.e. firstly, the impermeable nature of the crystalline rocks which cause rain to run off the surface, and secondly, the high rainfall over much of the island and especially on the edge of the hills where most of the rivers arise.

But although rivers are plentiful none of them are navigable in their upper reaches, either because of the rocky nature of their beds or their swift current. In the lower reaches some of the rivers, notably the Kelani Ganga*, contain sufficient water for navigation, but only by flat-bottomed boats, as nearly all the rivers are very shallow. During certain periods of each year many of the rivers of the northern, eastern and south-eastern plains are reduced to mere trickles of water, and many of the feeder streams dry up completely or are reduced to a series of pools. The rivers of the western and southern parts of the island, on the other hand, carry a much greater volume of water for longer periods of the year and many of them do not pool in times of drought as extensively as those mentioned above. Of the rivers that find an outlet on the west coast, the Deduru Oya* and the Maha Oya* and their catchment areas form part of the subject of this paper. These two rivers pool extensively whenever there is an absence of rain in the catchment, particularly in January, February and March and again in July, August and September each year. All the rivers of the island are converted into raging torrents during the monsoons and extensive flooding often occurs.

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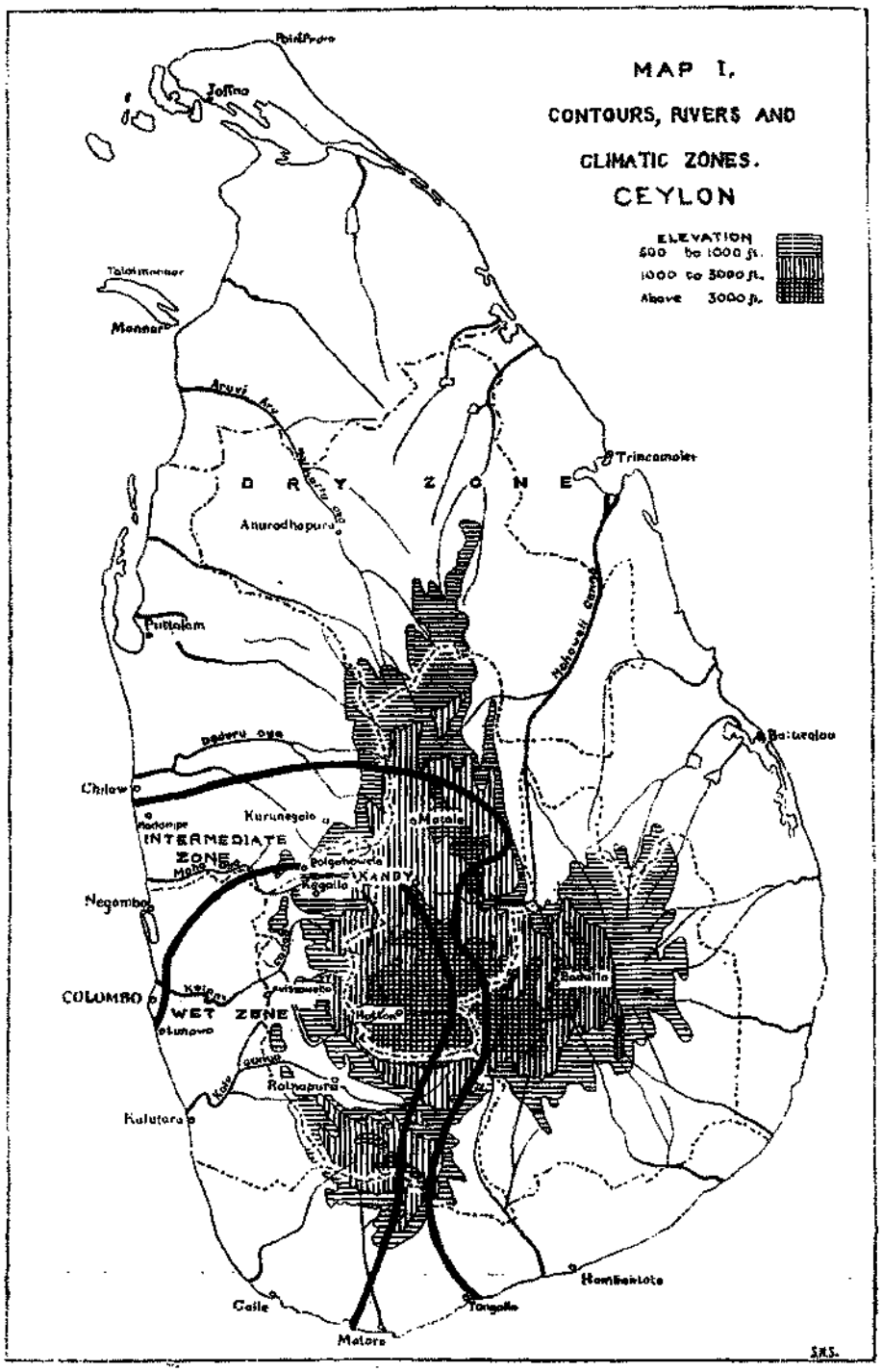
* Ganga and Oya are Sinhalese words both meaning river. The Tamil equivalent is Aru.


MAP I.
CONTOURS, RIVERS AND
CLIMATIC ZONES.
CEYLON
ELEVATION
500 to 1000 ft.
1000 to 3000 ft.
Above 3000 ft.
Jaffna
Talaimannar
Mannar
Trincomalee
DRY ZONE
Anuradhapura
Puttalam
Batticaloa
Chilaw
Kurunegala
Matale
INTERMEDIATE ZONE
KANDY
Negombo
COLOMBO
WET ZONE
Badulla
Ratnapura
Kalutara
Hambantota
Galle
Tangalle
Matara

Temperature, humidity and rainfall.—There is little seasonal variation in temperature, for not only is the island situated near the Equator but the influence of the sea is also probably considerable. Thus along the south-west and south-east coastal plains the average temperature is 80-81°F., while in the northern plain it is 81–83°F. The distribution of temperature depends largely on the relief of the land and isothermal lines can be drawn to coincide with the contour lines. Thus at 1,000 feet the isotherm would be 77°F., at 3,000 feet 70°F., and at 6,000 feet 60°F., there being a fall of 1°F. for every 300 feet of ascent above sea-level.

During March and April there is a rise of temperature all over the island, as these are the months when the skies are generally cloudless and the highest temperatures for the year are recorded. A fall in temperature is associated with the monsoon rains, cooler conditions especially being experienced during the north-east monsoon, which commences to blow in October-November.

Although seasonal variations in temperature are not great in the coastal areas, there may occur considerable daily ranges of temperature. At Colombo in February there is a daily range of 15°F., while in June and July, it is only 8-9°F. At Anuradhapura, an inland dry zone town situated about 40 miles from the north-west coast, the daily range of temperature in March is 20°F., with a humidity of 60 per cent; in November-December the daily range is 13°F., and the humidity 77 per cent.

The air in Ceylon is always moist, even in the so-called dry zone, as no part of the island is more than 65 miles from the sea. The average humidity varies but little from month to month. At Colombo on the west coast it ranges from 68 per cent in February, March and December to 78 per cent in June and July. At Anuradhapura the lowest average humidities are recorded in March and April (62 and 63 per cent respectively), and the highest in November (77 per cent). The daily ranges of temperature are correlated with humidity; high daily ranges being associated with low humidity and *vice versa*.

The island as a whole has a high rainfall caused by local thunderstorms, cyclones or by the monsoons. Most of this rain is concentrated in the south-western lowlands and the edges of the hill country, on each side of which it steadily decreases. The lowest rainfall, under 50 inches a year, is experienced in two narrow coastal strips in the north-western and south-eastern corners of the island.

There are two monsoons, blowing from the south-west and north-east respectively. The former begins to blow in May, strengthens in June and July and begins to weaken in August. By the end of September it has almost spent its force. During this monsoon the rain is chiefly confined to the south-western quadrant, partly in the coastal regions and partly on the windward slopes of the hill country.

The north-east monsoon rains begin in October-November. In these months the rains are often augmented by cyclones moving in a north-westerly direction from the southern part of the Bay of Bengal. When these atmospheric disturbances come close to Ceylon, heavy rains ensue. These cyclonic rains are important in providing the flat lands of the north and east with additional rain, particularly in the months of October and November. The north-east monsoon continues into December, but begins to weaken in January, while some activity

is still experienced rather feebly in February and March. While this monsoon brings rain to the south-western quadrant as well, it is the chief monsoon of the dry zone. Unfortunately, it is a relatively weak monsoon originating in the north-eastern part of India and what rain it precipitates in Ceylon is what it gathers on its passage over the Bay of Bengal. This monsoon is often a failure, causing hardship and misery in the dry zone.

In between the south-west and north-east monsoons in the months of April and October, are two periods of thunderstorm activity caused by convectional rains and these rains, especially in April, often provide the first relief from a prolonged spell of drought.

The amount of rainfall recorded during the south-west monsoon (May to September) has been of use in demarcating three climatic zones, namely, dry, intermediate and wet (Map 1). The dry and intermediate zones correspond approximately to hyperendemic and endemic areas, while most of the wet zone is largely free from malaria. During the south-west monsoon the dry zone receives less than 20 inches of rain, the intermediate zone receives 20–40 inches and the wet zone receives over 40 inches. In Table I, the average monthly rainfall for six stations in each zone is given.

It may also be mentioned that nowhere in the island is the temperature and humidity adverse enough at any time for a cessation of development of *Anopheles culicifacies*, and the uninterrupted development of the mosquito and malarial parasites can take place throughout the year.

DISTRIBUTION AND PREVALENCE OF MALARIA.

The most important factor which determines the distribution of malaria in Ceylon is the extent to which conditions are favourable for the production of the vector *A. culicifacies*. The humidity and temperature throughout the country, except at high altitudes, are usually favourable for malaria transmission. The density of *A. culicifacies* is dependent on suitable breeding places being available, and these may be natural or man-made.

Climatic zones.—In the dry zone a rainfall sufficient to allow water to stagnate for about a fortnight is adequate to facilitate the breeding of this species. It is only the failure or absence of rainfall that reduces the density of the vector in the dry zone. Where an area in the dry zone is under irrigation and wet cultivation, *A. culicifacies* breeding is perennial. As such, malaria transmission with seasonal variations can take place throughout the year, thus making the dry zone endemic to hyperendemic. There are only two exceptions to this: firstly, the portion of the Jaffna Peninsula to the west of the Jaffna-Point Pedro Road, and secondly, the dry zone hill country above an elevation of 1,000 feet. Thus the whole of the districts of Vavuniya, Mannar, Anuradhapura, Trincomalee, Batticaloa, Puttalam, Hambantota and the major portions of the districts of Matale, Kurunegala and Badulla and small portions of the Jaffna, Ratnapura and Chilaw districts, which come within the dry zone, are highly endemic with spleen rates varying between 26 and 50 per cent, or hyperendemic with spleen rates of over 50 per cent (Table II). The seasonal rise of malaria is during the period of the north-east monsoon from about October to March.

As the wet zone, under normal conditions, does not afford suitable breeding facilities for the vector, it remains healthy with spleen rates well below 10 per cent. This zone has some of the principal rivers arising on the western slopes of the hills. Both monsoons affect this area and the annual rainfall here is usually over 100 inches; the rivers are full and fastflowing throughout the year. This is particularly so with the rivers in the Kalutara and Galle districts. It is only when there is a failure of one or both monsoons that sand and rock pools form in the beds of rivers and the *A. culicifacies* density may become very high. Since this is an abnormal feature occurring only once in about three to six years, and since the area is normally healthy, conditions favourable for an outbreak of epidemic malaria are created. The districts of Kalutara and Galle are so situated that the bigger rivers and streams are always full and do not lend themselves to pool formation. As a result, they are healthy and not subject to regional epidemics. A few localized epidemics have been reported. But, these are due mostly to man-made conditions in the villages and are purely localized. The rest of the wet zone area including portions of Colombo, Negombo, Ratnapura, Kegalle, Kandy and Nuwara Eliya districts are subject to very severe epidemic malaria.

The intermediate zone with its annual rainfall of between 75 and 100 inches is healthy or moderately endemic with spleen rates varying from under 10 to 25 per cent. The more the area approximates to the dry zone, the higher the spleen rate and *vice versa*. In the moderately endemic parts of this area there are two malaria seasons, a mild one commencing in April and a more marked one coinciding with the north-east monsoon period. Included within this zone are portions of the Colombo, Negombo, Matara, Ratnapura and Kandy districts which are normally healthy and portions of the Kurunegala, Chilaw and Matale districts which are moderately endemic. The whole of this zone is subject to severe epidemic malaria.

Endemicity rates.—Sivalingam and Rustomjee (1941) in an island-wide spleen and parasite survey carried out in February and March 1939 and 1940 respectively considered that the pre-epidemic figures obtained in 1939 would be the lowest that could ever be recorded. These rates for the twenty-one districts are given in Table II together with the spleen rates in 1936, 1947, 1948 and 1949. The year 1936 has been selected to demonstrate post-epidemic conditions. The rates for 1947–49 show the progressive decline in the spleen and parasite rates consequent on the island-wide campaign of residual spraying of houses with D.D.T. The reduction in the rates for these years is discussed more fully in Part II of this paper, which deals with the hyperendemic areas. No surveys were carried out during the period 1941–46 owing to war conditions.

There is one disadvantage in considering the spleen rates by districts because such a classification does not record what Sivalingam and Rustomjee (*loc. cit.*) describe as the diversity in malaria endemicity which necessitates each localized area to be studied separately. A revenue district may be healthy in one part, moderately endemic in another and highly endemic in yet another part. Such differences cannot be pictured clearly when the endemicity of the district as a whole is considered. But, it serves the purpose of this paper to consider the district as a whole and to note the changes in endemicity resulting from an epidemic or from residual spraying. There is also the advantage that these rates can be correlated

with the vital statistics of the different districts as contained in the annual reports of the Registrar-General.

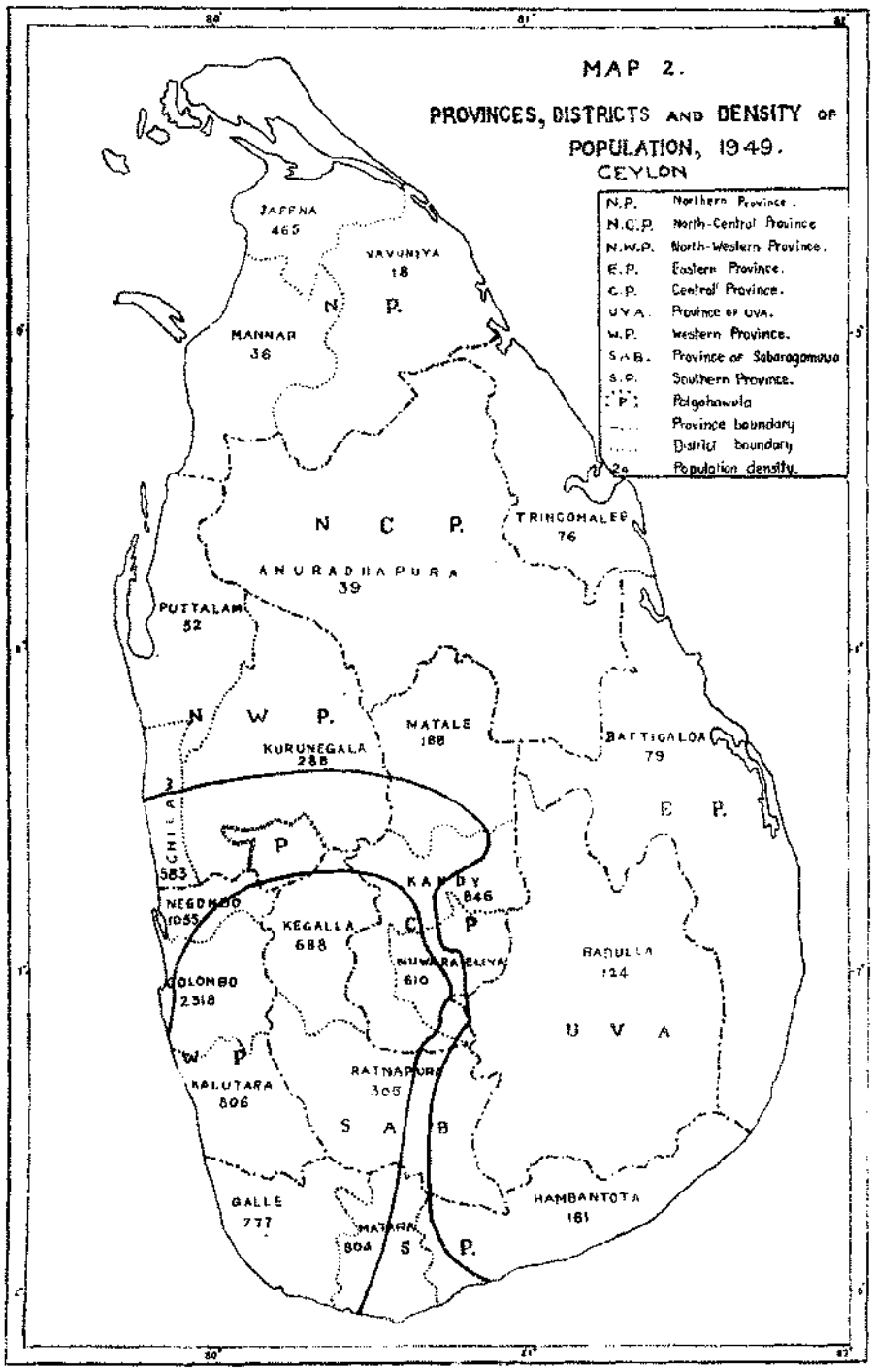
Using the spleen rates of 1939 as the index of the normal endemicity of malaria, the districts can be classified into (*a*) Healthy, with spleen rates not over 10 per cent; (*b*) Moderately endemic, with spleen rates between 10 and 25 per cent; (*c*) Highly endemic, with spleen rates between 25 and 50 per cent; and (*d*) Hyperendemic, with spleen rates of over 50 per cent. Accordingly, the districts of Kalutara, Galle, Colombo, Negombo, Matara, Kegalle, Nuwara Eliya, Jaffna and Kandy are healthy. But, except for Kalutara and Galle districts, the others suffer from epidemic malaria as seen from the spleen rates of 1936. The districts of Ratnapura, Chilaw and Badulla are moderately endemic and are also subject to epidemic malaria. The five districts of Matale, Kurunegala, Mannar, Puttalam and Batticaloa are highly endemic. Except for Batticaloa, the rest have been affected by epidemic malaria, particularly the districts of Kurunegala, Puttalam and Matale. The rest of the districts are hyperendemic. The 1936 spleen rates indicate that even the normally highly endemic and hyperendemic districts have been affected by the 1934-35 epidemic, though not so intensely as the normally healthy or moderately endemic areas were affected. In the subsequent epidemics of 1939-40 and of 1945-46, the highly endemic and hyperendemic districts were not affected.

Altitude.—Altitude has an important influence on malaria prevalence. *A. culicifacies* is a mosquito of the plains and the foothills. This species has not been known to breed at elevations of over 3,000 feet. Hence there is no malaria in the hills above that elevation. But the valleys of the hill country may go down to 1,000 feet or less, in which case, malaria becomes moderately endemic or even highly endemic as in the case of certain small portions of the Kandy, Nuwara Eliya, Badulla and Ratnapura districts. Between elevations of 1,000 and 3,000 feet it is only when either or both monsoons fail and drought conditions are present that the upper reaches of the rivers and streams start pooling and give rise to conditions favourable for the increased breeding of *A. culicifacies*, which in turn leads to epidemic malaria.

It is worth noting that the epidemiology of malaria in Ceylon is such that two contrasted conditions like deficient rainfall in the wet and intermediate zones, and excess of rainfall in the dry zone, result in increased malaria transmission, as both these conditions cause increased breeding of *A. culicifacies*. Ceylon has the problem of epidemic malaria in one part and of endemic malaria in another part. The only areas which have practically no malaria problem are the small sector comprising the Kalutara and Galle districts in the south-western corner, the hill country over 3,000 feet and a portion of the Jaffna Peninsula.

VITAL STATISTICS, 1901–49.

The population of Ceylon which at the 1946 census was 6,657,339 has been estimated by Davies (1949) to be 7,193,000 at the end of 1948. The population has approximately doubled itself in the last fifty years and is steadily increasing, more especially since 1947.


MAP 2.
PROVINCES, DISTRICTS AND DENSITY OF POPULATION, 1949.
CEYLON
N.P. Northern Province.
N.C.P. North-Central Province
N.W.P. North-Western Province.
E.P. Eastern Province.
C.P. Central Province.
UVA. Province of Uva.
W.P. Western Province.
S.A.B. Province of Sabaragamuwa
S.P. Southern Province.
P. Polgahawela
Province boundary
District boundary
Population density.
JAFFNA 465
VAVUNIYA 18
MANNAR 36
N P.
N C P.
TRINCOMALEE 76
ANURADHAPURA 39
PUTTALAM 52
N W P.
KURUNEGALA 288
MATALE 188
BATTICALOA 79
E P.
CHILAW 583
P
NEGOMBO 1055
KEGALLA 688
KANDY 846
C P
NUWARA ELIYA 610
BADULLA 124
COLOMBO 2318
U V A
W P
KALUTARA 806
RATNAPURA 305
S A B
GALLE 777
MATARA 804
S P.
HAMBANTOTA 161

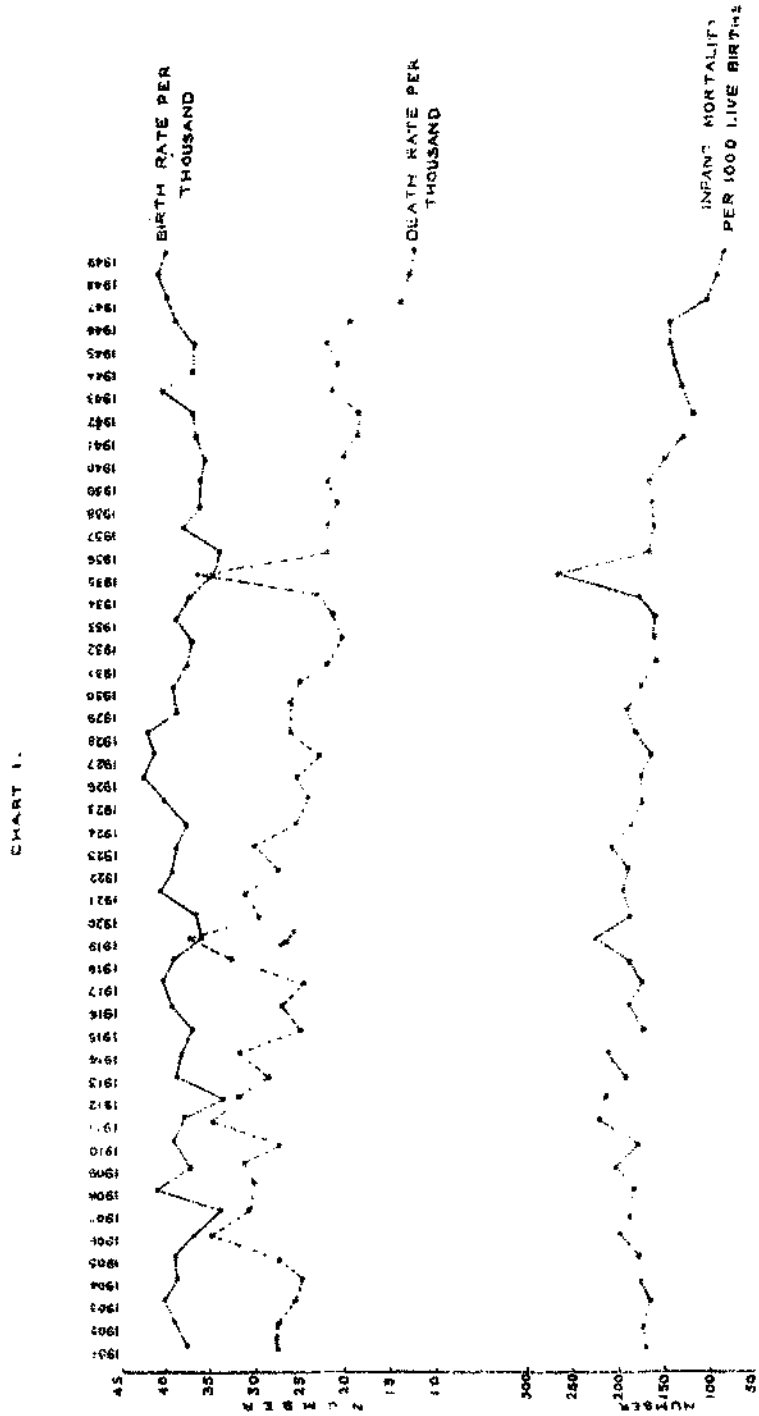
At the 1946 census, the Sinhalese were found to constitute 69·4 per cent, Ceylon Tamils 11·0 per cent, Indian Tamils 11·8 per cent, and the Ceylon Moors 5·5 per cent of the total population. The Ceylon Tamils occupy mainly the Northern and Eastern Provinces (Map 2), while the Sinhalese are widely distributed over the rest of the country. The Indian Tamils are mostly employed as plantation labourers, while the Moors are found along the coast, particularly in the northern half of the island and the east coast.

The density and distribution of population as estimated in 1949 by Davies (*loc. cit.*) is given in Table III and Map 2. The nine provinces of Ceylon have been divided into twenty-one revenue districts, which vary in their malarial endemicity. Of the healthy districts, Kalutara and Galle are wet zone areas in which only very mild, sporadic and localized epidemics have occurred. The two groups of districts classified as healthy and moderately endemic contain the bulk of the population, the density ranging from 305 in the Ratnapura District, which contains large tracts of virgin forest, to 2,318 in the Colombo District. The distribution of the population in the rest of the districts has been governed largely by malaria. Once heavily populated areas in ancient times in the Anuradhapura District in the North-Central Province now carry a population of only 39 per square mile and much of the land, once heavily cultivated, has turned into jungle. Most of these highly malarial and hyperendemic districts are sparsely populated and very largely covered with jungle. The exception being the Kurunegala District, which in spite of being highly endemic and even hyperendemic in parts, has managed to attract large numbers of people engaged primarily in coconut cultivation.

The vital statistics in so far as they relate to the crude birth rate, crude death rate and infant mortality rate for the years 1901-49 are given in Table IV and Chart I. Up to the year 1945 the death rate has shown a steady decline, except for the malaria epidemic years of 1906, 1911, 1914, 1923, 1928-29, 1935, 1939, 1943 and 1945, and the pandemic of influenza in 1919, with which was also associated an epidemic of malaria. In the course of this half century the worst epidemics were of 1919, in which nearly 67,000 lives were lost, and of 1934-35. The latter was an explosive and widespread outbreak of malaria involving the catchment areas of several important rivers in the Western, Central, North-Western and Sabaragamuwa Provinces. This epidemic caused nearly 80,000 deaths.

The crude birth, crude death and infant-mortality rates have been summarized in Table V. Up to 1940, these statistics have been given for decennial periods. In what would have been the fifth decade the considerable effect of D.D.T. residual spraying of houses has to be taken into consideration. This spraying which was commenced in November 1945 at Anuradhapura (North-Central Province) and other parts of the country in 1946 would only have begun to exert its full effect in 1947. By this time the spraying had become well organized. Further in 1945-46 there was a sharp, localized epidemic in the middle and lower catchments of the Maha Oya. Thus the figures for 1941-49 have been divided into two periods; the first for the years 1941-46 and the second for the period 1947-49.

The crude death rate has shown a steady decline from the decade 1911-20 when it was 30·6 to the period 1941-46 when it had fallen to 20·4, while the crude birth rate has been maintained more or less at a steady level.



Vital statistics, 1901–49.

The infant-mortality rate has not shown the same steady decline as the crude death rate during the years 1921–40. In the period 1921–30, it ranged from 160 to 212 per thousand with an average of 182, while in the decade 1931–40, the range was 149 to 263 with an average of 171. The highest rates in each decade coincided with an epidemic of malaria. During the periods 1941–46, in spite of severe localized epidemics in the Maha Oya basin in 1943 and 1945, the infant-mortality rate averaged 133, ranging from 120 to 141. Not only was this an improvement on previous comparable periods but also in 1942 the rate of 120 was the lowest ever recorded up to that date.

According to Rustomjee (1944) 'death rates return rapidly to normal within two years of a major outbreak, however devastating the intensity of the epidemic may have been. Infant-death rates return to stability exceptionally quickly by the end of one year after an outbreak, while the birth rates are high just before an epidemic and return to normal within two years after an outbreak'.

From 1947 onwards the vital statistics have taken a truly dramatic turn. The death rate has declined from 20·3 in 1946 to 12·6 in 1949, while the infant-mortality rate has declined from 141 in 1946 to 87 in 1949. Since the outbreak in 1945-46 in the Maha Oya basin, Ceylon has been free from malaria epidemics, although conditions in areas liable to epidemics were quite favourable in 1948 and also in 1950 at the time this paper was being written. All were years of deficient rainfall.

A detailed study of the crude death rate and infant-mortality rate classified according to districts given in Table VI indicates that these rates have shown a progressive decline from 1938–49. But it is only in the years 1947, 1948 and 1949 that an appreciable reduction in these death rates has been recorded in the hyperendemic and highly endemic districts. It is the reduction in these districts that has caused the figures for the whole of Ceylon to show a marked decline since 1947.

The rate of natural increase of the population may be summarized as follows:

Period.	Rate of natural increase per 1,000 population.
1901–10 ...	9·3
1911–20 ...	7·2
1921–30 ...	13·2
1931–40 ...	13·4
1941–46 ...	17·2
1947–49 ...	26·6

These figures indicate that at present Ceylon has one of the highest rates in the world and that the sharp upward trend first noted in 1947 has been maintained. The rates for 1947, 1948 and 1949 were 25·3, 27·4 and 27·3 respectively. The

highest rate previously recorded was 19·2 in 1913, while in 1919 and 1935, the years of major epidemics, negative rates of 1·5 and 2·2 were recorded.

THE ANOPHELINE MOSQUITOES OF CEYLON.

Twenty species of anopheline mosquitoes have been recorded in Ceylon. They are :—

Group A. THE VECTOR :—

1. *A.* (*Myzomyia*) *culicifacies* Giles, 1901.

Group B. IMPORTANT CARRIER OUTSIDE CEYLON :—

2. *A.* (*M.*) *maculatus* Theobald, 1901.

Group C. VECTORS OF LOCAL IMPORTANCE IN CERTAIN AREAS OUTSIDE CEYLON :—

3. *A.* (*M.*) *aconitus* Dönitz, 1902.
4. *A.* (*M.*) *annularis* Van der Wulp, 1884.
5. *A.* (*Anopheles*) *barbirostris* Van der Wulp, 1884.
6. *A.* (*A.*) *hyrcanus* var. *nigerrimus* Giles, 1900.
7. *A.* (*M.*) *leucosphyrus* Dönitz, 1901.
8. *A.* (*M.*) *tessellatus* Theobald, 1901.
9. *A.* (*M.*) *varuna* Iyengar, 1924.

Group D. PROBABLY OF NO PRACTICAL IMPORTANCE :—

10. *A.* (*A.*) *aitkeni* James, 1903.
11. *A.* (*A.*) *annandalei* var. *interruptus* Puri, 1929.
12. *A.* (*A.*) *gigas* var. *refutans* Alcock, 1913.
13. *A.* (*A.*) *insulaflorum* Swell. and Swell., 1920.
14. *A.* (*M.*) *jamesi* Theobald, 1901.
15. *A.* (*M.*) *karwari* James, 1903.
16. *A.* (*M.*) *pallidus* Theobald, 1901.
17. *A.* (*A.*) *pseudobarbirostris* Ludlow, 1902.
18. *A.* (*M.*) *ramsayi* Covell, 1927.
19. *A.* (*M.*) *subpictus* Grassi, 1899.
20. *A.* (*M.*) *vagus* Dönitz, 1902.

Out of twenty species of anopheline mosquitoes recorded in Ceylon, eleven have proved to be innocuous wherever they have been found and merit no further discussion. A brief account of each of the other species follows.

VECTOR SPECIES OF OTHER COUNTRIES FOUND IN CEYLON.

A. maculatus is not an important carrier in India (Covell, 1944) but is a vector of major importance in Malaya and the East Indies. In Ceylon, it is essentially

a hill and foothill species, where it is found breeding in relatively large number in streams. The adult is rarely found in houses but can be taken in cattle-traps in moderate numbers. Senior White, (1920), basing his conclusion on the fact that the larva formed 85 per cent of the total anophelines at an elevation of 1,000-1,600 feet, considered it to be the chief carrier in Ceylon. At this time, and for quite ten years after, this species was generally suspected to be one of the important vectors in Ceylon and whenever the adult was found, careful dissections were carried out. Whenever epidemics occurred in the hill-country, a special attempt was made to find and dissect this mosquito. At Passara, about 10 miles from Badulla (Map 1) at a height of 2,000 feet, during an epidemic only one female *A. maculatus* was found, the catches being almost exclusively *A. culicifacies*. It was also found in this district that there was no correlation between its period of maximum larval prevalence and malaria incidence, as the latter took place from July to November, while the larvæ were only plentiful from December onwards.

A. aconitus has been found infected in India, Indo-China, Malaya and the East Indies. In Indonesia, it appears to be a vector of real importance in the west. But according to Covell (*loc. cit.*) it is generally zoophilic in habit and appears to feed on man where cattle are scarce. In Ceylon, it is essentially a hill or foothill species and is relatively uncommon in the upper catchment of the Deduru Oya. It is a little more plentiful in the Maha Oya basin and is caught in moderate numbers in cattle-traps; but this has not exceeded a catching rate of 0·84 per hour in any one year. The larvæ are not found breeding in pools or along the banks of rivers in any great extent, and when present, are often found in ricefields.

A. annularis is a vector of some importance in certain parts of India, such as Orissa, Bengal and Assam. Natural infections have also been recorded in Burma, South China, Malaya and the East Indies. Although the adult is said to be taken frequently in houses in India, it is very rarely so in Ceylon; when found it is more likely to be taken in a cattle-trap. The larvæ are often found in ricefields but are less common in rivers and streams.

A. barbirostris has been found naturally infected in Malaya and the East Indies but not in India. In the East Indies the variety *vana* is of very great local importance in Sumatra. In Ceylon, it is a swamp species and generally found along with *A. hyrcanus*, particularly in ricefields which are more than half-grown. The larva is not found commonly in rivers and streams. The adult is more often found in cattle-traps than in houses but is never very common.

A. hyrcanus var. *nigerrimus* is not a vector in India or Burma, but natural infections have been recorded in Indo-China, Malaya and the East Indies. The larva is found breeding in very large numbers in ricefields which are more than half-grown. In such situations it is the dominant species. It is far less common in rivers and streams. The adult is rarely found in houses but is quite often taken in cattle-traps in fair numbers. Along with *A. subpictus* and *A. vagus*, it is the species most commonly found in traps.

A. leucosphyrus has fairly recently been incriminated as a vector of some importance at Digboi, Assam, by Clark and Choudhury (1941). It is also the vector in the Shingbwiyang area of North Burma. It has also been found infected in Indo-China. More recently, McArthur (1947) has found that it is the chief carrier

in Borneo and not *A. maculatus* as hitherto believed. In Ceylon, both larvæ and adults are rather rare but as this mosquito is most elusive, and according to McArthur only feeds in the early hours of the morning, it has probably been missed here and elsewhere in houses. Larvæ are more often found in very small numbers in the Maha Oya basin than in the Deduru Oya catchment. Adults are never taken in cattle-traps.

A. tessellatus is the vector in the Maldive Islands, where it is the only anopheline mosquito so far recorded. Although natural infections have been recorded in the East Indies, Borneo, Indo-China, South China and Formosa, it is not considered to be an important vector in any of these countries. In Ceylon, it is not taken in any great numbers in houses but is found more often in cattle-traps. The larva is not found often in rivers and streams but is generally found in various village breeding places in association with *A. hyrcanus*, particularly in ricefields.

A. varuna is a proved vector in certain parts of India, such as Travancore and near Calcutta, and quite harmless in others such as Vizagapatam and the Orissa coastal belt. In Ceylon, the larvæ are most commonly found in the intermediate zone, breeding prolifically in rivers and streams, particularly along the margins and in wells, drains and irrigation channels. In Ceylon, unlike in India, the adults are not domestic and the numbers caught in cattle-traps are not commensurate with the prolific numbers of larvæ found. Even night catches in houses have failed to reveal this elusive mosquito. Clemesha (1934) set up special cow-houses on the banks of streams where the larvæ were plentiful but found the catches most disappointing. The biting preferences of this mosquito in Ceylon have still to be elucidated.

Of the eight species of mosquitoes described above, only a single, doubtful gut infection in *A. hyrcanus* has been recorded up to now. It is not likely that any one of them is of importance as a vector in Ceylon.

THE VECTOR IN CEYLON—*A. CULICIFACIES*.

History of relation to malaria.—*A. culicifacies* alone is the proved vector of malaria in Ceylon. Chalmers (1905) first drew attention to its association with epidemics of malaria in North Colombo, Uva Province, Hambantota and near Trincomalee. It was first incriminated as a vector by James and Gunasekara (1913), who found one gland infection out of 36 mosquitoes dissected at Talaimannar in Mannar. Another fifteen years were to elapse before Carter (1928) recorded the next infections at Ridigama in the Kurunegala District (12 guts out of 421 dissections—infection rate 2·85 per cent; 5 glands out of 398 dissections—infection rate 1·28 per cent) and at East Lynne Estate, Chilaw (4 guts out of 197 dissections—infection rate 2·03 per cent; 12 glands out of 196 dissections—infection rate 6·12 per cent). In the meantime, as a result of Carter's (1927) pioneer work on the anopheline mosquitoes of Ceylon, *A. culicifacies*, *A. varuna*, to a less extent *A. maculatus*, *A. annularis* and *A. subpictus* were regarded as being dangerous potential vectors. *A. subpictus* was, however, excluded in spite of its great prevalence in certain areas as it was of no importance in India. During Carter's investigations no infected adults were found and his work dealt mainly with larval

stages. Earlier, Senior White (*loc. cit.*) had come to the conclusion that *A. maculatus* was the chief carrier in the hills but no infections had been recorded, and by 1931 it was considered to be of no importance as a vector. *A. annularis* and *A. varuna* were also by this time ruled out as vectors, owing to their non-domestic habit, the latter species in spite of being the dominant anopheline larva in the intermediate zone. The prevalence of *A. annularis* was, however, never very great in any part of the island. By 1932, *A. culicifacies* alone was regarded as being the chief vector as no infections in any of the other suspected carriers had been found. This holds good to this day, except for the single, doubtful gut infection in *A. hyrcanus*.

Daytime resting places.—In Ceylon, *A. culicifacies* is domestic in habit and rests in dwellings after its blood feed. Except for dairies in urban and suburban areas, cattlesheds are not common in Ceylon and the prevailing habit is to tether cattle in the open. In coconut-growing districts, it is customary to tether cattle in pairs round trees for manuring. In any case the adult is far more readily taken in dwellings than in cattle-traps (Table XVII). Clemesha (*loc. cit.*) with his experience of this mosquito in India says that it is an intensely domestic species, which accords with the views of most observers. It is found generally in dark corners of houses and if there is a fire burning inside, it is generally caught near the door. Out of doors, except for certain earth drains at Kurunegala where a few specimens were found by Carter (1928), places which might have been expected to yield adults such as culverts and cool moist places on the sides of drains and undergrowth in close proximity to houses, proved negative.

Comparison of spray catches and live catches in dwellings.—The catches of adults made in dwellings are probably no reflection on the actual numbers present (Table XVII), as this mosquito is peculiar in secreting itself in cracks and crevices as James and Liston (1911) first pointed out. In rural Ceylon, the wattle and daub houses, thatched with the interwoven leaf of the coconut palm, afford ample opportunity for the adults to secrete themselves. This is amply borne out by the results given in Table VII which represent a comparison of spray catches at six villages in the intermediate zone, where the mosquito was found in relatively large numbers compared with live (hand) catches in three relatively favourable villages in the same zone. Both spray and live catches were made after 7 in the morning. Although only 814 houses were sprayed before examination, 1,695 were examined for live mosquitoes. Thus less than half the number of sprayed houses yielded nearly thirteen times as many *A. culicifacies* adults (both males and females).

Nocturnal movements.—There is ample evidence that in Ceylon the adult enters houses in large numbers from dusk till about 9.30 p.m. At Ponparankulama, near Anuradhapura, catches as high as 48·38 per hour have been recorded. But the evidence for the other hours of the night are not very conclusive. Carter (1933) basing his conclusion on the total anopheline night catch at Badulla, of which *A. culicifacies* represented only 30·3 per cent, concluded that this mosquito was also active from 5 to 6 a.m.

Flight range.—The maximum flight range of this species is generally regarded as being about half a mile (Stephens and Christophers, 1902; Christophers, 1904; James, 1903). Carter (1934) found that the catch fell away in dwellings situated

beyond 500 yards from the Deduru Oya at Chilaw. His results may be summarized as follows:

Distance of huts from river.	Catching rate per hour.
50-200 yards ...	16·3
500 ,, ...	9·9
1,000-1,300 ,, ...	0·7
2,000-2,200 ,, ...	0·5
2,500-3,000 ,, ..	0·06

Seasonal prevalence of larvæ and their breeding places.—The larva has always been regarded as being a sun-loving stage. Carter (1930) found that along the banks of the Deduru Oya near Chilaw, the larval rate (the number of larvæ per 100 dips from a standard ladle) was 25·0 in the shade and 77·4 in the sun, and in pools it was 10·9 and 116·0 respectively. But recently Rajendram *et al.* (1950) have found the larvæ breeding in shaded situations in streams in thick jungle up to a distance of six miles from human habitation. Adults too were caught in human-baited traps in the jungle far away from the nearest dwelling. The presence of larvæ breeding in jungle was not surprising, for whenever jungle had been cleared in the past in connection with colonization schemes, outbreaks of malaria had always followed.

The larva shows a preference for fresh clear water and the principal breeding places of this species in Ceylon are:—

(1) Sand and rock pools in rivers and streams; also to a less extent the margins of rivers and streams.
(2) Wells of all types, built and unbuilt.
(3) Brick, quarry and coral pits, also coconut pits.
(4) Borrowpits, particularly when freshly dug.
(5) Irrigation channels; also seepages from them.
(6) Roadside drains.
(7) Rain pools.
(8) Ricefields, particularly fallow and up to quarter-grown.

The above list of breeding places covers most of the situations in which larvæ of *A. culicifacies* are found in the intermediate and the wet zones, when conditions are favourable. Favourable conditions in these zones are brought about by a failure of one or both monsoons. The heaviest breeding undoubtedly takes place in sand and rock pools in the rivers and streams and in all other possible situations, which can be classed as village breeding places, the larvæ are present in negligible numbers (Table XIII). Of these, brick pits and coconut pits (pits in which coconut fronds are soaked prior to weaving) are more prolific than others, while in certain towns such as Kurunegala and Trincomalee, drinking wells have been a source of perennial breeding.

Normally in the intermediate zone there are two periods when rainfall is scanty, the first in February and March and the second in August and September. These are the periods in normal years when breeding is most pronounced in rivers and

streams. Each dry period is generally brought to a conclusion by inter-monsoonal thunderstorms followed by the monsoons. The heavy rain received tends to flush out the pools in the river beds and the frequent rain received in normal years tends to keep the breeding down to a minimum.

In the wet zone, even during the months of scanty rainfall, breeding in normal years is confined to specialized breeding places such as brick and quarry pits. Rivers normally do not pool in this zone. It is only in years when the monsoon rains have been very deficient that pooling occurs.

In the dry zone, mosquito prevalence is largely seasonal and coincides with the north-east monsoon rains. The prevalence of adults and larvæ are greatest in the months of November, December and January. A certain amount of breeding in May and June is associated with the south-west monsoon rains, sparse though they be in this zone. During the rainy season from November to January, larvæ are found in large numbers in almost every puddle of water and when these begin to dry up, breeding is continued in the more permanent situations such as wells, irrigation channels and rivers and streams. The smaller streams tend to dry up more or less completely in the dry season but the larger rivers contain water for long periods. When, however, the north-east monsoon is deficient, pooling of these rivers takes place and larvæ breed heavily in the pools.

HISTORY OF MALARIA CONTROL.

A. 1905–20.

Although the period under review was not characterized by any marked attention paid to the control of malaria, yet certain important events occurred which were not without significance at the time. The investigations of Chalmers in 1905 already referred to represent the first attempt made to study the anopheline mosquitoes of Ceylon in relation to malaria. Perry (1908) inaugurated the first spleen census among school children and young adults up to the age of 15. Unfortunately, this inclusion of children and young adults introduced an error which makes a strict comparison with more recent results impossible. These spleen censuses were carried out annually until 1918.

Perhaps it was a paper read by Fernando (1910) stressing the importance of research followed by the introduction of organized malaria control measures which prompted Government to inaugurate an antimalaria campaign in the capital city of the North-Western Province, Kurunegala, in 1911. The subsequent report of Gunasekara (1913) is the record of the pioneer malaria control work done in Ceylon, which consisted, apart from studies on the bionomics of the anopheline mosquitoes, mainly in antilarval measures such as oiling of water collections and minor filling, clearing and drainage.

Bahr (1913) also reported on the conditions existing at the time of his visit to Kurunegala and on the control work already in progress. Other important investigations during this period were by James and Gunasekara (*loc. cit.*) at Talaimannar, during which, as already mentioned, the first infected *A. culicifacies* was found and by James (1914) in the Jaffna Peninsula and at Kalmunai in the Eastern Province. But it does not appear that any control work was undertaken in any of these places even after these investigations.

B. 1921–36.

In 1921, a malariologist was appointed to carry out island-wide studies on the anopheline mosquitoes of Ceylon, to determine the vectors of malaria in various districts and to recommend control measures. While the early years of this period were devoted mainly to research, opportunities were soon made available to carry out antimosquito measures under the immediate supervision of the malariologist. These measures consisted mainly in the introduction of larvivorous fish into different waters, particularly wells, of minor drainage works, clearing drainage channels and oiling various water collections which could not be drained or filled in. It was at Mahara Prison, twelve miles north-east of Colombo, that these malaria control measures were first put into effect in 1922. Also in this year antimosquito measures of a minor nature were begun at Kurunegala with the financial assistance of the local authorities. In 1923, well-staffed antimalaria campaign centres were established at Anuradhapura and Trincomalee, in which antilarval measures of various kinds such as drainage, filling, clearing and oiling of actual and potential breeding places were commenced after the preliminary surveys had been carried out. In addition spleen surveys were to be a regular feature at these campaign centres.

In 1925, an Antimalaria Advisory Board with the Colonial Secretary as Chairman was set up to receive reports from experts and consider the practical and financial implications of their recommendations and advise Government on the most suitable measures to be adopted. The members of the Board included several elected members of the Legislative Council and a number of heads of departments, including the Director of Medical and Sanitary Services. In 1926, it was proposed by the Board that a Departmental Committee on Malaria (of the Medical Department) should be formed with the Assistant Director of Sanitary Services as Chairman, and the Medical Entomologist and Sanitary Engineer as members, with Dr. K. J. Rustomjee as Executive Officer of the Committee. The function of the Committee was to inaugurate and administer the antimalaria campaigns in progress and advise on new proposals put up by the Executive Officer in regard to their practicability and cost. The Executive Officer was appointed Superintendent, Antimalaria Campaigns in 1927 and thereafter became a member of the Committee. Thus the necessary machinery for the planning, organizing and executing of antimalaria works in Ceylon was created.

Great importance was paid at this time to malaria control measures in towns in hyperendemic areas, which were liable to seasonal epidemics every year. Thus in addition to the centres at Anuradhapura and Trincomalee, campaign centres were created, after preliminary larval surveys, at Chilaw and Kurunegala in 1928, at Puttalam and Badulla in 1930 and Maho in 1931. In 1928, control work was also commenced at a number of railway stations in malarial areas.

The Ceylon Estates' Proprietary Association's Malaria Control Scheme was inaugurated in September 1926, with Dr. H. C. Ross (a brother of Sir Ronald Ross) as the first malariologist, seconded by the Ross Institute of Tropical Hygiene, London. He was succeeded a few months later by Colonel W. W. Clemesha, I.M.S. (retd.), to whom credit must be given for first pointing out 'the pernicious influence of rivers on malaria' in 1928, in a circular to estates (personal communication from Dr. O. J. S. Macdonald). He was also responsible for the earliest attempt at protecting a population by oiling a main river.

Oiling of rivers and streams as an emergency antilarval measure was begun in 1935 during the great epidemic which commenced late in 1934. The rivers and streams thus brought under control included sections of the Mahaveli Ganga, Deduru Oya, Maha Oya and the Kelani Ganga. Spray-killing of adult mosquitoes in dwellings with pyrethrum was first commenced in October 1934 at the Minneriya Colonization Scheme in the North-Central Province.

In 1935, the Sanitary Engineering Division commenced a series of investigations which were to last some years on the most suitable methods to be employed in reducing pooling conditions in river beds, and of training and controlling dry weather channels. Boulder and rock-bottomed sections were dealt with by draining and sealing; those rock pools which could not be effectively drained were filled and sealed with cement. The prevention of scour of sand bars and banks was dealt with by movable concrete protection blocks cast on the site from tetrahedron moulds, whilst the gradual deviation of dry weather channels was effected by spur dykes composed of hog wire fencing and rubble filling.

After the epidemic of 1934-35, it was apparent that it would not be possible to continue malaria control work only at campaign centres in hyperendemic areas. The time was therefore opportune to commence investigations in epidemic areas and with this end in view twenty-five malaria observation stations were opened in the intermediate zone and eight for contrast in non-epidemic areas in the wet zone. At these stations, all of which were closely associated with a main river or its tributary, it was proposed to carry out monthly investigations on the bionomics of larvæ and adult anopheline mosquitoes. Selected stretches of the river were to be examined for anopheline larvæ, while all types of breeding places in the selected village were also to be examined. Dwellings were to be examined for adult anopheline mosquitoes, while trapping with both human and cattle bait was to be carried out at night from dusk till about 8.30 p.m. This work which was inaugurated in June 1935 continues with modifications to this day. Only a greater area is being covered with far more staff than was at first available.

C. 1937–45.

This period, following as it did the most disastrous epidemic within living memory, was one in which every effort was made to avert by every possible means another such calamity. Accordingly, a new malaria control and health scheme was brought into operation in 1937. It provided for the carrying out of malaria control measures as part of an intensive general health scheme based on the principles of health unit work. Such work had been in progress for nearly fifteen years at various campaign centres. In the new scheme, which was meant primarily for rural areas, malaria was to be given special attention. But no less importance was to be given to maternal and child welfare, school health work, mass hookworm treatment, treatment for yaws, control of communicable diseases, general sanitary work and health education. Field Medical Officers (later designated Medical Officers of Health) were assigned areas with an average population of 40,000 and they were given a staff of sanitary inspectors, midwives and nurses. The training of the necessary staff, however, could only be done gradually, so that this was an initial disadvantage, which however did not delay the launching of the scheme. The training in malariology was facilitated by the opening of a Malaria Field Training Centre at Kurunegala in December 1939. At

this centre comprehensive courses in malariology were given to medical and sanitary personnel.

Oiling of rivers and streams, which was first instituted as a control measure in 1935 during the epidemic, was continued as and when necessary until 1939 when it was placed on a permanent basis as the chief malaria control measure in the intermediate and the wet zones. Government also took over the oiling of streams running through estates which were over 10 feet wide, thus relieving the proprietors of this heavy financial burden. In 1940, a very large number of larval dipping stations called subsidiary observation sites, situated on rivers and streams in the wet and the intermediate zones, were inaugurated. These sites, which were to be examined fortnightly or weekly in times of drought by sanitary inspectors, were to act as 'detector' stations for the presence of *A. culicifacies*. Whenever *A. culicifacies* larvæ were reported from such stations, a programme of emergency river oiling was put into operation. This involved oiling of all rivers and streams within a three-mile radius of the site. Such emergency oiling continued for several years until it was decided to put whole areas under generalized oiling in times of drought even though the vector species had not been reported from any site in some of the areas. These were areas that were liable to severe epidemics in times of drought and it was considered worth while putting whole areas under oil at such times as a precautionary measure. Thus by 1945, a year of intense drought, oiling was continued almost without interruption for the greater part of the year and 543,000 gallons of malariol were sprayed as against 81,000 in 1935.

Spray-killing of adult mosquitoes in houses, which would undoubtedly have been given much importance, was seriously interfered with by the lack of supplies in the early years of the war. The available supplies were used mainly in agricultural and colonization schemes until 1943, when ample supplies were made available by the military authorities. This was an epidemic year in the Maha Oya basin and upper catchment of the Deduru Oya and in this area disinsectization was vigorously carried out for the first time over a wide area. By 1944, the spray-control of adult mosquitoes had been further extended to thirty-eight rural malaria control centres and there was no lack of pyrethrum during the epidemic of 1945, although this method of control gave way to residual spraying of houses with D.D.T. in 1946 when the epidemic was on the wane.

Automatic flushing devices in rivers and streams were first installed by Government in 1938. Worth and Subrahmanyam (1940) described the main types of siphons that were installed and their effect in the controlling of anopheline breeding. Macdonald (1939) reported considerable success in the control of *A. culicifacies* with a cheap automatic siphon discharging 475 gallons a minute. These were erected in large numbers on estates where conditions were suitable.

In spite of all these activities, epidemics could not be averted in 1939-40 (covering a more extensive area than in 1934-35 but less severe in its effect), 1943 and 1945-46. But there is little doubt that if it were not for the control measures adopted in these years these epidemics might have assumed much larger proportions.

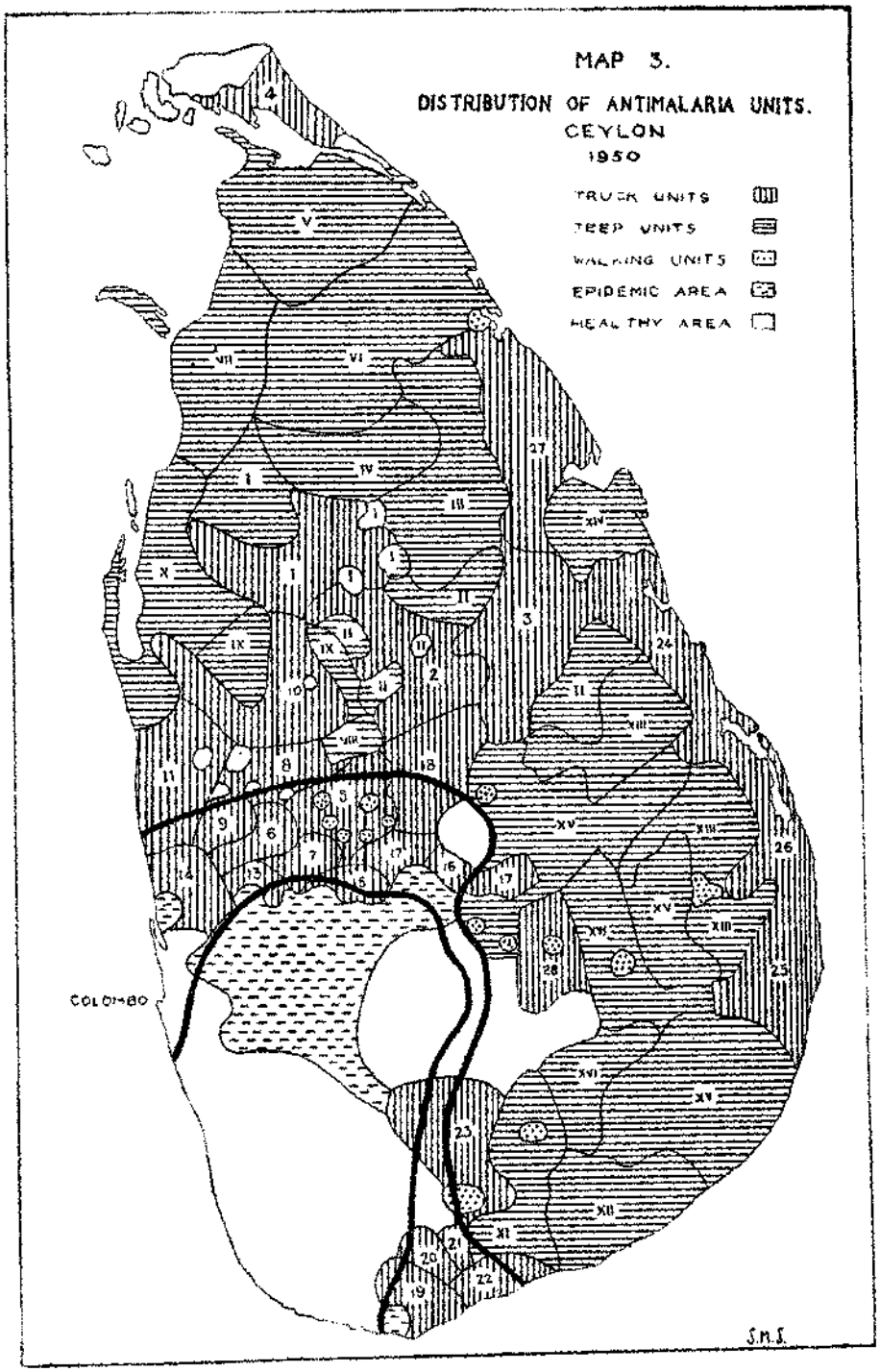
D. 1946–49.

With nearly two-thirds of the country subject to endemic malaria, and with the thickly populated normally healthy areas subject to severe regional epidemics

which break out very frequently, the control measures so far tried were, if anything, just enough to modify the severity of the outbreaks. Malaria retarded the development and progress of the country. There was need to take more effective steps against malaria, particularly in view of the epidemic which was raging in 1945. With a small quantity of D.D.T. that became available through the courtesy of the military authorities, then having their headquarters in Ceylon, a few preliminary experiments were made in three small areas. The experience gained in the use of D.D.T. solutions for residual spraying of houses in these trials showed that the malaria problem of this country could be successfully dealt with by this method. A complete scheme for controlling both endemic and epidemic malaria was submitted on September 9, 1945. The difficulty of obtaining sufficient D.D.T. spraying equipment and transport prevented the simultaneous introduction of the scheme over the whole country. Therefore, the scheme was implemented by stages as material became available. The first two units were set up in the first week of November 1945 in the Anuradhapura District. By September 1946, the entire endemic area was taken up in time to control the seasonal rise usually associated with the north-east monsoon rains towards the end of the year. The first year in which the complete scheme was in operation was 1947.

Area of operation.—This residual spraying campaign extends to all areas where the spleen rates were over 10 per cent and to a part of the healthy areas which are liable to epidemics, particularly the south bank of the Maha Oya, a portion of the catchment of the Mahaveli Ganga round Kandy and Matale, and a portion of the Matara District coming within the catchment of the lower reaches of the Nilwala Ganga. In the case of the other areas which are normally healthy and are subject to epidemic malaria, no residual spraying is undertaken as a routine. To prevent epidemic outbreaks, the scheme envisages the residual spraying of all houses situated within a distance of half a mile from the rivers and streams running through areas liable to epidemics. Whenever pre-epidemic conditions set in, the spraying is commenced either in February or in August as the case may be. As a result of the failure of the north-east monsoon in 1947, there were indications that an epidemic would break out in April 1948. This was dealt with by diverting some of the spraying units from the dry zone and by setting up a few additional units to operate in the areas liable to epidemics. After doing two rounds of spraying the units were withdrawn. No epidemic occurred that year. There are in all twenty-seven truck units, seventeen jeep units and fifteen walking units covering the entire area (Map 3).

Types of houses.—In the rural areas, the houses are of poor construction. The average hut consists of two rooms approximately 12 × 15 × 10 feet with a front and a back verandah. Besides, there may be a small kitchen about 10 × 10 feet. The walls have a wooden framework covered over with mud on the outside and inside. The roof is covered with thatch made from leaves of the coconut palm. The rooms are poorly ventilated and are dark. Very often the inmates, particularly the older males, sleep outside in the open verandahs. There are usually no cowsheds, as cattle are tethered in the open at some distance from the dwelling house. In Ceylon, one does not see that close contact between man and animals as seen in many parts of India or Egypt. The residual spraying is limited to the usual resting places of the vector, namely, the walls, roof and ceiling and eaves of houses. Hangings and furniture are not sprayed. The walls are such that some of


MAP 3.
DISTRIBUTION OF ANTIMALARIA UNITS.
CEYLON
1950
TRUCK UNITS
JEEP UNITS
WALKING UNITS
EPIDEMIC AREA
HEALTHY AREA
COLOMBO
S.M.S.

the insecticide is absorbed. Further, the mud walls, unless of good clay, break up readily and have got to be patched up from time to time. In some cases, the wall is mud-plastered frequently. A few of the better houses in the rural areas and the majority of the houses in urban areas have brick walls and tiled roofs which are about 14-15 feet high and are not attractive to *A. culicifacies*. In such cases the roofs are not sprayed.

Technique.—Until 1949, D.D.T. continued to be the only insecticide used. To begin with, a 5 per cent solution of technically pure D.D.T. in kerosene oil was used at an approximate dosage of 120 mg. per square foot. This was replaced by a 40 per cent emulsion concentrate of D.D.T. in xylene with Triton X-100, diluted in the field to make a 5 per cent emulsion. From about the end of 1948, water-wettable D.D.T. powders replaced all the D.D.T. formulations. With this change, the dosage was reduced to about 100 mg. per square foot. But, since October 1949, it was further reduced to about 50 mg. per square foot.

The spraying was done at intervals of six weeks when kerosene solutions and emulsions were used. With the introduction of the wettable powder, different units spray at different intervals depending on the endemicity of malaria in the area and the type of houses found. Some units spray at intervals of six weeks, others at seven, eight and ten weeks. The kerosene oil solution was very popular with the people. But the watery emulsions and suspensions, particularly the latter, are not popular even though these formulations are equally effective while being cheaper and more handy to transport into interior and remote villages away from the motorable road. Even with the reduced dosage of 50 mg., the efficiency has not suffered.

Benzene hexachloride has been used since 1949 in a few areas at a dosage of 10-11 mg. per square foot. The preparation used is Gammexane P. 520 which is a wettable powder. Contrary to one's expectation that the people would object to the odour given out by this preparation, they preferred and, in some cases, demanded that their houses should be sprayed with Gammexane in preference to D.D.T. wettable powder. The interval of spraying is six weeks, by the end of which period the residual effect appeared to deteriorate.

Stirrup pumps were tried at the commencement. Later they were replaced by knapsack sprayers (Four Oaks, Ross pattern) as the latter were more economical in labour and were particularly suited for use in rural areas where houses are scattered about. There is considerable saving in labour as the knapsack sprayer, unlike stirrup pumps, needed only one labourer to operate it. Power-driven sprayers are unsuitable as they cannot be conveniently transported into the interior of the villages. There was no difficulty experienced with these knapsack sprayers when a 5 per cent solution or emulsion was used. But, with the introduction of the suspension, the spraying material was made up to a D.D.T. content of 2·5 per cent in order to avoid blocking of nozzles. The nozzle fitted to these sprayers gives a conical spray. But as a flat fan spray is preferable, such a nozzle can be made suitable by using an adaptor. The rate of discharge is about 30 oz. per minute at a pressure of about 40 lb. per square inch. At the request of one of the authors (S. R.), The Four Oaks Spraying Machine Company have manufactured a standard model which incorporates an agitator. This modification

keeps the D.D.T. powder in suspension leaving hardly any residue at the bottom of the tank. The labourers were trained to spray the surface so as just to wet it without any run-off from the surface. Particular care was taken to clean the sprayer and nozzles, etc., at the end of each day's work.

Field organization.—A truck unit has a sanitary inspector specially trained in malaria work in charge. Under him there are two or three supervisors (overseers) and twelve to fifteen labourers. Each labourer is able to spray about 25–30 houses during an eight-hour working day. In hilly districts and in areas where the houses are scattered, the number of houses sprayed by each labourer will be about 20–25. On this basis, a truck unit can spray about 300–400 houses daily.

A jeep unit has either a sanitary inspector or a driver-overseer in charge. Under him there is another overseer and not more than six labourers. This unit can cover about 120–150 houses daily. Jeep units work in those areas to which the bigger trucks cannot be taken and where there are only cart tracks and no proper roads. Invariably, these villages are scattered and are in the interior of jungle areas.

There are certain small localized areas where neither a truck nor a jeep can be taken. In such places a walking unit in charge of an overseer does the spraying. The number of labourers in the unit will depend on the number of houses allotted to the unit. Each labourer can spray only about ten houses a day. These units are not so efficient, particularly as they work in inaccessible areas which are difficult for supervising officers to reach without notice. They are also comparatively more expensive per house sprayed.

The supervision of the units in the field is expected to be done by the respective Medical Officers of Health and Divisional Medical Superintendents in whose areas the unit works at any time. Frequent field inspections are made by the Superintendent, Antimalaria Campaigns and his two assistants who are ultimately responsible for the efficient functioning of these units.

Headquarters organization.—The planning, direction and inspections of the work are done from the headquarters in Colombo where there is a laboratory in which both entomological and parasitological work is done, a workshop in which transport vehicles and spraying equipment are repaired and maintained, as well as the office which deals with matters relating to the personnel, stores, accounts, etc.

The present organization consists of the following full-time staff :—

(1)	The Superintendent, Antimalaria Campaigns.	
(2)	Medical Officers, Antimalaria Campaigns ...	2
(3)	Clerks	9
(4)	Sanitary Inspectors	40
(5)	Entomological Assistants	14
(6)	Laboratory Assistants	9
(7)	Transport Foreman	1
(8)	Driver-Overseers	15
(9)	Overseers	80
(10)	Drivers	41
(11)	Labourers	650

besides mechanics, peons, laboratory attendants, field attendants, watchers, etc. In addition, one has to take into account the part-time work of the respective Medical Officers of Health.

Cost.—The total cost of the antimalaria campaigns in the three years 1947, 1948 and 1949 is given below :—

Total cost in three years 1947, 1948 and 1949	Rs. 87,75,448
Average cost per annum	Rs. 29,25,149
Number of houses sprayed per annum	4,98,880
Total population resident in houses sprayed	26,64,000
Total number of applications in three years	86,84,497
Total number of applications per annum	28,94,832
Cost of spraying per house per annum	Rs. 5·86
Cost per annum per head of population directly benefited	Rs. 1·10
Total population indirectly benefited	45,00,000

Of the total cost, about 70 per cent has been spent on the purchase of insecticides and spraying equipment.

MALARIA IN THE UPPER CATCHMENT OF THE DEDURU OYA AND MAHA OYA BASINS, 1938-49 (THE EPIDEMIC ZONE).

For the sake of convenience the upper catchment of the Deduru Oya and the Maha Oya basins will be referred to as the epidemic zone. Although these areas may be regarded as being the chief epidemic areas in Ceylon, there are other river catchments, notably those of the Kelani Ganga, Kalu Ganga and Mahaveli Ganga where epidemics have occurred regularly in the past. But it is not proposed to deal with malaria in these river catchments here.

The rivers and their catchments.—The Deduru Oya (Map 1), which is 87 miles long, rises at a height of a little more than 1,000 feet in the Kandy and Kurunegala districts. For the greater part of its length the river and its tributaries flow through flat land between 100 and 500 feet contour lines. In its higher reaches, the bed is rocky but for the rest the river is wide, with a sandy bed. Only the upper third of the river lies within the intermediate zone, the rest of the river flowing through the dry zone. The upper third of the catchment area, 325 square miles in extent, is fairly well populated with rubber grown in the hills, and coconut and rice cultivation, which are the principal agricultural activities, over the rest of its extent. The rest of the catchment, which is 650 square miles in extent, lies in the dry zone. Here the river flows through thinly populated, poorly cultivated and highly malarious country. Considerable pooling of the river takes place in dry weather ; also the flow is much reduced because water is diverted into several dry zone tanks (irrigation reservoirs) from the upper part of the river.

The catchment area of the Maha Oya is approximately 562 square miles in extent. The river, which is 78 miles long, rises in the hills east of a line joining Kurunegala and Kegalle at elevations from 500 to 1,000 feet, situated partly in the wet zone. The river too runs partly in the wet zone and partly in the intermediate zone. The

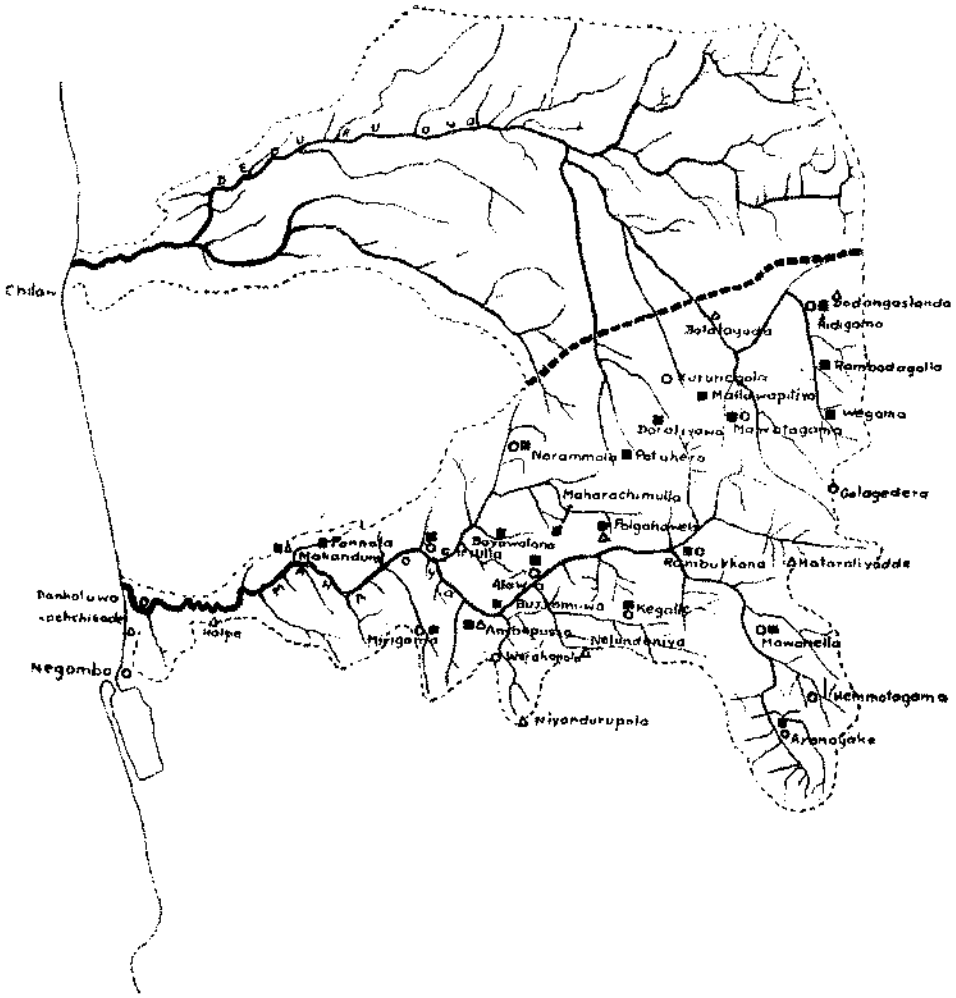
MAP 4.

EPIDEMIC ZONE

■ MALARIA OBSERVATION STATIONS

O HOSPITALS

Δ DISPENSARIES



first part of the river is rocky and runs through an area which is relatively healthy or at most moderately endemic. When it descends into the plains the river runs along a wide, flat, sandy channel with the bed becoming rocky at several points. The land thickly covered with coconut and rice is extensively cultivated, but malaria is endemic up to a short distance of the coast. In dry weather, considerable pooling of the bed occurs with the flow of water severely curtailed. The last few miles of the river always contain water.

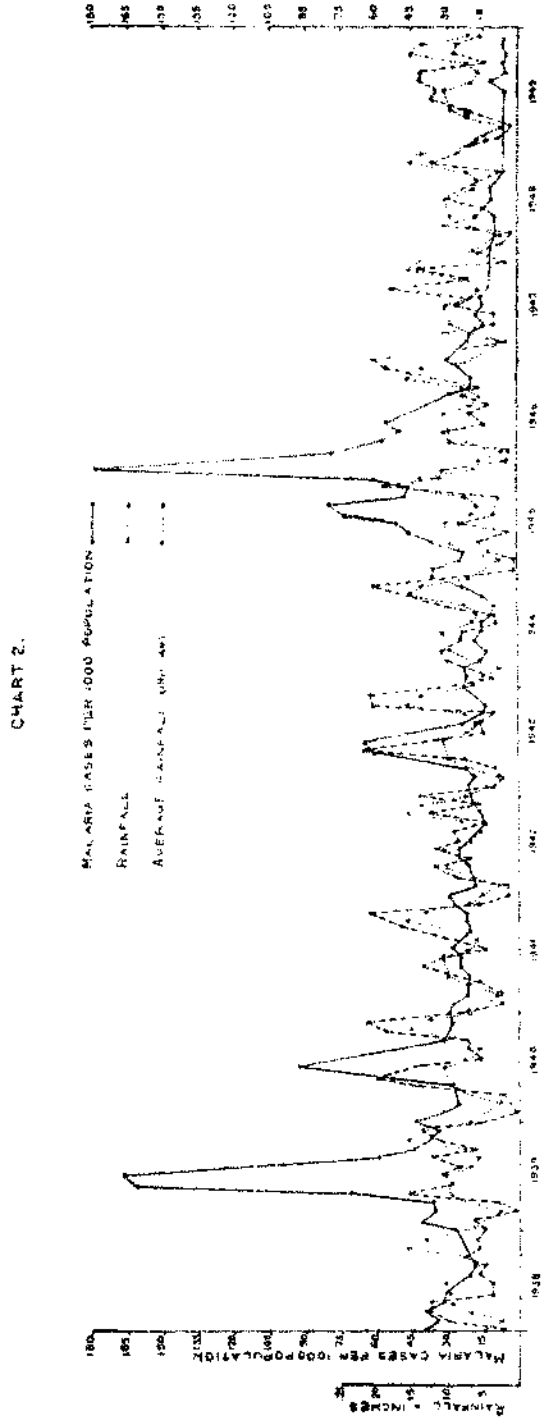
The two areas thus combined as the epidemic zone contain fourteen health areas, each under a Medical Officer of Health, who is provided with a staff of sanitary inspectors, apothecaries, midwives, health nurses and other personnel necessary for the discharge of his duties. Map 4 shows the distribution of hospitals, dispensaries and malaria observation stations in the epidemic zone. The latter are manned by personnel called Entomological Assistants, who have been trained in entomology. Their duties are mainly confined to the collection of data in the field.

Annual rainfall.—The monthly average rainfall at Delwita (490 feet above sea-level, Table VIII), may be taken as typical of the rainfall received in the upper catchment of the Deduru Oya. Here more rainfall is received from October to January (41·88 inches) during the north-east monsoon than during the south-west monsoon from May to September (28·88 inches). Both monsoons often tend to fail. The resultant pooling of the river bed gives rise to favourable conditions for the breeding of *A. culicifacies* and severe outbreaks of malaria ensue. There are two normal periods of relatively low rainfall; the first beginning when the north-east monsoon commences to weaken about the middle of January and lasts till the intermonsoon rains late in March or April and the second commencing in August lasts till the intermonsoon rains of October set in.

The Maha Oya receives more rain in the catchment than the Deduru Oya and the rainfall recorded at Kegalle (Table I) indicates that the rainfall received during the south-west monsoon (42·88 inches) is approximately the same as that received during the north-east monsoon (41·75 inches) in a normal year. Unfortunately, although the river is often in flood during the height of each monsoon, it tends to subside very rapidly at other times, so rapidly in fact that pooling may occur within a short time of the river being in spate. Both monsoons are often erratic in behaviour and tend to fail. The periods of low rainfall are approximately the same as for the Deduru Oya.

There are five meteorological stations in the upper catchment of the Deduru Oya and fourteen in the Maha Oya catchment. In Table IX is summarized the average monthly rainfall for the years 1938–49 for both catchments combined, while Chart 2 shows the monthly rainfall and the average for the years 1911–46. Over the combined catchment forming the epidemic zone, the average rainfall received during both monsoons is approximately the same, being 39·55 inches during the south-west and 39·79 inches over the north-east monsoon.

Periodicity of malaria.—The catchment areas of the Maha Oya and the upper Deduru Oya are moderately endemic with two fever seasons each year. The first of these is relatively mild in intensity and commences generally in April immediately after the intermonsoon rains late in March which bring the dry period of



Monthly morbidity and rainfall in the epidemic zone, 1938–49.

late January and February to a close. The fever lasts till about June and begins to decline in July. It therefore lasts during the period of the south-west monsoon. The second fever season also follows the relatively dry period of August and September, which is broken by the heavy intermonsoonal rains of October. It is generally more marked than the first fever season and commencing in November it lasts till about January. Both fever seasons follow dry periods when the pooling in the river beds is at its greatest and most favourable to *A. culicifacies*.

Apart from these seasonal rises of malaria, the combined catchments are subjected to epidemics of malaria of varying intensity every three, four, five or six years. Such epidemics have for certain been known to occur since 1877 and their distribution, duration and intensity up to the epidemic of 1934-35 are discussed by Briercliffe (*loc. cit.*). Every one of these epidemics has followed as a consequence of the failure of one or both monsoons. The failure of the south-west monsoon is generally followed by an epidemic commencing at the end of the year, while a failure of the north-east monsoon generally results in an epidemic commencing in April of the following year. The greatest of these was that of 1934-35 (an autumnal epidemic), which was extensive in its distribution (far beyond the borders of the epidemic zone under discussion), explosive in its onset and devastating in its intensity. Nearly 80,000 lives were lost during the course of the epidemic. Briercliffe (*loc. cit.*), Gill (*loc. cit.*), Briercliffe and Dalrymple-Champneys (1935) and Dunn (1936), among others, have discussed this epidemic very thoroughly.

Malarial morbidity in relation to rainfall, 1938-49.—Table X summarizes the monthly dispensary and hospital attendances of patients in the epidemic zone for the years 1938–49 clinically diagnosed as malaria, while in Chart 2 the monthly morbidity rate per 1,000 of population is plotted along with the monthly rainfall and the average monthly rainfall calculated for the period 1911–46. There are seventeen hospitals and nine dispensaries in the zone. The latter are manned by apothecaries and their diagnoses clinically made are subject to error. It is a common practice for them to diagnose almost any type of fever as 'malaria' in these endemic areas. The malaria attendances therefore tend to be too high at non-epidemic times, but during epidemics the error due to incorrect diagnosis is at a minimum.

In this zone, just as the seasonal rises of malaria follow after a period when rainfall is normally deficient, epidemics are a consequence of a failure of the monsoons. The considerable pooling of river beds that takes place when a monsoon is deficient affords abundant facilities for *A. culicifacies* to breed in very large numbers. And just as the seasonal rise of malaria follows the first inter-monsoonal rains, the fever index according to Rustomjee (*loc. cit.*) begins to mount three weeks after the first heavy rains which follow a long dry period. The peak is reached in about seven weeks.

The failure of a monsoon is not of infrequent occurrence in the epidemic zone. Such failures may be offsets from the average rainfall for the whole period of the monsoon when they may be referred to as complete failures or for one or more months when they may be called partial failures. Thus in 1938 after heavy rain well above the average in February, March and April (Table IX), both

monsoons were almost complete failures; only in September 1938 and January 1939 was rainfall above the average recorded. These are the final months of each monsoon and slight excesses of rainfall above the average are insufficient to flush the rivers out and prevent breeding of *A. culicifacies* for any length of time. In April 1939 following very heavy intermonsoonal rains, a severe epidemic broke out and lasted till July, and not until August did the morbidity begin to decline. In 1939, after the early cessation of the north-east monsoon late in November, a long period of drought followed, which was not broken until April when the intermonsoonal rains were again very heavy. A second wave of malaria followed in May, attained its peak in June and began to decline in July.

No further epidemic occurred until April 1943. The monsoons in 1940, 1941 and the south-west monsoon of 1942 were up to average. The north-east monsoon of 1942 was erratic in behaviour. The October rainfall was rather below normal, while in November it was considerably below average. To make up for this, the December rainfall was more than twice the average. A dry period followed in January, February and March to be broken as usual by the intermonsoon rains of April, which this year were slightly below average. The epidemic which followed was by previous standards only a minor one and as the south-west monsoon was above average, the breeding of *A. culicifacies* in rivers was constantly being interrupted and the epidemic died down by August.

The north-east monsoon of 1944 was above average but following it came a long dry spell which was first broken by intermonsoonal rains in April 1945 which this year were well below normal. But the epidemic which followed in April was long drawn out. It is curious that the peak of the first wave of the epidemic was not reached until August. The south-west monsoon this year was a complete failure and the breeding of *A. culicifacies* was almost uninterrupted and small bursts of rain were sufficient to keep the humidity at such a level that uninterrupted transmission went on. With the onset of the north-east monsoon rains, a second and much larger wave of the epidemic commenced in November and attained its peak in December. In February the morbidity figures began to decline and by September had become normal.

No further epidemics have been experienced up to the end of 1949. But the weather conditions in 1947, 1948 and 1949 have been very adverse. In all three years the north-east monsoons have been partial or total failures. In 1947, the south-west monsoon was a partial failure, although unprecedented rain was experienced on two consecutive days in August causing devastating floods. In 1948, the May rainfall was about half normal but otherwise the monsoon was up to average. In spite of this the year 1948 was a very dry one.

After the epidemic of 1945-46, the morbidity figures have steadily declined, although weather conditions have on the whole been most advantageous for the prolific breeding of *A. culicifacies*. In 1947–49, even the seasonal rises of malaria have not been experienced, while the morbidity rate of 78 recorded in 1949 is the lowest ever known in the epidemic zone.

Spleen and parasite rates.—A spleen survey of school children in a selected number of schools in the epidemic zone was done in March 1938, 1939 and 1940 and in September 1940. No survey was done thereafter owing to the war until

September 1946 and has since been carried out bi-annually in March and September during the three years 1947, 1948 and 1949. A summary of these findings is given in Table XI.

In March 1938, three years after the big epidemic of 1934-35, the spleen rate was 27·7 per cent, this went down to 19·8 per cent in March 1939. Sivalingam and Rustomjee (*loc. cit.*) considered this to be the lowest level to which spleen rates in this zone could come down to.

The epidemic of 1939-40 raised the spleen rate to 23·1 per cent in March 1940 and 26·6 per cent in September 1940.

In 1945 and early part of 1946, there was another epidemic. But, in the meantime, residual spraying of D.D.T. had been carried out in certain localized sections of this zone, but not throughout. This, perhaps, explains the comparatively low rate of 18·3 per cent in September 1946. Since then, there has been a progressive and steady decline in the spleen rate. From 13·5 per cent in March 1947 it has come down to 2·4 per cent in September 1949.

The parasite rates among school children examined each year in March 1938-41, 1948 and 1949 and in September 1946-49 are given in Table XII. In the earlier March surveys, in 1938 and 1941, the rates were 3·6 per cent and 3·8 per cent respectively. The rate rose to 4·6 per cent and 5·3 per cent in 1939 and 1940 indicating the influence of the epidemic in these years. One would have expected a higher parasite rate during and immediately after the epidemic. But it may be that many of the children were still acutely ill or convalescing from malaria and had kept away from school while the survey was done. The higher rate of 9·1 per cent in September 1946 gives a better picture of the epidemic conditions which existed in 1945 and 1946. In fact, this figure might have been higher still if not for the partial residual spraying that had already been undertaken in some sections of this zone.

By 1947, the residual spraying of houses had become thoroughly organized in the epidemic zone and the parasite rate had gone down steadily from 1·2 per cent in September 1947 to 0·04 per cent in September 1949. This reduction in the parasite rate coincides with a similar reduction in the spleen rate for the same period, indicating the practical absence of malaria transmission in the years 1947, 1948 and 1949.

Prevalence of Anopheles culicifacies.—The entomological data presented in this section record data gathered at Malaria Observation Stations situated at key places in the epidemic zone. They have varied in number from 11 to 18 during the period under review. The number of stations has usually varied between 11 and 13; extra stations were examined in connection with certain special investigations in 1947, 1948 and 1949 and while this data are included here, they will be dealt with in more detail in the next section.

All malaria observation stations are closely associated with a main river or one of its tributaries and observations are made once a month at each station. These observations include examination of dwellings in the village for adult anopheline mosquitoes for three hours each morning for four consecutive days (reduced to three days from 1943 and increased to five days in 1949); setting up cattle-baited traps

at a selected site in the village for two and a half hours from dusk for three consecutive nights; examination of a selected one-mile stretch of the river for two days; and finally examination of breeding places in the village for two days. All material collected, including live adults, were sent to the Central Laboratories in Colombo for identification and report.

Larval prevalence has been estimated as a larval rate which is defined as the number of larvæ caught per 100 dips of a standard ladle. In Tables XIII, XIV, XV and XVI are summarized the prevalence of *A. culicifacies* and all other anopheline larvæ caught in sand and rock pools in rivers and streams, along the margins (banks) of rivers and streams and in all types of village breeding places. The data summarized here are for all malaria observation stations in the epidemic zone for the years 1939–49. It is evident that *A. culicifacies* is of negligible importance in various types of village breeding places and in the epidemic zone no control of these situations has ever been attempted. The larvæ most usually met with in breeding places in villages are *A. varuna*, *A. hyrcanus* and *A. vagus* (Table XVI).

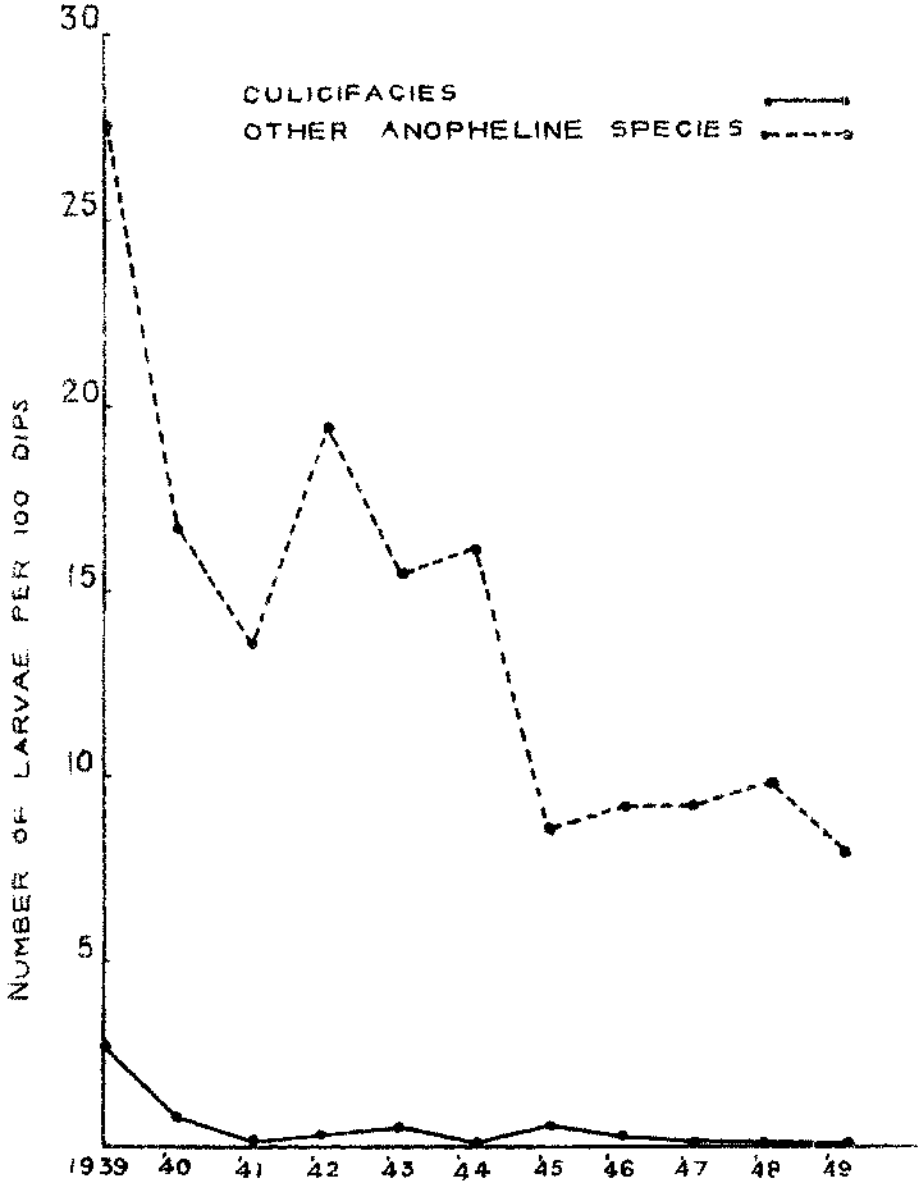
Along the banks or margins of rivers, *A. culicifacies* is slightly more plentiful (Table XV, Chart 3) but even here after the 1939-40 epidemic the larval rate has never exceeded 0·56. *A. varuna* breeds prolifically along the banks of rivers (Chart 4).

It is in sand and rock pools in the beds of rivers and streams that *A. culicifacies* is most commonly found, and the variation in prevalence of this species in comparison with all other anopheline species during the years 1939–49 is shown in Table XIII and Chart 5. *A. varuna* and *A. vagus* are once again found breeding in large numbers in sand and rock pools (Table XIV, Chart 6). In fact, *A. varuna* is the dominant anopheline larva of the epidemic zone breeding in vast numbers in every type of situation in rivers as well as in village breeding places.

The fluctuations in the larval rates of *A. culicifacies* in sand and rock pools for the years 1939–49 (Table XIII) show peaks in 1939, 1943 and 1945 coinciding with the malaria epidemics which broke out in these years. Thus in 1939, 1943 and 1945 larval rates of 19·84, 11·14 and 3·55 were recorded, while in the non-epidemic year of 1944 the larval rate was as low as 0·08. It has to be borne in mind that from 1939 to the early part of 1946, river oiling as a control measure was steadily increased in intensity in the epidemic zone until in 1945 it was in almost continuous operation, except for two weeks in June, from January till September. Oiling gangs under the supervision of sanitary inspectors were well aware of the existence of the malaria observation stations and special attention would naturally have been paid to the selected mile of river that was examined monthly by the entomological staff. Thus these larval rates would possibly tend to be rather low at times.

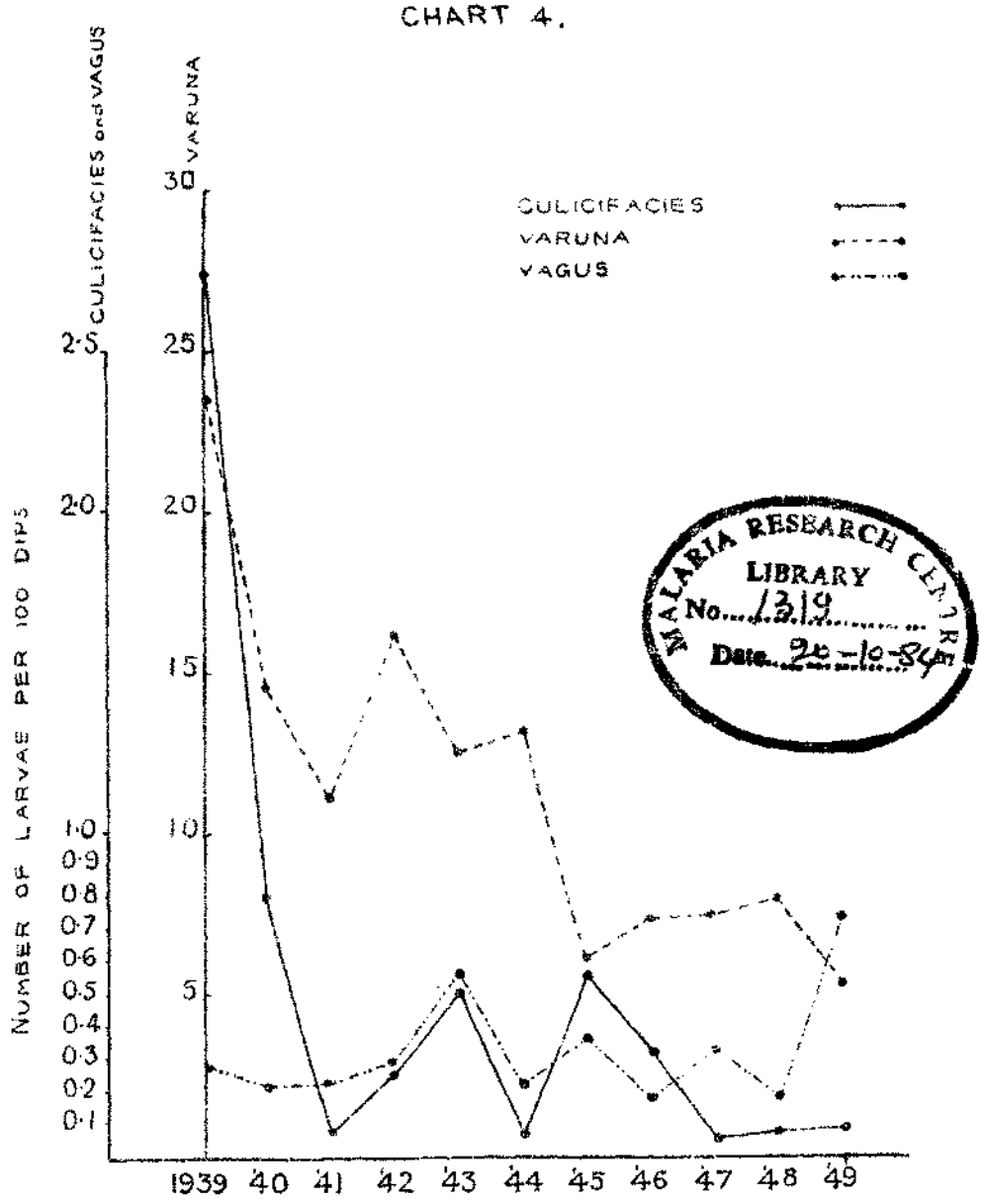
River oiling as a control measure was gradually abandoned in 1946 when the residual spraying of houses with D.D.T. had commenced. By the end of 1946 river oiling had been completely abandoned, as by that time the spraying of D.D.T. had become well organized in the epidemic zone. Since the residual spraying of houses was put into operation as the only control measure, the larval rate in sand and rock pools was reduced to 0·28 in 1947 and 1948 and 0·45 in 1949. Table XIV

CHART 3.



Larval rates for *A. culicifacies* and the total of all other anopheline species found in the margins of rivers and streams in the epidemic zone during 1939–49.

CHART 4.



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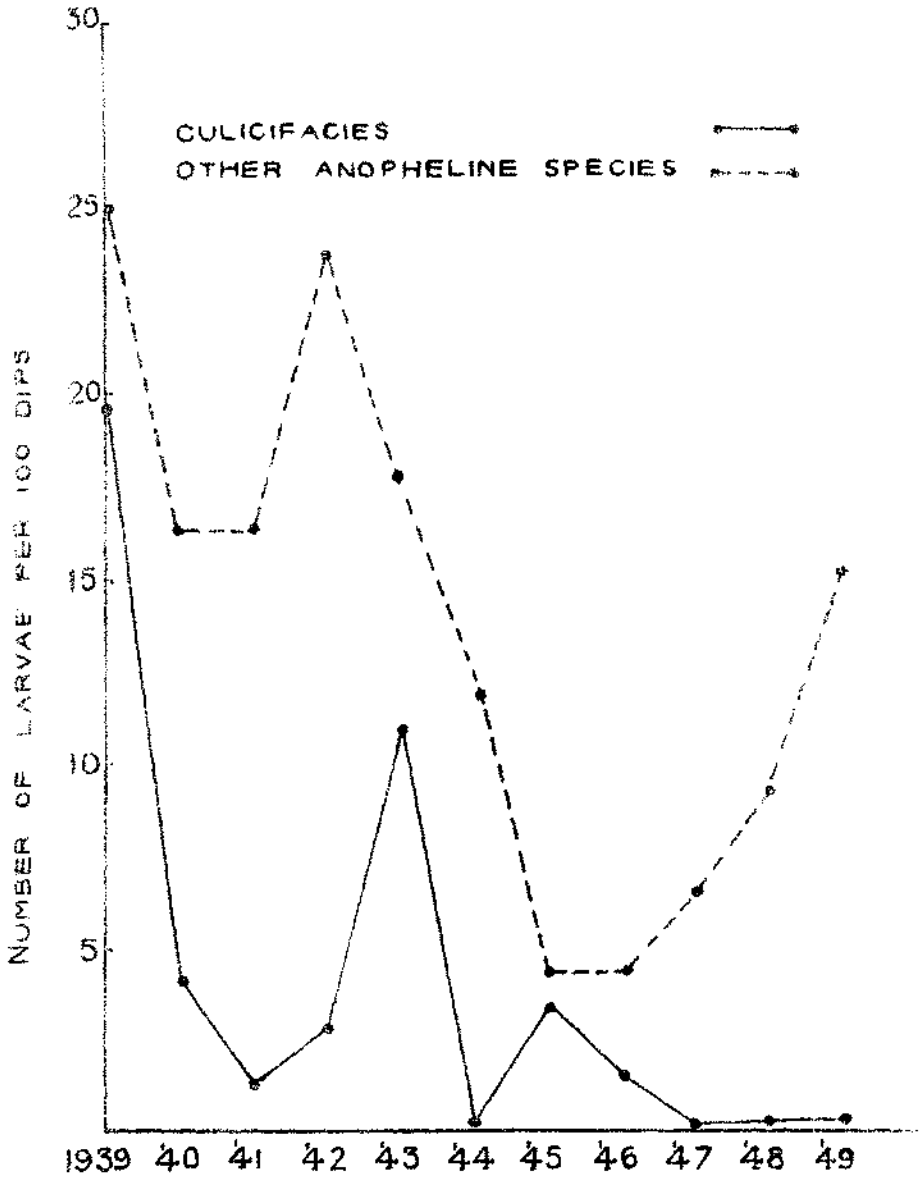
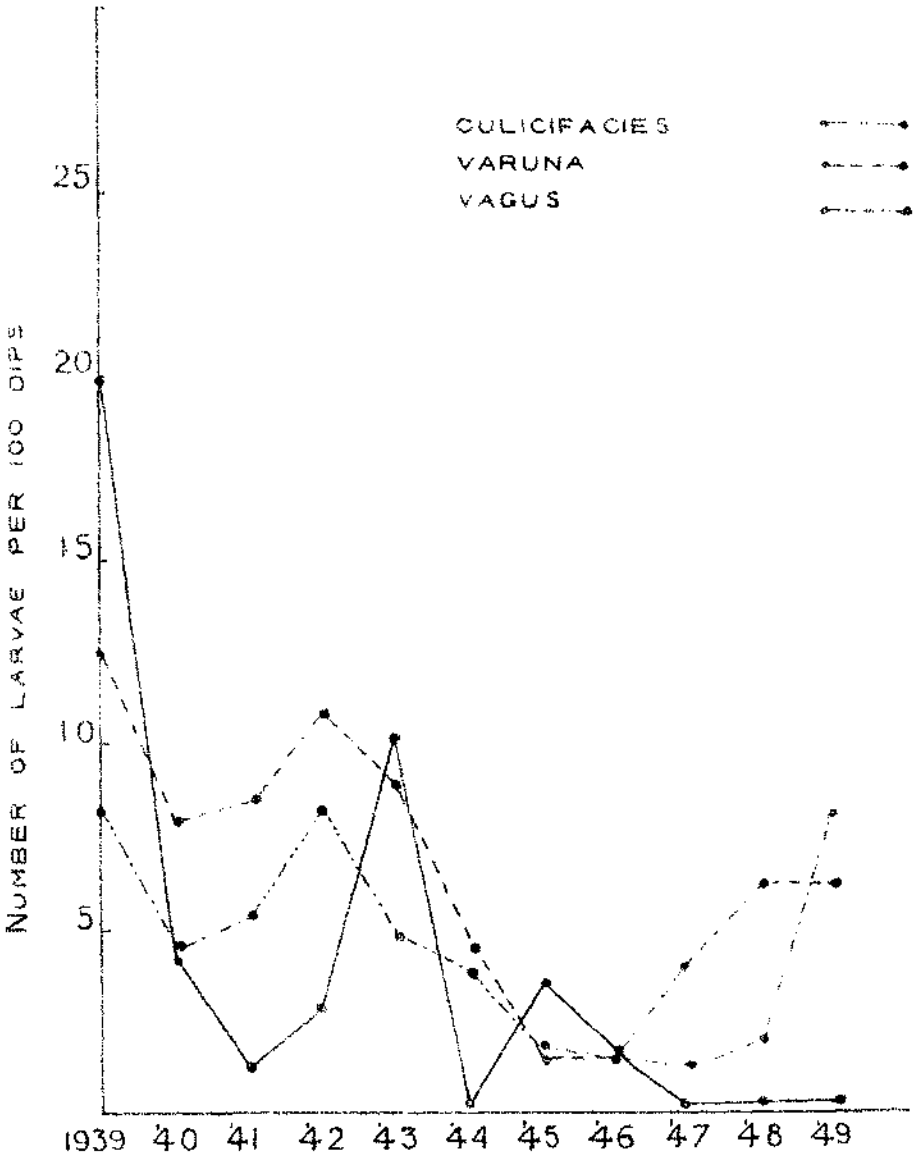
Variation in the larval rates for certain species of anopheline larvæ found in the margins of rivers and streams in the epidemic zone during 1939–49.

CHART 5.



Larval rates for *A. culicifacies* and the total of all other anopheline larvæ found in sand and rock pools in rivers and streams in the epidemic zone during 1939–49.

CHART 6.



Variation in the larval rates for certain species of anopheline larvæ caught in sand and rock pools in rivers and streams in the epidemic zone during 1939–49.

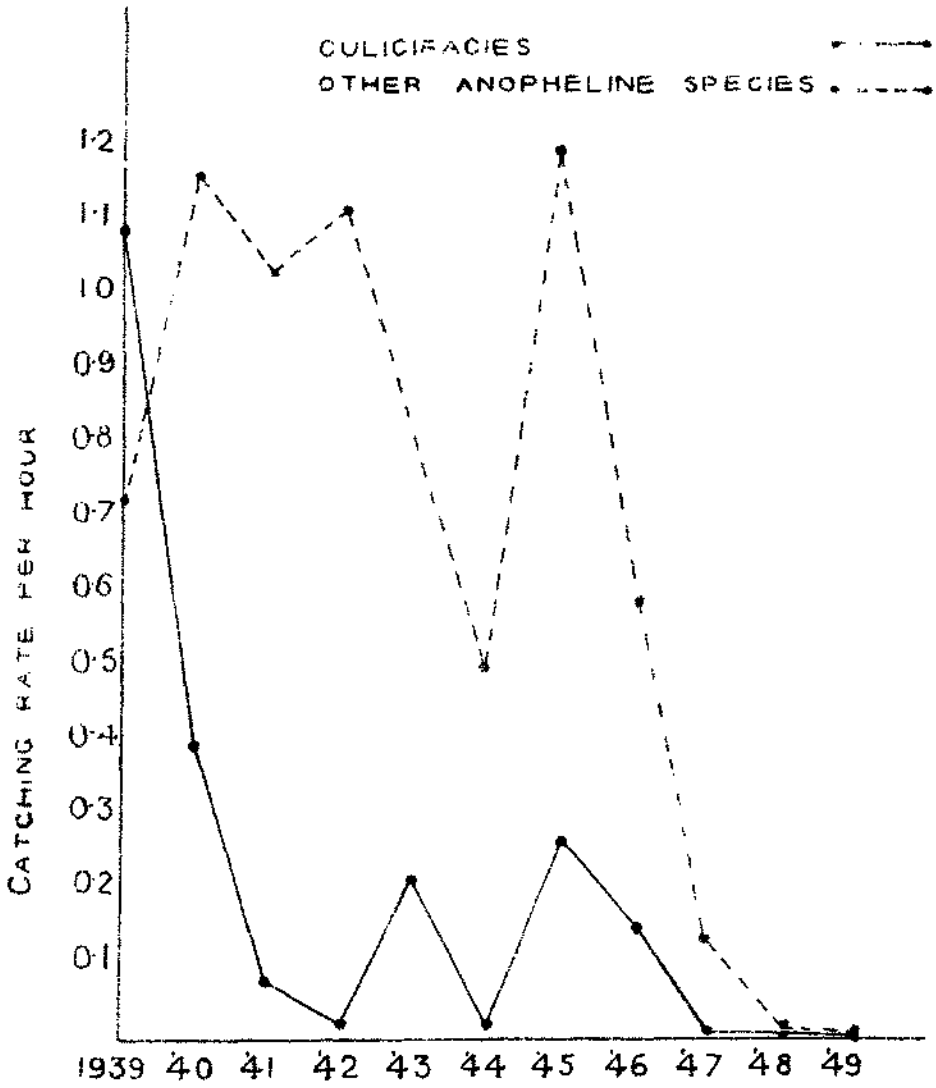
shows that while the prevalence of *A. culicifacies* has come down since 1947, the larval rates of *A. varuna* and *A. vagus* have gone steadily up.

Adult prevalence and natural infections.—The results of the hand-catching of adult *A. culicifacies* in houses during the years 1938-49 is summarized in Tables XVII and XVIII, together with the results of mosquito trapping in cattle-baited traps. *A. culicifacies* is scarcely ever caught in cattle-traps, the anopheline mosquitoes most frequently caught being *A. hyrcanus* and *A. vagus* (Table XIX). In houses it has already been pointed out that, as the adults of *A. culicifacies* have the habit of hiding in cracks and crevices, the numbers actually caught by hand are only a fraction of the numbers probably present (Charts 7 and 8). Hence the catching rates tend to be low, but in spite of this, during the epidemic years, they are higher than in non-epidemic years. The catching rates per hour were 1·07 and 0·41 during 1939 and 1940 respectively, 0·22 in 1943 and 0·27 and 0·16 in 1945 and 1946 respectively. The total infections (Table XX) too tend to show peaks during epidemic years. Infection rates of 4·97 in 1939, 2·16 in 1940, 3·77 in 1943, 3·57 in 1944 (a single infection in a non-epidemic year), and 2·56 in 1945 have been recorded. From 1947 onwards, there has been a great reduction in the numbers of adults caught in houses. This reduction will be discussed later.

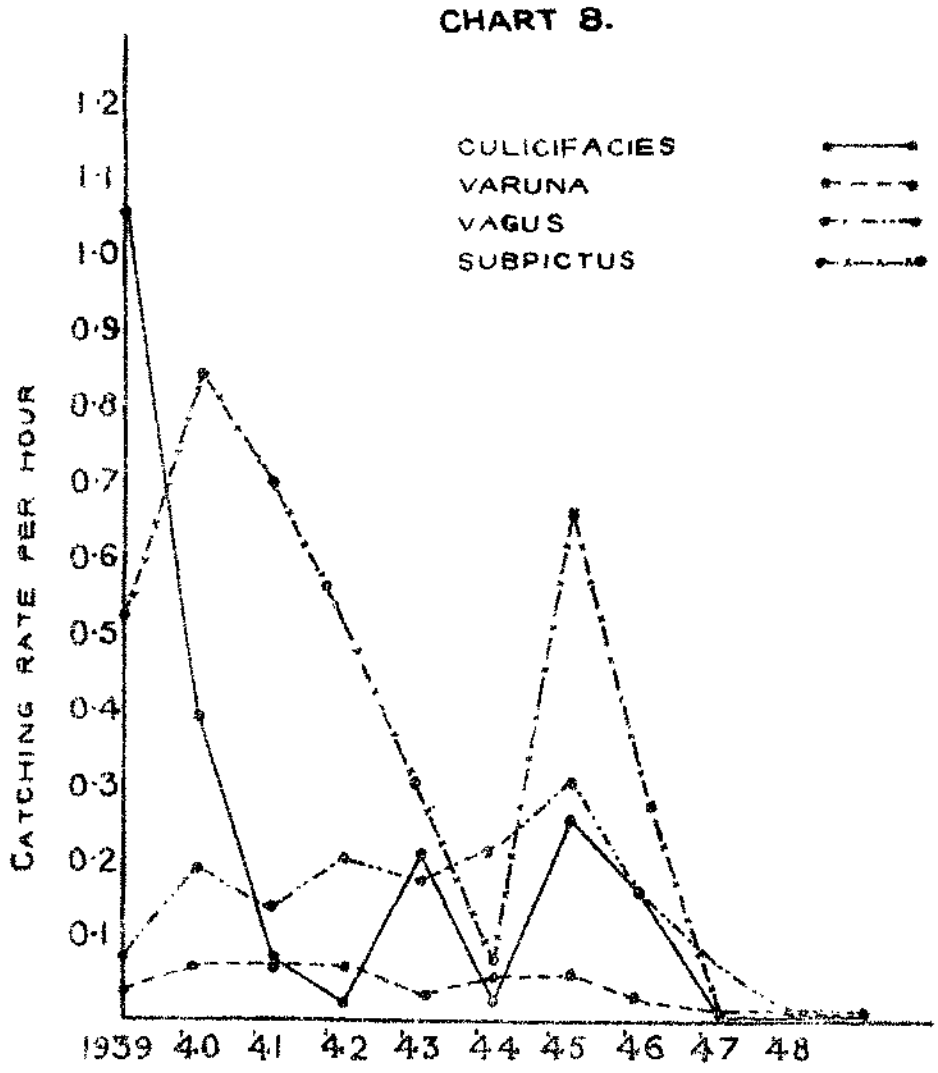
Observations on the prevalence of Anopheles culicifacies *in the Polgahawela Health Area, 1947-49.*—The Polgahawela Health Area, which is 93 square miles in extent and has a population of 66,876, forms part of the Dambadeni Hatpattu (a Divisional Revenue Officer's area—Map 2, 'P') situated in the Maha Oya basin of the epidemic zone. From about the second quarter of 1946, when the malaria epidemic which broke out in this zone in 1945 was on the wane, the houses in this area had been sprayed with D.D.T. At first a 5 per cent solution in kerosene was used and the interval between successive spraying was six weeks. From the end of 1947, a 5 per cent D.D.T. emulsion was also sprayed once in six weeks. (A 40 per cent D.D.T. emulsion concentrate was made up according to the following formula: 188 lb. Technical D.D.T.; 30 gallons xylene; 2·4 gallons emulsifier—Craft or Triton X-100.) Finally from the end of 1948 until October 1949, a 5 per cent D.D.T. suspension (50 per cent water-wettable D.D.T. powder until October 1949 and thereafter a 70 per cent powder) was sprayed also every six weeks. The dosage used at the commencement was 120 mg. per square foot with the 5 per cent D.D.T. solution in kerosene. The same dosage was used with the 5 per cent D.D.T. emulsion. When the 5 per cent D.D.T. suspension was sprayed, the dosage was reduced to 100 mg. per square foot and this was further reduced to 50 mg. per square foot from October 1949 when the concentration of the D.D.T. suspension was reduced to 2½ per cent. The type of equipment used and other details of the organization have already been described.

The widespread use of D.D.T. as the only control measure in all areas liable to hyperendemic as well as epidemic malaria made it imperative for careful entomological checking up of the new control measures. In the hyperendemic dry zone areas, Anuradhapura, where D.D.T. spraying was started in November 1945, was chosen as the most suitable place for entomological observations and the results of this checking up will follow in a subsequent communication. In the intermediate zone the Polgahawela Health Area was chosen as being suitable as this was an area liable to severe epidemics. Further, there was a considerable body of entomological

CHART 7.

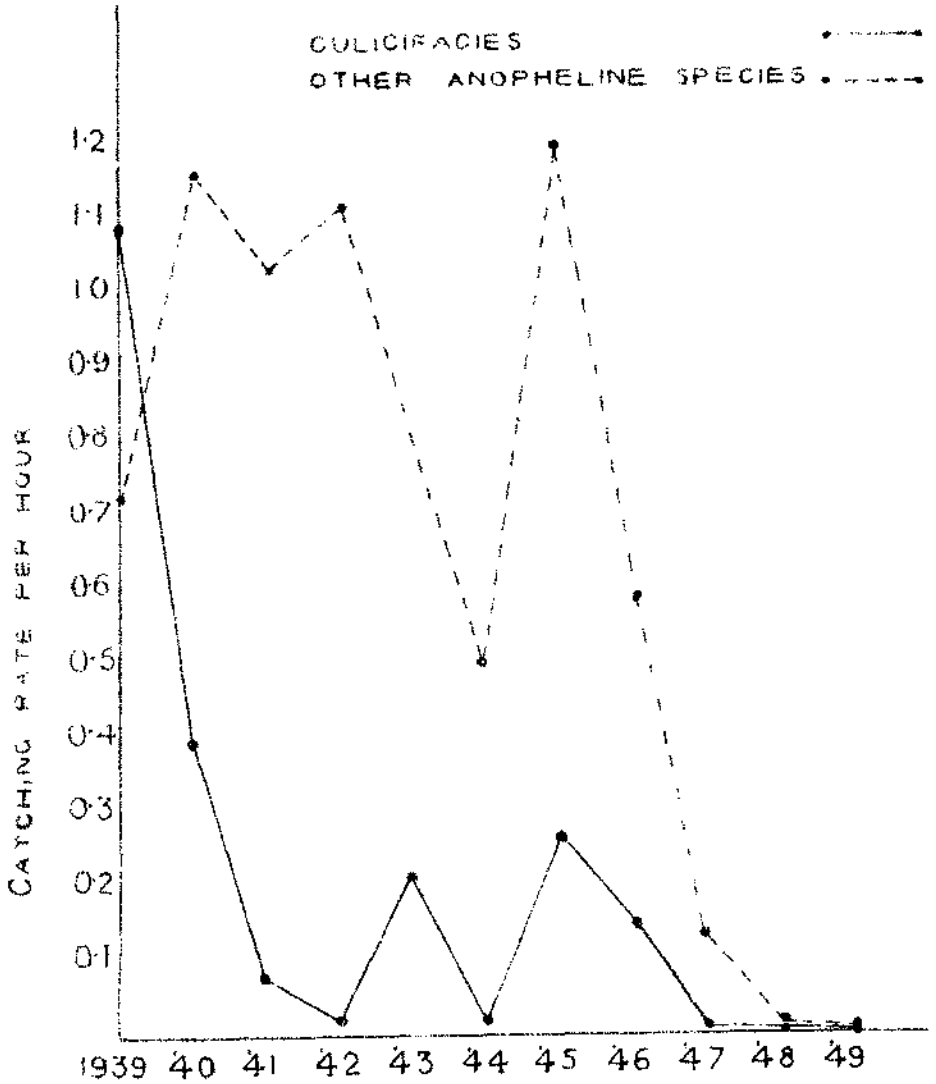


Catching rates for female *A. culicifacies* and the total of all other anopheline species caught in dwellings in the epidemic zone during 1939–49.



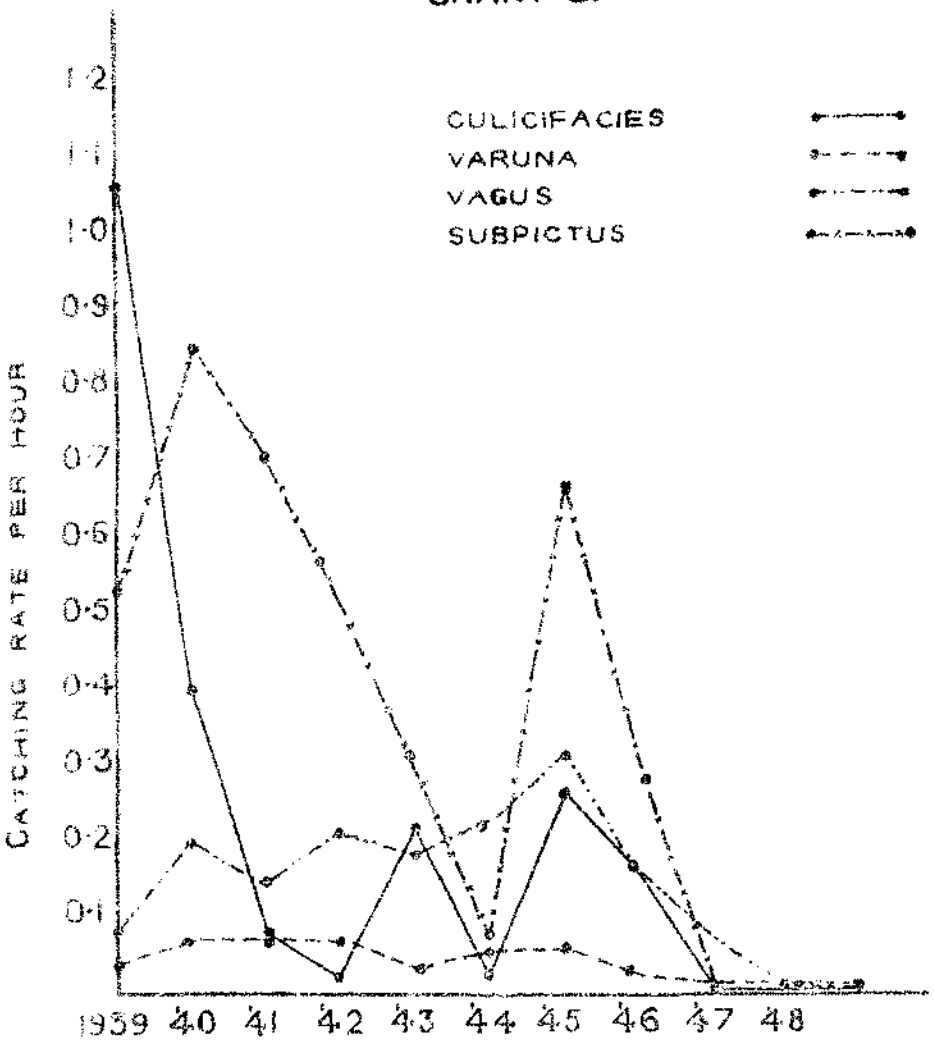
Variation in catching rates for certain species of female anopheline mosquitoes caught in dwellings in the epidemic zone during 1939–49.

CHART 7.



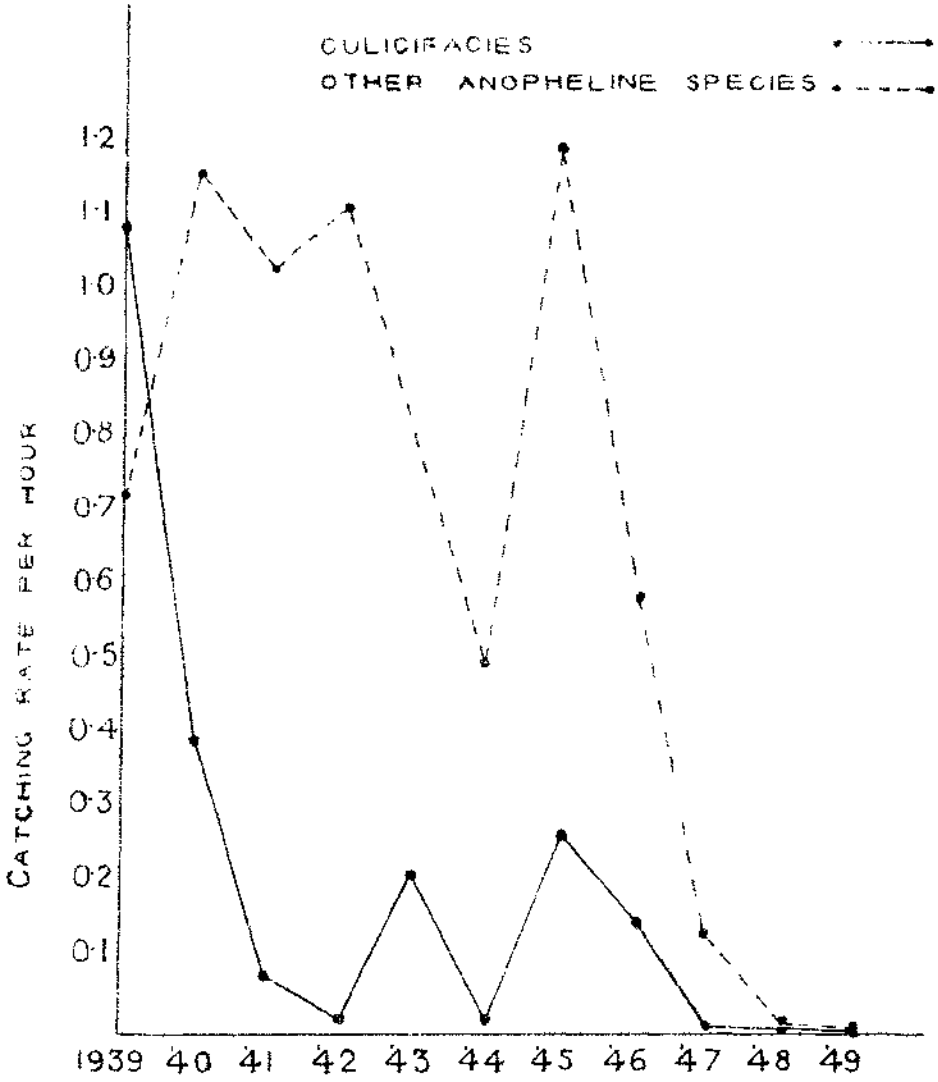
Catching rates for female *A. culicifacies* and the total of all other anopheline species caught in dwellings in the epidemic zone during 1939–49.

CHART 8.

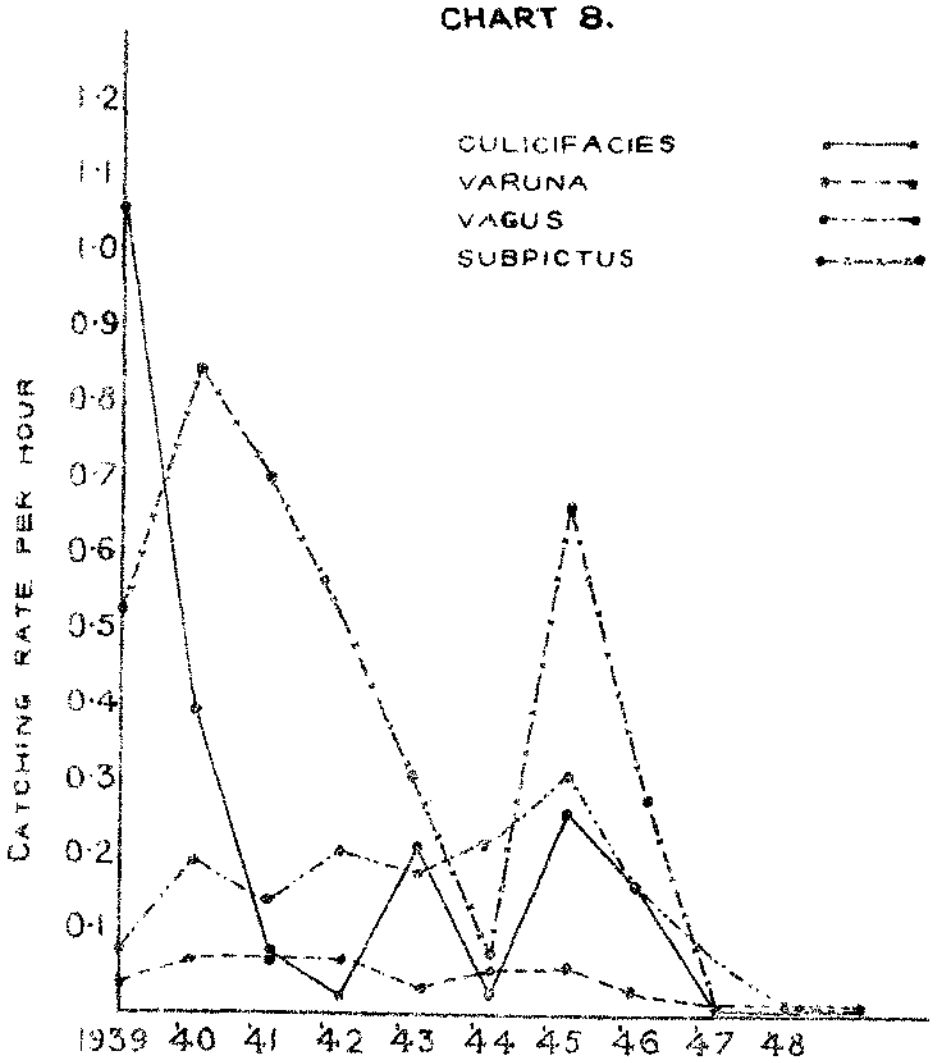


Variation in catching rates for certain species of female anopheline mosquitoes caught in dwellings in the epidemic zone during 1939–49.

CHART 7.



Catching rates for female *A. culicifacies* and the total of all other anopheline species caught in dwellings in the epidemic zone during 1939–49.



Variation in catching rates for certain species of female anopheline mosquitoes caught in dwellings in the epidemic zone during 1939–40.

data for both Anuradhapura and areas similar to Polgahawela situated on the Maha Oya which makes it possible to compare the present results with past conditions.

Accordingly, the Polgahawela Health Area was divided into two sections and an Entomological Assistant (with two Field Attendants each) was stationed at Polgahawela and Alawwa respectively. The monthly programme of the assistant stationed at Polgahawela, a small town, consisted of observations at Polgahawela itself for 3 days and in the following villages: Ganegoda (1 day), Oruliyadda (3 days), Potuhera (4 days), Pannala (4 days), four village groups in two lots of four villages each examined in alternate months (8 days). The assistant stationed at Alawwa, also a small town, examined this station for 4 days each month and the following villages: Werellagama (2 days), Maharachimulla (4 days), Boyawalana (4 days), Bujjomuwa (4 days), four village groups examined in two lots of four villages each in alternate months (8 days).

The actual work involved the examination of houses in the morning for anopheline mosquitoes; spray-catching of anopheline mosquitoes in houses; night-trapping of adult mosquitoes in houses; night-trapping of adult mosquitoes in cattle-baited traps; and the examination of rivers and streams for anopheline larvæ. The time spent each month by each assistant in the examination of houses was 19 hours; 45 hours for spray-catching; 18 hours for night-trapping and 64 hours for larval examination. These investigations commenced in January 1947, although the D.D.T. spraying had commenced about nine months earlier.

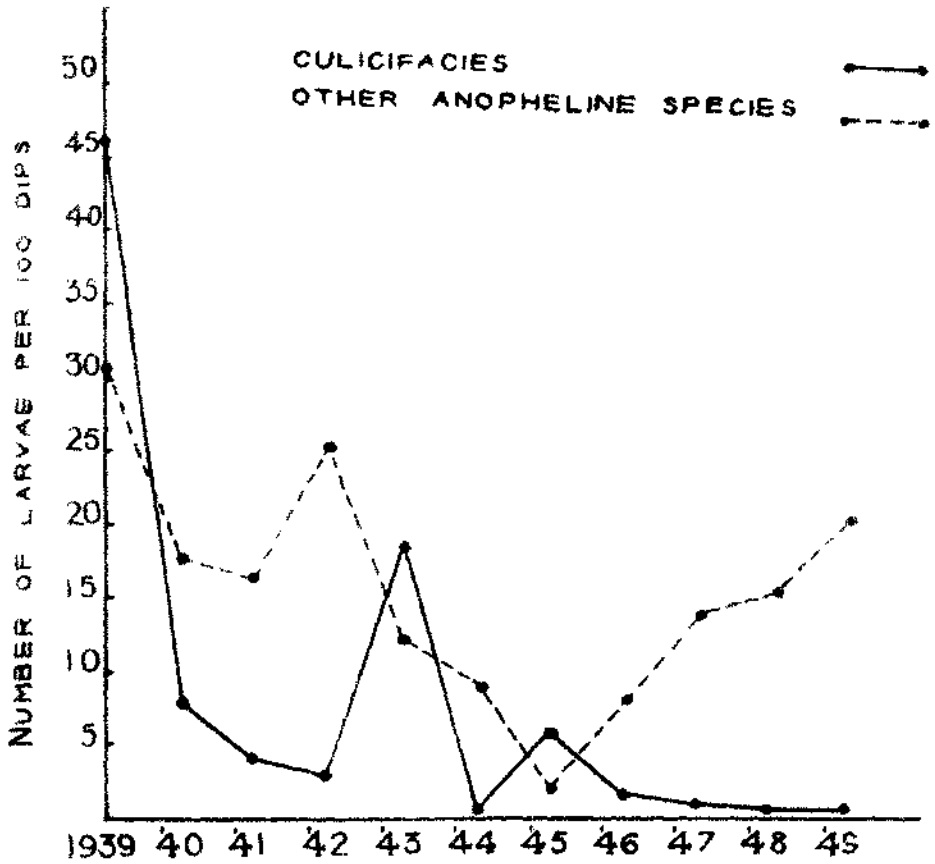
Five untreated villages outside the Polgahawela Health Area but contiguous to it were also kept under observation by other Entomological Assistants. They were Nakalagamuwa, Kudagammana, Kondapahwa, Pantanduwa and Pittawela. Some of the villages which were kept under observation are shown in Map 4. All these villages are within 15 miles of Polgahawela or the Malaria Observation Station at Alawwa.

The entomological data gathered during the years 1947-49 may be compared with the data collected over the period 1939-46 at the Malaria Observation Stations at Alawwa, Giriulla and Rambukkana. Alawwa is situated within the Polgahawela Health Area and Giriulla and Rambukkana are only just outside it. A reliable guide to the fluctuating conditions that existed prior to the residual spraying with D.D.T. from the second quarter of 1946 is therefore available.

Larval prevalence.—The larvæ of *A. culicifacies* breed prolifically at times in and around the Polgahawela Health Area. In Tables XXI and XXII is summarized the variation in the prevalence of the larvæ in sand and rock pools and in the margins of the Maha Oya and streams surrounding it from 1939–49. The variation in prevalence of *A. culicifacies* and other anopheline larvæ breeding in sand and rock pools is also shown in Charts 9 and 10. The highest larval rates of 45·82 in 1939, 18·16 in 1943 and 5·75 in 1945 in sand and rock pools are associated with the epidemics that occurred in these years. Similarly, even along the margins of the rivers, where *A. culicifacies* is less common, the rates were 6·78, 1·05 and 0·75 for these epidemic years (Chart 11). But from 1947 the larval rates in sand and rock pools have come down from 0·92 in 1947 to 0·55 in 1949. In these years the only control measure adopted was the residual spraying of houses with D.D.T.,

while prior to 1947 river oiling had been in force. Reference to Table XXI and Charts 9 and 10 shows the same interesting feature noticed when discussing larval prevalence in the epidemic zone. From 1947, while the prevalence of *A. culicifacies* larvæ in sand and rock pools has come down, the total larval rate for all other

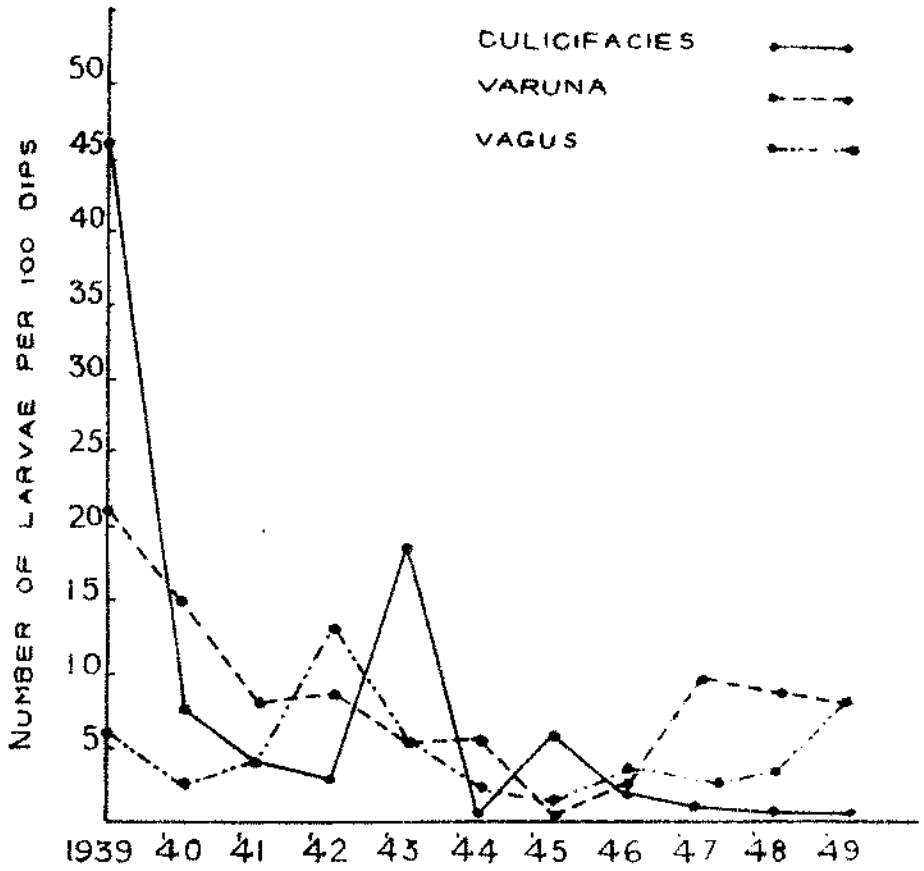
CHART 9.



Larval rates for *A. culicifacies* and the total of all other anopheline species found in sand and rock pools in rivers and streams at Alawwa, Giriulla and Rambukkana during 1939–46 and in the Polgahawela Health Area in 1947, 1948 and 1949.

anopheline larvæ has gone steadily up. The commonest larvæ found in the rivers and streams of the Polgahawela Health Area as in the epidemic zone are those of *A. varuna* with *A. vagus* next in order of importance.

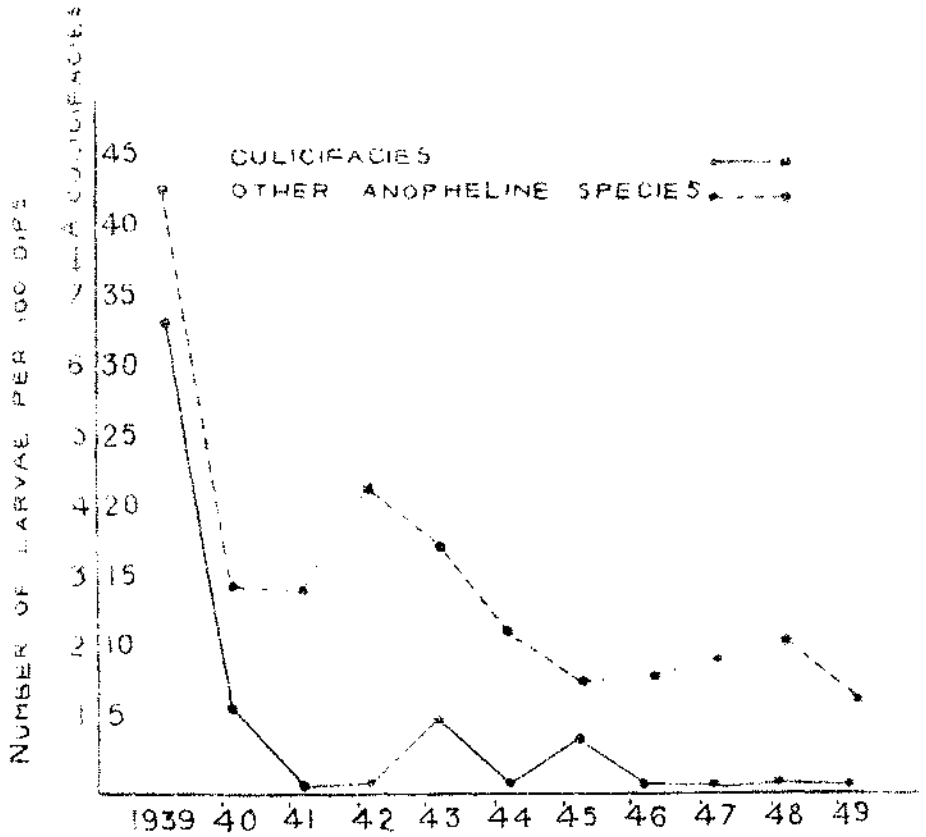
CHART 10.



Variation in larval rates for certain species of anopheline larvæ caught in sand and rock pools in rivers and streams at Alawwa, Giriulla and Rambukkana during 1939–46 and in the Polgahawela Health Area in 1947, 1948 and 1949.

Adult prevalence for the years 1939-46 has followed the same pattern seen when discussing the results for the epidemic zone (Table XXIII). While the catches of *A. culicifacies* adults as recorded here are probably no indication of the actual numbers that were present for reasons discussed before, the catching rate per hour for the epidemic years of 1939, 1943 and 1945 were 1·20, 0·79 and

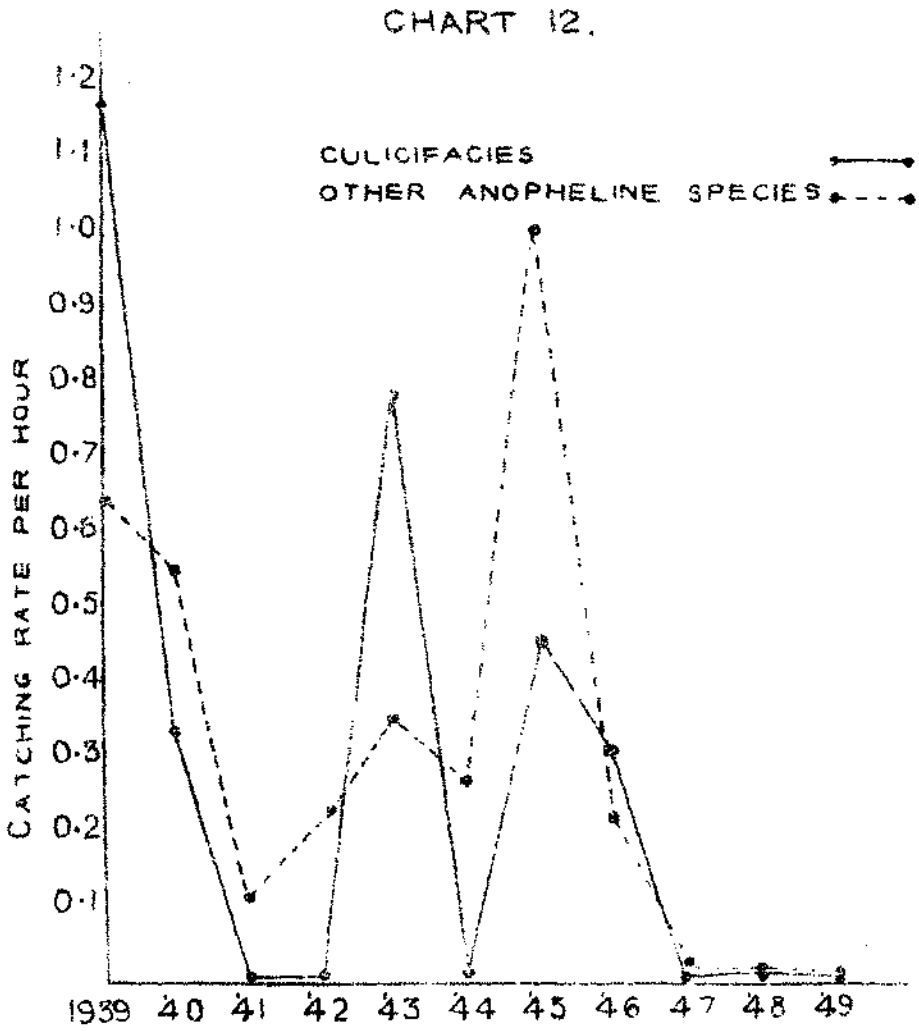
CHART 11.



Larval rates for *A. culicifacies* and the total of all other anopheline species found breeding in the margins of rivers and streams at Alawwa, Giriulla and Rambukkana during 1939-46 and in the Polgahawela Health Area in 1947, 1948 and 1949.

0·48 respectively (Chart 12). From 1947, the catching rate for *A. culicifacies* and all other anopheline mosquitoes has been reduced to zero or very negligible proportions. Even hand and spray catches carried out in the same villages (Table XXIV) failed to reveal any *A. culicifacies* and the catching rates for all other anopheline mosquitoes in 1947 and 1948 were only 0·08 and 0·06 respectively. Even in the

five comparison villages (Table XXV) the catching rate for *A. culicifacies* was 0 in 1947 and 0·002 in 1948, while the catching rate for all other anopheline mosquitoes was 0·06 in both years. The negligible catches of *A. culicifacies* in the untreated



Annual variation in the catching rate for *A. culicifacies* and the total of all other anopheline mosquitoes caught in dwellings at Alawwa, Giriulla and Rambukkana during 1939-46 and in the Polgahawela Health Area in 1947, 1948 and 1949.

villages were possibly due to the fact that all around them were the D.D.T. sprayed areas and this no doubt exerted its effect in reducing their mosquito populations.

Further, in this part of Ceylon it is difficult to say exactly where one village ends and where the next begins as there is considerable overlap.

As the low catches made in houses might have been due to the repellent effect of D.D.T. which Thomson (1947*a*, 1947*b*) had found in West Africa, it was decided to carry out hand catches in certain villages at night. These observations were begun in August 1948 and lasted till the end of 1949. The examination of houses at night is naturally resented by householders but with the willing co-operation of certain of them 501 houses were examined in 1948 and 910 in 1949 (Table XXIV). Not a single *A. culicifacies* adult was found coming into houses in the early part of the night, although conditions almost throughout the observation period in 1948 and in the first and fourth quarters of 1949 were favourable for the breeding of *A. culicifacies*. At the same time as these catches made at night were proving negative for *A. culicifacies* in the Polgahawela Health Area, catches also made at night at Ponnarankulama, a hamlet outside Anuradhapura, which had been left untreated with D.D.T. from the inception of spraying in November 1945, proved that this mosquito came into houses in large numbers from dusk till about 9.30 p.m. Observations were not made beyond this time.

Thus it is difficult to draw the conclusion that it was the repellent effect of the D.D.T. alone which resulted in scarcely any mosquitoes being caught in houses at night in the Polgahawela Health Area. It might be stated that although in this area *A. varuna* and *A. vagus* are the two most common anopheline larvæ, Table XXVI shows that these mosquitoes are not often taken in houses. *A. subpictus*, which next to *A. culicifacies* is the mosquito most usually taken in houses in Ceylon, does not breed to any great extent in the epidemic zone. The larva is most commonly present in village breeding places in the epidemic zone (Table XVI), but it is significant that since 1947 its prevalence even in these situations has decreased considerably. In this it parallels the behaviour of *A. culicifacies* larvæ in sand and rock pools in the river (the prevalence of *A. subpictus* in such breeding places is negligible—Table XIV), while both these domestic species were not found entering houses in the early part of the night.

DISCUSSION.

There is no question that malaria has been the chief public health problem in Ceylon for very many years. The infant-mortality rate, possibly the most sensitive index of adverse conditions, has invariably reached very steep heights in epidemic years. And so has the crude death rate (*vide* section on vital statistics).

The close correlation between epidemics of malaria in the epidemic zone and drought caused by a failure of the monsoon has been discussed earlier in some detail. There has never been an epidemic in this zone since the earliest recorded in 1877 in which this correlation did not hold good. Thus during the years 1938–39 there were epidemics in 1939-40, 1943 and 1945-46 which were preceded by conditions of drought. The absence of epidemics therefore after 1946 needs explanation as since 1947 at least one monsoon a year has been a partial or total failure. Thus in 1947 both south-west and north-east monsoons were failures.

In 1948, although the May rainfall was well below normal, the rest of the south-west monsoon was up to average. But the north-east monsoon was again a failure and the year was on the whole a very dry one. In 1949, the south-west monsoon was well above the average, but once again, for the third consecutive year, the north-east monsoon was a failure. In 1950, the south-west monsoon has been one of the poorest for many years. Thus of seven consecutive monsoons from 1947, only two, the south-west monsoons of 1948 and 1949, could be reckoned as being up to average. Such a long period of conditions favourable for the breeding of *A. culicifacies* has not been experienced for certain since the epidemic of 1934-35 and possibly even earlier. In 1948 and again in 1950 climatic conditions were absolutely set for spring epidemics of the sort that have been experienced for years in this zone. Up to 1946, in spite of very intensive malaria control measures no epidemic had ever been averted. At best one could say that their intensity might have been minimized. Even then although river oiling had been carried on almost continuously throughout 1945, it was impossible to prevent the epidemic which came in two waves in that year.

The only new factor to be reckoned with was the programme of residual spraying of houses with D.D.T. which was begun about the second quarter of 1946 when the epidemic was on the wane. By the end of the year this spraying had superseded all other forms of malaria control and was now being used as the sole malaria control measure throughout Ceylon. There appears to be little doubt that in Ceylon *A. culicifacies* has succumbed to the lethal effects of D.D.T. far more easily than other vectors, notably *A. gambiæ*, in West Africa. In Ceylon, there appears to be some evidence that D.D.T. repellency is not the reason why catches of *A. culicifacies* in houses have been so phenomenally low. Catches made at night in the epidemic zone have proved negative for adults of this mosquito while similar catches made in the dry zone in an untreated village showed that *A. culicifacies* came into houses in the early part of the evening in large numbers (results to be published later). Thus, if there were adults about, they would have been caught during the course of house examinations at night. It seems unlikely that the habits of the mosquito would be different in the epidemic zone.

The evidence is more convincing if one considers the larval rates. *A. culicifacies* larvæ are found most commonly in sand and rock pools in rivers and streams in the epidemic zone. In 1945, a year of intense river oiling, the larval rate for *A. culicifacies* could not be reduced below 3·55. Even with such a comparatively low figure in a favourable year for the vector it was not possible to avert an epidemic. From 1947 onwards when larvicidal control was superseded by the residual spraying of houses against adult mosquitoes the larval rates were brought down to 0·28 in 1947 and 1948 and 0·45 in 1949. It is obvious that the density of *A. culicifacies* with such low larval rates was insufficient for active transmission to take place. With our experience of river oiling it is difficult to believe that such low larval rates could ever have been achieved by oiling alone.

A significant fact is that while the larval rate for *A. culicifacies* in sand and rock pools has been coming down since 1947, the larval rate for the total of all other anopheline larvæ has been going steadily up. Now only *A. culicifacies* and *A. subpictus* can be reckoned to be domestic mosquitoes in the epidemic zone and of them *A. subpictus* is not found breeding to any extent in rivers. But it is

slightly more plentiful in various types of village breeding places. The commonest mosquito larvæ in rivers are *A. varuna* and *A. vagus*. Neither of these mosquitoes is domestic in habit. While *A. vagus* is taken in houses in small numbers it is more readily taken in cattle-traps, while the feeding habits of *A. varuna* are obscure. It is therefore not without significance that while the larval rates of the domestic mosquitoes have gone down since the residual spraying of houses was begun, the larval rates of the non-domestic mosquitoes have gone up.

Even if the correct interpretation of the entomological data is controversial, there are other criteria for judging the success or otherwise of malaria control measures. Thus no criterion could be more important than the health of the people so protected. And nothing has been quite so dramatic as the comparative absence of malaria since 1947 in this once unhealthy epidemic zone. A morbidity rate of 844 in 1939, 369 in 1943 and 706 in 1945 has been reduced to one of 78 per thousand of population in 1949. Since 1947, even the seasonal fever rises have not been experienced and as malaria cases are clinically diagnosed by personnel who are prone to regard almost any case of fever as 'malaria', it would not be incorrect to say that even the rate of 78 in 1949 is probably too high.

Even the spleen and parasite rates in this once malarious area have come down to a level which merits the epidemic zone being now called a healthy area. Thus while the pre-epidemic spleen rate in March 1938 was 27·7, in March 1949 it was only 4·7 and 2·4 in September. The parasite rate which in March 1938 was 3·6 has come down to 0·1 in March 1949 and 0·04 in September 1949.

One cannot help but conclude that the residual spraying of D.D.T. as a control measure against the vector has proved very successful in Ceylon up to now, and in the epidemic zone malaria transmission has virtually been brought to a standstill.

SUMMARY.

1. Two contrasted sets of conditions are responsible for malaria in Ceylon; rain in the hyperendemic dry zone and drought in the epidemic areas of the wet and intermediate zones.

2. In epidemic areas there are two fever seasons annually. In addition epidemics occur every three to six years.

3. The distribution and prevalence of malaria based on climatic zones, endemicity rates and altitude is described.

4. In the period under review epidemics occurred in 1939-40, 1943 and 1945-46.

5. Of the nine possible vectors only *Anopheles culicifacies* has been found infected in Ceylon.

6. The larvæ of *A. culicifacies* breed most heavily in sand and rock pools in the beds of rivers and streams in the epidemic zone. They breed to a much less extent along the margins (banks), while their presence in village breeding places is negligible.

7. Since the residual spraying of houses was begun in the epidemic zone, the larval rate for *A. culicifacies* in sand and rock pools has gone down to 0·28 in 1947

and 1948 and 0·45 in 1949. In the epidemic years of 1939, 1943 and 1945, the larval rates were 19·84, 11·14 and 3·55 respectively, but in the non-epidemic year of 1944 it had been as low as 0·08.

8. While the larval rate for *A. culicifacies* has gone down since 1947, the larval rate for the total of all other anopheline species has gone up to 6·50, 9·45 and 15·84 in 1947, 1948 and 1949 respectively.

9. The catching rate for the domestic mosquitoes in dwellings in the epidemic zone has gone down to negligible proportions in 1947–49 in keeping with the reduction in the larval rate.

10. The larval rates for the non-domestic species, *A. varuna* and *A. vagus*, have gone up in the period 1947–49.

11. The almost negligible catching rate for *A. culicifacies* in houses in the epidemic zone is not considered to be due to the repellent effect of D.D.T., but to a genuine fall in the density of the vector as shown by the absence in houses at night.

12. For the first time in the history of malaria control in Ceylon, an epidemic was averted in 1948 when climatic conditions were absolutely favourable for one.

13. This was due to the successful results achieved in reducing the density of the vector by the residual spraying of houses with D.D.T., first at a dosage of 120 mg., later at 100 mg., and finally at 50 mg. per square foot.

14. As a result of the reduction in the density of the vector a phenomenal reduction in the morbidity rate, spleen and parasite rates in the epidemic zone has been brought about in the years 1947–49.

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TABLE I.

Average monthly rainfall in inches in selected stations in the three climatic zones for the period, 1911–40.

Zone.	Station.	Height above mean sea-level, feet.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	TOTAL.
Dry ..	Anuradhapura ..	300	5·81	1·71	4·12	6·45	3·57	0·73	1·37	1·65	3·80	9·68	10·69	7·34	57·12
	Trincomalee ..	30	8·27	2·68	2·32	2·16	3·23	0·91	1·68	3·50	3·47	9·58	13·95	12·98	64·82
	Batticaloa ..	20	12·88	4·24	3·49	2·29	1·75	0·93	1·04	1·96	2·35	7·24	13·82	17·04	69·03
	Puttalam ..	10	3·41	1·41	3·10	4·89	3·75	1·43	0·94	0·69	1·55	7·42	10·09	5·59	44·27
	Chilaw ..	10	3·11	1·44	4·22	6·11	7·10	3·24	1·77	1·47	3·00	9·50	10·22	4·40	55·58
	Hambantota ..	60	4·00	1·48	3·40	3·83	4·31	2·11	2·05	1·48	2·74	4·79	7·53	5·61	43·33
Intermediate	Negombo ..	10	3·00	1·96	4·91	8·14	10·79	6·84	3·95	2·95	5·28	11·95	12·78	4·98	77·53
	Polgahawela * ..	240	5·20	1·45	6·87	10·05	9·87	10·18	6·55	5·56	6·63	16·42	14·87	6·54	100·59
	Kurunegala ..	380	5·10	2·00	6·31	9·90	7·51	6·75	3·92	3·58	5·56	14·89	12·42	6·70	84·70
	Matale ..	1,210	8·20	2·59	4·45	6·82	6·39	6·29	5·05	4·15	5·21	11·96	11·38	8·60	81·18
	Kandy ..	1,610	6·30	2·28	5·84	6·48	7·58	8·82	7·31	5·71	6·48	10·50	10·97	8·39	86·66
	Tangalla ..	70	3·05	1·60	3·08	4·38	7·44	4·01	3·89	3·15	4·55	5·94	7·34	5·08	55·51
Wet ..	Avissawela ..	100	7·05	5·53	10·15	14·55	21·55	17·61	11·49	9·48	13·40	20·31	19·28	9·49	159·89
	Ratnapura ..	130	6·21	5·34	10·39	11·53	20·95	18·56	12·80	11·34	14·51	17·98	14·77	9·14	153·52
	Kalutara ..	10	4·83	3·53	6·51	12·45	17·68	9·27	7·29	5·11	9·23	14·07	15·43	7·11	112·51
	Galle ..	70	3·84	3·29	5·28	8·75	12·69	8·43	6·39	6·11	8·90	12·03	11·97	7·31	94·39
	Kegalle ..	650	4·66	2·70	7·98	9·62	10·25	11·28	7·47	5·90	7·98	16·62	12·99	7·48	105·02
	Hatton ..	4,140	4·47	2·65	6·56	9·07	14·75	21·41	21·39	15·50	13·46	13·30	10·99	5·96	140·01

* Average for 19 years.

TABLE II.

Spleen and parasite rates of school children by districts.

Districts.	Climatic zone.	Endemicity (1939).	Spleen rates.					Parasite rates.		
			1939	1946	1947	1948	1949	1939	1948	1949
Kalutara ...	W. ...	Healthy	1·6	1·7	0·3	0·2	0·01	1·1	0·0	0·0
Galle ...	W. ...	,,	4·4	1·4	0·5	0·4	0·3	1·2	0·0	0·0
Colombo ... / Negombo ...	I. ...	,,	4·8	13·0	2·3	1·2	0·7	1·4	0·04	0·0
Matara ..	W. and I. ...	,,	6·8	15·1	9·2	2·6	0·5	5·5	0·0	0·0
Kegalle ...	W. and I. ...	,,	8·1	59·4	4·0	2·9	1·1	3·2	0·2	0·1
Nuwara Eliya	W., I. and D.	,,	8·4	22·1	9·1	2·2	2·7	1·1	0·4	0·2
Jaffna ...	D. ...	,,	8·5	19·4	2·3	1·1	1·2	2·2	0·1	0·0
Kandy ...	W., I. and D.	,,	8·9	33·1	2·2	1·4	1·1	2·3	0·1	0·2
Ratnapura ...	W., I. and D.	Moderate	11·5	26·7	8·7	2·9	1·0	6·7	0·1	0·2
Chilaw ...	I. and D. ...	,,	17·5	43·8	6·3	4·1	2·4	5·2	0·6	0·1
Badulla ...	D. ...	,,	20·2	35·9	9·6	7·8	4·5	3·2	0·9	0·6
Matale ...	I. and D. ...	High	33·0	55·2	15·0	8·9	2·6	6·3	0·5	0·4
Kurunegala ...	I. and D. ...	,,	34·8	75·1	34·8	30·8	7·8	8·3	1·6	0·6
Mannar ...	I. and D. ...	,,	43·5	51·3	33·7	12·0	4·3	6·1	1·5	0·3
Puttalam ..	D. ...	,,	46·0	77·7	31·7	17·5	4·5	7·0	1·7	0·0
Batticaloa ...	D. ...	,,	46·2	43·2	16·7	9·4	3·8	8·4	2·2	1·0
Anuradhapura	D. ...	Hyperendemic	57·5	77·6	36·9	18·3	7·1	11·4	1·8	0·8
Trincomalee	D. ...	,,	57·7	53·4	21·1	15·2	11·6	6·7	1·2	0·0
Hambantota	I. and D. ...	,,	58·6	63·6	47·4	30·0	9·7	12·8	0·7	0·2
Vavuniya ...	D. ...	,,	66·7	84·1	52·2	23·3	9·1	10·3	1·2	0·0
AVERAGE ...	...	...	18·2	30·6	10·3	5·8	2·7	5·1	0·6	0·2

W. = wet zone. I. = intermediate zone. D. = dry zone.

TABLE III.

Density of population.

Districts.	Climatic zone.	Endemicity (1939).	Extent in sq. miles.	Population in 1949 (estimated).	Density.
Kalutara ...	W. ...	Healthy	625·8	503,000	806
Galle ...	W. ...	,,	652·3	507,000	777
Colombo ...	I. ...	,,	560·8	1,300,000	2,318
Negombo ...	I. ..	,,	247·5	261,000	1,055
Matara ...	W. and I. ...	,,	481·3	387,000	804
Kegalle ..	W. and I. ...	,,	642·0	442,000	688
Nuwara Eliya ...	W., I. and D.	,,	473·9	289,000	610
Jaffna ...	D. ...	,,	998·6	464,000	465
Kandy ...	W., I. and D.	,,	913·8	773,000	846
Ratnapura ...	W., I. and D.	Moderate	1,250·5	381,000	305
Chilaw ...	I. and D. ...	,,	262·3	153,000	583
Badulla ...	D. ...	,,	3,277·1	407,000	124
Matale ...	I. and D. ...	High	902·4	170,000	188
Kurunegala ...	I. and D. ...	,,	1,843·8	532,000	289
Mannar ...	D. ...	,,	964·2	35,000	36
Puttalam ...	D ...	,,	909·9	47,000	52
Batticaloa ..	D. ...	,,	2,792·1	220,000	79
Anuradhapura	D. ...	Hyperendemic	4,008·5	157,000	39
Trincomalee ...	D. ...	,,	1,048·0	80,000	76
Hambantota ...	I. and D. ...	,,	1,012·8	163,000	161
Yavuniya ...	D. ...	,,	1,466·5	26,000	18
AVERAGE	...	...	25,332·0	7,207,000	288 (mean)

W. = wet zone. I. = intermediate zone. D. = dry zone.

TABLE IV.

Vital statistics (rates per thousand), 1901–49.

Year.	Birth rate.	Death rate.	Infant-mortality rate.	Year.	Birth rate.	Death rate.	Infant-mortality rate.
1901	37·5	27·6	170	1926	42·0	25·3	174
1902	38·5	27·5	173	1927	41·0	22·6	160
1903	40·0	25·9	164	1928	41·9	26·0	177
1904	38·5	24·9	174	1929	38·3	26·1	187
1905	38·7	27·7	176	1930	39·0	25·4	175
1906	36·5	35·1	198	1931	37·4	22·1	158
1907	33·6	30·7	186	1932	37·0	20·5	162
1908	41·0	30·1	185	1933	38·6	21·2	157
1909	37·5	31·0	202	1934	37·2	22·9	173
1910	39·0	27·3	176	1935	34·4	36·6	263
1911	38·0	34·8	218	1936	34·1	21·8	166
1912	33·3	32·4	215	1937	37·8	21·7	158
1913	38·6	28·4	189	1938	35·9	21·0	161
1914	38·1	32·2	213	1939	36·0	21·8	166
1915	37·0	25·2	171	1940	35·8	20·6	149
1916	39·0	26·8	184	1941	36·5	18·8	129
1917	40·1	24·7	174	1942	36·7	18·6	120
1918	39·2	31·9	188	1943	40·6	21·4	132
1919	36·0	37·6	223	1944	37·1	21·3	135
1920	36·5	29·6	182	1945	36·7	22·0	140
1921	40·7	31·2	192	1946	38·4	20·3	141
1922	39·1	27·5	188	1947	39·4	14·3	101
1923	38·7	30·3	212	1948	40·6	13·2	92
1924	37·5	25·8	186	1949	39·9	12·6	87
1925	39·9	24·3	172				

TABLE V.

Analysis of vital statistics (rates per thousand), 1901–49.

Period.	Birth rate.	Death rate.	Infant-mortality rate.
1901–10 ...	38·1	28·8	180
1911–20 ...	37·9	30·6	196
1921–30 ...	39·8	26·5	182
1931–40 ...	36·4	23·0	171
1941–46 ...	37·6	20·4	133
1947–49 ...	39·9	13·2	93

TABLE VI.

Crude death rates and infant-mortality rates.

Districts.	Endemicity (1938).	Crude death rates.					Infant-mortality rates.				
		1938	1946	1947	1948	1949	1938	1946	1947	1948	1949
Kalutara ...	Healthy	16·6	14·7	12·6	11·4	11·2	108	90	92	77	74
Galle ...	,,	20·7	17·4	14·2	13·2	13·1	131	104	103	84	79
Colombo ...	,,	18·9	18·5	14·9	14·3	14·5	139	107	99	100	97
Negombo ...	,,	15·3	16·4	10·9	10·2	10·5	142	134	92	85	77
Matara ...	,,	22·9	17·7	11·5	11·1	11·3	132	105	71	69	72
Kegalle ...	,,	15·3	17·2	11·2	10·5	10·2	126	124	83	75	75
Nuwara Eliya ...	,,	17·0	16·2	13·6	15·3	13·8	176	127	105	115	109
Jaffna ...	,,	23·3	17·0	15·3	12·5	12·7	166	106	91	77	73
Kandy ...	,,	16·0	18·0	14·2	14·0	13·0	152	140	97	97	94
Ratnapura ...	Moderate	19·8	18·4	13·1	12·6	12·2	144	127	94	84	82
Chilaw ...	,,	18·5	18·6	11·7	10·1	9·0	151	144	92	68	62
Badulla ...	,,	21·2	17·7	18·9	15·1	13·8	157	124	94	99	99
Matale ...	High	25·1	29·6	17·2	15·0	13·1	186	222	109	104	89
Kurunegala ...	,,	26·3	32·0	16·2	12·8	10·5	229	297	123	93	78
Mannar ...	,,	35·4	32·1	17·8	15·3	12·7	288	320	164	125	123
Puttalam ...	,,	39·2	24·7	19·7	12·5	11·9	342	192	161	100	95
Batticaloa ...	,,	30·0	30·9	18·2	17·2	16·1	213	201	131	128	116
Anuradhapura	Hyperendemic	37·0	27·2	19·5	14·1	12·1	263	207	150	102	90
Trincomalee ...	,,	31·4	25·5	16·6	12·6	11·4	174	201	146	120	109
Hambantota ...	,,	48·8	41·3	18·4	13·6	12·7	292	322	113	89	81
Vavuniya ...	,,	35·3	26·3	19·0	15·8	12·2	267	176	148	121	87
AVERAGE .	...	21·8	20·3	14·3	13·2	12·6	161	141	101	92	87

TABLE VII.

Anopheles culicifacies—*comparison of spray and live catches.*

Period.	Spray catch.		Live catch.	
	Total catch.	Number per 100 houses.	Total catch.	Number per 100 houses.
August 1945 to April 1946 ...	1,744	214·2	293	17·3

TABLE VIII.

Monthly average rainfall at Delwala (inches).

January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total.
9·50	2·36	5·48	8·37	6·87	7·26	4·87	4·41	5·47	14·24	12·60	8·54	87·07

TABLE IX.

Monthly rainfall in inches for the years 1938–49 calculated as an average for all meteorological stations situated in the epidemic zone.

Year.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total.
1938	2·17	9·87	12·50	12·52	3·90	3·76	5·51	4·80	7·61	5·48	5·96	4·88	278·96
1939	0·33	0·23	3·02	15·47	9·57	10·17	8·08	12·23	5·94	11·65	13·86	4·78	101·33
1940	0·02	0·95	1·94	14·68	19·69	14·87	5·03	6·92	6·98	18·46	21·26	6·81	117·61
1941	2·21	2·85	4·35	8·58	13·44	10·74	4·14	9·48	9·13	17·74	20·64	6·12	109·42
1942	1·31	1·59	8·34	11·92	6·79	11·67	8·06	9·00	4·63	12·20	5·71	13·01	95·13
1943	3·96	2·12	3·00	8·50	21·96	10·43	4·64	5·39	3·38	20·10	20·79	9·10	113·37
1944	3·23	6·71	9·85	10·42	8·22	7·77	2·94	3·43	10·17	15·19	20·21	8·56	106·70
1945	0·39	0·31	4·22	5·60	1·08	8·25	3·75	2·94	2·64	18·83	11·23	10·41	69·65
1946	1·41	1·25	9·41	10·26	4·82	7·02	4·01	7·90	5·90	11·38	18·60	20·06	102·02
1947	8·76	1·61	10·58	3·65	3·45	8·41	6·85	15·06	7·05	14·38	1·94	5·70	90·44
1948	2·62	0·90	10·57	5·98	4·71	10·53	6·90	5·31	2·41	12·16	9·02	7·62	78·73
1949	2·75	1·02	3·79	8·15	12·04	11·21	13·74	13·49	4·13	9·39	8·52	5·20	93·43
AVERAGE 1911–46	50·4	2·41	6·80	9·44	9·87	10·29	6·66	5·56	7·17	15·48	13·43	6·84	98·99

TABLE X.

Monthly dispensary and hospital attendance clinically diagnosed as malaria in the epidemic zone, 1938–49.

Year.	January.		February.		March.		April.		May.		June.		July.	
	Attendance.	Rate.	Attendance.	Rate.	Attendance.	Rate.	Attendance.	Rate.	Attendance.	Rate.	Attendance.	Rate.	Attendance.	Rate.
1938	35,182	39	30,353	34	34,298	38	29,098	32	27,822	31	23,893	26	19,489	21
1939	36,137	40	31,688	35	34,069	37	63,397	70	145,303	161	150,684	167	100,272	111
1940	29,846	32	23,192	25	23,786	26	25,288	27	51,745	56	85,330	93	65,179	71
1941	25,415	27	19,909	21	18,969	20	19,460	21	22,129	23	22,349	24	25,821	27
1942	26,205	28	16,608	17	18,601	19	19,366	20	22,778	24	23,363	24	20,216	21
1943	19,713	20	17,653	18	20,698	21	38,995	41	58,599	61	62,343	65	43,148	45
1944	20,293	21	21,666	22	15,046	15	12,519	13	16,577	17	15,235	15	14,962	15
1945	34,876	35	23,849	24	23,002	23	34,577	35	43,959	45	47,752	49	71,158	73
1946	126,405	128	78,787	79	59,239	60	50,158	50	56,153	56	49,039	49	39,734	40
1947	26,094	26	22,075	22	20,107	20	14,820	14	18,265	18	15,395	15	16,873	16
1948	11,462	11	10,019	9	10,535	10	11,075	10	14,232	14	11,814	11	11,402	11
1949	6,844	6	6,438	6	6,548	6	6,219	6	6,366	6	6,088	5	10,501	10

Rate = malaria attendance per thousand population.

TABLE X—*concld.*

Year.	August.		September.		October.		November.		December.		Total attendance.	Population.	Rate for the year.
	Attendance.	Rate.	Attendance.	Rate.	Attendance.	Rate.	Attendance.	Rate.	Attendance.	Rate.			
1938	17,523	19	18,673	21	21,122	23	21,825	24	24,754	27	304,032	886,713	342
1939	54,266	60	40,005	44	33,268	37	30,546	33	39,724	44	759,359	899,170	844
1940	42,496	46	28,373	31	27,283	29	26,083	28	26,051	28	454,652	911,627	498
1941	21,907	23	18,934	20	19,685	21	19,767	21	24,503	26	258,851	924,084	280
1942	15,117	16	13,404	14	14,041	15	16,893	18	18,462	19	225,654	936,541	240
1943	22,421	23	15,425	16	12,757	13	17,476	18	21,712	22	350,940	948,998	369
1944	11,135	11	9,765	10	14,170	14	21,536	22	34,972	36	207,876	961,455	216
1945	77,577	79	46,245	47	45,663	46	64,318	66	175,123	179	688,099	973,912	706
1946	29,650	30	20,496	20	20,042	20	23,324	23	30,461	30	583,488	986,369	592
1947	13,798	13	12,389	12	12,252	12	12,713	12	12,439	12	197,220	998,826	197
1948	9,098	9	6,851	6	6,740	6	6,948	6	6,574	6	116,759	1,011,283	115
1949	6,136	5	6,234	6	5,755	5	6,581	6	6,385	6	80,095	1,023,740	78

Rate = malaria attendance per thousand population.

TABLE XI.

Spleen rates among school children in the epidemic zone, 1938–49.

Year.	Month.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Month.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.
1938	March	14,823	4,099	27·7	September	...	...	...
1939	,,	15,269	3,023	19·8	,,	...	...	...
1940	,,	15,830	3,654	23·1	,,	15,124	4,022	26·6
1946	,,	...	...	...	,,	14,777	2,704	18·3
1947	,,	15,519	2,090	13·5	,,	17,800	1,568	8·8
1948	,,	19,599	1,299	6·6	,,	22,225	1,078	4·9
1949	,,	19,201	898	4·7	,,	21,894	533	2·4

TABLE XII.

Parasite rates among school children in the epidemic zone, 1938–49.

Year.	Month.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.	Month.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.
1938	March	1,795	65	3·6	September	...	...	...
1939	,,	1,754	80	4·6	,,	...	...	...
1940	,,	1,721	92	5·3	,,	...	...	...
1941	,,	1,750	67	3·8	,,	...	...	...
1946	,,	...	...	...	,,	1,658	151	9·1
1947	,,	...	...	...	,,	1,842	22	1·2
1948	,,	2,052	13	0·6	,,	2,369	11	0·5
1949	,,	2,320	3	0·1	,,	2,264	1	0·04

TABLE XIII.

Annual variation in the prevalence of A. culicifacies *larvæ and the total of all other anopheline larvæ found in pools, in the margins of rivers and streams and in village breeding places in the epidemic zone, 1939–49.*

Year.	SAND AND ROCK POOLS.					MARGINS.					VILLAGE BREEDING PLACES.				
		A. culicifacies.		OTHER ANOPHELINE SPECIES.			*A. culicifacies.*		OTHER ANOPHELINE SPECIES.			*A. culicifacies.*		OTHER ANOPHELINE SPECIES.	
	Number of dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number of dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number of dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.
1939	19,995	3,968	19·84	4,993	24·97	63,495	1,720	2·70	17,605	27·73	70,607	1,198	1·68	43,528	61·63
1940	27,560	1,241	4·34	4,521	16·40	79,235	662	0·80	13,347	16·84	98,339	573	0·57	45,379	46·13
1941	17,865	242	1·35	2,954	16·53	81,070	61	0·08	11,006	13·58	108,872	210	0·18	45,337	41·64
1942	19,925	588	2·95	4,719	23·68	82,210	213	0·26	15,934	19·38	112,103	79	0·08	46,452	41·43
1943	18,020	2,009	11·14	3,150	17·48	86,610	444	0·51	13,422	15·49	121,498	137	0·11	43,295	35·61
1944	21,810	17	0·08	2,723	12·48	95,715	72	0·08	15,516	16·20	114,990	61	0·05	46,087	40·07
1945	40,661	1,446	3·55	1,783	4·38	84,360	476	0·56	7,231	8·57	103,243	983	0·95	39,525	38·27
1946	44,563	715	1·60	1,962	4·40	81,705	273	0·33	7,542	9·23	103,874	215	0·20	33,930	32·67
1947	48,025	133	0·28	3,124	6·50	101,675	65	0·06	9,444	9·28	165,967	7	0·004	37,100	22·35
1948	52,024	149	0·28	4,921	9·45	103,735	89	0·08	10,314	9·94	141,081	58	0·04	30,419	21·56
1949	11,535	52	0·45	1,769	15·34	52,965	50	0·09	4,269	8·06	135,045	3	0·002	21,043	15·59

TABLE XIV.

Specific variation in the prevalence of anopheline larvæ found in sand and rock pools in rivers and streams in the epidemic zone, 1939–49.

Year.	Number of dips.	*A. culicifacies.*		*A. varuna.*		*A. subpictus.*		*A. ragus.*		OTHER ANOPHELINE SPECIES.*	
		Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.
1939	19,995	3,968	19·84	2,513	12·56	33	0·17	1,616	8·08	831	4·16
1940	27,560	1,241	4·34	2,219	8·05	168	0·61	1,239	4·49	895	3·25
1941	17,865	242	1·35	1,498	8·39	24	0·13	974	5·45	458	2·56
1942	19,925	588	2·95	2,167	10·87	12	0·05	1,661	8·35	879	4·41
1943	18,020	2,009	11·14	1,627	9·03	10	0·06	900	5·00	613	3·40
1944	21,819	17	0·08	1,049	4·81	4	0·01	844	3·87	826	3·79
1945	40,661	1,446	3·55	640	1·57	198	0·49	782	1·92	163	0·40
1946	44,563	715	1·60	760	1·70	161	0·36	689	1·54	352	0·79
1947	48,025	133	0·28	2,014	4·19	20	0·05	619	1·28	471	0·98
1948	52,024	149	0·28	3,256	6·25	51	0·10	1,020	1·96	594	1·14
1949	11,535	52	0·45	721	6·25	76	0·65	800	6·94	172	1·49

**A. aconitus, A. aitkeni, A. annularis, A. barbirostris, A. hyrcanus, A. jamesi, A. karwari, A. leucosphyrus, A. maculatus, A. pallidus* and *A. vessellatus.*

TABLE XV.

Specific variation in the prevalence of anopheline larvæ found in the margins of rivers and streams in the epidemic zone, 1939–49.

Year.	Number of dips.	*A. culicifacies.*		*A. varuna.*		*A. subpictus.*		*A. vagus.*		OTHER ANOPHELINE SPECIES.*	
		Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.
1939	63,485	1,720	2·70	16,093	23·76	18	0·02	181	0·28	1,308	2·06
1940	79,235	662	0·80	11,574	14·60	78	0·09	176	0·22	1,519	1·92
1941	81,070	61	0·08	9,093	11·21	9	0·01	185	0·23	1,719	2·19
1942	82,210	213	0·26	13,307	16·19	117	0·14	247	0·50	2,263	2·75
1943	86,610	444	0·51	10,934	12·62	19	0·02	492	0·57	1,977	2·28
1944	95,715	72	0·08	12,670	13·24	57	0·06	223	0·23	2,560	2·68
1945	84,300	476	0·56	5,249	6·22	207	0·24	311	0·37	1,464	1·74
1946	81,705	273	0·33	5,957	7·29	85	0·10	144	0·18	1,356	1·66
1947	101,675	65	0·06	7,652	7·52	36	0·03	337	0·33	1,419	1·39
1948	103,735	89	0·08	8,333	8·04	7	0·006	201	0·19	1,773	1·71
1949	52,965	50	0·09	2,865	5·41	34	0·06	393	0·74	977	1·84

* *A. aconitus, A. aitkeni, A. annularis, A. barbirostris, A. hyrcanus, A. insulæflorum, A. leucosphyrus, A. maculatus, A. pallidus* and *A. tessellatus.*

TABLE XVI.

Specific variation in the prevalence of anopheline larvæ found in village breeding places in the epidemic zone, 1939–49.

Year.	Number of dips.	*A. culicifacies.*		*A. varuna.*		*A. subpictus.*		*A. vagus.*		OTHER ANOPHELINE SPECIES.*	
		Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.
1939	70,697	1,198	1·68	14,045	19·90	922	1·30	5,657	8·01	22,895	32·42
1940	98,339	573	0·57	15,305	15·56	1,777	1·80	5,721	5·81	22,576	22·96
1941	108,872	210	0·18	13,026	11·96	908	0·83	5,361	4·92	26,042	23·92
1942	112,103	79	0·08	14,887	13·28	1,420	1·26	5,545	4·94	24,690	21·94
1943	121,498	137	0·11	14,709	12·10	321	0·26	5,053	4·14	23,212	19·11
1944	114,990	61	0·05	15,718	13·67	779	0·67	5,691	4·94	23,899	20·78
1945	103,243	983	0·95	16,397	15·86	2,589	2·51	4,660	4·51	15,879	15·38
1946	103,874	215	0·20	14,162	13·63	1,298	1·25	2,636	2·54	15,834	15·24
1947	165,967	7	0·004	11,923	7·18	38	0·02	6,506	3·92	18,633	11·22
1948	141,081	58	0·04	13,418	9·51	78	0·06	4,177	2·96	12,746	9·03
1949	135,045	3	0·002	6,281	4·65	114	0·08	4,211	3·12	10,437	7·73

* *A. aconitus, A. aitkeni, A. annularis, A. barbirostris, A. hyrcanus, A. insulæflorum, A. jamesi, A. karwari, A. leucosphyrus, A. maculatus* and *A. pallidus.*

TABLE XVII.

Annual variation in the prevalence of female A. culicifacies *and the total of all other anopheline mosquitoes caught in dwellings and in cattle-baited traps in the epidemic zone, 1939-49.*

Year.	DWELLINGS.						CATTLE-BAITED TRAPS.				
	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		OTHER ANOPHELINE MOSQUITOES.		Number of trapping hours.	*A. culicifacies.*		OTHER ANOPHELINE MOSQUITOES.	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.		Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1939	5,819	874	943	1·07	614	0·70	1,474	158	0·10	12,710	8·62
1940	6,696	1,003	413	0·41	1,176	1·16	1,711	73	0·04	14,783	8·64
1941	6,420	967	79	0·08	1,012	1·04	1,773	4	0·002	15,091	8·51
1942	6,954	1,052	26	0·02	1,180	1·12	1,432	3	0·002	14,248	9·95
1943	8,878	1,458	325	0·22	1,174	0·80	1,166	7	0·006	11,088	9·50
1944	10,337	1,956	49	0·02	970	0·50	1,127	3	0·002	8,520	7·56
1945	11,132	2,034	560	0·27	2,426	1·20	1,122	32	0·02	8,434	7·51
1946	9,572	1,593	261	0·16	1,018	0·64	1,084	5	0·004	7,301	6·73
1947	8,309	1,344	5	0·003	188	0·14	895	0	0	7,436	8·30
1948	9,904	1,585	9	0·005	34	0·02	596	0	0	4,515	7·57
1949	12,995	1,995	0	0	17	0·008	172	21	0·12	1,126	6·54

TABLE XVIII.

Specific variation in the prevalence of female anopheline mosquitoes caught in dwellings in the epidemic zone, 1939–49.

Year.	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		*A. varuna.*		*A. subpictus.*		*A. vagus.*		Other anopheline species.*	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1939	5,819	874	943	1·07	33	0·04	458	0·52	68	0·08	55	0·06
1940	6,696	1,003	413	0·41	74	0·07	863	0·85	201	0·20	38	0·03
1941	6,420	967	79	0·08	63	0·07	688	0·71	162	0·15	99	0·10
1942	6,954	1,052	26	0·02	79	0·07	545	0·52	225	0·21	331	0·31
1943	8,878	1,458	325	0·22	65	0·04	441	0·30	276	0·19	394	0·27
1944	10,337	1,956	49	0·02	115	0·06	142	0·07	453	0·23	260	0·13
1945	11,132	2,034	560	0·27	122	0·06	1,357	0·67	631	0·32	316	0·16
1946	9,572	1,593	261	0·16	44	0·03	496	0·31	256	0·16	222	0·14
1947	8,309	1,344	5	0·003	33	0·02	8	0·006	105	0·08	42	0·03
1948	9,904	1,585	9	0·005	2	0·001	1	0·001	16	0·01	15	0·01
1949	12,995	1,995	0	0	2	0·001	0	0	4	0·002	7	0·003

* *A. aconitus, A. annularis, A. barbirostris, A. hyrcanus, A. insulæflorum, A. jamesi, A. karwari, A. maculatus, A. pallidus* and *A. tessellatus.*

TABLE XIX.

Anopheline mosquitoes caught in cattle-traps in the epidemic zone, 1939-49.

Year.	Number of trapping hours.	*A. culicifacies.*		*A. varuna.*		*A. subpictus.*		*A. vagus.*		*A. hyrcanus.*		OTHER ANOPHELINE SPECIES.*	
		Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1939	1,474	158	0·10	1,032	0·70	377	0·26	4,128	2·80	4,176	2·83	2,997	2·03
1940	1,711	73	0·04	675	0·39	2,079	1·22	5,414	3·16	3,678	2·15	2,937	1·71
1941	1,773	4	0·002	988	0·56	1,683	0·95	4,251	2·40	4,640	2·61	3,529	1·99
1942	1,432	3	0·002	634	0·44	1,389	0·98	4,315	3·01	4,727	3·30	3,183	2·22
1943	1,166	7	0·006	377	0·32	1,289	1·10	3,959	3·40	3,488	2·99	1,975	1·69
1944	1,127	3	0·002	255	0·23	410	0·36	3,495	3·10	3,107	2·76	1,253	1·11
1945	1,122	32	0·02	194	0·17	1,796	1·61	2,789	2·48	1,865	1·66	1,790	1·60
1946	1,084	5	0·004	31	0·03	930	0·85	1,820	1·68	2,448	2·26	2,072	1·91
1947	895	0	0	51	0·06	315	0·35	2,961	3·30	3,261	3·66	848	0·94
1948	596	0	0	12	0·02	70	0·12	1,720	2·89	2,337	3·92	376	0·63
1949	172	21	0·12	79	0·45	25	0·14	414	2·41	485	2·82	123	0·71

* *A. aconitus, A. annularis, A. barbirostris, A. jamesi, A. karwari, A. leucosphyrus, A. maculatus, A. pallidus* and *A. tessellatus.*

TABLE XX.

Natural infections recorded in A. culicifacies *caught in dwellings in the epidemic zone, 1939-49.*

Year.	Number dissected.	Gut infections.	Oöcyst rate, per cent.	Gland infections.	Sporozoite rate, per cent.	Total infections.	Infection rate, per cent.
1939	382	11	2·88	9	2·36	19	4·97
1940	277	3	1·08	3	1·08	6	2·16
1941	46	0	0·0	0	0·0	0	0·0
1942	18	0	0·0	0	0·0	0	0·0
1943	212	5	2·36	5	2·36	8	3·77
1944	28	1	3·57	0	0·0	1	3·57
1945	339	2	0·06	6	1·77	8	2·36
1946	130	0	0·0	1	0·07	1	0·07
1947	0	0	0·0	0	0·0	0	0·0
1948	3	0	0·0	0	0·0	0	0·0
1949	0	0	0·0	0	0·0	0	0·0

TABLE XXI.

Annual variation in the prevalence of A. culicifacies *and other anopheline larvæ found in sand and rock pools in the Maha Oya and streams surrounding it at Alawwa, Giriulla and Rambukkana from 1939–46 and in the Polgahawela Health Area in 1947, 1948 and 1949.*

Year.	Number of dips.	SAND AND ROCK POOLS.				Number of dips.	MARGINS.			
		A. culicifacies.		OTHER ANOPHELINE SPECIES.			*A. culicifacies.*		OTHER ANOPHELINE SPECIES.	
		Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.		Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.
ALAWWA, GIRIULLA AND RAMBUKKANA.										
1939	5,900	2,704	45·82	1,781	30·18	18,600	1,262	6·78	8,063	43·35
1940	7,280	561	7·71	1,299	17·84	18,740	235	1·25	2,825	15·08
1941	4,655	172	3·69	651	16·32	18,400	10	0·05	2,666	14·49
1942	3,895	110	2·82	969	24·88	16,010	21	0·13	3,471	21·68
1943	5,155	936	18·16	637	12·36	18,080	190	1·05	3,202	17·71
1944	4,390	8	0·18	411	9·36	24,370	26	0·11	2,843	11·67
1945	14,415	829	5·75	351	2·43	23,830	179	0·75	1,956	8·21
1946	10,115	175	1·73	788	7·79	21,380	26	0·12	1,840	8·61
POLGAHAWELA HEALTH AREA.										
1947	10,710	99	0·92	1,450	13·54	48,675	12	0·02	4,671	9·60
1948	20,829	102	0·49	3,180	15·31	50,035	45	0·08	5,461	10·91
1949	9,420	52	0·55	1,879	19·95	42,885	50	0·11	3,086	7·20

TABLE XXII.

Variation in the prevalence of anopheline larvæ found in sand and rock pools and in the margins of rivers and streams at Alawwa, Giriulla and Rambukkana from 1939–46 and in the Polgahawela Health Area in 1947, 1948 and 1949.

Year	Number of dips.	SAND AND ROCK POOLS. *A. culicifacies.* Number collected.	*A. culicifacies.* Larval rate per 100 dips.	*A. varuna.* Number collected.	*A. varuna.* Larval rate per 100 dips.	*A. vagus.* Number collected.	*A. vagus.* Larval rate per 100 dips.	OTHER ANOPHELINE SPECIES.* Number collected.	OTHER ANOPHELINE SPECIES.* Larval rate per 100 dips.	Number of dips.	MARGINS. *A. culicifacies.* Number collected.	*A. culicifacies.* Larval rate per 100 dips.	*A. varuna.* Number collected.	*A. varuna.* Larval rate per 100 dips.	OTHER ANOPHELINE SPECIES.* Number collected.	OTHER ANOPHELINE SPECIES.* Larval rate per 100 dips.
ALAWWA, GIRIULLA AND RAMBUKKANA.																
1939	5,900	2,794	45·82	1,233	20·89	351	5·95	197	3·33	18,800	1,262	6·78	7,462	40·01	601	3·23
1940	7,280	561	7·71	1,077	14·80	192	2·64	30	0·41	18,740	235	1·25	2,619	13·97	206	1·10
1941	4,655	172	3·69	384	8·25	196	4·21	71	1·52	18,400	10	0·05	2,306	12·53	360	1·96
1942	3,895	110	2·82	345	8·84	500	12·83	114	2·93	16,010	21	0·13	3,230	20·23	232	1·45
1943	5,155	936	18·16	286	5·55	308	5·97	43	0·83	18,080	190	1·05	2,897	16·03	305	1·68
1944	4,390	8	0·18	246	5·60	108	2·46	57	1·30	24,370	26	0·11	2,601	10·68	242	0·99
1945	14,415	829	5·75	91	0·63	221	1·53	39	0·27	23,830	179	0·75	1,791	8·51	165	0·69
1946	10,115	175	1·73	272	2·69	371	3·68	145	1·43	21,380	26	0·12	1,753	8·20	87	0·41
POLGAHAWELA HEALTH AREA.																
1947	10,710	99	0·92	1,031	9·63	329	3·07	90	0·84	48,675	42	0·02	3,653	7·51	1,018	2·09
1948	20,829	102	0·49	1,868	8·96	932	3·55	389	1·87	50,035	45	0·03	4,565	9·12	896	1·79
1949	9,420	52	0·55	753	7·99	800	8·49	326	3·46	42,885	50	0·11	1,937	4·52	1,149	2·68

* *A. aitkeni, A. aconitus, A. annularis, A. barbirostris, A. hyrcanus, A. insulæflorum, A. jamesi, A. karwari, A. leucosphyrus, A. maculatus, A. pallidus, A. subpictus, A. tessellatus* and *A. vagus.*

TABLE XXIII.

Annual variation in the prevalence of female A. culicifacies *and the total of all other anopheline mosquitoes caught in dwellings at Alawwa, Giriulla and Rambukkana from 1939–46 and in the Polgahawela Health Area in 1947, 1948 and 1949.*

Year.	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		Other anopheline species.	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
ALAWWA, GIRIULLA AND RAMBUKKANA.						
1939	1,799	286	343	1·20	191	0·67
1940	1,677	276	107	0·35	155	0·56
1941	1,487	248	3	0·01	30	0·12
1942	1,606	266	2	0·007	61	0·22
1943	2,027	300	237	0·79	87	0·36
1944	1,815	349	1	0·002	93	0·27
1945	2,028	369	182	0·48	377	1·02
1946	1,878	299	91	0·30	69	0·23
POLGAHAWELA HEALTH AREA.						
1947	4,622	695	0	0·0	13	0·02
1948	6,737	1,033	9	0·008	8	0·007
1949	13,745	2,582	0	0·0	4	0·001

TABLE XXIV.

Prevalence of A. culicifacies *and other female anopheline mosquitoes as shown by day and night hand catches and daytime spray catches in houses treated with D.D.T. in the Polgahawela Health Area in 1947, 1948 and 1949.*

Year.	HAND CATCHES (DAYTIME).						SPRAY CATCHES (DAYTIME).						HAND CATCHES (NIGHT-TIME).					
	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		OTHER ANOPHELINE SPECIES.		Number of house examinations.	Number of catching hours.	*A. culicifacies.*		OTHER ANOPHELINE SPECIES.		Number of house examinations.	Number of catching hours.	*A. culicifacies.*		OTHER ANOPHELINE SPECIES.	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1947	4,622	695	0	0	13	0·02	2,437	788	0	0	72	0·08	...	...	...	...	...	...
1948	6,737	1,033	9	0·008	8	0·007	2,129	730	0	0	49	0·06	501	169	0	0	7	0·04
1949	13,745	2,582	0	0	4	0·001	...	...	...	...	...	...	910	306	0	0	0	0

| | | |

TABLE XXIV.

Prevalence of A. culicifacies *and other female anopheline mosquitoes as shown by day and night hand catches and daytime spray catches in houses treated with D.D.T. in the Polgahawela Health Area in 1947, 1948 and 1949.*

Year.	HAND CATCHES (DAYTIME).						SPRAY CATCHES (DAYTIME).						HAND CATCHES (NIGHT-TIME).					
	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		OTHER ANOPHELINE SPECIES.		Number of house examinations.	Number of catching hours.	*A. culicifacies.*		OTHER ANOPHELINE SPECIES.		Number of house examinations.	Number of catching hours.	*A. culicifacies.*		OTHER ANOPHELINE SPECIES.	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1947	4,622	695	0	0	13	0·02	2,437	788	0	0	72	0·08	...	...	...	...	...	...
1948	6,737	1,033	9	0·008	8	0·007	2,129	730	0	0	49	0·06	501	169	0	0	7	0·04
1949	13,745	2,582	0	0	4	0·001	...	...	...	...	...	...	910	306	0	0	0	0

TABLE XXV.

Prevalence of A. culicifacies *and other female anopheline mosquitoes as shown by day and night hand catches and daytime spray catches in dwellings in five untreated villages contiguous to the Polgahawela Health Area in 1947 and 1948.*

Year.	HAND CATCHES (DAYTIME).						SPRAY CATCHES (DAYTIME).					
	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		OTHER ANOPHELINE SPECIES.		Number of house examinations.	Number of catching hours.	*A. culicifacies.*		OTHER ANOPHELINE SPECIES.	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1947	2,896	393	0	0	28	0·07	1,762	594	2	0·003	36	0·06
1948	3,088	440	1	0·002	17	0·04	1,223	380	0	0	29	0·06

TABLE XXVI.

Variation in the prevalence of female anopheline mosquitoes caught in dwellings at Alawwa, Giriulla and Rambukkana from 1939–46 and in the Polgahawela Health Area in 1947, 1948 and 1949.

Year.	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		*A. varuna.*		*A. subpictus.*		*A. vagus.*		OTHER ANOPHELINE SPECIES.*	
			Number caught.	Catching rate.	Number caught.	Catching rate.	Number caught.	Catching rate.	Number caught.	Catching rate.	Number caught.	Catching rate.
ALAWWA, GIRIULLA AND RAMBUKKANA.												
1939	1,799	286	343	1·20	24	0·08	115	0·40	27	0·09	25	0·08
1940	1,677	276	107	0·35	16	0·05	103	0·37	30	0·11	6	0·02
1941	1,487	248	3	0·01	3	0·01	0	0	14	0·05	13	0·05
1942	1,666	266	2	0·007	4	0·01	0	0	28	0·10	29	0·11
1943	2,027	300	237	0·79	25	0·08	1	0·003	32	0·11	29	0·09
1944	1,815	349	1	0·002	40	0·11	0	0	26	0·07	27	0·07
1945	2,028	369	182	0·48	36	0·09	155	0·42	110	0·29	76	0·20
1946	1,878	299	91	0·30	12	0·04	17	0·05	20	0·06	20	0·06
POLGAHAWELA HEALTH AREA.												
1947	4,622	695	0	0	3	0·004	5	0·007	4	0·005	1	0·001
1948	6,737	1,033	9	0·008	1	0·0009	0	0	5	0·004	2	0·001
1949	13,745	2,582	0	0	0	0	0	0	4	0·001	0	0

* *A. aconitus, A. annularis, A. barbirostris, A. hyrcanus, A. jamesi, A. karwari, A. pallidus* and *A. tessellatus.*

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MALARIA IN CEYLON.

Part II.

THE CONTROL OF ENDEMIC MALARIA AT ANURADHAPURA BY THE RESIDUAL SPRAYING OF HOUSES WITH D.D.T.

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[November 6, 1950.]

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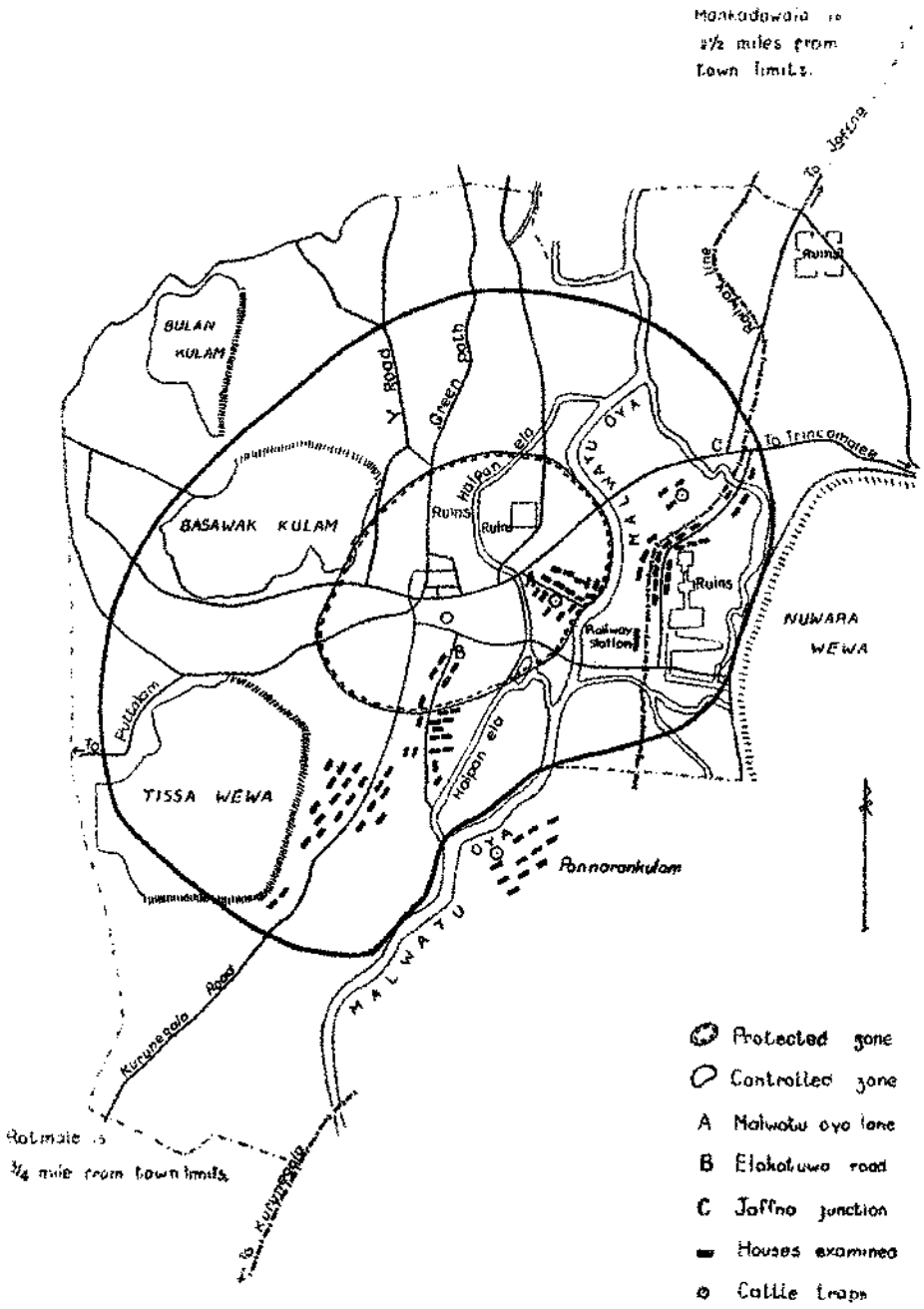
INTRODUCTION.

MALARIA control at Anuradhapura, capital of the North-Central Province (Rajendram and Jayewickreme, 1951—Map 1), dates back to 1923 in which year a Campaign Centre was opened. It was the second town in Ceylon, Kurunegala having been the first, where malaria control measures were begun on an intensive scale and it was perhaps fitting that this ancient and historic town with a population of 13,200 in 1949, should have been the first place where D.D.T. was used as a residual spray against the adult of the vector, *Anopheles culicifacies*. Anuradhapura receives one major monsoon, the north-east each year. The annual seasonal periodicity of malaria associated with this monsoon at Anuradhapura is

MAP 1.

ANURADHAPURA TOWN



typical of the dry zone of Ceylon lying below an elevation of 1,000 feet. Climatic conditions similar to those to be described for Anuradhapura are to be found all over this extensive area. The malaria control measures which have been in operation since 1923 have also been carried out at Campaign Centres in the dry zone towns of Puttalam, Chilaw, Trincomalee, Badulla and Maho. *A. culicifacies* is found breeding in similar situations in most of these towns. But there is no riverine problem causing epidemics as described in Part I of this paper in the dry zone except at elevations above 1,000 feet. In all these towns, intensive checking-up of the control measures has been carried out from the inception of the campaigns until 1945. But when the residual spraying of D.D.T. was first introduced in November 1945 at Anuradhapura, it was decided to regard the control work here as an experiment and entomological investigations were planned and carried out from its inception, a discussion of which forms the major part of this paper. All references to adult mosquitoes refer to the female only.

PHYSICAL FEATURES AND CLIMATIC CONDITIONS.

Topography.—Anuradhapura, first founded as a capital city by King Pandukabhaya in 247 B.C. according to Codrington (1929), is a city sacred to Buddhists. The following description of it is taken from Carter (1927), as it can scarcely be bettered. 'Anuradhapura ..... is situated near the centre of the great northern plain in slightly undulating, forest-clad, country at an elevation of approximately 300 feet. The area comprised within the town limits (Map 1) is extensive, being about 9½ square miles, and consists largely of well-wooded park lands and paddy fields with occasional patches of dense jungle. Most of the park lands are archæological reserves ....., they contain many depressions, pits and blind trenches most of which are capable of acting as breeding places of mosquitoes for a longer or shorter period during the wet season. Several low-lying areas of varying sizes —which from time to time are transformed into swamps—occur, and innumerable small pools, rills and swampy areas, due to seepage from tanks or irrigation channels and overflow from paddy fields, of a more or less permanent nature are invariably present. Borrowpits of both public and private construction are also numerous, and in addition a considerable number of ancient baths or water reservoirs, built of rock ..... are scattered throughout the town ..... the majority retain water only from four to eight months of the year.'

'The river Malwatu-oya flows in a northerly direction through the eastern part of the town, and near the south-eastern boundary gives rise to a stream or channel—the Halpan-ela—which after maintaining a roughly parallel course, westwards of the river, bends sharply eastwards and rejoins the latter near the northern boundary. The river is perennial and contains a moderate volume throughout the year, but towards the end of the dry period the water level is usually not sufficiently high to prevent the exposure of sand banks and their associated pools or to allow of connection with the Halpan-ela. In the wet season the river often rises with great rapidity and overflows its banks, flooding the ela and its adjacent lands together with a considerable section of the eastern portion of the town. The Halpan-ela functions as an accessory to the river only during the three or four months of the wet season, and throughout the rest of the year merely serves as an outlet for waste irrigation water.'

'The water supply is derived from four large tanks or reservoirs situated wholly or partially within the town limits; they are in order of size Nuwarawewa, Tissawewa, Basawakulam and Bulankulam. Nuwarawewa tank is situated on the eastern boundary of the town and supplies water to the lands lying east of the Malwatu-oya; these are to a great extent outside the town area. Tissawewa, and through it Basawakulam and Bulankulam, receive most of their water supplies via ancient waterways from two very extensive tanks Kalawewa and Nachchaduwa, situated respectively 35 miles south and twelve miles south-east of Anuradhapura. Tissawewa supplies water for domestic purposes to the three smaller reservoirs or "pokunas"—the so-called drinking, bathing and washing pokunas—referred to above, and, together with Basawakulam and the smaller Bulankulam, is the main source of irrigation water throughout the greater portion of the town area. From these tanks issue a number of major irrigation channels, which are under the control of Government, and from which innumerable smaller channels lead to the paddy fields, banana plantations and gardens. The minor channels are maintained by the landowners or cultivators under supervision of irrigation headmen (*vel-vidanes*) appointed by the Government agent; they form a network throughout a large portion of the town area and in the aggregate must extend a very considerable number of miles. Water for drinking is also obtained from ..... wells.'

Temperature, humidity and rainfall.—For the greater part of the year, in this inland dry zone town, the climate is hot and dry, the seasonal variation in temperature being about 8°F. The elevation above mean sea level is 300 feet. But although the seasonal variation in temperature is not very great there may be a considerable daily range of temperature. This may amount to as much as 20°F. in March, when the humidity (60 per cent) is the lowest for the year. In August and September, the daily range is about 15°F., with humidity about 61-62 per cent. In May and June the daily range drops to 13°F., while the humidity, influenced by the south-west monsoon, rises to 68 per cent. In November and December when the north-east monsoon blows, the daily range is also about 13°F., and in these months the humidity is the highest for the year (77 per cent).

Anuradhapura receives the major portion of its rain from October to January during the north-east monsoon (Table I). During these four months the average annual rainfall, calculated for the period 1911–40, amounts to as much as 33·72 inches, out of the average annual rainfall of 57·12 inches. In October, which is a month of intermonsoonal thunderstorms, and in November the rainfall is often augmented by cyclonic depressions originating in the Bay of Bengal. In May and June an average rainfall of 6·45 and 3·57 inches respectively are received as feeble manifestations of the south-west monsoon which at this time is active in the south-western quarter of the island. It is characteristic of Anuradhapura and all other dry zone areas that the rainfall received during the south-west monsoon period from May to September is less than 20 inches a year.

MALARIA CONTROL MEASURES.

A. 1923–45.

Malaria control in this Campaign Centre, as it was called, began in May 1923 with a labour force of 30 convicts under the supervision of an Entomological

Assistant. The town was divided into 10 sections to facilitate work of which sections 1, 2, 3 and 5 comprised the more densely populated central part of the town. It has to be remembered that at this time *A. culicifacies* had not been definitely incriminated as a vector and therefore the control work carried out was not species sanitation but directed against a number of possible vectors. This consisted of clearing and burning jungle and low-growing vegetation and clearing and canalizing drains and the margins of the more extensive pools and elas (streams). This work was carried out during the dry season but, with the rains drainage, filling and levelling operations were vigorously carried out. Oiling was introduced in December 1923 and this was to become eventually the most important control measure adopted. As the work became more organized, a well-supervised system of oiling and the checking-up of its efficiency was set in operation and continued without interruption until October 1945. In fact during the period under review the emphasis was on larval control by every possible means. The introduction of larvivorous fish into wells was organized in the very first year and continued without interruption.

In 1938, the town was divided into two zones (Map 1), a central protected zone and an outer controlled zone, with the outside zone lying beyond it in open country and acting as an untreated comparison zone. Malaria control work of all kinds was vigorously carried out in the protected and controlled zones, and the former was so named, because the antimosquito measures carried out were supposed to protect the central protected zone, which was the most densely populated area of the town. The outside zone was far too sparsely populated to act as a satisfactory control. However, in spite of all the antimosquito work done throughout the period, the breeding of *A. culicifacies* was never satisfactorily controlled at any time in this town.

B. 1946-49.

On November 11, 1945, D.D.T. in kerosene was first used in a 5 per cent solution as a residual spray against the adult vector and all forms of larval control were abandoned. In November and December, the dosage used was as high as 200 mg. per square foot but this was reduced to 100 mg. per square foot from January 1946. The interval between successive sprayings was four weeks for the first two months and thereafter up to the present day the interval has been six weeks. On December 1, 1948, the kerosene solution was abandoned and a D.D.T. emulsion was used instead in 5 per cent strength diluted in the field from a 40 per cent D.D.T. emulsion concentrate prepared according to the following formula: 188 lb. technical D.D.T., 30 gallons xylene, 2·4 gallons emulsifier—craft or Triton X-100. In March 1949, a 50 per cent water-wettable powder was first used at a dosage of 50 mg. per square foot. But as many householders objected to the use of this suspension, the emulsion was also used at a similar dosage when requested. Dilutions of D.D.T.-xylene concentrate were used in kerosene if the latter was supplied by the householder.

For the first two spraying months of 1945, a truck unit was used consisting of 14 labourers and 2 overseers under the supervision of a Sanitary Inspector. In January 1946, this unit was withdrawn and a walking unit of 7 labourers under the supervision of a Sanitary Inspector was substituted. But from April 1949, the truck unit was once more used for the work in the town.

The total cost of malaria control work each year at Anuradhapura from 1938–49 is given below. This includes, in addition to materials and equipment used, the personal emoluments of the staff engaged:

		Rs.
1938-39	...	24,777
1939-40	...	15,570
1940-41 } 1941-42 }	...	18,647
1943 ...	...	17,972
1944 ...	...	18,001
1945 ...	...	34,933
1946 ...	...	47,870
1947 ...	...	55,194
1948 ...	...	38,262
1949 ...	...	22,328

ANOPHELINE PREVALENCE AND SEASONAL VARIATION.

In the dry zone in which Anuradhapura is situated, the breeding habits of *A. culicifacies* are somewhat different to those previously described for the upper catchment of the Deduru Oya and the Maha Oya basin (Rajendram and Jayewickreme, *loc. cit.*). At Anuradhapura in the wet season, breeding takes place in every conceivable collection of ground water and during the rest of the year, when these temporary breeding places have dried up, *A. culicifacies* still continues to breed in the more permanent situations such as the Malwatu Oya, irrigation channels, wells and paddy fields up to a quarter grown. Apart from *A. culicifacies*, the most prevalent anopheline species is *A. subpictus*, while *A. varuna*, *A. hyrcanus* and *A. vagus* are also found breeding to a lesser extent. *A. varuna* is not very prevalent in the town but tends to breed prolifically at times in areas outside it. *A. subpictus* is the dominant mosquito of the area and this species and *A. culicifacies* are the only domestic mosquitoes present.

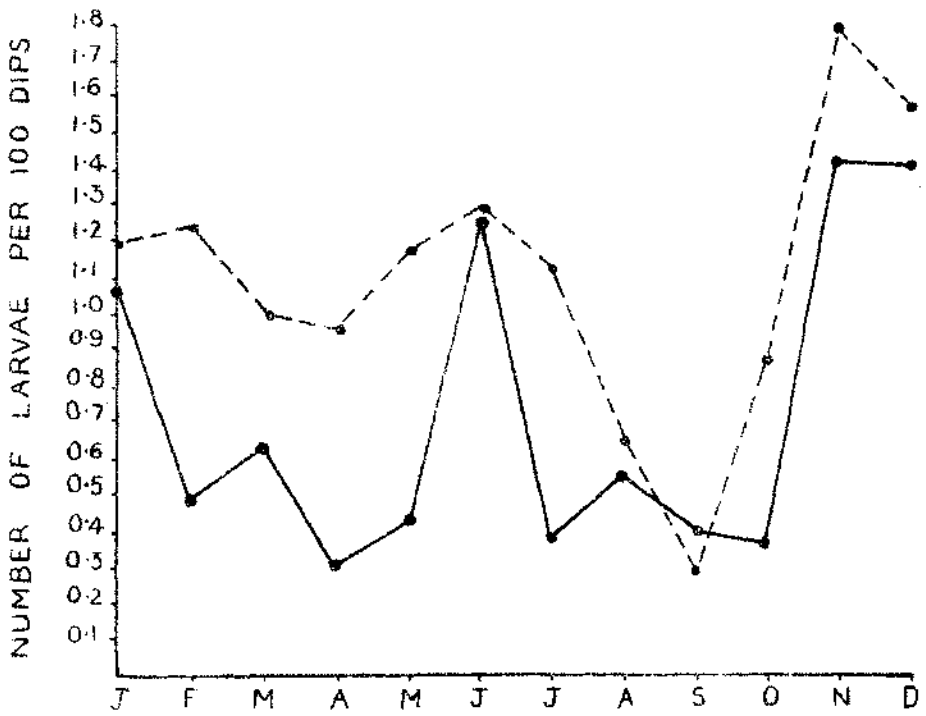
ANOPHELINE PREVALENCE PRIOR TO D.D.T. SPRAYING, 1940–45.

Larval prevalence.—The boundaries of the protected and the controlled zones are indicated in Map 1, the outside zone (untreated area) lying beyond the periphery of the controlled zone. For the purposes of this paper the larval and adult figures for the protected and the controlled zones have been combined as both represent areas in which malaria control measures were carried out in the past. Larval examinations were carried out at Elakattuwa Road and Green Path in the protected and the controlled zone respectively and at Bulankulam and Alankulam (about 2 miles from the Urban Council limits on the Kurunegala Road) in the outside zone. Examinations of water collections were made with a standard ladle and the larval rate is expressed as the number of larvæ per 100 dips.

The average monthly larval rates for *A. culicifacies*, *A. subpictus*, *A. vagus* and the combined rate for all the other anopheline species found from 1940–45 are given in Table II. It will be seen that while anopheline mosquitoes are found

breeding throughout the year, the prevalence of *A. culicifacies* is greatly increased during the north-east monsoon months of November, December and January. The larval rates in these months were 1·41, 1·40 and 1·09 respectively in the protected and controlled zones and 1·78, 1·57 and 1·20 respectively in the outside

CHART I.



Average monthly larval rate for *A. culicifacies* in the protected and controlled zones and in the outside zones (untreated area) at Anuradhapura for 1940–45.

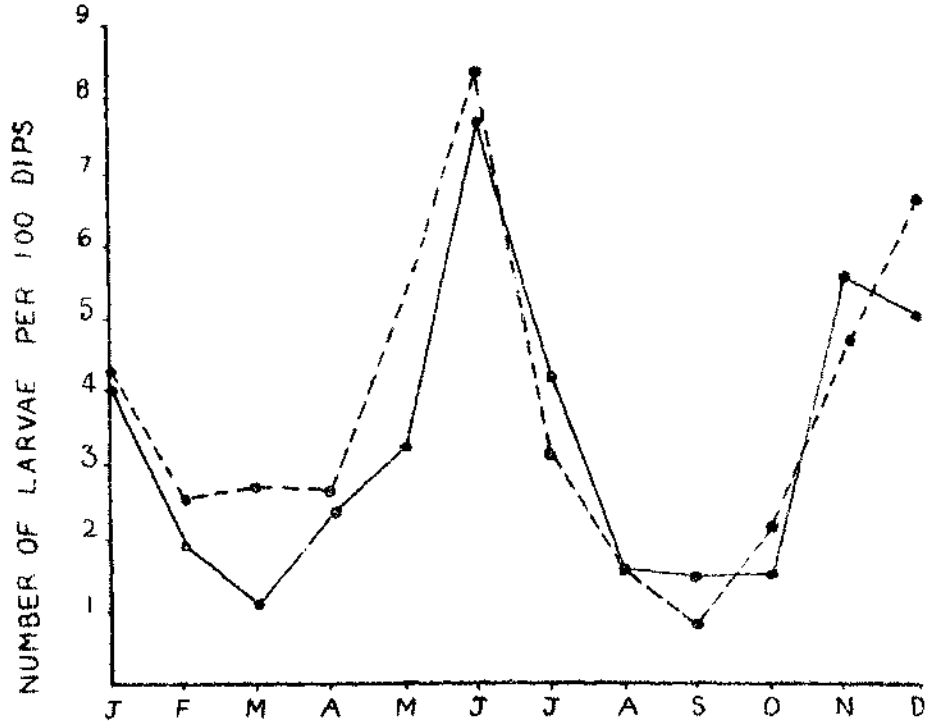
zone. Thus in spite of all the control work done, once the rains arrived, breeding of *A. culicifacies* continued unabated. The main reason for this was that it was quite impossible to oil the innumerable temporary breeding places that were created in the rainy season. The same thing occurred with *A. subpictus*, only here the

density of the mosquito was conside ably greater. In November, December and January the larval rates for *A. subpictus* were 5·55, 5·05 and 4·15 respectively in the protected and controlled zones and 4·42, 6·54 and 4·32 respectively in the outside

CHART 2.

PROTECTED AND CONTROLLED ZONE ———

OUTSIDE ZONE ... ...



Average monthly larval rate for *A. subpictus* in the protected and controlled zones and in the outside zone (untreated area) at Anuradhapura for 1940–45.

zone. After January the prevalence of *A. culicifacies* falls away until June when the irrigation water let into the paddy fields in May for the minor (Yala) crop of

the year facilitates the breeding of the larva until the paddy is quarter grown. South-west monsoonal rains received in these months also cause a certain amount of generalized breeding. From then on, a dry period ensues and *A. culicifacies* once more begins to breed precariously in the more permanent situations until the advent of the north-east monsoon rains. Charts 1 and 2 show this seasonal rhythm clearly for *A. culicifacies* and *A. subpictus* in the protected and controlled zones and outside zone respectively.

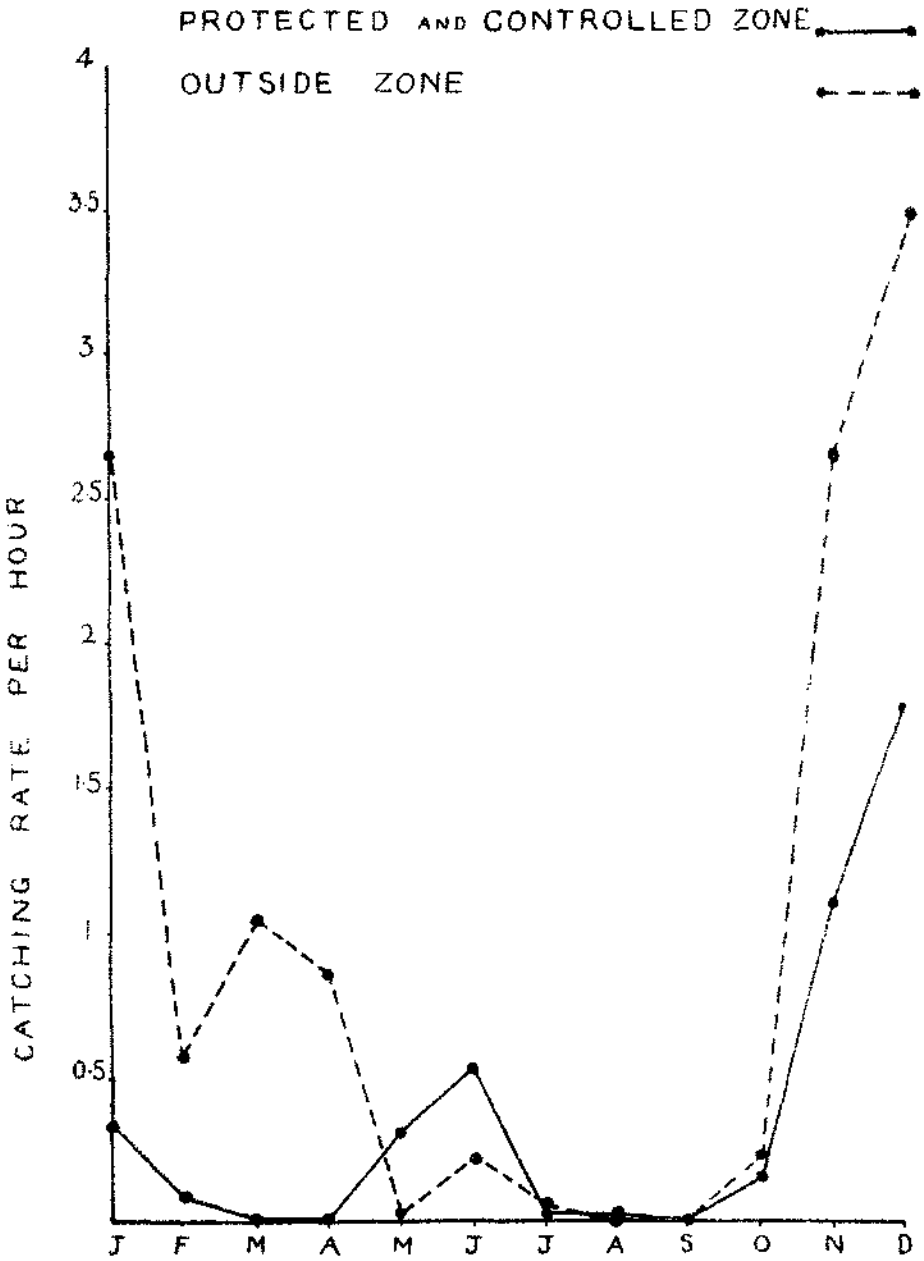
Adult prevalence.—Daytime examination of houses for adults, generally from 7 to 11 a.m., was carried out at Elakattuwa Road in the protected zone, at Jaffna Junction, Y Road and Arippu Road in the controlled zone and at Bulankulam and Alankulam in the outside zone. The results for the period 1940–45 expressed as an average are given in Table III. The adult prevalence of *A. culicifacies* and *A. subpictus* in houses (hardly any other species were taken) follows the same seasonal rhythm discussed in connection with the larval rates. Thus for *A. culicifacies* in the protected and controlled zones, the catching rate per hour in November, December and January was 1·09, 1·76 and 0·33 respectively, while it was 2·66, 3·48 and 2·75 respectively in the outside zone. For *A. subpictus* the catching rates were 21·74, 20·24 and 3·28 respectively in the protected and controlled zones and 22·38, 17·40 and 9·91 in the outside zone. In June, the catching rate for *A. culicifacies* increases slightly to 0·54 in the protected and controlled zones and 0·22 in the outside zone as a result of the breeding that takes place in ground water collections created by the light south-west monsoonal rains and by the irrigation of the paddy fields. The annual seasonal rhythm of adult prevalence of *A. culicifacies* and *A. subpictus* in the protected and controlled zones and outside zone are depicted in Charts 3 and 4.

Cattle baited traps have also been used for trapping adult anopheline mosquitoes. They were set up at Elakattuwa Road in the protected zone at Green Path or Basawakulam in the controlled zone and at Bulankulam in the outside zone. The results of these examinations for the period 1938–42 are given as mean catching rates in Table IV. *A. culicifacies* is not often caught in traps but in spite of it shows a catching rate of 1·23 per hour in December in the protected and controlled zones. *A. subpictus* is however caught in fair numbers unlike in the upper catchment of the Deduru Oya and the Maha Oya basin probably because it breeds so heavily at Anuradhapura. In the protected and controlled zones the catching rate in December was as high as 17·73, while in May the rate was 3·49. The catching rate in traps was considerably lower than in houses for this species throughout the year.

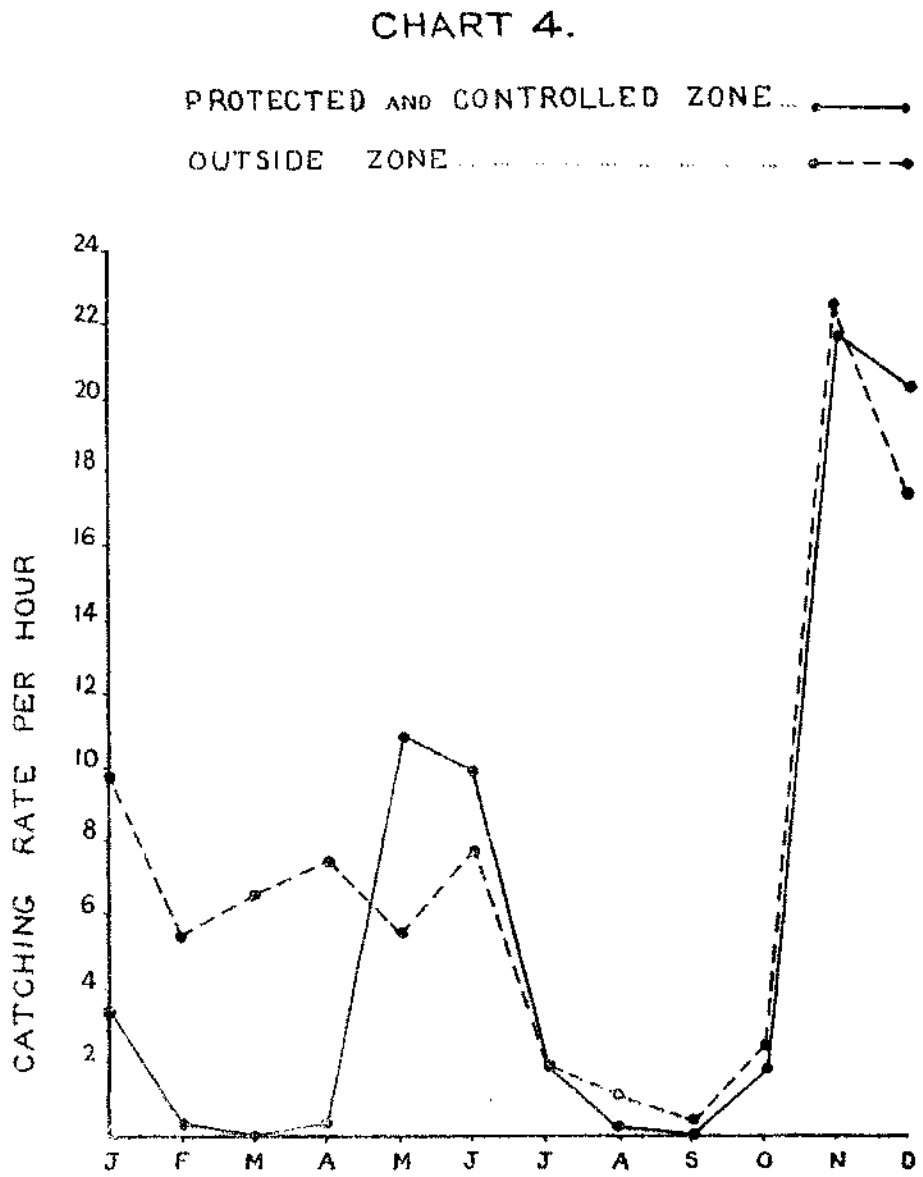
ANOPHELINE PREVALENCE AFTER THE INCEPTION OF D.D.T. SPRAYING, 1946–49.

Once the residual spraying of houses with D.D.T. was inaugurated in November 1945 as the sole malaria control measure, the old zones had to be abandoned and a new series of observations were begun with a view to checking-up on the efficiency of the new method of control. As at this time little was known about the duration of the residual action of D.D.T., much attention was paid to the daytime examination of dwellings for adults of *A. culicifacies*. Thus in January 1946 a group of about 25 houses in Elakattuwa Road (all the place names mentioned are

CHART 3.



Average monthly catching rate per hour for *A. culicifacies* caught in dwellings in the protected and controlled zones and in the outside zone (untreated area) at Anuradhapura for 1940–45.



Average monthly catching rate per hour for *A. subpictus* caught in dwellings in the protected and controlled zones and in the outside zones (untreated area) at Anuradhapura for 1940–45.

indicated in Map 1) were examined on 15 days for mosquitoes and Ponnarankulama, on the south-eastern outskirts of the town, beyond the Malwatu Oya was kept for comparison. Trapping of mosquitoes with cattle-baited traps at these two sites was carried out as well as larval examinations. In March, daytime house examinations were begun at Jaffna Junction for 5 days when about 15 houses were examined at each visit. As time went on and scarcely any anopheline mosquitoes were being found in dwellings, the programme of work was more evenly distributed at Elakattuwa Road, Jaffna Junction and Ponnarankulama. Thus by November 1946, house examinations were being carried out on 4 days each month at Elakattuwa Road, 8 days at Jaffna Junction and 4 days at Ponnarankulama; cattle-traps were set up for two hours each night on 4 nights at Elakattuwa Road, 3 nights at Jaffna Junction and 2 nights at Ponnarankulama. Larval examinations were being carried out on 3 days at Elakattuwa Road, but on the 3 days allotted to Ponnarankulama no work could be done owing to heavy monsoonal rains.

In February 1947, the programme of work was further reorganized and two villages, Mankadawela, 2½ miles north-east of the town on the Jaffna Road, and Ratmale Colony, ¾ mile south-west of the town on the Kurunegala Road, were also examined three times a month for adult mosquitoes in dwellings, while larval examinations were also carried out at each visit. In the town, in addition to the houses at Elakattuwa Road and Jaffna Junction, houses were selected at Kurunegala Road and Malwatu Oya Lane and each group of houses was examined on two occasions each month. The cattle-trap at Elakattuwa Road was discontinued at the end of January 1947 and from February 1947 traps were set up on 2 nights each week at Jaffna Junction and Malwatu Oya Lane. The larval examinations at Elakattuwa Road were also discontinued at the end of January 1947, and from February 1947 examinations were made at Kurunegala Road and Malwatu Oya Lane on 2 days each month. At Ponnarankulama from February 1947, house examinations were carried out on 2 days each month, cattle-traps were set up on 2 nights each month and a monthly larval examination was carried out. This programme continued until July 1948 when the cattle-trapping in the town was reduced to one night a month at Jaffna Junction and Malwatu Oya Lane and the trapping at Ponnarankulama was abandoned. Instead, night-time catches, lasting from dusk till about 9 p.m., were commenced in houses at Mankadawela, Jaffna Junction and Ponnarankulama on 4 nights each month.

Ponnarankulama, the untreated comparison centre, lies to the south-east of Anuradhapura and was very difficult of access at the commencement of these investigations in November 1945. It is the site of the new town and a new road is being constructed but has not reached as yet the group of houses which have been examined since 1945. There were in all about 70 houses scattered about, but only about 18 of them were within about a quarter of a mile of the river and were therefore selected for examination. For comparison, it was not the most satisfactory but no other village was available, in which the people would have readily acceded to the authors' request that no D.D.T. spraying should be done. The river was the main source of anopheline breeding, apart from four or five wells, which soon after the rains run dry. The breeding of *A. culicifacies* in rivers only becomes pronounced when pooling occurs. The Malwatu Oya is generally full of water, from bank to bank, after a normal north-east monsoon, and the volume

of water generally begins to subside only about September each year. When, however, the monsoon fails as it did in 1947, pooling becomes pronounced much earlier and intense breeding of *A. culicifacies* takes place with relatively high catches of the adult as occurred from July onwards in 1948. In normal years of rainfall there are few breeding places except the river, and in the months of November and December when anopheline larvæ should be breeding in ground water collections, Ponnarankulama is virtually cut off by the flooding of the river.

Larval prevalence in Anuradhapura Town.—The results of larval examinations carried out at Elakattuwa Road from January 1946 to January 1947 and thereafter at Kurunegala Road and Malwatu Oya Lane for the period 1946–49 are summarized in Table V. It will be seen that the seasonal rhythm of *A. culicifacies* breeding, already referred to, has been completely upset (Table II, Chart 1). The presence of *A. culicifacies* in 1946 and 1947 was negligible, but heavy breeding occurred in July, August and September 1948, when larval rates of 9·09, 21·19 and 16·16 respectively were recorded. These larval rates are higher than any recorded during the period 1940–45, even in the untreated outside zone in the months of November and December, when breeding was at its greatest. This heavy breeding of *A. culicifacies* was the direct result of the failure of the north-east monsoon of 1947, as a result of which considerable pooling of the Malwatu Oya, which runs through the town, took place. In these months, the larvæ of *A. culicifacies* were collected entirely from the river. The partial failure of the monsoon of 1948 again caused pooling of the Malwatu Oya in 1949 and as a result a larval rate of 19·78 for *A. culicifacies* was recorded in the town in September.

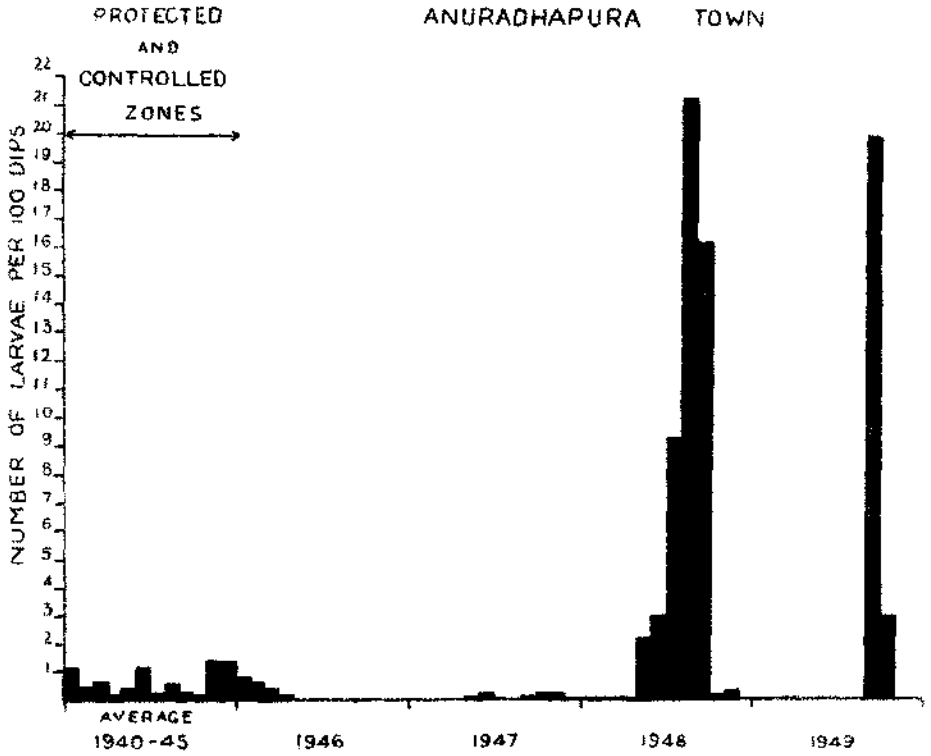
But even more remarkable has been the greatly reduced incidence of *A. subpictus*, normally the most prolific breeding mosquito at Anuradhapura. In December 1946, a larval rate of 6·41 was recorded for this mosquito. Since then the highest larval rate recorded was only 1·88 in November 1948. On the other hand the larval rate for the total of all other anopheline species, during the period 1946–49, has been consistently high, often very much higher, particularly in November and December each year, than the average larval rates recorded for the protected and controlled zones and the outside zone (Table II). These mosquitoes are the non-domestic species rarely found in houses.

The general decline in the prevalence of *A. culicifacies* larvæ at Anuradhapura during the period 1946–49 is clearly shown in Chart 5, where the larval prevalence in these years is compared with the average monthly larval rates recorded for the protected and controlled zones for the years 1940–45. Only in 1948 and 1949, when the Malwatu Oya pooled heavily owing to the drought, was *A. culicifacies* at all prevalent, but the normal seasonal rhythm has not been in evidence at all. In fact the high figures recorded in 1948 and 1949 were for months which in normal years yielded the lowest larval rates.

Larval prevalence outside Anuradhapura Town.—At Mankadawela, larval examinations commenced in February 1947 and the results for the years 1947–49 are summarized in Table VI. Throughout 1947, no *A. culicifacies* were recorded but in 1948, after D.D.T. spraying was deliberately stopped in July, larval rates of 8·14 and 1·89 were recorded in October and November respectively. However, owing to protests from the villagers, spraying was resumed at the end of November and thereafter larval rates of 0·95 and 0·19 were recorded in October and November

1949. It is significant that the larval rates for *A. subpictus* were also high during the period when the spraying of houses was temporarily abandoned, being 1·36 in October and 22·08 in November 1948. After the resumption of spraying, this larva was only recorded again in October 1949, when the larval rate was 0·57. Once again the larval rates for the total of all other anopheline species has been consistently high, and far higher than those recorded for the town.

CHART 5.



Larval rate for *A. culicifacies* by months at Anuradhapura for 1946–49 compared with the average monthly prevalence of *A. culicifacies* in the protected and controlled zones for 1940–45.

At Ratmale Colony, similar larval conditions have been experienced. The typical seasonal rhythm displayed by *A. culicifacies* and *A. subpictus* has once more not been experienced, and in only 5 out of 35 months in which larval examinations were made, were larvæ of *A. culicifacies* recorded, the highest rates being 1·92 in June 1948 and 1·20 in November 1949 (Table VII).

Larval prevalence at Ponnarankulama (untreated area) outside Anuradhapura Town.—It has already been pointed out that Ponnarankulama is not an area with

breeding places typical of the town and district. Therefore, although *A. culicifacies* has been recorded in 18 out of the 43 months in which examinations were made, the normal seasonal rhythm usually displayed by *A. culicifacies* has not been experienced (Table VIII). The virtual absence of *A. subpictus* in this untreated area is probably due to lack of suitable breeding places. *A. subpictus* does not normally breed to any extent in rivers (Rajendram and Jayewickreme, *loc. cit.*), and the Malwatu Oya is the chief breeding place at Ponnarankulama. The larval rates for the total of all other anopheline species has been on a very high level except on twelve occasions. Larval rates as high as 137·92 in September 1947 and 88·07 in September 1949 have been recorded.

Chart 6 shows the prevalence of *A. culicifacies* at Ponnarankulama during 1946–49, compared with the average larval rates recorded in the outside zone (untreated area) for the period 1940–45. The seasonal rhythm seen in the larval rates for the outside zone has not been seen at Ponnarankulama due chiefly to the fact that the only real source of breeding has been the river. But whenever the river pooled high catches of *A. culicifacies* were recorded, the larval rates being particularly high in June 1947 (11·95), September and October 1948 (16·88 and 34·81 respectively) and September 1949 (11·92).

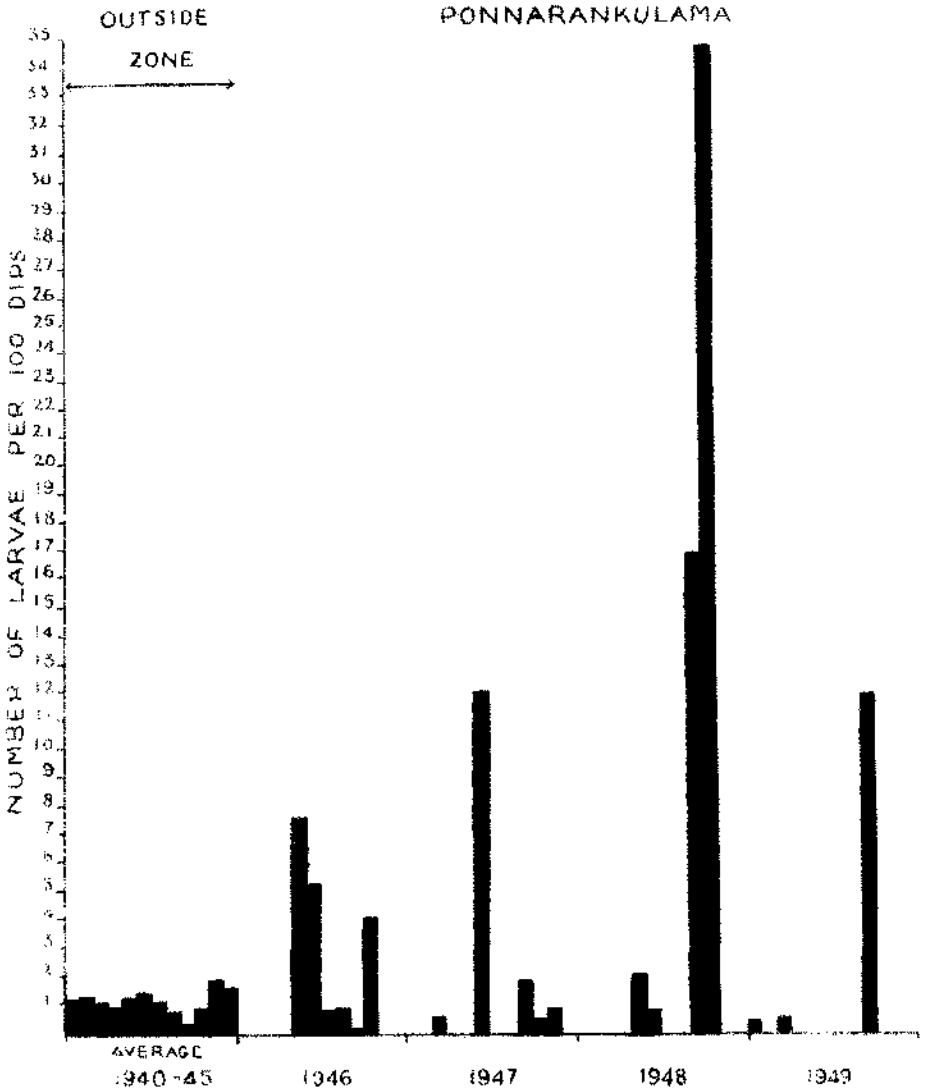
Daytime adult catches in dwellings at Anuradhapura.—The results of the daytime hand catches of adult anopheline mosquitoes are summarized in Table IX. They relate only to female anopheline mosquitoes and during the four years in which observations were made *A. culicifacies* was only once recorded in November 1947. The catches of *A. subpictus* were also scanty and the normal seasonal rhythm seen for the period 1940–45 has not been in evidence. The catching rate for the total of all other anopheline species caught has also been very low, except in the month of May 1947 when unusually high catches of *A. vagus* and *A. jamesi* species, that are not often found in dwellings, raised the catching rate to 4·12 per hour.

Daytime adult catches in dwellings outside Anuradhapura.—The results for Mankadawela from February 1947 to December 1949 are summarized in Table X. Only once was *A. culicifacies* found and that too in October 1948 (catching rate 0·14), when the D.D.T. spraying had been temporarily suspended. During this period catching rates of 6·48 and 19·33 respectively were recorded for *A. subpictus* in October and November 1948, otherwise this mosquito was also rarely caught. The catching rates for the total of all other anopheline mosquitoes were also very low.

At Ratmale Colony (Table XI), *A. culicifacies* and *A. subpictus* were not recorded at all in 1948 and 1949. In 1947, *A. culicifacies* was caught in February and March only (catching rates 0·09 and 0·11 respectively) and *A. subpictus* in April, May and November only (catching rates 0·11, 0·43 and 0·14 respectively). The catches of all other anopheline mosquitoes were also negligible.

Daytime adult catches in dwellings at Ponnarankulama (untreated area).—These catches are summarized in Table XII. In 1946 and 1947, when the Malwatu Oya did not pool and the larval density of *A. culicifacies* was very low, catches of adults at Ponnarankulama were also low, but there was some indication of the normal seasonal rhythm. The highest catches, 3·33 per hour in November 1946 and 1·71 in December 1947, were associated with the north-east monsoon, while in 1947

CHART 6.



Larval rate for *A. culicifacies* at Ponnarankulama (untreated area) from 1946–49 compared with the average monthly prevalence of *A. culicifacies* in the outside zone (untreated area) at Anuradhapura for 1940–45.

there were catching rates of 0·28 and 0·10 recorded in May and June. In 1948 when pooling occurred quite early in the Malwatu Oya, *A. culicifacies* adults were found in every month of the year except March. The catching rates from July to November were particularly high, ranging from 26·27 in July to 14·72 in November. No *A. culicifacies* adults were recorded in the town during these months (Table IX). *A. subpictus* was rather scanty, except in November and December 1947 when catching rates of 6·25 and 10·85 were recorded and in November and December 1948 when the catching rates were 5·12 and 7·20 respectively. The other anopheline species caught were negligible.

Night-time adult catches in dwellings at Anuradhapura.—These observations were carried out from July 1948 till December 1949 at Jaffna Junction and are summarized in Table XIII, and no *A. culicifacies* or *A. subpictus* were found in dwellings. Only in July 1948 were any mosquitoes caught at all, these being *A. hyrcanus* and *A. varuna*.

Night-time adult catches in dwellings outside Anuradhapura.—These observations were carried out at Mankadawela from July 1948 till December 1949 and revealed no adult *A. culicifacies* in houses in the early part of the night (Table XIII). The catches both here and at Jaffna Junction were made from dusk until 9 p.m.

Night-time adult catches in dwellings at Ponnarankulama (untreated area).—At Ponnarankulama catches were also made from July 1948 till December 1949 (Table XIV). From July 1948 to February 1949 catches of *A. culicifacies* were recorded every month. These ranged from 37·80 per hour in September 1948, when pooling in the Malwatu Oya was intense, to 1·73 in February 1949. From March to August 1949 no catches were made but once again from September onwards catches of *A. culicifacies* were made each month till November. The catching rates recorded were 20·00 in September, 8·00 in October and 4·57 in November.

Adults caught in cattle-baited traps at Anuradhapura.—Traps were set at Elakatturwa Road, Jaffna Junction and Malwatu Oya Lane from 1946–49. The trap at Elakatturwa Road was discontinued in January 1947. The results summarized in Table XV show that *A. culicifacies* was caught only twice in the trap in June 1946 and April 1948, when the catching rates were only 0·06 and 0·25 respectively.

A. subpictus was caught more often in traps than in dwellings, particularly in 1947. But from January 1948 the catches were relatively poor, except in December 1946 and April 1949, when catching rates of 15·08 and 15·50 respectively were recorded. Thus these trap figures indicate that *A. subpictus* adults were gradually diminishing at Anuradhapura. During the north-east monsoon month of December 1949, no *A. subpictus* were caught at all. Table IV shows that this was the month, prior to the use of D.D.T., in which the highest catches of *A. subpictus* were recorded in the period 1940–45.

Adults caught in cattle-baited traps at Ponnarankulama (untreated area).—No *A. culicifacies* were caught in traps at Ponnarankulama from January 1946 to June 1948, after which trapping was abandoned at this site. The catches of *A. subpictus* were not so heavy as in the town, probably because satisfactory breeding places for this mosquito were not available (Table XVI).

MALARIAL MORBIDITY.

Malarial morbidity in this town for the period prior to 1946 closely followed the seasonal rhythm of *A. culicifacies* prevalence depicted in Charts 1 and 3. Table XVII summarizes the actual malaria attendance clinically diagnosed at the Civil Hospital for the period 1938-49.

The monthly malaria attendance at the Civil Hospital and the rainfall at Anuradhapura for the period 1940-49 are shown in Chart 7. It will be noticed that each year the incidence of malaria begins to rise in November, coinciding with the arrival of the north-east monsoon, and reaches its peak in January and thereafter begins to decline. This is the main seasonal wave of malaria each year and from 1938 to 1945 it has always occurred. Actually, records going back to the inception of the Campaign Centre in 1923 show that this seasonal rhythm has never been interrupted. There is a smaller wave in June, coinciding with the south-west monsoon and the irrigation of the paddy fields in connection with the Yala rice crop. The morbidity in May and June is also probably augmented by the influx of Buddhist pilgrims from all parts of the island for the celebration of the *Wesak* and *Poson* festivals. For the period 1940-45 depicted in Chart 7, these seasonal rises have occurred regularly. From 1946, the incidence of malaria has been steadily on the decline and the normal seasonal rhythm has been completely absent.

The total number of malaria attendances at the Civil Hospital has also shown a considerable decline. For the four years 1938-41, this has exceeded 18,000 attendances per year. From 1942, when 14,700 cases were treated, there was a steady decline in the morbidity up to 1945, when 10,034 cases were treated. From 1946, the decline in malaria attendances has been most marked and from 4,380 attendances in 1946 the figure has reached the extremely low one of 1,797 in 1949.

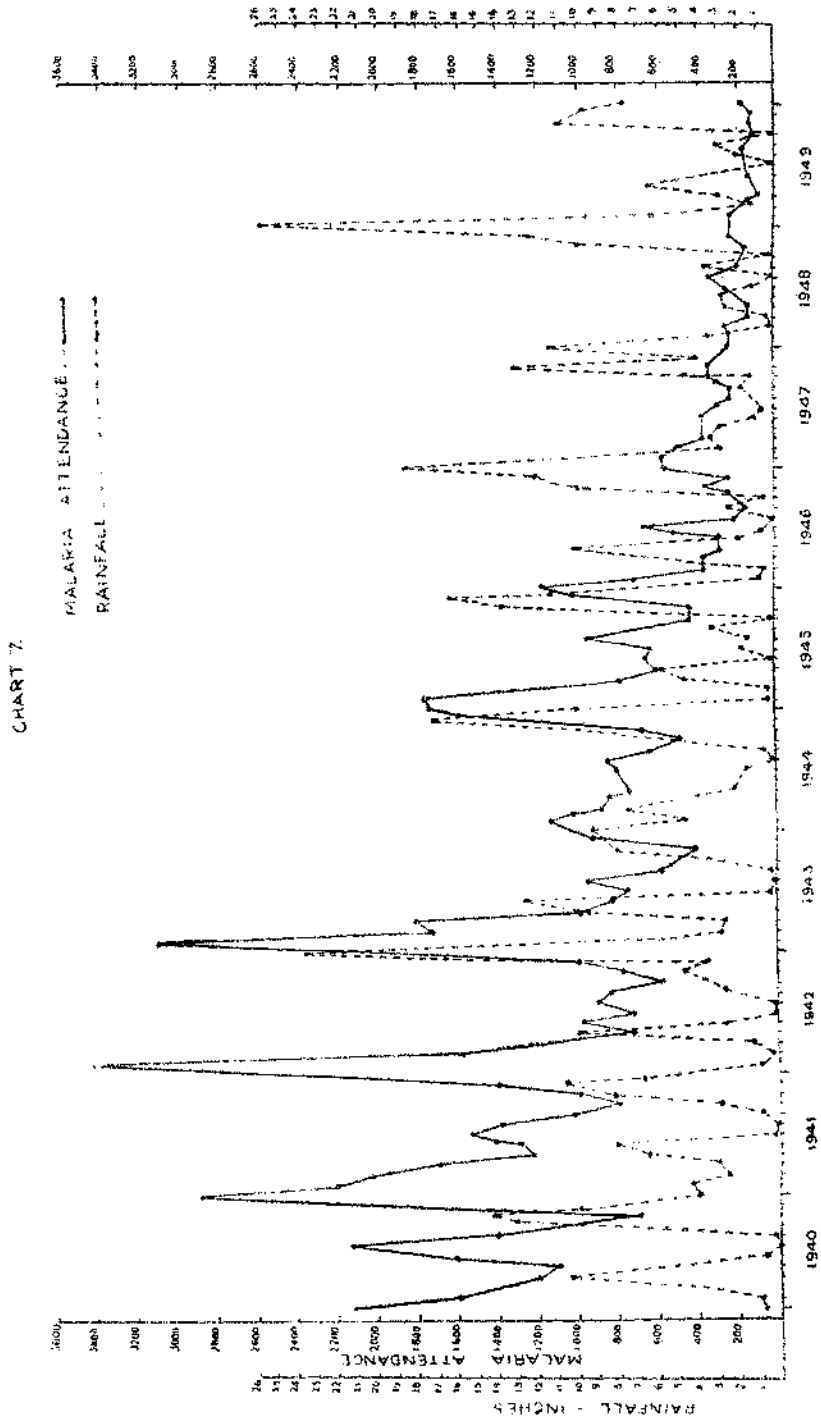
SPLEEN AND PARASITE RATES.

Spleen examinations of school children have been carried out each March from 1936 to 1949, except in 1942, and in September from 1943 to 1949, and these results are summarized in Table XVIII. For the period prior to 1946 the spleen rates have always been high. In March 1936, after the malaria epidemic of 1934-35 which also affected this hyperendemic area, the spleen rate was 70·2 per cent. It gradually declined to 58·8 in March 1939 and rose again to 60·8 in 1940. From 1941 onwards, there was an improvement till in March 1944 the spleen rate had come down to 29·1, the lowest figure recorded up to then. In September of this year the spleen rate was even lower (23·2). From 1946, the spleen rates have steadily declined till in March 1949 it was 2·6 and in September 1949 it had declined to 1·1.

The parasite rates for the period 1939-49 are given in Table XIX. They range from 16·4 in 1939 to 4·0 in 1944, while in 1945 the rate was 6·6. From 1946 onwards the rate has declined, except in 1948 when it was 2·4. But in 1949 the rate was only 0·3 with only a single quartan infection recorded.

DISCUSSION.

From the inception of the residual spraying of houses with a 5 per cent solution of D.D.T. in kerosene in November 1945, the most noticeable feature was the virtual



Monthly malaria attendance at the Civil Hospital, Anuradhapura, and rainfall for 1940–49.

absence of adult mosquitoes in houses examined in the day time. It was therefore assumed that this was due to the successful control of both domestic anopheline species found at Anuradhapura, *A. culicifacies* and *A. subpictus*. As the morbidity due to malaria was considerably on the decline in 1946, the total number of attendances at the Civil Hospital being 4,380 in 1946 as against 10,034 in 1945, there was no reason to doubt that the control of the vector had been achieved for the first time in the history of antimalaria operations in the town. This was further confirmed by the larval catches of *A. culicifacies* in the town in 1946-47, when for the first time the normal seasonal rhythm of increased prevalence in November, December and January and again the subsidiary rise in June were absent. Larval catches of *A. subpictus* too from May 1946 were exceedingly low and showed that the density of this domestic mosquito had also been affected by the residual spraying of houses.

Outside the town too at Ratmale Colony and Mankadawela, the larval catches of *A. culicifacies* and *A. subpictus* were also negligible, except during the month of October and November 1948 when the houses at Mankadawela were left unsprayed. But the larval catches of the non-domestic mosquitoes, *A. varuna*, *A. hyrcanus*, *A. vagus* and others, were generally higher than in previous years. The complete absence of larvicidal control which had been in operation since 1923 in the town, and the lack of competition from *A. subpictus* and *A. culicifacies* had evidently permitted these mosquitoes to breed in greater numbers than ever before both inside and outside the town.

At Ponnarankulama, the untreated area, fairly high larval catches of *A. culicifacies* had been recorded in 1946 and also in June 1947. Unfortunately, a strict comparison with conditions prior to the residual spraying of houses with D.D.T. is not possible, as the principal breeding place was the Malwatu Oya and *A. culicifacies* generally breeds heavily only when pooling occurs in rivers. No work had ever been done in this area before owing to its inaccessibility. It is probable that no suitable breeding places normally exist for *A. subpictus* as most of the wells run dry a short time after the rains, while no borrowpits or any semi-permanent ponds are found here. In 1946 and 1947, adult catches of *A. culicifacies* were negligible at Ponnarankulama.

Thomson (1947) first showed that in West Africa the absence of *A. gambiae* in houses was due to the repellent effect of D.D.T. and that the absence of adults in the day time was due to this fact alone. Accordingly, it was felt that in spite of the great success of the control experienced up to June 1948, as reflected in the markedly reduced incidence of malaria in the town, the almost complete absence of adult mosquitoes in dwellings both inside and outside the town, and the comparative absence of the larva of *A. culicifacies*, some attempt should be made to establish the presence of the adult if it were breeding in adequate numbers. For this purpose it was decided to examine houses at night for adult mosquitoes at Jaffna Junction in the town, at Mankadawela outside the town and at Ponnarankulama, which was taken up for comparison. The results were surprising. At Ponnarankulama from July 1948 to February 1949, adults of *A. culicifacies* were obtained at every visit except one (the last examination made in December). House examinations at each visit lasted from dusk till about 9 p.m. and those made at Jaffna Junction and Mankadawela yielded no adult *A. culicifacies* at all. At

Ponnarankulama no adults were captured between March and August 1949, but thereafter in September, October and November, adult *A. culicifacies* were found at night in dwellings. The absence of *A. culicifacies* at Jaffna Junction and Mankadawela was probably due to a reduction in the larval density and not due to the repellent effect of D.D.T. At Mankadawela in October and November 1948 larvæ of *A. culicifacies* were found breeding, particularly in October when the larval rate was 8·14, as the houses there had been left unsprayed from July till the end of November. Yet the density of *A. culicifacies* was apparently not very high for no adults were coming into the houses in the early part of the night as at Ponnarankulama. In the town, heavy breeding of the vector, far greater than in any previous year prior to 1946, was taking place from May to September 1948, the breeding being confined entirely to the Malwatu Oya, which because of the drought was a series of isolated pools. In spite of this, no adults were caught at Jaffna Junction even at night; the houses examined here were within a quarter of a mile from the river. In September and October 1949, larvæ of *A. culicifacies* were found breeding in the Malwatu Oya again but no adults could be taken at Jaffna Junction at night. At Ponnarankulama heavy breeding was also taking place in September 1949 and adults were caught in houses, situated less than 50 yards from the river, in September, October and November 1949.

In normal years of rainfall prior to the use of D.D.T. as a residual insecticide in November 1945, the larval and adult densities of *A. culicifacies* rose in November, December and January each year with the advent of the monsoonal rains. A subsidiary rise in June each year also occurred. Since the use of D.D.T., the density of *A. culicifacies* has evidently been reduced to such a low level that malaria transmission has been virtually halted. Otherwise the heavy increase in the larval rates of *A. culicifacies* recorded in July, August and September 1948, far greater than any larval rates for this species known before at Anuradhapura, and again in September 1949 should have resulted in an increase in the incidence of malaria in the town. That this did not occur shows that D.D.T. was quite effective in destroying the adult in spite of the great increase of the density of the larva breeding in restricted situations in the Malwatu Oya. Thus the absence of *A. culicifacies* in house examinations made during the day time, is probably not due to a repellent effect. Instead, it is probable that *A. culicifacies* is effectively destroyed by D.D.T. and a significant reduction in the density of the vector has been achieved.

The other predominantly domestic species, *A. subpictus*, has also been controlled to a very great extent. It has been absent from houses examined in the day time as well as at night. Its nocturnal movements in Ceylon are not well known but its absence at Ponnarankulama at night merely means that it was not breeding there at all. In the usual types of breeding place examined in the town and outside it at Ratmale Colony and Mankadawela, it has been found, when present, in very small numbers. Prior to 1946, this species was the most prolific of all mosquitoes at Anuradhapura. Although adults had been taken in fair numbers in cattle-traps in 1946 and 1947, in 1948 and 1949 the catches were very low, except in April 1949, when a catching rate of 15·50 per hour was recorded. It is apparent that the density of this domestic mosquito is in the process of being reduced to very negligible proportions in the dry zone.

In this hyperendemic dry zone area the annual seasonal periodicity of malaria associated with the north-east monsoon with a rise in the fever index in November, December and January and again in June each year has been completely brought under control. Thus from 1946 onwards, no such seasonal periodicity has been observed and the annual figure of 1,797 malaria attendances at the Civil Hospital is the lowest on record. Similarly, the spleen and parasite rates have now been brought to such a low level that Anuradhapura can now be truly considered a healthy town and this has been achieved after only four years of residual spraying of houses with D.D.T.

But it is not only in Anuradhapura that the residual spraying of houses has been so successful in rendering a once hyperendemic area into a healthy one. In Appendix I is given the crude death rates and infant-mortality rates for the years 1938 and 1946–49 and in Appendix II the spleen and parasite rates of school children are given by districts for 1939 and 1946–49. In addition to Anuradhapura, there are three other wholly hyperendemic districts in Ceylon — Trincomalee, Hambantota and Vavuniya. The crude death rate which ranged from 31·4 to 48·8 per 1,000 population in these districts in 1938 has been reduced to a range of 11·4 to 12·7 in 1949. The infant-mortality rate which ranged from 174 to 292 per 1,000 live births in 1938 has been lowered to a range of 81 to 109 in 1949. The spleen and parasite rates are equally striking. Spleen rates ranging from 57·5 to 66·7 per cent in 1939 came down to a range of 7·1 to 11·6 in 1949, and the parasite rates which ranged from 6·7 to 12·8 per cent in 1939 in these four districts, ranged from 0 to 0·8 in 1949.

There are five districts, namely Matale, Kurunegala, Mannar, Puttalam and Batticaloa, which used to be areas of high endemicity (Table VI of Rajendram and Jayewickreme, *loc. cit.*). In 1938, the crude death rates in these five districts ranged from 25·1 to 39·2 per 1,000 population. By 1949, these had been reduced to a range of 11·9 to 16·1. The infant-mortality rates which in 1938 ranged from 186 to 342 per 1,000 live births had by 1949 been reduced to a range of 89 to 125. The spleen and parasite rates given in Table II of Rajendram and Jayewickreme (*loc. cit.*) have also shown similar reductions. Thus, in 1939, the spleen rates ranged from 33·0 to 46·2 but in 1949 they had come down to a range of 2·6 to 7·8, while the parasite rates which had ranged from 6·3 to 8·4 in 1938, had come down to 0 to 1·0 in 1949.

Nothing further need be said on the extraordinary success achieved in the control of *A. culicifacies* by the residual spraying of houses with D.D.T. in the hyperendemic and highly endemic areas of Ceylon. For the first time in these greatly afflicted areas, in which malaria had for centuries retarded the agricultural progress of the peasants, malaria transmission has apparently been brought to a halt.

SUMMARY.

MALARIA CONDITIONS PRIOR TO THE USE OF D.D.T. AS A RESIDUAL INSECTICIDE.

1. The larval and adult densities of *A. culicifacies* increased each year in November, December and January with the arrival of the north-east monsoon.

A smaller, subsidiary rise occurred in June. *A. subpictus*, the other domestic species, behaved in a similar manner.

2. The annual, seasonal increase in the larval and adult densities of *A. culicifacies* and *A. subpictus* occurred in the former protected and controlled zones, where antimalaria measures had been adopted, as well as in the outside zone which was an untreated area.

3. The incidence of malaria followed the same trend as the density of *A. culicifacies*, beginning to rise in November, reaching its peak in January and thereafter declining. A smaller rise occurred in June.

4. The total malaria attendances at the Civil Hospital ranged from 18,939 in 1939 to 10,034 in 1945.

5. Spleen rates among school children varied between 70·2 in March 1939 and 23·2 in September 1944.

6. Parasite rates varied between 16·4 in 1939 and 4·0 in 1944.

MALARIA CONDITIONS AFTER THE USE OF D.D.T. AS A RESIDUAL INSECTICIDE.

7. The annual, seasonal increases in the larval and adult densities of *A. culicifacies* and *A. subpictus* have not occurred.

8. Daytime adult catches of *A. culicifacies* and *A. subpictus* at Anuradhapura and outside it at Mankadawela and Ratmale Colony have generally been zero. At Ponnarankulama, the untreated area, catches of *A. culicifacies* have been made but these have not generally been in November, December and January as one would have expected, but during the drier months of the year, when pooling of the Malwatu Oya resulted in an increased density of *A. culicifacies* larvæ.

9. High catches of adult *A. culicifacies* were made in houses examined from dusk till about 9 p.m. at Ponnarankulama from July 1948 to February 1949 and again in September to November 1949. Corresponding catches made at Jaffna Junction in Anuradhapura and Mankadawela revealed no adults entering houses at night, although in the Malwatu Oya, only a quarter of a mile away from Jaffna Junction, *A. culicifacies* was breeding heavily.

10. D.D.T. as a residual insecticide, used at dosages ranging from 50–100 mg. per square foot, has evidently reduced the density of *A. culicifacies* to such a low level that no transmission appears to be taking place at Anuradhapura. The greatly increased larval density of the vector which occurred in September and October 1948 and in September 1949 did not result in an increase in the incidence of malaria.

11. The greatly reduced incidence of malaria could not have been achieved unless D.D.T. has effectively destroyed the adults of *A. culicifacies* entering houses.

12. The total malaria attendance at the Civil Hospital, Anuradhapura, was in 1949 only 1,797.

13. Spleen rates in March and September 1949 were 2·6 and 1·1 respectively.

14. The parasite rate in 1949 had been reduced to 0·3 per cent.

15. Considerable reductions in the crude death rates, infant-mortality rates, spleen and parasite rates have occurred in the four hyperendemic districts of Anuradhapura, Trincomalee, Hambantota and Vavuniya, and in the five highly endemic districts of Matale, Kurunegala, Mannar, Puttalam and Batticaloa.

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TABLE I.

Monthly rainfall in inches at Anuradhapura from 1940–49.

Year.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	TOTAL.
1940	0·70	0·99	3·75	10·37	5·33	0·67	0·00	0·10	9·78	14·20	7·47	4·06	56·79
1941	4·33	2·53	3·09	6·30	8·00	0·22	0·00	0·82	2·78	8·35	10·65	5·31	52·38
1942	0·76	0·37	1·22	9·90	2·55	0·12	0·06	2·31	3·31	4·67	3·49	23·57	52·33
1943	6·42	2·89	2·45	9·37	12·67	0·16	0·02	0·09	3·23	7·98	8·65	9·21	63·14
1944	4·76	7·30	5·14	2·26	1·87	1·55	0·00	0·57	5·30	10·43	17·18	9·64	66·00
1945	0·24	0·32	4·42	5·78	0·00	1·83	1·41	3·21	0·00	11·79	16·20	7·39	52·59
1946	0·92	0·66	6·15	10·27	1·73	0·60	0·13	2·51	0·53	9·00	12·12	18·54	64·99
1947	8·51	2·71	3·32	2·75	1·27	0·73	1·17	1·65	1·30	13·98	3·99	11·24	52·62
1948	3·47	0·12	0·38	2·57	2·72	1·27	0·03	3·45	0·00	8·91	12·56	25·77	61·25
1949	6·03	1·33	3·08	6·39	3·22	0·04	1·69	2·93	0·03	10·95	9·92	7·79	53·40
AVERAGE ... 1911–40	5·80	1·72	4·15	6·43	3·54	0·73	1·32	1·62	3·76	9·72	10·67	7·52	56·98

TABLE II.

Prevalence of anopheline larvæ in the protected and controlled zones and the outside zone at Anuradhapura expressed as an average for the years 1940–45.

Month.	Number of dips.	*A. culicifacies*		*A. subpictus.*		*A. rugus.*		Other anopheline species.*	
		Average number collected.	Larval rate per 100 dips.	Average number collected.	Larval rate per 100 dips.	Average number collected.	Larval rate per 100 dips.	Average number collected.	Larval rate per 100 dips.
Protected and controlled zones.									
Jan.	2,653	28·80	1·09	109·00	4·15	0·00	0·00	113·20	4·26
Feb.	2,118	10·20	0·48	40·40	1·91	4·40	0·21	120·00	5·66
Mar.	2,106	13·00	0·62	23·30	1·11	2·00	0·09	115·00	5·49
Apr.	2,155	6·33	0·29	50·99	2·36	2·50	0·12	76·86	3·57
May	2,428	10·00	0·41	79·46	3·26	7·00	0·28	81·28	3·34
Jun.	2,652	32·83	1·24	203·99	7·69	4·33	0·16	40·71	3·64
Jul.	2,807	10·32	0·37	119·00	4·24	2·16	0·08	133·13	4·72
Aug.	1,899	10·32	0·54	29·83	1·57	0·99	0·05	109·30	5·76
Sep.	1,713	6·66	0·39	24·99	1·47	0·00	0·00	79·06	4·51
Oct.	1,871	6·83	0·36	28·16	1·50	0·00	0·00	53·85	3·14
Nov.	2,546	35·99	1·41	141·32	5·55	2·00	0·08	115·30	4·51
Dec.	2,886	40·40	1·40	145·80	5·05	1·16	0·04	85·40	2·96
Outside (untreated area) zone.									
Jan.	1,412	17·00	1·20	61·00	4·32	0·00	0·00	72·11	5·09
Feb.	788	9·80	1·24	19·00	2·54	9·40	1·19	95·40	12·12
Mar.	1,225	12·25	1·00	32·75	2·68	0·75	0·06	106·75	8·70
Apr.	1,113	10·66	0·96	29·33	2·64	7·00	0·63	108·65	9·76
May	1,040	12·16	1·17	54·83	5·27	2·66	0·25	82·41	6·94
Jun.	1,218	15·83	1·29	100·33	8·23	0·83	0·06	93·65	7·68
Jul.	1,145	12·83	1·12	35·50	3·10	0·00	0·00	117·02	10·20
Aug.	700	4·50	0·64	10·83	1·55	0·00	0·00	59·15	8·46
Sep.	510	1·50	0·29	3·75	0·73	0·00	0·00	38·75	7·59
Oct.	812	7·00	0·86	16·25	2·00	0·00	0·00	47·00	5·67
Nov.	1,334	23·80	1·78	59·00	4·42	6·80	0·51	79·80	5·97
Dec.	1,494	23·40	1·57	97·60	6·54	0·00	0·00	73·80	4·97

* *A. aconitus, A. aitkeni, A. annularis, A. barbirostris, A. hyrcanus, A. jamesi, A. pallidus* and *A. varuna.*

TABLE III.

Average daytime catch of female anopheline mosquitoes in houses for the period 1940–45 in the protected and controlled zones and in the outside zone at Anuradhapura.

Month.	Average number of house examinations.	Average number of catching hours.	*A. culicifacies.*		*A. subpictus.*		OTHER ANOPHELINE SPECIES.*	
			Average number caught.	Mean catching rate per hour.	Average number caught.	Mean catching rate per hour.	Average number caught.	Mean catching rate per hour.
Protected and controlled zones.								
Jan.	92	9¼	3·13	0·33	30·40	3·28	0·80	0·08
Feb.	94	8½	0·80	0·09	3·00	0·35	0·20	0·02
Mar.	100	9¼	0·00	0·00	0·00	0·00	0·00	0·00
Apr.	103	9	0·00	0·00	2·00	0·22	0·00	0·00
May	107	8½	2·66	0·30	92·60	10·89	0·50	0·05
Jun.	108	9¾	4·99	0·54	96·83	9·93	11·16	1·14
Jul.	108	9¼	0·32	0·03	17·66	1·90	1·48	0·16
Aug.	111	9½	0·16	0·01	0·16	0·01	0·16	0·01
Sep.	100	10	0·00	0·00	0·00	0·00	0·00	0·00
Oct.	105	9¼	1·33	0·14	15·16	1·63	0·16	0·01
Nov.	99	9¼	10·66	1·09	211·99	21·74	0·00	0·00
Dec.	108	10	17·60	1·76	202·40	20·24	0·00	0·00
Outside zone.								
Jan.	50	4½	12·40	2·75	44·60	9·91	0·80	0·17
Feb.	45	4¼	2·40	0·56	23·00	5·41	8·00	1·88
Mar.	53	4¾	5·00	1·05	30·75	6·47	0·75	0·15
Apr.	49	4¼	3·66	0·86	31·50	7·41	0·00	0·00
May	53	4¾	0·16	0·03	26·33	5·54	0·00	0·00
Jun.	56	5¼	1·16	0·22	40·00	7·61	1·00	0·18
Jul.	54	4¾	0·33	0·06	9·33	1·96	0·16	0·03
Aug.	49	4¼	0·00	0·00	4·50	1·00	0·00	0·00
Sep.	51	4½	0·00	0·00	1·33	0·29	0·00	0·00
Oct.	53	5	1·16	0·23	12·00	2·40	0·16	0·03
Nov.	48	4¼	12·66	2·66	106·33	22·38	0·16	0·03
Dec.	54	5	17·40	3·48	87·00	17·40	0·20	0·04

* *A. aitkeni, A. annularis, A. barbirostris, A. hyrcanus, A. pallidus, A. vagus* and *A. varuna.*

TABLE IV.

Average catching rate for anopheline mosquitoes caught in cattle-traps in the protected and controlled zones and in the outside zone at Anuradhapura for the years 1938-42.

Month.	Average number of trapping hours.	*A. culicifacies.*		*A. subpictus.*		*A. vagus.*		OTHER ANOPHELINE SPECIES.*	
		Average number caught.	Mean catching rate per hour.	Average number caught.	Mean catching rate per hour.	Average number caught.	Mean catching rate per hour.	Average number caught.	Mean catching rate per hour.
Protected and controlled zones.									
Jan. ...	4	0·00	0·00	7·00	1·75	2·37	0·59	23·49	5·88
Feb. ...	4¼	0·25	0·06	2·77	0·65	2·12	0·49	6·42	1·50
Mar. ...	4	0·00	0·00	2·77	0·69	0·25	0·06	5·37	1·34
Apr. ...	4½	0·00	0·00	4·12	0·91	0·62	0·14	1·74	0·39
May ...	4½	0·40	0·09	15·70	3·49	7·20	1·60	0·90	0·19
Jun. ...	4	0·00	0·00	11·20	2·80	1·80	0·45	5·70	1·43
Jul. ...	4	0·00	0·00	1·60	0·40	0·30	0·07	1·90	0·48
Aug. ...	4½	0·10	0·02	0·30	0·07	0·00	0·00	4·80	1·06
Sep. ...	4½	0·00	0·00	0·20	0·04	0·00	0·00	2·50	0·56
Oct. ...	4½	0·00	0·00	0·00	0·00	0·00	0·00	2·30	0·50
Nov. ...	3½	0·70	0·20	19·70	5·63	1·30	0·38	0·60	0·17
Dec. ...	3¾	4·60	1·23	51·50	17·73	1·90	0·51	6·90	1·83
Outside zone.									
Jan. ...	4½	3·50	0·82	9·00	2·12	0·00	0·00	4·25	1·00
Feb. ...	4¼	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
Mar. ..	3¾	0·00	0·00	15·50	4·13	0·50	0·13	8·25	2·20
Apr. ...	4¾	0·00	0·00	11·00	2·32	3·66	0·77	1·66	0·35
May ...	3½	0·80	0·23	15·60	4·46	0·20	0·07	7·00	2·00
Jun. ...	4¼	0·00	0·00	28·00	5·90	4·80	1·00	10·20	0·44
Jul. ...	3¾	0·00	0·00	7·80	2·08	1·00	0·27	9·80	2·61
Aug. ...	4½	0·00	0·00	7·80	1·73	0·00	0·00	10·20	2·27
Sep. ...	4	0·00	0·00	5·80	1·45	0·00	0·00	0·60	0·15
Oct. ...	4	0·00	0·00	0·75	0·19	0·00	0·00	11·50	2·88
Nov. ...	3½	0·80	0·23	6·80	1·94	0·00	0·00	0·75	0·21
Dec. ...	4½	0·75	0·17	21·80	4·84	0·00	0·00	0·20	0·04

* *A. annularis, A. barbirostris, A. hyrcanus* and *A. pallidus.*

TABLE V.

Prevalence of anopheline larvæ at Anuradhapura† for 1946–49.

Month.	Number of dips.	*A. culicifacies.*		*A. subpictus.*		*A. vagus.*		OTHER ANOPHELINE SPECIES.*	
		Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected	Larval rate per 100 dips.
1946									
Jan. ...	3,700	31	0·84	341	9·21	0	0·00	306	8·26
Feb. ...	3,865	27	0·69	161	4·16	3	0·07	273	7·33
Mar. ...	3,530	17	0·48	242	6·85	33	0·93	54	1·52
Apr. ...	3,055	2	0·06	103	3·37	23	0·65	4	0·13
May ..	4,075	0	0·00	0	0·00	661	16·22	113	2·76
Jun. ...	2,975	0	0·00	0	0·00	565	18·99	217	6·65
Jul. ...	4,347	0	0·00	47	1·08	135	3·10	189	4·33
Aug. ...	4,186	0	0·00	21	0·52	160	3·82	107	2·54
Sep. ...	1,199	0	0·00	0	0·00	5	0·42	21	1·75
Oct. ...	2,919	0	0·00	0	0·00	251	8·59	258	8·82
Nov. ...	1,462	0	0·00	0	0·00	46	3·14	114	7·08
Dec. ...	1,485	0	0·00	95	6·41	142	9·56	134	9·01
1947									
Jan. ..	2,079	0	0·00	1	0·04	128	6·15	245	11·77
Feb. ...	2,257	0	0·00	0	0·00	400	17·72	275	12·17
Mar. ...	1,638	0	0·00	0	0·00	123	7·50	95	5·79
Apr. ...	1,506	0	0·00	0	0·00	42	2·78	216	14·32
May ...	1,850	1	0·05	0	0·00	146	7·89	174	9·39
Jun. ...	2,698	4	0·14	0	0·00	340	12·60	142	5·25
Jul. ...	2,202	0	0·00	0	0·00	85	3·78	280	12·77
Aug. ...	1,692	0	0·00	0	0·00	160	9·45	162	9·61
Sep. ...	1,167	1	0·08	0	0·00	9	0·77	12	1·02
Oct. ...	1,340	2	0·14	0	0·00	8	0·59	73	5·44
Nov. ...	2,521	3	0·11	0	0·00	26	1·03	498	19·74
Dec. ...	2,090	0	0·00	0	0·00	10	0·47	418	19·98

* *A. aconitus, A. annularis, A. barbirostris, A. hyrcanus, A. jamesi, A. pallidus, A. tessellatus* and *A. varuna.*

† Examinations at Elakattuwa Road from January 1946 to January 1947 and thereafter at Kurunegala Road and Malwatu Oya Lane.

TABLE V.—*concld.*

Month.	Number of dips.	*A. culicifacies.*		*A. subpictus.*		*A. vagus.*		OTHER ANOPHELINE SPECIES.*	
		Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected	Larval rate per 100 dips.
1948									
Jan.	1,910	0	0·00	0	0·00	30	1·57	508	26·59
Feb.	1,587	0	0·00	0	0·00	37	2·33	331	20·84
Mar. ..	1,362	0	0·00	0	0·00	28	2·05	207	15·19
Apr. ...	642	0	0·00	0	0·00	3	0·46	207	5·54
May ..	803	17	2·11	0	0·00	27	3·36	35	8·21
Jun. ...	876	25	2·85	0	0·00	76	8·44	96	10·95
Jul. ...	627	57	9·09	0	0·00	24	3·82	162	25·82
Aug. ...	420	89	21·19	0	0·00	9	2·14	89	21·18
Sep. ...	625	101	16·16	5	0·80	11	1·76	173	27·68
Oct. ...	709	1	0·14	0	0·00	18	2·53	41	5·77
Nov. ...	1,060	3	0·28	20	1·88	89	8·39	31	2·91
Dec. ...	422	0	0·00	1	0·23	9	2·14	78	18·47
1949									
Jan. ...	904	0	0·00	0	0·00	2	0·22	62	6·86
Feb. ..	787	0	0·00	0	0·00	5	0·64	216	27·45
Mar. ...	1,391	0	0·00	0	0·00	126	9·06	157	11·29
Apr. ...	1,575	0	0·00	0	0·00	50	3·17	45	2·86
May ..	1,966	0	0·00	0	0·00	22	1·12	225	11·44
Jun. ...	1,332	0	0·00	0	0·00	7	0·53	263	19·74
Jul. ...	1,344	0	0·00	0	0·00	1	0·07	161	11·98
Aug. ...	1,162	0	0·00	0	0·00	0	0·00	71	6·11
Sep. ...	632	125	19·78	0	0·00	40	6·33	32	5·06
Oct. ...	613	18	2·94	3	0·49	0	0·00	36	5·87
Nov. ...	1,382	0	0·00	0	0·00	23	1·66	349	25·25
Dec. ..	1,603	0	0·00	9	0·56	88	5·49	522	32·56

* *A. aconitus, A. annularis, A. barbirostris, A. hyrcanus, A. jamesi, A. pallidus, A. tessellatus* and *A. varuna.*

TABLE VI.

Prevalence of anopheline larvæ at Mankadawela for 1947–49.

Month.	Number of dips.	*A. culicifacies.*		*A. subpictus.*		*A. vagus.*		OTHER ANOPHELINE SPECIES.*	
		Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.
1947									
Feb. ...	2,719	0	0·00	0	0·00	112	4·12	532	18·99
Mar. ...	1,637	0	0·00	0	0·00	52	3·16	494	30·16
Apr. ...	1,058	0	0·00	0	0·00	27	2·74	299	28·26
May ...	1,305	0	0·00	0	0·00	75	5·75	272	20·83
Jun. ...	1,185	0	0·00	0	0·00	2	0·17	371	31·30
Jul. ..	1,900	0	0·00	0	0·00	2	0·11	320	16·84
Aug. ...	838	0	0·00	0	0·00	0	0·00	226	26·68
Sep. ...	715	0	0·00	0	0·00	0	0·00	239	33·43
Oct. ...	570	0	0·00	0	0·00	2	0·35	225	39·49
Nov. ...	1,245	0	0·00	0	0·00	17	1·36	415	33·33
Dec. ...	1,430	0	0·00	0	0·00	39	2·72	518	35·31
1948									
Jan. ..	1,285	0	0·00	0	0·00	2	0·15	374	29·09
Feb. ..	905	0	0·00	0	0·00	7	0·77	361	39·96
Mar. ...	495	0	0·00	0	0·00	3	0·61	144	31·22
Apr. ...	370	0	0·00	0	0·00	19	5·14	109	29·43
May ...	804	0	0·00	0	0·00	2	0·25	122	15·17
Jun. ...	460	0	0·00	0	0·00	0	0·00	183	39·78
Jul.† ...	460	0	0·00	0	0·00	0	0·00	98	21·30
Aug. ...	388	0	0·00	0	0·00	1	0·26	173	44·58
Sep. ...	114	0	0·00	0	0·00	0	0·00	0	0·00
Oct. ...	295	24	8·14	4	1·36	2	0·68	17	5·76
Nov. ...	317	6	1·89	70	22·08	19	5·98	155	48·90
Dec. ...	164	0	0·00	0	0·00	0	0·00	37	28·64
1949									
Jan. ...	433	0	0·00	0	0·00	5	1·13	197	45·49
Feb. ..	393	0	0·00	0	0·00	2	0·50	158	23·13
Mar. ...	683	0	0·00	0	0·00	12	1·46	244	35·72
Apr. ...	488	0	0·00	0	0·00	1	0·20	73	14·95
May ...	437	0	0·00	0	0·00	0	0·00	114	26·08
Jun. ...	599	0	0·00	0	0·00	0	0·00	45	7·50
Jul. ...	736	0	0·00	0	0·00	0	0·00	124	16·84
Aug. ...	472	0	0·00	0	0·00	0	0·00	100	21·18
Sep. ...	291	0	0·00	0	0·00	0	0·00	97	33·33
Oct. ...	522	5	0·95	3	0·57	0	0·00	32	6·13
Nov. ...	522	1	0·19	0	0·00	0	0·00	134	25·67
Dec. ...	817	0	0·00	0	0·00	86	10·52	92	11·26

* *A. aconitus, A. annularis, A. barbirostris, A. hyrcanus, A. jamesi, A. pallidus, A. tessellatus* and *A. varuna.*

† Unsprayed from July 5 to November 28, 1948.

TABLE VII.

Prevalence of anopheline larvæ at Ratmale Colony for 1947–49.

Month.	Number of dips.	*A. culicifacies.*		*A. subpictus.*		*A. vagus.*		OTHER ANOPHELINE SPECIES.*	
		Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.
1947									
Feb.	2,775	0	0·00	0	0·00	28	1·01	462	16·63
Mar. ...	1,487	0	0·00	0	0·00	2	0·14	372	25·03
Apr. ...	685	0	0·00	0	0·00	11	1·61	217	31·12
May ...	1,146	5	0·44	3	0·26	36	3·14	244	21·29
Jun. ...	1,529	0	0·00	0	0·00	81	5·28	191	12·51
Jul. ..	1,779	0	0·00	0	0·00	0	0·00	172	9·70
Aug. ...	820	0	0·00	0	0·00	31	3·78	193	23·55
Sep. ...	411	0	0·00	0	0·00	0	0·00	75	18·22
Oct. ...	489	0	0·00	0	0·00	0	0·00	83	16·93
Nov. ...	1,258	0	0·00	9	0·72	48	3·82	300	23·84
Dec. ...	1,470	0	0·00	0	0·00	3	0·20	280	19·04
1948									
Jan. ...	982	0	0·00	0	0·00	9	0·92	66	6·72
Feb. ..	1,122	0	0·00	0	0·00	3	0·27	163	14·25
Mar. ...	776	0	0·00	0	0·00	12	1·54	139	17·93
Apr. ...	311	0	0·00	0	0·00	0	0·00	20	6·42
May ...	206	0	0·00	0	0·00	0	0·00	26	12·62
Jun. ...	156	3	1·92	0	0·00	9	5·77	46	29·49
Jul. ...	96	0	0·00	21	21·87	3	3·12	0	0·00
Aug. ...	30	0	0·00	0	0·00	0	0·00	0	0·00
Sep. ..	78	0	0·00	0	0·00	0	0·00	0	0·00
Oct. ...	48	0	0·00	0	0·00	0	0·00	0	0·00
Nov. ...	256	2	0·77	0	0·00	2	0·78	28	11·93
Dec. ...	333	2	0·60	3	0·90	0	0·00	72	21·62
1949									
Jan. ..	594	0	0·00	0	0·00	0	0·00	129	21·71
Feb. ...	331	0	0·00	0	0·00	0	0·00	23	6·94
Mar. ...	728	0	0·00	0	0·00	80	10·98	101	13·87
Apr. ...	414	0	0·00	0	0·00	0	0·00	126	30·43
May ...	246	0	0·00	0	0·00	0	0·00	20	8·17
Jun. ...	712	0	0·00	1	0·14	0	0·00	55	7·72
Jul. ...	566	0	0·00	0	0·00	0	0·00	80	14·13
Aug. ...	490	0	0·00	2	0·40	1	0·20	82	16·73
Sep. ...	97	0	0·00	0	0·00	0	0·00	20	20·61
Oct. ...	324	0	0·00	1	0·30	3	0·92	53	16·17
Nov. ...	248	3	1·20	0	0·00	2	0·80	194	78·22
Dec. ...	160	0	0·00	0	0·00	0	0·00	331	206·87

* *A. annularis, A. barbirostris, A. hyrcanus, A. jamesi, A. pallidus, A. tessellatus* and *A. varuna.*

TABLE VIII.

Prevalence of anopheline larvæ at Ponnarankulama (untreated area) for 1946–49.

Month.	Number of dips.	*A. culicifacies.*		*A. subpictus.*		*A. vagus.*		OTHER ANOPHELINE SPECIES.*	
		Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.
1946									
Apr.	570	0	0·00	0	0·00	0	0·00	0	0·00
May ...	1,000	75	7·50	0	0·00	9	0·90	418	41·80
Jun. ...	900	47	5·22	30	3·33	0	0·00	286	31·78
Jul.	850	6	0·71	9	1·06	15	1·76	41	4·83
Aug. ...	1,000	8	0·80	0	0·00	0	0·00	183	18·30
Sep. ..	820	1	0·11	18	2·19	0	0·00	261	31·74
Oct. ..	940	38	4·04	0	0·00	24	2·56	165	17·51
Nov.	18	0	0·00	0	0·00	0	0·00	0	0·00
Dec. ...	...	...	...	...	...	...	...	...	...
1947									
Jan.	..	...	...	...	..	...	..	..	...
Feb. ..	293	0	0·00	0	0·00	0	0·00	125	42·67
Mar.	358	2	0·56	0	0·00	165	46·09	11	3·07
Apr. ..	268	0	0·00	0	0·00	0	0·00	122	45·52
May ...	243	0	0·00	0	0·00	0	0·00	182	74·91
Jun. ..	318	38	11·95	0	0·00	18	5·65	11	3·46
Jul. ..	124	0	0·00	0	0·00	0	0·00	0	0·00
Aug. ..	224	0	0·00	0	0·00	0	0·00	15	6·70
Sep. .	224	4	1·78	2	0·89	0	0·00	309	137·92
Oct. .	224	1	0·45	0	0·00	0	0·00	126	56·25
Nov. ...	118	1	0·85	1	0·85	0	0·00	52	44·07
Dec. ...	118	0	0·00	0	0·00	0	0·00	9	7·62

* *A. barbirostris, A. hyrcanus, A. jamesi, A. pallidus, A. tessellatus* and *A. varuna.*

TABLE VIII—*concld.*

Month.	Number of dips.	*A. culicifacies.*		*A. subpictus.*		*A. vagus.*		OTHER ANOPHELINE SPECIES.*	
		Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.	Number collected.	Larval rate per 100 dips.
1948									
Jan.	218	0	0·00	0	0·00	0	0·00	63	28·92
Feb.	267	0	0·00	0	0·00	0	0·00	137	51·31
Mar.	136	0	0·00	0	0·00	0	0·00	74	54·38
Apr.	222	0	0·00	0	0·00	0	0·00	62	27·89
May	142	3	2·11	0	0·00	0	0·00	45	31·64
Jun.	242	2	0·82	0	0·00	0	0·00	50	20·81
Jul.	130	0	0·00	0	0·00	0	0·00	49	37·67
Aug.	142	0	0·00	0	0·00	0	0·00	0	0·00
Sep.	154	26	16·88	0	0·00	0	0·00	124	80·52
Oct.	92	32	34·81	0	0·00	0	0·00	8	8·69
Nov.	186	0	0·00	2	1·07	4	2·14	6	3·23
Dec.	185	0	0·00	0	0·00	0	0·00	11	6·05
1949									
Jan.	236	1	0·42	0	0·00	2	0·84	50	21·18
Feb.	205	0	0·00	0	0·00	0	0·00	85	41·46
Mar.	192	1	0·52	0	0·00	0	0·00	89	46·30
Apr.	230	0	0·00	0	0·00	0	0·00	60	26·08
May	136	0	0·00	0	0·00	5	3·67	50	36·76
Jun.	136	0	0·00	0	0·00	0	0·00	26	19·11
Jul.	180	0	0·00	0	0·00	0	0·00	11	6·11
Aug.	159	0	0·00	0	0·00	0	0·00	19	11·87
Sep.	218	26	11·92	0	0·00	4	1·83	192	88·07
Oct.	150	0	0·00	0	0·00	0	0·00	75	50·00
Nov.	184	0	0·00	0	0·00	0	0·00	80	43·42
Dec.	135	0	0·00	0	0·00	0	0·00	53	39·24

* *A. barbirostris, A. hyrcanus, A. jamesi, A. pallidus, A. tessellatus* and *A. varuna.*

TABLE IX.

Results of the daytime examination of houses for anopheline mosquitoes at Anuradhapura for 1946–49.

Month.	Number of house examinations.†	Number of catching hours.	*A. culicifacies.*		*A. subpictus.*		OTHER ANOPHELINE SPECIES.*	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1946								
Jan. ..	375	48½	0	0·00	1	0·02	0	0·00
Feb. .	350	47¼	0	0·00	0	0·00	0	0·00
Mar. ...	350	45¾	0	0·00	0	0·00	0	0·00
Apr. ..	345	45	0	0·00	0	0·00	0	0·00
May ...	320	45	0	0·00	0	0·00	0	0·00
Jun. ..	185	26¼	0	0·00	0	0·00	0	0·00
Jul. ...	363	52¼	0	0·00	0	0·00	0	0·00
Aug. .	252	37¾	0	0·00	0	0·00	0	0·00
Sep. ...	130	20	0	0·00	0	0·00	0	0·00
Oct. ..	260	39½	0	0·00	0	0·00	0	0·00
Nov. ..	212	31¾	0	0·00	2	0·06	1	0·03
Dec. ..	203	31¼	0	0·00	1	0·03	0	0·00
1947								
Jan. ..	246	38½	0	0·00	2	0·05	11	0·29
Feb. ...	468	62¾	0	0·00	0	0·00	14	0·22
Mar. ...	399	57½	0	0·00	0	0·00	5	0·09
Apr. .	407	57¾	0	0·00	8	0·13	7	0·12
May ...	386	55¾	0	0·00	17	0·30	230	4·12
Jun. ..	433	60	0	0·00	31	0·52	28	0·47
Jul. .	379	45½	0	0·00	2	0·04	1	0·02
Aug. ...	401	53¾	0	0·00	0	0·00	0	0·00
Sep. ...	362	48½	0	0·00	0	0·00	0	0·00
Oct. .	282	36¾	0	0·00	0	0·00	1	0·03
Nov. ..	237	37¼	2	0·05	4	0·10	8	0·20
Dec. ...	231	27½	0	0·00	0	0·00	0	0·00

* *A. annularis, A. barbirostris, A. hyrcanus, A. jamesi, A. pallidus, A. tessellatus, A. varuna* and *A. vagus.*

† At Elakattuwa Road, Jaffna Junction, Malwatu Oya Lane and Kurunegala Road.

TABLE IX—*concld.*

Month.	Number of house examinations.†	Number of catching hours.	*A. culicifacies.*		*A. subpictus.*		OTHER ANOPHELINE SPECIES.*	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1948								
Jan. ...	264	31¼	0	0·00	0	0·00	1	0·03
Feb. ..	226	25	0	0·00	0	0·00	0	0·00
Mar. ..	277	30¾	0	0·00	0	0·00	0	0·00
Apr. ...	190	20½	0	0·00	0	0·00	3	0·22
May ..	300	32¼	0	0·00	1	0·03	0	0·00
Jun. ..	351	40½	0	0·00	0	0·00	4	0·10
Jul. ...	347	36	0	0·00	0	0·00	0	0·00
Aug.	305	37	0	0·00	0	0·00	0	0·00
Sep. .	319	31	0	0·00	0	0·00	0	0·00
Oct.	385	48¼	0	0·00	0	0·00	0	0·00
Nov. .	283	33¾	0	0·00	2	0·06	0	0·00
Dec. .	134	16	0	0·00	0	0·00	0	0·00
1949								
Jan. ...	336	38¼	0	0·00	0	0·00	0	0·00
Feb. .	281	25¾	0	0·00	0	0·00	1	0·03
Mar. ..	388	41½	0	0·00	0	0·00	15	0·34
Apr. ...	357	39¾	0	0·00	0	0·00	0	0·00
May ..	269	28¼	0	0·00	0	0·00	0	0·00
Jun. ...	283	27½	0	0·00	0	0·00	0	0·00
Jul. ...	313	30	0	0·00	0	0·00	0	0·00
Aug. ...	237	22½	0	0·00	0	0·00	0	0·00
Sep. ...	276	26¼	0	0·00	0	0·00	0	0·00
Oct. ...	357	33	0	0·00	0	0·00	0	0·00
Nov. .	319	29¾	0	0·00	0	0·00	0	0·00
Dec. ..	212	21¼	0	0·00	0	0·00	3	0·14

* *A. annularis, A. barbirostris, A. hyrcanus, A. jamesi, A. pallidus, A. tessellatus, A. varuna* and *A. vagus.*

† At Elakattuwa Road, Jaffna Junction, Malwatu Oya Lane and Kurunegala Road.

TABLE X.

Results of the daytime examination of houses for anopheline mosquitoes at Mankadawela for 1947–49.

Month.	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		*A. subpictus.*		OTHER ANOPHELINE SPECIES.*	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1947								
Feb. ...	60	9¾	0	0·00	0	0·00	0	0·00
Mar. ...	60	9½	0	0·00	0	0·00	0	0·00
Apr. ...	62	9¼	0	0·00	0	0·00	0	0·00
May ...	61	9	0	0·00	0	0·00	0	0·00
Jun. ...	60	8¾	0	0·00	0	0·00	7	0·80
Jul. ...	60	8¼	0	0·00	2	0·24	1	0·12
Aug. ...	44	6½	0	0·00	2	0·31	0	0·00
Sep. ...	59	7¾	0	0·00	1	0·13	0	0·00
Oct. ...	56	7	0	0·00	0	0·00	0	0·00
Nov. ...	54	7	0	0·00	1	0·14	0	0·00
Dec. ...	57	7¼	0	0·00	2	0·27	0	0·00
1948								
Jan. ...	56	7	0	0·00	0	0·00	0	0·00
Feb. ...	57	6½	0	0·00	0	0·00	0	0·00
Mar. ...	48	5½	0	0·00	0	0·00	0	0·00
Apr. ...	23	2¼	0	0·00	0	0·00	0	0·00
May ...	52	4¾	0	0·00	1	0·21	0	0·00
Jun. ...	48	5¼	0	0·00	0	0·00	0	0·00
Jul.† ..	53	6¼	0	0·00	0	0·00	0	0·00
Aug. ...	42	5¼	0	0·00	0	0·00	0	0·00
Sep. ...	56	7¼	0	0·00	0	0·00	0	0·00
Oct. ...	55	7¼	1	0·14	47	6·48	0	0·00
Nov. ..	49	7¼	0	0·00	140	19·33	4	0·55
Dec. ...	26	3½	0	0·00	0	0·00	1	0·29
1949								
Jan. ...	34	4¾	0	0·00	0	0·00	1	0·21
Feb. ...	37	4¾	0	0·00	0	0·00	0	0·00
Mar. ...	50	5¾	0	0·00	0	0·00	0	0·00
Apr. ..	24	2¾	0	0·00	0	0·00	0	0·00
May ..	38	4¼	0	0·00	0	0·00	0	0·00
Jun. ...	43	4½	0	0·00	0	0·00	0	0·00
Jul. ...	59	5½	0	0·00	0	0·00	0	0·00
Aug. ...	43	4¼	0	0·00	0	0·00	0	0·00
Sep. ...	43	4¼	0	0·00	0	0·00	0	0·00
Oct. ...	44	3¼	0	0·00	0	0·00	0	0·00
Nov. ...	44	4½	0	0·00	0	0·00	0	0·00
Dec. ...	30	3	0	0·00	0	0·00	2	0·66

* *A. pallidus, A. vagus* and *A. varuna.*
† Unsprayed from July 5 to November 28, 1948.

TABLE XI.

Results of the daytime examination of houses for anopheline mosquitoes at Ratmale Colony for 1947–49.

Month.	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		*A. subpictus.*		OTHER ANOPHELINE SPECIES.*	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1947								
Feb.	69	10¾	1	0·09	0	0·00	0	0·00
Mar.	61	9½	0	0·00	0	0·00	0	0·00
Apr.	59	9¼	1	0·11	1	0·11	0	0·00
May	61	9¼	0	0·00	4	0·43	8	0·86
Jun.	58	8½	0	0·00	0	0·00	9	1·06
Jul.	60	8¼	0	0·00	0	0·00	2	0·24
Aug.	37	5¾	0	0·00	0	0·00	0	0·00
Sep.	60	8¼	0	0·00	0	0·00	0	0·00
Oct.	56	7½	0	0·00	0	0·00	0	0·00
Nov.	54	7	0	0·00	1	0·14	0	0·00
Dec.	52	6¾	0	0·00	0	0·00	0	0·00
1948								
Jan.	32	4	0	0·00	0	0·00	0	0·00
Feb.	51	5¾	0	0·00	0	0·00	0	0·00
Mar.	47	5½	0	0·00	0	0·00	0	0·00
Apr.	41	4¾	0	0·00	0	0·00	0	0·00
May	46	5¼	0	0·00	0	0·00	0	0·00
Jun.	50	5¼	0	0·00	0	0·00	0	0·00
Jul.	30	3¾	0	0·00	0	0·00	0	0·00
Aug.	15	2	0	0·00	0	0·00	0	0·00
Sep.	43	5¼	0	0·00	0	0·00	0	0·00
Oct.	23	4½	0	0·00	0	0·00	0	0·00
Nov.	45	5½	0	0·00	0	0·00	1	0·18
Dec.	32	4	0	0·00	0	0·00	0	0·00
1949								
Jan.	49	6¼	0	0·00	0	0·00	0	0·00
Feb.	32	6	0	0·00	0	0·00	0	0·00
Mar.	44	5¼	0	0·00	0	0·00	0	0·00
Apr.	30	3¼	0	0·00	0	0·00	0	0·00
May	18	2	0	0·00	0	0·00	0	0·00
Jun.	48	5	0	0·00	0	0·00	0	0·00
Jul.	45	4¾	0	0·00	0	0·00	0	0·00
Aug.	44	4½	0	0·00	0	0·00	0	0·00
Sep.	31	3¼	0	0·00	0	0·00	0	0·00
Oct.	50	4½	0	0·00	0	0·00	0	0·00
Nov.	49	4¼	0	0·00	0	0·00	0	0·00
Dec.	35	3½	0	0·00	0	0·00	1	0·28

* *A. barbirostris, A. tessellatus, A. vagus* and *A. varuna.*

TABLE XII.

Results of the daytime examination of houses for anopheline mosquitoes at Ponnarankulama (untreated area) for 1946–49.

Month.	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		*A. subpictus.*		OTHER ANOPHELINE SPECIES.*	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1946								
Jan. ...	84	11½	5	0·43	8	0·69	0	0·00
Feb. ...	64	8¾	0	0·00	0	0·00	0	0·00
Mar. ...	60	8¼	0	0·00	0	0·00	0	0·00
Apr. ...	60	8¼	0	0·00	0	0·00	0	0·00
May ...	75	10¾	0	0·00	0	0·00	0	0·00
Jun. ...	45	6½	0	0·00	0	0·00	0	0·00
Jul. ...	75	11	0	0·00	0	0·00	0	0·00
Aug. ...	60	9¼	0	0·00	0	0·00	0	0·00
Sep. ...	45	6¾	0	0·00	0	0·00	0	0·00
Oct. ...	75	12	0	0·00	0	0·00	0	0·00
Nov. ...	58	9	3	3·33	16	1·78	3	3·33
Dec. ...	...	...	...	...	...	...	...	...
1947								
Jan.	15	2½	0	0·00	0	0·00	0	0·00
Feb. ..	61	9¾	1	0·10	0	0·00	1	0·10
Mar. ...	57	8¾	0	0·00	0	0·00	1	0·11
Apr. ..	71	10	0	0·00	0	0·00	0	0·00
May ..	72	10¾	3	0·28	6	0·56	9	0·84
Jun. ...	72	10	1	0·10	0	0·00	2	0·20
Jul. ..	72	9½	0	0·00	0	0·00	1	0·10
Aug. ..	72	9¾	0	0·00	0	0·00	0	0·00
Sep.	71	9	0	0·00	0	0·00	0	0·00
Oct. .	54	6½	2	0·31	0	0·00	0	0·00
Nov. .	30	4	0	0·00	25	6·25	3	0·75
Dec. .	14	1¾	3	1·71	19	10·85	0	0·00

* *A. annularis, A. barbirostris, A. hyrcanus, A. tessellatus, A. varuna* and *A. vagus.*

TABLE XII—*concld.*

Month.	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		*A. subpictus.*		OTHER ANOPHELINE SPECIES.*	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught	Catching rate per hour.
1948								
Jan.	31	4	3	0·75	8	2·00	0	0·00
Feb.	28	3¾	1	0·27	0	0·00	0	0·00
Mar.	30	3½	0	0·00	0	0·00	1	0·29
Apr.	25	2¾	4	1·45	0	0·00	0	0·00
May	33	4¼	17	4·00	4	0·94	0	0·00
Jun.	36	4¼	8	1·88	3	0·69	1	0·23
Jul.	50	7½	197	26·27	4	0·53	2	0·27
Aug.	51	7¼	111	15·31	4	0·56	0	0·00
Sep.	52	7¼	183	25·24	2	0·28	1	0·14
Oct.	54	7½	113	15·07	7	0·93	0	0·00
Nov.	45	6¼	92	14·72	32	5·12	6	0·96
Dec.	91	11¼	40	3·55	81	7·20	30	2·67
1949								
Jan.	13	1½	0	0·00	0	0·00	0	0·00
Feb.	45	8¾	17	1·94	0	0·00	1	0·11
Mar.	61	7	0	0·00	0	0·00	14	2·00
Apr.	41	4	0	0·00	0	0·00	0	0·00
May	40	4½	0	0·00	0	0·00	0	0·00
Jun.	40	4½	0	0·00	0	0·00	0	0·00
Jul.	43	4½	1	0·22	0	0·00	0	0·00
Aug.	26	2½	0	0·00	0	0·00	0	0·00
Sep.	43	5	46	9·20	0	0·00	0	0·00
Oct.	29	2¾	10	3·63	0	0·00	0	0·00
Nov.	38	3½	19	5·42	1	0·28	1	0·28
Dec.	...	...	...	...	...	...	...	...

* *A. annularis, A. barbirostris, A. hyrcanus, A. tessellatus, A. varuna* and *A. vagus.*

TABLE XIII.

Examination of houses at night for female anopheline mosquitoes at Jaffna Junction and Mankadawela in Anuradhapura in 1948 and 1949.

Month.	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		*A. subpictus.*		OTHER ANOPHELINE SPECIES. *	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
Jaffna Junction.								
1948								
Jul.	89	11¼	0	0·00	0	0·00	5	0·44
Aug.	74	9¼	0	0·00	0	0·00	0	0·00
Sep.	76	9½	0	0·00	0	0·00	0	0·00
Oct.	69	9	0	0·00	0	0·00	0	0·00
Nov.	59	7¾	0	0·00	0	0·00	0	0·00
Dec.	24	3	0	0·00	0	0·00	0	0·00
1949								
Jan.	57	6½	0	0·00	0	0·00	0	0·00
Feb.	60	5	0	0·00	0	0·00	0	0·00
Mar.	90	9¾	0	0·00	0	0·00	0	0·00
Apr.	118	13¼	0	0·00	0	0·00	0	0·00
May	35	4	0	0·00	0	0·00	0	0·00
Jun.	52	5½	0	0·00	0	0·00	0	0·00
Jul.	30	3	0	0·00	0	0·00	0	0·00
Aug.	41	4	0	0·00	0	0·00	0	0·00
Sep.	52	5	0	0·00	0	0·00	0	0·00
Oct.	50	4	0	0·00	0	0·00	0	0·00
Nov.	60	5¾	0	0·00	0	0·00	0	0·00
Dec.	30	3	0	0·00	0	0·00	0	0·00

* *A. hyrcanus* and *A. varuna.*

TABLE XIII—*concld.*

Month.	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		*A. subpictus.*		OTHER ANOPHELINE SPECIES.*	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
Mankadawela.								
1948								
Jul.	53	5½	0	0·00	0	0·00	0	0·00
Aug. ..	14	5¾	0	0·00	0	0·00	0	0·00
Sep. .	54	6¾	0	0·00	0	0·00	0	0·00
Oct.	46	6	0	0·00	0	0·00	0	0·00
Nov. ..	52	7½	0	0·00	2	0·26	1	0·13
Dec. .	13	1½	0	0·00	0	0·00	0	0·00
1949								
Jan.	22	2¼	0	0·00	0	0·00	0	0·00
Feb.	38	4	0	0·00	0	0·00	0	0·00
Mar.	37	4¼	0	0·00	0	0·00	0	0·00
Apr. .	38	4¼	0	0·00	0	0·00	0	0·00
May	13	1¼	0	0·00	0	0·00	0	0·00
Jun. ..	37	3¾	0	0·00	0	0·00	0	0·00
Jul. ...	50	5¼	0	0·00	0	0·00	0	0·00
Aug. ...	38	4	0	0·00	0	0·00	0	0·00
Sep. .	24	2½	0	0·00	0	0·00	0	0·00
Oct. ..	29	2¾	0	0·00	0	0·00	0	0·00
Nov. .	26	2½	0	0·00	0	0·00	0	0·00
Dec. .	30	3	0	0·00	0	0·00	0	0·00

* *A. hyrcanus* and *A. varuna.*

TABLE XIV.

Examination of houses at night for anopheline mosquitoes at Ponnarankulama (untreated area) in 1948 and 1949.

Month.	Number of house examinations.	Number of catching hours.	*A. culicifacies.*		*A. subpictus.*		OTHER ANOPHELINE SPECIES.*	
			Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1948								
Jul.	62	9¾	327	33·53	0	0·00	13	1·33
Aug.	35	4¾	114	24·00	0	0·00	0	0·00
Sep.	51	7¾	293	37·80	0	0·00	0	0·00
Oct.	61	9	127	14·11	0	0·00	0	0·00
Nov.	12	1¾	30	17·14	0	0·00	0	0·00
Dec.	35	12¼	42	3·42	14	1·14	14	1·14
1949								
Jan.	13	1½	3	2·00	0	0·00	0	0·00
Feb.	44	7½	13	1·73	0	0·00	1	0·13
Mar.	60	7	0	0·00	0	0·00	3	0·42
Apr.	28	3¼	0	0·00	0	0·00	0	0·00
May	39	4¼	0	0·00	0	0·00	0	0·00
Jun.	39	4	0	0·00	0	0·00	0	0·00
Jul.	36	3¾	0	0·00	0	0·00	0	0·00
Aug.	14	1¼	0	0·00	0	0·00	0	0·00
Sep.	36	4¼	85	20·00	0	0·00	0	0·00
Oct.	14	1¼	10	8·00	0	0·00	0	0·00
Nov.	20	1¾	8	4·57	0	0·00	0	0·00
Dec.	...	...	...	...	...	...	...	...

* *A. barbirostris, A. hyrcanus, A. pallidus, A. vagus* and *A. varuna.*

TABLE XV.

Anopheline mosquitoes caught in cattle-traps at Elakattuwa Road, Jaffna Junction and Malwatu Oya Lane in Anuradhapura in 1946, 1947 and 1948.

Month.	Number of trapping hours.	*A. culicifacies.*		*A. subpictus.*		*A. vagus.*		OTHER ANOPHELINE SPECIES.*	
		Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1946									
Jan.	16	0	0·00	75	4·69	92	5·75	91	5·69
Feb.	14	0	0·00	16	1·14	39	2·79	48	3·42
Mar.	16	0	0·00	5	0·31	0	0·00	12	0·76
Apr.	22	0	0·00	4	0·18	0	0·00	1	0·05
May	20	0	0·00	0	0·00	47	2·35	15	0·75
Jun.	16	1	0·06	4	0·25	30	1·87	11	0·60
Jul.	26	0	0·00	2	0·08	13	0·50	120	4·61
Aug.	26	0	0·00	0	0·00	0	0·00	42	1·62
Sep.	14	0	0·00	0	0·00	0	0·00	0	0·00
Oct.	14	0	0·00	3	0·00	0	0·00	0	0·00
Nov.	14	0	0·00	181	0·21	21	1·50	7	0·50
Dec.	12	0	0·00	75	15·08	3	0·25	21	1·75
1947									
Jan.	20	0	0·00	42	3·75	256	12·80	58	2·90
Feb.	8	0	0·00	42	5·25	0	0·00	258	32·25
Mar.	8	0	0·00	34	5·25	22	2·75	57	7·12
Apr.	8	0	0·00	47	4·25	126	15·75	25	3·13
May	8	0	0·00	21	5·87	330	41·25	67	8·39
Jun.	8	0	0·00	31	2·62	115	14·38	29	3·63
Jul.	8	0	0·00	2	3·88	51	6·38	28	3·50
Aug.	8	0	0·00	5	0·25	4	0·50	17	2·13
Sep.	8	0	0·00	8	0·63	5	0·63	7	0·87
Oct.	8	0	0·00	0	1·00	5	0·63	30	3·75
Nov.	8	0	0·00	10	0·00	81	10·13	98	12·25
Dec.	8	0	0·00	29	1·25	61	7·63	121	15·14

* *A. annularis, A. barbirostris, A. hyrcanus, A. karwari, A. pallidus* and *A. varuna.*

TABLE XV—*contd.*

Month.	Number of trapping hours.	*A. culicifacies.*		*A. subpictus.*		*A. vagus.*		OTHER ANOPHELINE SPECIES.*	
		Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1948									
Jan. ..	8	0	0·00	0	3·63	0	0·00	277	34·63
Feb. ..	8	0	0·00	3	0·00	6	0·75	181	22·63
Mar. ..	8	0	0·00	0	0·38	29	3·63	41	5·12
Apr. ..	8	2	0·25	1	0·00	19	2·38	17	2·13
May ...	8	0	0·00	0	0·13	178	22·25	30	3·75
Jun. .	8	0	0·00	0	0·00	112	14·00	112	14·01
Jul. ...	4	0	0·00	0	0·00	0	0·00	9	1·88
Aug. ...	4	0	0·00	0	0·00	0	0·00	0	0·00
Sep. ...	4	0	0·00	0	0·00	2	0·50	4	1·00
Oct. ..	4	0	0·00	3	0·75	0	0·00	0	0·00
Nov. ..	4	0	0·00	3	0·75	14	3·50	17	4·25
Dec. ...	2	0	0·00	9	4·50	4	2·00	4	1·75
1949									
Jan. .	4	0	0·00	0	0·00	31	7·75	24	6·00
Feb. ..	...	...	...	...	...	...	...	...	...
Mar. ...	4	0	0·00	0	0·00	14	3·50	96	24·00
Apr. ..	2	0	0·00	31	15·50	3	1·50	0	0·00
May ...	4	0	0·00	24	6·00	0	0·00	92	23·00
Jun. .	4	0	0·00	0	0·00	0	0·00	0	0·00
Jul. ..	4	0	0·00	2	0·50	0	0·00	0	0·00
Aug. ...	2	0	0·00	0	0·00	0	0·00	0	0·00
Sep. ...	4	0	0·00	0	0·00	0	0·00	4	1·00
Oct. ..	4	0	0·00	0	0·00	2	0·50	9	2·25
Nov. ...	4	0	0·00	15	3·75	3	0·75	106	26·50
Dec. ...	2	0	0·00	0	0·00	14	7·00	42	21·00

* *A. annularis, A. barbirostris, A. hyrcanus, A. karwari, A. pallidus* and *A. varuna.*

TABLE XVI.

Anopheline mosquitoes caught in cattle-traps at Ponnarankulama (untreated area) from 1946–48.

Month.	Number of trapping hours.	*A. culicifacies.*		*A. subpictus.*		*A. vagus.*		OTHER ANOPHELINE SPECIES.*	
		Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.	Number caught.	Catching rate per hour.
1946									
Jan. ...	8	0	0·00	21	2·63	12	1·50	35	4·30
Feb. ...	8	0	0·00	1	0·12	0	0·00	35	4·38
Mar. ...	6	0	0·00	1	0·17	0	0·00	25	4·17
Apr. ...	6	0	0·00	3	0·50	0	0·00	2	0·33
May ...	8	0	0·00	0	0·00	0	0·00	5	0·62
Jun. ...	6	0	0·00	0	0·00	2	0·33	0	0·00
Jul. ...	10	0	0·00	0	0·00	0	0·00	1	0·10
Aug. ...	4	0	0·00	0	0·00	0	0·00	2	0·50
Sep. ...	4	0	0·00	0	0·00	0	0·00	0	0·00
Oct. ...	8	0	0·00	0	0·00	0	0·00	0	0·00
Nov. ...	4	0	0·00	0	0·00	1	0·25	1	0·25
Dec. ...	..	...	...	...	...	...	..	...	...
1947									
Jan. ..	..	...	...	...	...	...	...	..	...
Feb. ..	4	0	0·00	0	0·00	56	14·00	47	11·75
Mar. ...	4	0	0·00	33	8·25	20	5·00	28	7·00
Apr. ...	4	0	0·00	33	8·25	35	8·75	14	3·50
May ...	4	0	0·00	12	3·00	209	52·25	100	25·00
Jun. ...	4	0	0·00	1	0·25	40	10·00	137	34·25
Jul. ...	4	0	0·00	11	2·75	41	10·25	167	41·75
Aug. ...	4	0	0·00	10	2·50	2	0·50	80	20·00
Sep. ...	4	0	0·00	2	0·50	1	0·25	37	9·25
Oct. ...	2	0	0·00	0	0·00	1	0·50	5	2·50
Nov. ...	4	0	0·00	2	0·50	24	6·00	120	30·00
Dec. ...	4	0	0·00	12	3·00	76	19·00	92	23·00
1948									
Jan. .	4	0	0·00	19	4·75	5	1·25	184	46·00
Feb. .	4	0	0·00	4	1·00	3	0·75	133	33·25
Mar. ...	4	0	0·00	0	0·00	18	4·50	31	7·75
Apr. ...	4	0	0·00	0	0·00	6	1·50	82	20·50
May ...	4	0	0·00	6	1·50	3	0·75	23	5·75
Jun. ...	4	0	0·00	31	7·75	23	5·75	38	9·50
Jul.–Dec.				No trapping.					

* *A. annularis, A. barbirostris, A. hyrcanus, A. jamesi, A. pallidus* and *A. varuna.*

TABLE XVII.

Monthly malaria attendance at the Civil Hospital, Anuradhapura, for 1938–49.

Year.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total.
1938	3,409	2,083	1,843	1,560	1,496	1,586	1,551	1,229	931	872	1,049	1,294	18,939
1939	1,897	1,396	1,220	1,090	1,887	2,801	2,203	1,145	961	747	1,063	1,794	18,201
1940	2,119	1,609	1,411	1,193	1,116	1,644	2,120	1,510	995	694	1,397	2,883	18,691
1941	2,238	2,005	1,707	1,226	1,290	1,529	1,381	1,036	797	974	1,387	2,503	18,274
1942	3,413	1,597	1,247	700	971	712	898	858	578	735	967	2,024	14,700
1943	3,090	1,727	1,801	973	794	745	961	560	478	410	863	1,047	13,449
1944	1,143	880	844	733	771	807	848	607	466	699	1,474	1,729	10,992
1945	1,775	1,151	754	599	653	645	966	682	430	430	787	1,172	10,034
1946	694	355	366	278	286	661	223	160	203	354	234	566	4,380
1947	571	463	366	367	365	316	232	237	322	323	284	238	4,086
1948	235	252	143	142	186	256	327	203	156	150	226	221	2,497
1949	225	183	93	112	143	143	185	153	121	126	127	186	1,797

TABLE XVIII.

Spleen rate among school children at Anuradhapura for 1936–49.

Year.	Month.	Number examined.	Number with enlarged spleen.	Spleen rate per cent.	Month.	Number examined.	Number with enlarged spleen.	Spleen rate per cent.
1936	Mar.	413	290	70·2	Sep.	...	...	...
1937	,,	305	207	67·9	,,	...	...	...
1938	,,	324	198	61·1	,,	...	...	...
1939	,,	343	202	58·8	,,	...	...	...
1940	,,	337	205	60·8	,,	...	...	...
1941	,,	301	147	48·8	,,	...	...	...
1942	,,	...	...	...	,,	...	...	...
1943	,,	590	281	47·6	,,	751	294	39·1
1944	,,	900	262	29·1	,,	825	192	23·2
1945	,,	840	331	40·0	,,	1,039	323	31·0
1946	,,	922	240	26·0	,,	555	86	15·5
1947	,,	646	107	16·6	,,	638	75	10·2
1948	,,	731	46	6·3	,,	657	17	2·6
1949	,,	686	18	2·6	,,	766	9	1·1

TABLE XIX.

Parasite rate among school children at Anuradhapura for 1939–49.

Year.	Number examined.	Number with malaria parasites.	Parasite rate per cent.	Species.*		
				V.	F.	M.
1939	377	62	16·4	3	14	45
1940	277	21	7·6	1	3	17
1941	256	29	11·3	7	7	15
1942	...	...	...	...	...	...
1943	210	26	12·4	11	10	5
1944	329	13	4·0	1	4	8
1945	315	21	6·6	18	3	...
1946	410	6	1·4	2	4	...
1947	260	1	0·3	...	1	...
1948	247	6	2·4	2	1	3
1949	265	1	0·3	...	...	1

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

APPENDIX I *

Crude death rates and infant-mortality rates.

Districts.	Endemicity (1938).	Crude death rates.					Infant-mortality rates.				
		1938	1946	1947	1948	1949	1938	1946	1947	1948	1949
Kalutara ...	Healthy	16·6	14·7	12·6	11·4	11·2	108	90	92	77	74
Galle ...	..	20·7	17·1	14·2	13·2	13·1	131	104	103	84	79
Colombo ...	..	18·9	18·5	14·9	14·3	14·5	139	107	99	100	97
Negombo ..	..	15·3	16·1	10·9	10·2	10·5	142	134	92	85	77
Matara ...	..	22·9	17·7	11·5	11·1	11·3	132	105	71	69	72
Kegalle ...	..	15·3	17·2	11·2	10·5	10·2	126	124	83	75	75
Nuwara Eliya ...	..	17·0	16·2	13·6	15·3	13·8	176	127	105	115	109
Jaffna ...	..	23·3	17·0	15·3	12·5	12·7	166	106	91	77	73
Kandy ...	..	16·0	18·9	14·2	14·0	13·0	152	140	97	97	94
Ratnapura ...	Moderate	19·8	18·4	13·1	12·6	12·2	144	127	94	84	82
Chilaw ...	..	18·5	18·6	11·7	10·1	9·0	151	144	92	68	62
Badulla ...	..	21·2	17·7	18·9	15·1	13·8	157	124	94	99	99
Matale ...	High	25·1	29·6	17·2	15·0	13·1	186	222	109	104	89
Kurunegala ...	..	26·3	32·0	16·2	12·8	10·5	224	297	123	93	78
Mannar ...	..	35·4	32·1	17·8	15·3	12·7	288	320	164	125	123
Puttalam ...	..	39·2	24·7	19·7	12·5	11·9	342	192	161	100	95
Batticaloa ...	..	30·0	30·9	18·2	17·2	16·1	213	201	131	128	116
Anuradhapura ...	Hyperendemic	37·0	27·2	19·5	14·1	12·1	263	207	150	102	90
Trincomalee ...	..	31·4	25·5	16·6	12·6	11·4	174	201	146	120	109
Hambantota ...	..	48·8	41·3	18·4	13·6	12·7	292	322	113	89	81
Vavuniya ...	..	35·3	26·3	19·9	15·8	12·2	267	176	148	121	87
Average ...	...	21·8	20·3	14·3	13·2	12·6	161	141	101	92	87

* Reproduced from Rajendram and Jayewickreme (1951).

APPENDIX II.*

Spleen and parasite rates of school children by districts.

Districts.	Climatic zone.†	Endemicity (1939).	Spleen rates.					Parasite rates.		
			1939	1946	1947	1948	1949	1939	1948	1949
Kalutara ...	W.	Healthy	1·6	1·7	0·3	0·2	0·01	1·1	0·0	0·0
Galle ...	W.	..	4·4	1·4	0·5	0·4	0·3	1·2	0·0	0·0
Colombo ... / Negombo ..	I.	..	4·8	13·0	2·3	1·2	0·7	1·4	0·04	0·0
Matara ..	W. & I.	..	6·8	15·1	0·2	2·6	0·5	5·5	0·0	0·0
Kegalle ...	W. & I.	..	8·1	59·4	4·0	2·9	1·1	3·2	0·2	0·1
Nuwara Eliya	W., I. & D.	..	8·4	22·1	9·1	2·2	2·7	1·1	0·4	0·2
Jaffna ..	D.	..	8·5	19·4	2·3	1·1	1·2	2·2	0·1	0·0
Kandy ...	W., I. & D.	..	8·9	33·1	2·2	1·4	1·1	2·3	0·1	0·2
Ratnapura ...	W., I. & D.	Moderate	11·5	26·7	8·7	2·9	1·9	6·7	0·1	0·2
Chilaw ...	I. & D.	..	17·5	43·8	6·3	4·1	2·4	5·2	0·6	0·1
Badulla ...	D.	..	20·2	35·9	9·6	7·8	4·5	3·2	0·9	0·6
Matale ...	I. & D.	High	33·0	55·2	15·0	8·9	2·6	6·3	0·5	0·4
Kurunegala ...	I. & D.	..	34·8	75·1	34·8	30·8	7·8	8·3	1·6	0·6
Mannar ...	I. & D.	..	43·5	51·3	33·7	12·0	4·3	6·1	1·5	0·3
Puttalam ...	D.	..	46·0	77·7	31·7	17·5	4·5	7·0	1·7	0·0
Batticaloa ...	D.	..	46·2	43·2	16·7	9·4	3·8	8·4	2·2	1·0
Anuradhapura	D.	Hyperendemic	57·5	77·6	30·9	18·3	7·1	11·4	1·8	0·8
Trincomalee ...	D.	..	57·7	53·4	21·1	15·2	11·0	6·7	1·2	0·0
Hambantota ...	I. & D.	..	58·6	63·6	47·4	30·0	9·7	12·8	0·7	0·2
Vavuniya ...	D.	..	66·7	84·1	52·2	23·3	9·1	10·3	1·2	0·0
Average ..	...	...	18·2	30·6	19·3	5·8	2·7	5·1	0·6	0·2

* Reproduced from Rajendram and Jayewickreme (1951).

† W = Wet zone; I = Intermediate zone; D = Dry zone.

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MALARIA AND THE ANCIENT CITIES OF CEYLON.

BY

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[October 30, 1950.]

INTRODUCTION.

A NUMBER of writers have put forward their views in books and scientific papers with regard to the relationship of malaria and the ancient cities of Ceylon.

Briercliffe (1935) quotes from the Government archives that in 1638 the Dutch colonists published a map which indicated that the Southern Province was depopulated and deserted some 300 years previously by 'fever sickness' and that the North Central Province is marked as an area 'depopulated by sickness'.

Nicholls (1921) considers that the collapse of the ancient civilization of Ceylon was due to the importation of malaria from India. He says 'the North Central areas of Ceylon could not have bred or supported the vast numbers of the active race that built and developed its ancient cities had malaria existed there at the time, and the gradual fall of these people was due to the importation of malaria from India, and possibly also the importation of the vector species. When once malaria was established the people would drift to less malarious parts and that is what happened'.

Codrington (1939) contributes to the same theme by asserting 'Hereafter we hear little of irrigation: foreign disturbances and to a much greater degree, the appearance of malaria, account for the collapse of the old works.'

Dunn (1936) quotes 'Carter, on the other hand, thinks that malaria was probably already present, and that depopulation was primarily due to the repeated Tamil invasions as a result of which a large number of Sinhalese bread-winners were slain and agriculture disorganized. This resulted in great poverty and distress and a large addition to the suitable breeding grounds for anophelines, which increased the previously manageable prevalence of the disease into highly endemic proportions'.

Toynbee (1948) maintains 'As a matter of fact it can be demonstrated that the malaria is a consequence of the ruins of the irrigation system and therefore posterior to its construction.'

The purpose of this paper is to elucidate the problem under the following main headings :—

1. The Indic civilization.
2. The influence of the Indic civilization on the building of tanks in Ceylon.
3. The destruction of the tanks and the consequent introduction of malaria in endemic and hyperendemic form.
4. The influence of climatic changes.

THE INDIC CIVILIZATION.

The earlier history of Ceylon is inextricably woven with that which Toynbee calls the Indic civilization. He in his introduction to the 'Study of History' argues that the only intelligible units of historical study are societies, not nations. The examination of any nation is unintelligible in itself but begins to make sense when taken as a part of a larger whole. The construction of the amazing system of tanks and irrigation channels and their eventual neglect and disintegration has led to the erroneous conclusions, as set out in the introduction to this paper, because the concept of the tanks was localized to Ceylon, and not taken as an integral part of a society which once flourished and finally disintegrated.

But what was this Indic society and how came it into existence ?

One of the main concepts of Toynbee's monumental work is that present-day societies or civilizations are apparented to older and extinct civilizations. The marks of this relationship are (*a*) a universal state or an empire which itself is the outcome of a time of troubles, consisting of internal strife and civil war, followed by (*b*) an interregnum in which appear a church and an invasion of barbarians in a heroic age.

It is of importance to trace back the affiliation of the present Hindu civilization to the Indic civilization, since both have left their mark on the subject of this paper.

The universal state of the present Hindu society is the Empire of the Guptas (circa A.D. 375–475). Hinduism became the universal church, supplanting Buddhism after the latter had been dominant on the sub-continent for about 8 centuries. The barbarians who overran the Gupta Empire at its fall were the Huns from the Eurasian Steppe. The period occupied by their activity is approximately 300 years, from A.D. 475 to 775, after which the present Hindu society emerged (circa A.D. 800).

In searching back for the society to which the Hindu is affiliated, there is the complicating factor of an Hellenic intrusion beginning with the invasion of Demetrius, the Greek King of Bactria, circa 183-182 B.C., and which ends about A.D. 390, which in fact may be taken as the approximate date of the establishment of the Gupta Empire.

The Empire of the Mauryas, established by Chandragupta I, circa 323 B.C., is the universal state of which the Gupta Empire may be regarded as the post-Hellenic resumption.

The Mauryan Empire arose from a time of troubles covering in its span the lifetime of the Gautama Buddha. His life and philosophy are a mark of the troubled times in which he lived. But behind this time of strife there was a period of growth which Toynbee identifies as the Indic civilization. The original home of this civilization lay in the Ganges and Indus valleys from which it spread over most of the continent.

The Empire of the Mauryas was made illustrious by the reign of Emperor Dharmasoka, who was contemporary with Devanampiya Tissa, circa 247–207 B.C.

Dharmasoka, or Asoka, as he is commonly called, inherited an extensive empire from his grandfather Chandragupta. On the north-west his frontier roughly corresponded to the so-called scientific frontier of the 19th century—the Kabul-Ghazni-Kandahar Line—and in the south it extended to Nellore. The sole conquest attributed to Asoka himself was that of the kingdom of Kalinga. It is said that it was miseries caused by him in the conquest of Kalinga that drove Asoka to seek refuge in Buddhism.

To the south of Nellore were the independent kingdoms of the Cholas, Pandyans and the Cheras.

THE INFLUENCE OF THE INDIC CIVILIZATION ON THE BUILDING OF TANKS IN CEYLON.

The island which is known by the name of Lanka, and to the western world as Ceylon, has an area of 25,332 square miles, being 270 miles long and 140 miles wide. The long axis is mainly north-south. A mountainous mass rising to over 8,000 feet is situated in the southern portion of the island. Most of the rivers take their origin above the 3,000-foot level: the area of this mass is roughly 40 miles by 30 miles.

It is this mountainous mass which is the determining factor in the main meteorological features of the island, and its position and height are chiefly responsible for the variations in rainfall over the whole island.

The earliest map of the island in existence is that of Ptolemy, circa A.D. 100. In this, the east coast with the Mahaweliganga is plainly recognizable. The hill country or Malaya is shown under this name. The two chief cities were Anurogrammon or Anuradhapura and Maagrammon, the modern Alutnuwara. It is interesting to note the termination grammon, meaning village, instead of pura, meaning city, tending to show that in Ptolemy's time these had not reached the high degree of development which they certainly did later.

To-day most of the population is found in the west and south-west, but in the olden days the greater part of the population was in the 'King's Country' or Pihiti, including most of the land north of the Dedura Oya and the Mahaweliganga. The rest of the island was divided into the Maya Rata or the country of the sub-king and the Rubuna.

The real history of Ceylon begins with the reign of Devanampiya Tissa, circa 247–207 B.C. He was contemporary with, and there is some evidence to support a relationship to, Asoka. Having embraced Buddhism, Asoka used his immense power and sent missions to various countries. His elder son, Mahinda, went to

Ceylon where he found Devanampiya Tissa a ready convert. With Asoka's power and Tissa's willing and ready acquiescence, most of the Sinhalese accepted the Indic philosophy.

It was from the reign of Devanampiya Tissa that there arose in the northern parched plains of Ceylon that amazing irrigation system and a wealth of cities which to-day lie hidden in the jungle.

Those lost cities, those neglected tanks and irrigation channels are in that area of the island which to-day is ravaged with endemic and hyperendemic malaria, with an insufficient rainfall to allow of adequate crop cultivation but which nevertheless provides plenty of breeding grounds for the vector species. And although, as will be shown later in this paper, the rainfall would appear to have been more plentiful in the past than it is at present, the northern part of the island receives only one monsoon and hence gets nothing like so much rainfall as that with which the south-west quadrant is blessed. Yet it was to these northern drier plains that the Indic civilization carried its philosophy. It is of course true that the king's country was in the north of the island, but it is legitimate to surmise from what follows that the tanks would have been built nevertheless.

The Indic civilization arose in the valleys of the Indus and Ganges. 3,000 years ago these valleys presented a picture which is completely different from that which is seen there to-day. These valleys were covered with primeval jungle. These untouched forests offered no easy environmental conditions. It was the challenge of this adverse country which gave birth to the Indic civilization.

In Ceylon, there was the equally arduous task of conquering the parched plains. What was the impetus that set in motion the colossal tank building era ? Toynbee gives a double answer : the stimulus of new ground and the virtues of adversity.

THE STIMULUS OF NEW GROUND.

After drawing different analogies, both mythological and historical, Toynbee says : 'In the history of Buddhism it is the same story, for the decisive victories of this Indic faith are not won in the old ground of the Indic world. The Hinayana first finds an open road in Ceylon, which was a colonial annexe of the Indic civilization. And the Mahayana starts its long and roundabout journey towards its future domain in the Far East by capturing first the Syriacized and Hellenized Indic province of the Punjab. It is in the new grounds of these alien worlds that the highest expressions of both the Syriac and the Indic religious genius eventually bear fruit '.

India was the cradle of Buddhism, a system of religion inaugurated by Gautama Buddha (623–543 B.C.), yet of the 140,000,000 adherents to-day only 10,000,000 are in India ; the rest are principally in Ceylon, Tibet, Siam, China and Japan.

THE VIRTUES OF ADVERSITY.

The popular assumption has been that civilizations emerge when environments offer easy conditions of life, but this is the complete antithesis to the known facts. Abnormally difficult environmental conditions have produced the genesis of the various civilizations.

The Mayan civilization emerged in the tropical jungles of Mexico. Here the pioneers of this now extinct civilization won, if only for a time, their battle with primeval jungle. The ruined cities of Copal, Tikal and Palenque bear testimony to their brief conquest of the jungle.

Similarly the Syriac civilization battled against the opposite extreme, the Afrasian Steppe. These pioneers knew how to bring water to the arid wastes. In the height of their greatness, Palmyra and Petra were situated in the midst of an oasis of irrigated gardens. 'Their present state reveals not only the final victory of the desert over man but the dimensions of the previous victory of man over desert' (Toynbee, *loc. cit.*).

In Ceylon there was the equally hard task of conquering the parched plains for agricultural purposes, particularly the growing of rice. Numerous tanks and reservoirs with their conduits between each other and to the fields were a necessary perquisite.

'To realise how such tanks came into being one must know something of the history of Lanka. The idea underlying the system was very simple but very great. It was intended by the tank building kings that none of the rains which fell in such abundance in the mountains should reach the sea without paying tribute to man on the way. In the middle of the southern half of Ceylon is a wide mountain zone, but to the east and north dry plains cover thousands of square miles, and at present are very sparsely populated. In the height of the monsoon when armies of storm swept clouds rush over day after day to match their strength against the hills, there is a line drawn by nature that the rains are unable to pass..... The line curves from sea to sea and appears to be stable and unaffected by the operations of man, such as the felling of forests' (Still, 1947).

But the missionaries of the Indic civilization and their converts in Ceylon had not only the skill, but also the strength—there could have been no malaria in endemic or hyperendemic form then—of compelling the highland rains to give water, health and wealth to the plains.

'Hill streams were tapped and their water guided into giant tanks below, some of them 4,000 acres in extent; and, from those, channels ran on to other larger tanks farther from the hills and from them to others still more remote, and below each great tank and each great channel were hundreds of little tanks, each a nucleus of a village, all, in the long run, fed from the wet mountain zone. So gradually the ancient Sinhalese conquered all, or nearly all, the plains that are now so empty of men' (Still, *loc. cit.*).

The building of these tanks and irrigation channels extended over many long years. The first of the tank building kings appears to have been Devanampiya Tissa, who constructed Tissawewa. Saddha Tissa, circa 137 B.C., is reputed to have built Padawiya.

Vasabha, circa A.D. 66, did much for irrigation. He constructed 11 tanks, including Kawduluwewa and also the Elahera channel connecting that tank to the Ambaganga.

In the 30 odd years between A.D. 267 and 304, 23 tanks were built; 6 are accredited to Jettha Tissa I, circa A.D. 267, and 17 to Mahasena which included

Rantisa and the great Minneri tanks which he fed by a channel from the Ambaganga. He may also have enlarged the Padawiya tank.

Upatissa, circa A.D. 370, constructed Topawewa and Dhatusena, circa A.D. 463, built Kalawewa.

The first 5 centuries of this era in Ceylon are marked by the construction of numerous irrigation works. One point must however be borne in mind, namely, that some of the tanks mentioned above may not have been new works, some may have been, in fact probably were, merely restored. There is also some doubt that all were not in working order throughout the period, nevertheless those actually in use must have been considerable.

THE DESTRUCTION OF THE TANKS AND THE CONSEQUENT INTRODUCTION OF MALARIA IN ENDEMIC AND HYPERENDEMIC FORM.

Separated as it is by a mere channel from the sub-continent of India, it is not unnatural to assume that the history of Ceylon is not uninfluenced by this close proximity.

In the earlier history of the island civil war was the rule rather than the exception.

The kings were polygamous and married the daughters of the rajas of the neighbouring kingdoms in southern India. As a result a king might have many sons, all of whom might be ambitious. There was the feudal system of land tenure, hence the overlord could command his tenants to follow him into war. There was thus every temptation to wage civil war, since a defeated prince could fly to his relations in India and there await another chance of recovering his lost position.

There can be little doubt but that the Sinhalese fighting among themselves respected the tanks and irrigation channels. They were, when all is said and done, one people embracing a common faith.

There were a number of Tamil invasions from the earliest times. The first occurred about 237 B.C., followed later by the Tamil King Elara, who ruled the whole of the king's country for about 42 years. There can be little doubt that Elara's reign was a beneficent one, and although a Hindu, his rule was just and he was as a consequence beloved by the people.

From 103 to 88 B.C., five Pandyanas maintained their hold on the capital for 14 years. From A.D. 436 there was a short rule by six Tamil kings.

These were however short reigns and by no means affected the whole country. The destruction of tanks and channels would appear to have been negligible since their construction continued. It is of course true that some of the new constructions were in fact replacements, as, for instance, some of the 14 tanks accredited to Aggabhodi XI, circa A.D. 598, but after the last of the above-mentioned invasions, there was the construction of Kurunduwewa in the reign of Aggabhodi I, circa A.D. 564, and Kalawewa by Dhatusena, circa A.D. 463, neither of which are mentioned before. The continued building would therefore appear

to be intelligible only on the ground that no previous great destruction had taken place.

The abortive and minor Tamil invasions up to the 5th century A.D. were in no way a prelude to the annexation of Lanka to their territory. The conquests were short-lived and brought little destruction in their wake. The annexation of Ceylon to a part of India required first an empire on that sub-continent. The southern Indian kingdoms of the Cholas, Pandyans and Cheras became one under the Chola Empire, which then set about with the conquest of Ceylon. But whereas the annexation of Ceylon to the Mauryan Empire was one of peaceful penetration, enlightenment and the prelude to an era of prosperity that of the Cholan conquest was one of mass destruction. To the Cholan Emperors, Ceylon was a country of great riches, with a people with an alien creed. Attracted by the wealth of the monasteries their wish was to conquer the country in as short a time as possible.

The breaching of the channels was to them a necessary, though wholly unwise, tactics of war. The old city of Anuradhapura was supplied initially water from the tank Kalawewa, some 50 miles distant from the city. What easier means of isolating the city than by cutting the channel somewhere along its 50-mile conduit? Such tactical operations would be, and are carried out in modern warfare, and the Cholan Emperors were in no wit less wise.

These devastating wars commenced with the Cholan Emperor Parantaka I. He first invaded Ceylon about A.D. 918, but owing to trouble in his own country from the Rashtrakutas of the Deccan, he returned leaving the people of Lanka in great fear. After this time and before Ceylon became a province of the Cholan Empire under Raja Raja I about A.D. 1004-5, there were periods of counter-attacks by the Sinhalese and reprisals by the Cholans.

All this added to the destruction of the tanks and channels, and with some minor works by Parakrama I, there is no reference to any irrigation works. From this period onward there was a gradual decline.

The forest gradually crept in; the watercourses, with their larvicidal fish, gave way to intermittent pools. The vector species breeding places increased; the lowered health of the people as a result of their impoverished conditions led the way to the present state.

The lessons of the past were forgotten in the follies of the Cholan conquest. The genius of the Indic civilization which enabled a once parched land to be turned into a granary with a healthy and self-supporting people gradually declined. A war-worn people gave up the struggle and malaria began its terrible course.

THE INFLUENCE OF CLIMATIC CHANGES.

Since earliest geological time, change and not uniformity has been the rule. A few generations ago it was thought that the climate of the world had been uniform since the completion of a definite creative act. However, geologists have perceived at least 4 glacial periods, separated by interglacial periods. There has therefore been a pulsation back and forth between mild conditions and those that lead to glaciation.

There has also been a further change in thought. The last ice age occurred about 50,000 B.C. and, as the morains of this ice age were studied more carefully, it became evident that the melting of this ice sheet did not take place steadily. On more than one occasion the climate ceased to become milder and either remained uniform for a considerable time or reverted back to those conditions which induced glaciation. There are therefore post-glaciation 'stages' which show that after the main pulsations of the glacial epochs have passed away there are a series of minor pulsations of the same kind, but much less severe.

There is considerable evidence for these post-glacial pulsations in climate. In places like the dead sea valley in Palestine, there are a number of old strands, at least 15 in number. These lie above the present level of the water. They indicate that the level of the water has fallen very irregularly. At times it must have fallen more or less rapidly, then the level remained stationary for a time and may even have risen again, though direct evidence of the latter is usually concealed. These up and down movements indicate climatic changes.

There are in many parts of Africa and Asia ruins of ancient cities located in areas where to-day the water supply seems entirely inadequate. To account for these, two hypotheses, both untenable, have been put forward, those of deforestation and progressive desiccation. According to the first the indiscriminate felling of forests has actually led to a decrease in the rainfall. Measurements in the actual amount of precipitation before and after felling have however shown little difference.

Whereas more water in the past is more prominent, evidence of a contrary nature, though less spectacular, is none the less significant. Ruins have been located on the floor of lakes which must have been partially dry when the original buildings were first erected, as for example, the finding of a stone age village on the floor of a lake in Switzerland in 1854, as a result of the lowering of the lake waters by an exceptional drought. Such findings negative the idea of progressive desiccation.

What evidence is there of climatic change within historic time? The following is cited as one of many: The general belief has been that environmental conditions along the Nile are to-day the same as those that confronted the fathers of the Egyptiac civilization. The facts would however appear to be otherwise. It has been estimated that by 10,000 B.C. the ice sheet had left the Baltic and reached the present coast of Sweden. While northern Europe was covered with ice the Atlantic rain storms were deflected southward by the Arctic high pressure. The cyclones that to-day traverse central Europe then passed over the Mediterranean area. The parched Sahara then enjoyed a regular rainfall with the result that the northern parts of Africa were covered with grassland. But as the ice sheet retreated northwards so did the cyclonic rains and North Africa experienced a physical change in the direction of desiccation. When the grasslands overlooking the lower Nile changed into the Libyan desert, the pioneers of the Egyptiac civilization answered the challenge and went into the jungle swamps of the valley bottom.

All the evidence points to moist conditions prevailing in western Asia and northern Africa about 500 to 600 B.C., followed about 200 B.C. by a drier climate.

About A.D. 100 climatic conditions were again more favourable. During the next 5 centuries there was a gradual increase in aridity reaching its worst state about A.D. 650. An improvement then followed culminating about A.D. 1000, succeeded by another bad spell which reached its worst condition about the 13th century. There was a slight recovery in the 14th century from which time there has been a gradual increase in aridity.

In these areas therefore there has been a pulsation in climate and in order to prove this Huntington (1922) carried out an investigation in California. The thickness of the annual rings of trees furnish a reliable indication of variations in the water supply from year to year.

During the years 1911 and 1912, Huntington measured the thickness of the annual rings of the Sequoia tree, which varied from 250 to 3,250 years, and from the figures and graphs obtained there appeared to be a general agreement between the main climatic changes in western Asia and a similar region of the United States of America. This suggests that climatic changes are phenomena which are probably world wide.

If this is indeed the case then the pulsations in climate set forth above assume a considerable significance. From the 10th century A.D., with a minor improvement in the 14th, the general tendency has been towards arid conditions. The onset of these arid conditions occurred about the 10th century at a time when the Sinhalese tanks and channels were being subjected to the destruction due to the devastating wars mentioned earlier in this paper.

A prosperous, self-supporting and healthy people were henceforth to be denied the irrigation waters for their fields, and were also to be subjected to increasing arid conditions. The paddy field, which had hitherto given subsistence to the people from the 10th century onward, failed to maintain sufficient staple food owing to lack of water. The general health of the population therefore declined.

The vector species, with a comparatively low sporozoite rate, which depends upon numbers per unit of population for transmission, had hitherto not the facilities for abundant breeding. Combined with the increased pool formation in the once flowing channels and the once filled tanks there is to be added in the years succeeding the 10th century A.D. an increased breeding potential in the rivers. Normally, these rivers dry up after the rains with the increased formation of pools favoured by the vector species. With the increase in arid conditions, the duration of the river flow would decrease with a corresponding increase in the length of time that the pools remain.

There can be no doubt that the vagaries in climate did much to augment the difficulties resulting from the destruction of the irrigation system. With a lowering of the people's health due to impoverished conditions, the increase in breeding of the vector quickly resulted in those endemic malaria conditions which brought about the depopulation of districts referred to by the Dutch colonists.

SUMMARY.

1. The colossal tank and irrigation system of ancient Ceylon was the direct result of the influence of the Indic civilization which penetrated peacefully into the island during the rain of Devanampiya Tissa.

2. The impetus that set in motion the tank building era resulted from a double challenge—the stimulus of new ground and the virtues of adversity.

3. Minor Tamil invasions had taken place up to the 5th century A.D. but were coincident with the development of the irrigation system and could therefore not have been responsible for its decay.

4. The destruction of the irrigation system commenced with the Cholan conquest of the 10th century A.D.

5. The climate is not stable but pulsates from arid to moist conditions and that this phenomenon is world wide.

6. From the 10th century onward there has been a gradual increase in aridity.

7. This increased aridity is coincident with the commencement of the destruction of the irrigation system due to the Cholan conquest.

8. The lack of water for irrigation of the staple food together with the increased breeding potential of the vector species led the way to the introduction of endemic and hyperendemic malaria.

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DEFORESTATION AND EPIDEMIC MALARIA IN THE WET AND INTERMEDIATE ZONES OF CEYLON.

BY

T. W. TYSSUL JONES.

[October 30, 1950.]

INTRODUCTION.

MANY important contributions to epidemic malaria in Ceylon have appeared from time to time but its relationship to deforestation has not been fully appreciated. This paper is put forward as a contribution to that line of thought.

The subject will be treated under the following main headings :—

1. Influence of forests on the hydrologic cycle.
2. Climatic factors in Ceylon.
3. Mosquito vector in Ceylon.
4. Main epidemic zones.
5. Deforestation consequent upon the development of the rubber and tea industries.
6. Effect of deforestation on the rivers and consequently on epidemic malaria.

INFLUENCE OF FORESTS ON THE HYDROLOGIC CYCLE.

There is some difference of opinion with regard to the effect of forests on rainfall.

Thus, Huntington (1922) remarks ' . . . . . the reckless cutting of forests has not only allowed the rain to denude the mountains of soil but has caused an actual diminution of rainfall. This view has now practically ceased to be considered among scientists. Measurements of rainfall before and after deforestation show slight differences, too small to be of practical value '.

Walters (1910), commenting on the view of the older residents of Mauritius that deforestation has affected the climate of that island, says ' From a careful analysis of all the available statistics, there would appear to be some evidence in support of their views, but the effect has been greatly overestimated. So far as rainfall is concerned the utmost that can be said is that the distribution has been affected, the number of days in which the afternoon rainfall having been diminished by perhaps 40 to 45 per cent representing at the most a diminished fall of 6 to 10 inches '.

Pfeiffer (1947) contributes to the same theme in maintaining that hills, mountains and forests are the main water gatherers.

Whatever the difference of opinion existing with regard to the effect of forests on rainfall, most authorities are agreed on their effect on the discipline of rivers.

Thus, Walters (*loc. cit.*) remarks ' Whatever may be thought of the climatic effect of forests, their influence on the flow of rivers appears to be beyond dispute '.

Collis (1950) says ' They (the forests) exert considerable influence upon the fall and distribution of rain ; by virtue of sponging the earth around their feet they enormously influence the behaviour of floods, the discipline of rivers '.

The water in the world is constant in quantity, though there is considerable variation in its state, existing as it does in liquid solid and gaseous forms. There is a continual interchange between the liquid and gaseous states. Water is evaporated by solar energy from the oceans, lakes, etc., and is also transpired from the leaves of plants, into the atmosphere. This gaseous output forms clouds which are shifted by the winds. Due to physical causes the clouds precipitate their water in the form of rain. What happens to the rainfall will depend upon the type of surface upon which it falls.

If the land is flat and bare the water will quickly evaporate back into the atmosphere again. If on the other hand the flat land has a good cover of vegetation the water will be absorbed and will feed the underground water-table.

The presence or absence of forests on mountains and watersheds will determine the immediate fate of rain water falling on such areas.

A natural forest has a three-tiered vegetative complex. Near the ground are the small plants, usually annuals, which give an excellent cover. Between them and the leafy canopy of the main trees are the variety of shrubs. The fallen leaves of these different components of the forest add a natural return to the soil, and, in fact, act as a colossal sponge.

On a forested watershed the storm water, instead of falling and exerting its full force on the soil, is cushioned by the leafy canopy of the trees, and gradually trickles down to the ground where it is easily absorbed by the forest litter. It has been estimated that a single acre can absorb as much as 46 tons of water. The montane region of Ceylon is approximately 1,200 square miles in extent. It was originally covered with forest, so on the above basis approximately 35,000,000 tons of water were capable of being absorbed.

The capacity of this forest litter to absorb and to detain moisture is called seepage, which even on the steepest slopes has been found to be so marvellously absorptive that ' it creates conditions with regard to surface run-off such as obtains in level country ' (Sears, 1949).

It is this seepage that promotes the discipline of rivers by creating and maintaining springs which feed them.

When a watershed is deforested, seepage is eliminated or seriously curtailed so that the storm rains quickly find their way to the rivers thus causing flood conditions. When the storm subsides, the rivers quickly revert to a mere trickle. In

the forested areas floods are minimized and the continuous supply of spring water maintains a more even flow in the rivers.

CLIMATIC FACTORS IN CEYLON.

In respect of relative humidity, temperature and pressure, the climate of Ceylon is extremely equable.

There is a remarkable constancy from month to month and from year to year in the mean relative humidity, although there are variations between different parts of the island. In the low country of the south-west quadrant, the mean monthly relative humidity lies between 70 and 80 per cent. In the coastal districts, there is little deviation from the normal, even during periods of drought.

The temperature as a whole rarely shows a diurnal range exceeding 15°F., the minimum hardly ever drops below 65°F.

The environmental factors in respect of relative humidity and temperature would therefore appear to be most favourable for the transmission of malaria in the south-west quadrant and more particularly in the coastal areas where there is a mean temperature of 75 to 80°F., associated with a mean relative humidity of 80 per cent throughout the year.

There is however a remarkable variation in the rainfall in different parts of the island. This is due to the influence of the central montane tract on the south-west and north-east monsoons.

The precipitation of the south-west monsoon is chiefly confined to the south-west quadrant which however also receives rain from the north-east monsoon. Upon the variations in the rainfall, the country is divided into Wet, Intermediate and Dry Zones for purposes of malaria study (Map 1). These three zones have been defined from the amount of rain received from the south-west monsoon. In the Dry Zone the rainfall is below 20 inches, in the Intermediate between 20 and 40 inches and in the Wet Zone over 40 inches. In respect of the average annual rainfall, the Dry Zone receives from below 50 to 100 inches, the Wet Zone over 100 inches, whereas the Intermediate gets between 75 and 100 inches.

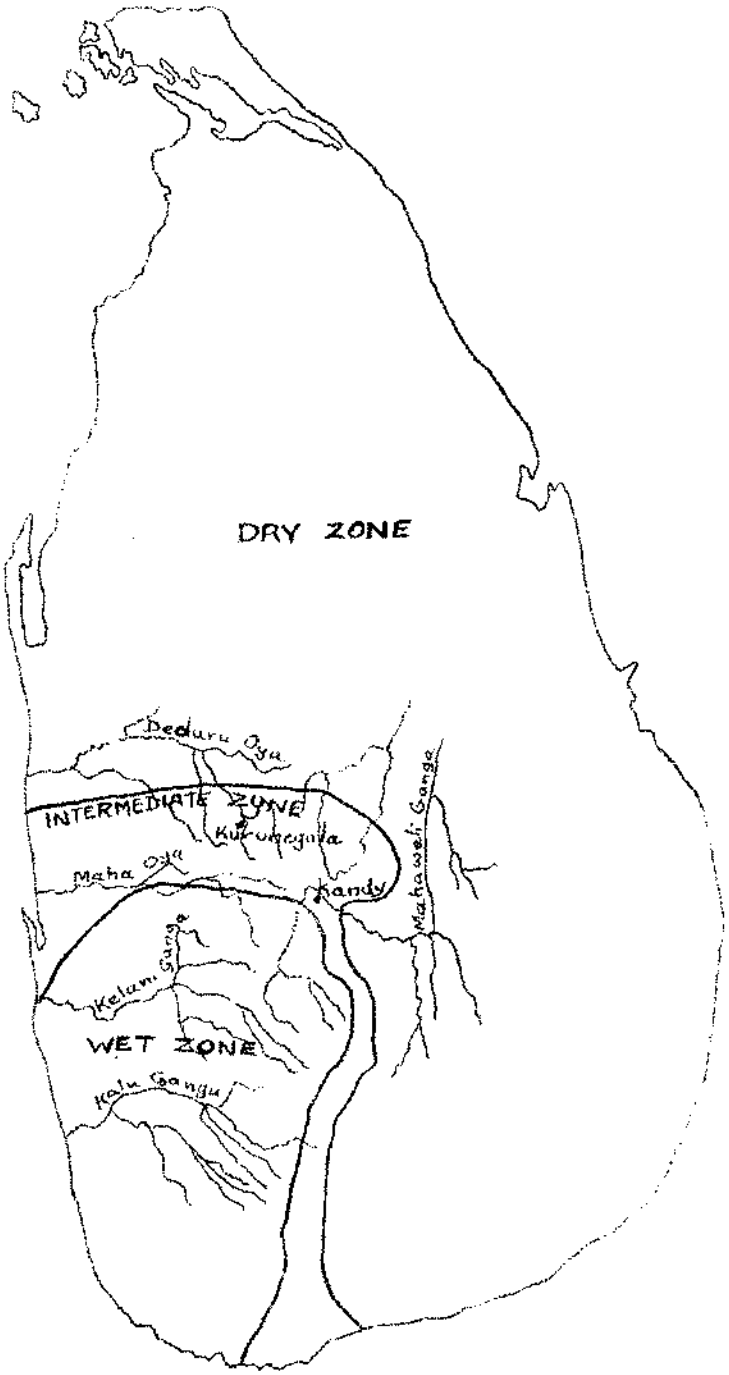
Coincident with the monsoon rains there is the attendant malaria. In the Dry Zone, there is one malaria season, whereas in the other two zones there are the spring and autumnal peaks of transmission. There is a close relationship between rainfall and malaria. In the Dry Zone, malaria morbidity rises after the rains, but epidemic malaria in the Wet and the Intermediate Zones does not follow heavy rain. A wet year is a healthy year particularly in the Wet Zone. It is the failure of the monsoon rains which gives rise to epidemic malaria.

MOSQUITO VECTOR IN CEYLON.

Prior to 1927 the species of mosquito responsible for malaria transmission was not definitely known. On epidemiological grounds three species were suspected —*A. culicifacies*, *A. maculatus* and *A. varuna*, all proven vectors in other areas. In 1927, one *A. culicifacies* only was found infected (Wickremesinghe, 1945). From that year onward, particularly as a result of work by the Government

MAP 1.

(From Rustomjee, 1944.)



entomologist. *A. culicifacies* was definitely incriminated. During and since the 1934-35 epidemic, nearly one-quarter of a million anopheline mosquitoes including many thousands of all species on the island have been dissected. Well over 1,000 have been found infected, and except two specimens of *A. hyrcanus* all have been *A. culicifacies*. The evidence is therefore indicative that *A. culicifacies* is the main vector, if not the only vector, in Ceylon. There can be little doubt however that in so far as epidemic malaria is concerned, *A. culicifacies* with its great prevalence and high infection rate during such periods is the only species actively concerned with the transmission of the disease.

Carter (1927) has shown that *A. culicifacies* is essentially a Dry Zone species and is widely prevalent in the north, east and south-east of the island. Its range is restricted by altitude, being rarely found above the 2,000-foot contour line.

In the Wet Zone, its distribution is much more erratic, but is usually present in small numbers throughout this part of Ceylon.

In the Intermediate Zone, its prevalence depends essentially on the distribution of the rainfall. In the years of normal rains, it is more prevalent towards the drier parts but in the years of drought it becomes more plentiful towards the wetter regions.

MAIN EPIDEMIC ZONES.

The main epidemic zone is concerned with the basins of the rivers, Deduru-oya, Maha-oya, Kelani-ganga and the Kalu-ganga (Map 1). These four rivers rise in the hills and flowing in a westerly direction through the foothills and the coastal plains drain the epidemic zone.

The Deduru-oya is approximately 87 miles long, arises in the low hills, little more than 1,000 feet high, in the Kandy and Kurunegala districts. There is a considerable drop in altitude within a comparatively short distance with the result that the greater part of the river flows through level country. The bed of upper reaches of the river is rocky but throughout the flat area the bed is wide and sandy. The river receives most of its rain from the north-east monsoon though the south-west monsoon contributes also. However, the contribution from the south-west monsoon, practically every other year, is feeble with the result that the flow of the river is poor. Added to which is the fact that much of the water from the upper reaches is drawn off to supply the water tanks in the Dry Zone.

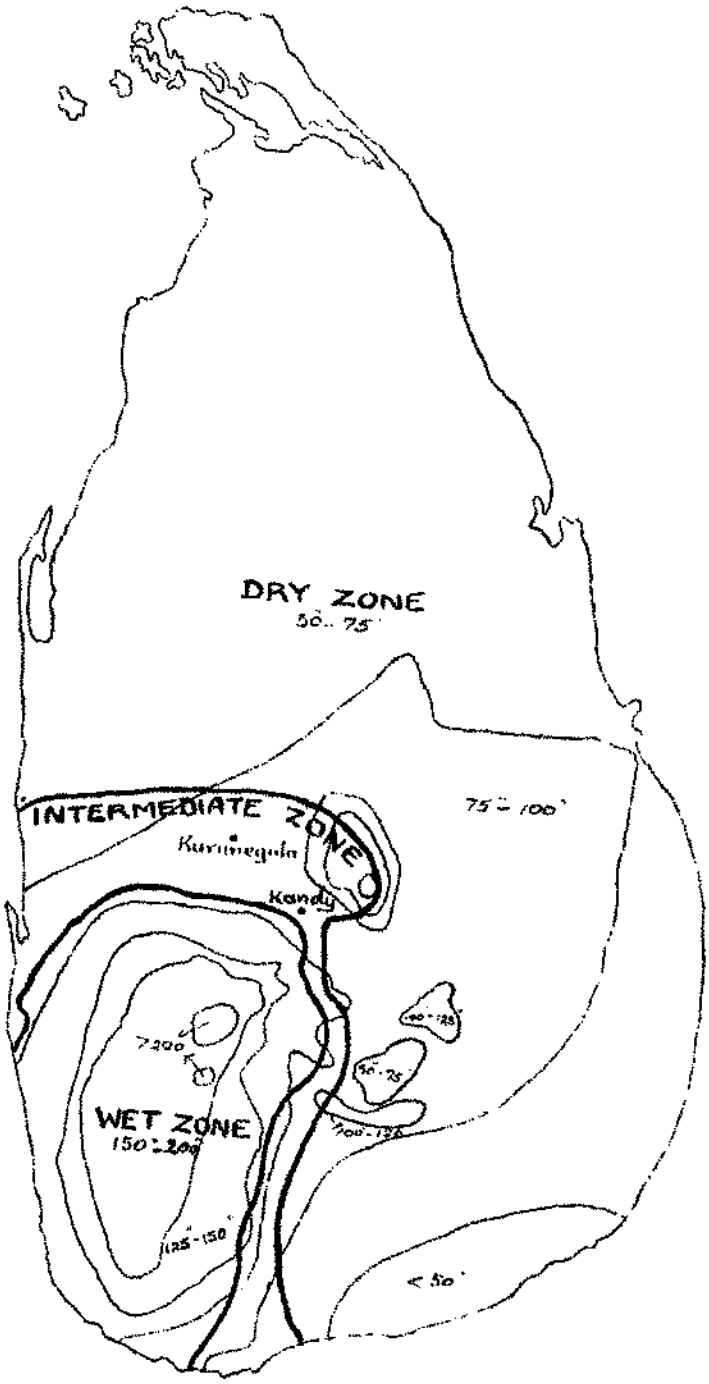
The Maha-oya is about 78 miles long. Its catchment area is within the 500- and 1,000-foot contour lines. As with the Deduru-oya, the bed of the upper reaches is rocky and that of the lower reaches is again wide and sandy. The last few miles of the river, even under drought conditions, always has a good flow.

The Kelani-ganga is the longest of the four rivers, having a total length of 94 miles. Its catchment area is above the 3,000-foot contour. This area is within the 100- to 200-inch rain belt and thus receives abundant rainfall which has the effect of keeping the river in good flow throughout most of the year.

The Kalu-ganga is 70 miles long and rises in the Adam's Peak Range where the rainfall is abundant throughout most of the year.

MAP 2.

(From Rustomjee, 1944.)



There are two important points to be noted with regard to these rivers :—

1. Their length is comparatively short, hence there is normally a sharp ebb and flow in the storm water.

2. It will be seen from Maps 1 and 2 that the Deduru-oya and the Maha-oya rise in the mountain range which has respectively 75 to 100 inches and 75 to 125 inches of rain. The Kelani-ganga and the Kalu-ganga have their source in the watershed which receives over 150 inches of rainfall. The catchment area of the first two mentioned rivers is at a much lower altitude than that of the latter two.

A. culicifacies has a predilection for sunlit pools in the dry river beds, though its breeding places also include many other situations. However, there is no doubt that it is the increased output of the vector from the pools in the dry river bed that produces the epidemics.

It is not the author's intention to go into the question of the health of the population prior to the onset of epidemic malaria, but rather to confine himself to question of increased breeding potential in the river beds during drought periods and particularly to the effect that deforestation has had on this.

All authors on epidemic malaria in Ceylon have noted the relationship between an epidemic and the failure of one or in some cases two monsoons. Failure of the south-west monsoon results in drought conditions during the months of May to September and that of the north-east monsoon to drought in the months of November to April. It has already been pointed out that droughts lead to pool formation in the river beds. The author is not aware of anyone having drawn attention to the possible relationship which exists between increased pool formation and the deforestation of the watersheds of the rivers concerned.

DEFORESTATION CONSEQUENT UPON THE DEVELOPMENT OF THE RUBBER AND TEA INDUSTRIES.

Before the advent of the European to the south-west quadrant, most of the hill country was covered with virgin forest. Little of that forest remains to-day and it will be shown subsequently that, in so far as it affects the main arguments of this paper, the rubber and tea plantations have been no substitute for the primeval jungle and its direct effect on the hydrologic cycle.

Coffee was the first commercial crop to be grown in Ceylon. Planting started in 1827 when many acres of virgin forest were cleared. By the year 1883, a scale insect and a fungoid disease attacked the crop simultaneously and wiped it out. There is little coffee growing in Ceylon to-day and the tens of thousands of acres that were once given to it are fast settling down to the state of patina land. Coffee has gone, but tea and rubber fill a larger area than coffee ever did.

The first commercial planting of tea dates back to 1867, with only 10 acres. It was from 1883 onwards that the vast felling of the forest for tea plantations really got under way. To-day Ceylon, second only to India as a tea exporting country, has half a million acres under this crop.

The original supply of rubber came from the wild rubber tree of the jungles of Brazil and the Belgian Congo. This supply was sufficient to meet the

requirements of the early 19th century markets. With the growing popularity of rubber goods in the European markets, the demand increased beyond the capabilities of the wild rubber tree and the organization concerned

The planting of rubber from seedlings grown in English nurseries started in the Malayan areas about 1880. In Ceylon, rubber was started on a commercial scale about 1883. From 1900 onwards with the coming of the car and the consequent demand for rubber tyres, consumption of this product increased at an enormous rate with the accompanying increase in production. By 1911, rubber was a most important factor in Ceylon's commerce, with at least 1,000,000 acres under cultivation.

These areas under tea and rubber, of which there are an estimated 2,000,000 acres to-day, have been possible only at the expense of the original virgin forests. This rapid development has led to untold damage to the soil. Erosion is widespread and in places far advanced.

EFFECT OF DEFORESTATION ON THE RIVERS AND CONSEQUENTLY ON EPIDEMIC MALARIA.

Good cultivation in both the tea and rubber plantations was taken to mean cultivation free from weeds. This left the soil completely unprotected. There is to-day considerable concern among both tea and rubber planters about soil erosion.

"The rapid development of commercial tea and rubber has had a disastrous effect on the soil. In particular, weeds and other forms of ground cover are coming to be regarded as indispensable" (Jacks and Whyte, 1939).

"The rubber research scheme (Ceylon) is therefore recommending that local estates should endeavour to establish leguminous ground cover, in the meantime retaining naturally occurring plants of a desirable type including rubber seedlings up to a height of 6 to 8 feet" (Jacks and Whyte, *loc. cit.*).

All this adds up to the fundamental fact that the storm water instead of soaking into the ground runs off rapidly carrying the soil with it. The lighter soil particles are actually carried out to the Indian Ocean to be deposited there. The heavier ones, particularly when the storm waters are subsiding, are deposited in the lower and middle reaches of the river's course thus adding to those conditions necessary for *A. culicifacies* breeding.

The substitution of tea and rubber has been no answer to the primeval forests from the point of view of the discipline of the rivers. Tea and rubber have substituted a single growth of plants for the three-tiered complex of the original forests. Particularly was this so when good cultivation in the plantations was taken to mean cultivation free from weeds. In the original forests there was the close net-work of ground weeds, at the top the very close canopy of the trees' leaves and in between the growth of shrubs and smaller trees. Instead of this natural and varied growth the tea and rubber trees are grown in regimental rows, which particularly in the tea plantations gives no protection to the ground between the rows.

In the primeval forests, leaves falling to the ground from the trees, shrubs and ground plants add a natural return to the soil in the form of humus, thus maintaining the soil's great absorptive properties. In such areas vast quantities of water are taken in, which after the storms have subsided, trickle into the rivers, thus maintaining a more even flow. This natural return is particularly absent in the tea plantations, since the commercial value lies in the leaves which are continually plucked off.

Note the conditions prevailing to-day, particularly in the northern basins of the epidemic zone. ' In the hill country the rush of water down subsidiary streams, after even a moderate fall, is sufficiently powerful to produce a beneficial clearing effect..... The smaller streams do generally get flushed after a moderate shower but unless the fall is heavy a great number of rock pools remain. *The sudden rise and rapid subsidence of rivers and streams is very marked.** A fall of just over 2 inches in the Maha-oya upper catchment area was sufficient to flush the entire width of the bed at Alawwa within 12 hours and equally rapid was the subsidence pools forming four days after flushing. The large tributary of the Mahaweli-ganga at Katugastota was full in the morning after local precipitation and by the evening the sandy edges were quickly reappearing ' (Briercliffe, 1935).

Again by the same author ' It is reasonable therefore to state that unless the rainfall is more or less uniformly distributed over a month, rivers and streams are favourable to anopheline production from time to time '.

That the spate of the rivers during heavy rains will increase is beyond dispute, and it is equally true that the spate will decrease after the rains. However, in well-forested areas, the flood water will not be so excessive and the prolonged seepage from the absorbent forest ground will maintain a steady flow over extended periods. In Ceylon, the rivers are small, below the level of the surrounding country and the flow of subsoil water will be into them. In well-forested areas, the flow of the rivers will not be so dependent on the distribution of rainfall.

As Dunn (1936) correctly states ' In Ceylon it is the drying of the rivers and not the overflow from rivers that produce extensive breeding grounds '.

Gill (1935) makes a remark pregnant with meaning ' thus ..... it will be seen that the area involved in the recent epidemic (1934-35), which corresponds closely with the areas affected in previous malaria epidemics, is also the area in which most of the estates are situated '. He might with equal truth have said areas which had been deforested.

Briercliffe (*loc. cit.*) says ' the districts most seriously affected were, of course, those situated in the upper catchment areas of the Maha-oya and the Deduru-oya, viz. Kegalla and Kurunegala '. Reference to the agricultural map of Ceylon will reveal the fact that the upper catchment areas of these two rivers have been heavily deforested for the purposes of planting tea and rubber.

Dunn (*loc. cit.*) remarks ' My study of the records impress me with the almost constant association of the failure of one monsoon at least with previous epidemics '.

* The present author's italics.

TABLE

Weekly attendances at each hospital and dispensary showing rise and

(From

19

Institutions.	August.			September.					Octo	
	11	18	25	1	8	15	22	29	6	13
Hospitals :										
Dambadeniya ...	794	680	714	655	616	498	546	700	759	637
Dandeganuwa ...	1,458	1,385	1,246	1,273	1,196	1,199	1,205	1,071	1,016	1,097
Kurunegala ...	1,136	1,074	1,081	1,048	1,046	1,028	1,020	986	1,072	964
Nikaweratiya ..	367	410	341	345	321	319	316	426	378	373
Rediganna ...	482	549	522	470	446	535	607	656	499	699
Dispensaries :										
Batalagoda .	167	195	160	158	173	193	213	213	192	184
Binziriya .	568	599	472	526	598	437	509	555	524	604
Dodangaslanda .	96	84	59	108	136	163	134	138	159	225
Galgamuwa ..	305	267	326	265	318	254	301	331	441	396
Hiripitiya ...	482	449	477	464	458	469	519	593	541	437
Maho	427	392	314	330	341	331	365	409	368	441
Makandura ...	378	360	367	399	304	277	362	292	351	338
Mawatagama ..	555	510	482	494	509	485	539	555	589	474
Narammala ...	748	737	635	536	531	493	535	522	519	565
Nikaweewa ..	181	162	124	109	124	127	171	223	203	181
Polgahawela ...	818	839	759	758	743	748	764	789	781	839
Wariyapola .	353	374	379	369	327	288	341	393	360	295
Uswewa ...	342	371	381	400	386	400	464	477	484	953*
Katupotha .	236	257	252	219	198	181	133	152	174	128
Hettipola ...	349	338	298	286	315	232	258	313	308	265

* Indicates start of epidemic.

I.

development of the epidemic in Kurunegala District.

Briercliffe, 1935.)

34

ber.			November.					December.		
20	27	3	10	17	24	1	8	15	22	29
849	892	1,863*	2,160	2,470	2,452	3,690	3,562	7,831	*10,392*	6,475
1,139	1,146	1,745*	1,956	2,341	2,319	3,097	5,259	9,794	*10,123*	7,931
1,143	1,385	2,763*	2,909	2,557	2,648	2,941	3,922	3,275	2,959	2,116
369	353	496*	922	1,332	1,200	1,269	1,462	1,577	*2,023*	1,759
697	559	1,011*	1,833	1,881	1,449	1,335	1,309	1,385	*1,980*	1,042
232	281	668*	1,270	1,458	1,341	1,156	1,504	1,407	*1,561*	889
624	607	814*	1,233	1,518	1,037	1,231	1,437	2,615	*4,367*	3,740
222	209	543*	700	724	556	551	885	818	*999*	755
372	390	498	875*	1,287	1,298	1,089	1,029	1,216	*1,435*	1,243
486	528	939*	1,375	1,616	1,460	1,174	1,189	1,378	*1,579*	1,543
449	396	668*	1,189	1,646	1,404	1,623	1,716	2,012	*2,201*	1,817
334	449*	667	987	1,337	1,235	2,072	2,177	3,194	*4,278*	2,708
681	777*	1,584	2,416	2,087	1,986	2,442	2,916	*3,461*	3,249	2,728
624	1,076	2,170	2,204	2,512	2,769	4,150	4,655	*5,076*	4,615	4,577
204	265	410*	772	996	813	619	593	815	*1,245*	1,036
1,045*	1,444	3,098	2,884	3,867	3,736	4,749	*5,598*	4,368	5,166	5,120
335	374	869*	1,239	1,460	1,141	1,210	*2,264*	2,009	1,944	1,663
858	1,625	3,402	2,582	2,487	3,233	3,689	3,340	2,841	*3,673*	2,819
207	172	412*	495	486	466	752	1,180	1,052	1,709	1,795	*1,996*
317	241	427*	686	850	690	792	398	1,869	*4,349*	4,235

Figures in italics denote peak.

TABLE

Weekly attendances at each hospital

(From

19

Institutions.	August.			September.					Octo	
	11	18	25	1	8	15	22	29	6	13
Hospitals:										
Aranayake ...	346	295	389	367	319	283	346	371	324	257
Ehelyagoda ...	745	712	677	698	619	661	584	683	585	446
Karawanella ...	535	627	550	633	518	508	504	566	551	457
Kegalla ...	785	600	654	589	618	651	703	707	702	639
Kitulgala ...	230	212	235	181	182	177	178	211	192	122
Undugoda ...	248	293	226	282	329	296	277	378	366	266
Dispensaries:										
Deraniyagala ..	130	129	107	144	159	161	124	135	122	85
Hematagama ..	372	345	312	277	321	245	253	261	261	274
Mawanella ..	356	363	354	346	376	354	409	382	385	382
Nedundeniya ..	272	270	233	279	316	356	374	384	453	578*
Nayadurupola ...	264	262	322	268	324	239	333	294	301	276
Pindeniya ..	163	168	175	174	260	215	217	179	214	159
Rambukkana ..	583	585	483	557	618	653	640	706	918*	934
Warakapola ...	220	206	241	231	221	286	273	245	400*	407

* Indicates start of epidemic.

II.

and dispensary, Kegalla District.

Briercliffe, 1935.)

34

ber.		November.				December.				
20	27	3	10	17	24	1	8	15	22	29
273	274	307	344	487*	491	697	1,783	1,691	1,734	1,682
477	558	649	501	606	630	770	1,235*	1,889	*2,175*	2,171
482	390	478	504	593	586	978*	3,228	4,465	3,809	4,898
385	470	856*	1,284	1,659	1,572	2,250	4,330	*9,230*	8,344	7,857
153	156	191	156	179	195	306*	1,166	*1,784*	1,376	1,418
270	236	254	319	381	455	581*	1,454	1,449	1,260	*1,649*
101	128	108	127	169	175	165	627*	*1,145*	957	801
216	124	189	276	178	276	610*	1,562	*2,531*	1,996	1,798
429	406	357	746*	1,011	1,014	1,502	3,251	*6,258*	6,205	5,348
715	658	1,051	2,122	2,472	2,169	2,884	4,862	4,676	*5,265*	5,208
264	268	354	499*	555	638	743	1,433	*2,646*	2,478	2,349
173	*146*	205	220	265	272	686*	2,868	*3,211*	2,723	2,794
958	1,114	1,756	2,840	2,836	2,388	2,895	3,068	*4,815*	4,286	4,238
406	515	702	1,206	1,449	1,224	1,475	2,627	3,668	*3,768*	3,302

Figures in italics denote peak.

However, it is not drought conditions *per se* (excessive drought preceding the 1934-35 epidemic was abnormal) that are all important so much as the distribution of the rainfall within that period. The various charts and graphs produced by different authors show that it is a deficiency of rain in the 2 months preceding the epidemics that is of importance and not deviation from the normal for the whole monsoon period. Even during non-drought years a lack of rain in these 2 months will produce epidemic conditions. In order to illustrate this point the autumn epidemic peaks in Kurunegala District is cited for the years 1928, 1929 and 1930. The 1929 epidemic followed a non-drought year, however there was no rainfall in August and reduced precipitation in September. A comparison of the rainfall figures for the epidemics preceding and succeeding the one in 1929 bring out some important points. The greater epidemic in 1928 was preceded by a drought period of 50 days compared with a drought extending over 65 days preceding the 1930 epidemic, but the 65-day drought was concerned mainly with the failure of the north-east monsoon, whereas the 50-day drought was concerned with the failure of the south-west monsoon, when the precipitation in August and September and particularly in the former month was very much less than that during the 65-day drought.

The 1934-35 epidemic was the first to which a considerable amount of detailed analysis was given, particularly by Gill (1935) and Bricrcliffe (*loc. cit.*).

Gill (1935, 1938) postulates that epidemic malaria in the Wet Zone is associated in the first place with an 'epidemic of relapses'. His main thesis is that epidemic malaria in Ceylon can only be accounted for on the assumption that they are initiated by relapses and to substantiate his hypothesis he brings forth the points that there was an 'explosive outbreak' which occurred over a wide area and that they reached their acme in 6 weeks. Study of Tables I and II will show that these assumptions are not maintained.

It will be seen from Table I that only in two instances was the acme reached in 6 weeks—Kurunegala and Wariyapola; the usual period was 8 weeks and in some cases much longer, as for instance, 11 weeks in Alawwa and 10 weeks in Katupotha. This Table also negatives his statement that an 'explosive outbreak' occurred over a wide area because, although it is true that in some hospitals the attendances were doubled, in the majority of hospitals and dispensaries the onset of the epidemic was gradual.

The same general picture is seen in Table II, except that in some cases the acme was reached in 3 weeks of the onset of the epidemic—Kitulgala. In Deraniyagala, the acme was reached in the week following the onset.

It is apparent that the outbreak of epidemic malaria did not occur simultaneously even with a district; there was at least a month between that of Alawwa and Katupotha (Table I).

Gill's explanation for the sudden onset of the 'epidemic of relapses' was the sudden increase of humidity from 51 to 70 per cent (he refers to Kurunegala). He says 'the rise of atmospheric humidity ..... may have exercised a direct influence upon the parasite in the human carrier'. However, he agrees that the onset of sickness at Alawwa started *before* the end of the drought, hence during a time when there was a low atmospheric humidity.

If epidemic malaria is precipitated by an 'epidemic of relapses', it is difficult to account for discrepancies between time of onset, severity of sickness and difference in the time before the acme is reached.

Gill's second main point is that the outbreak of malaria being due to relapses would account for the low mortality of the 0-10 age group. Gill (1935) says 'If the sudden onset of sickness was mainly due to new infections, it would be expected that there would have been a sudden sharp rise in the number of deaths in children under 10 years with a week of October 27, whereas the mortality in this age group was scarcely raised until the first week in December'.

It is characteristic of *P. vivax* malaria that it causes less clinical disturbance than that of *P. falciparum*; the intensity of the parasite infection of the former rarely exceeds that of 100,000 per cubic millimetre of blood. An important consequence of this is that benign tertian attacks are seldom fatal except to very young infants. It will be noted that up to December 1934 it was generally infants who died, though it was in early December that the main effect was felt in the 0-10 age group.

Reports from 9 laboratories during the 1934-35 epidemic were analysed for the three species of malaria parasite. Month by month details are given in Table III.

TABLE III.

Percentage of three species of malaria parasite.

Month.	*P. vivax* per cent.	*P. falciparum* per cent.	*P. malariae* per cent.
1934			
Nov.	72·2	24·7	3·1
Dec.	68·4	31·1	0·5
1935			
Jan.	55·0	43·4	1·6
Feb.	56·1	43·1	0·8
Mar.	71·2	28·0	0·8

It will be seen from this Table that the *falciparum* infection was very much increased during the months of December and January which in fact corresponds to the acme of the mortality wave. However, as Briercliffe (*loc. cit.*) remarks, there appeared to be some doubt about the correct diagnosis of the three species. It is therefore of some interest to note the findings of the pathologist at the Colombo General Hospital for the same period, in respect of the gametocyte percentages of the three species. These are given in Table IV.

TABLE IV.

Number of slides examined and the percentage of gametocytes for the two species from November to February 1934-35.

(From Briercliffe, 1935.)

Month.	Total slides positive for parasites.	Tertian gametocytes.	Crescents.	Percentage.
Nov. ...	68	1	1	2·9
Dec. ...	737	4	68	9·8
Jan. ...	822	30	133	19·8
Feb. ...	925	47	292	36·6

Briercliffe (*loc. cit.*) concludes ' the only definite conclusions which are justified from the figures are that tertian malaria was considerably more prevalent in the beginning of the epidemic, but that subtertian increased in prevalence during the months of December and January '.

Would not this account for the low infant mortality rate before mid-December ?

In an analysis of the epidemics in Ceylon, Carter (*loc. cit.*) stressed the important fact that *A. culicifacies* is essentially a Dry Zone species. This point would seem to have been overlooked. Epidemics in the Epidemic Zone may be looked upon as being due to the invasion of that zone by the vector species from a focus in the Dry Zone or the northern part of the Intermediate Zone. Further evidence to this effect may be gleaned from the following remarks of Briercliffe (*loc. cit.*) : ' The area of the epidemic (1934-35) was confined to the basins of the five rivers, Deduru-oya, Maha-oya, Kalu-ganga, Kelani-ganga and the Mahaweli-ganga. Malaria broke out early in October in the villages on the middle reaches of the Maha-oya and invaded rapidly and with intensity the regions drained by the upper and middle reaches of the Maha-oya and the Deduru-oya (Kegalla and Kurunegala districts). A little later, the epidemic appeared in the Kandy and Matale districts in the Mahaweli-ganga basin, and then at the beginning of December in the upper and middle reaches of the Kelani River. In the Mahaweli-ganga and the Kelani-ganga basins the intensity of the epidemic was moderately severe but it did not cause the same amount of suffering and devastation as in the Maha-oya and the Deduru-oya basins. It finally invaded the Kalu-ganga basin (Ratnapura and Kalutara districts) and the coast '. In other words there was a definitely southern trend from the north.

Malaria presupposes an increase in the vector species, which in turn involves an increase in the favoured breeding places. It is therefore of some importance to note the distribution of rainfall for various stations between Kurunegala in the north and Kalutara in the south which occurred during the year 1934. These figures are given in Table V.

From a scrutiny of this Table the following facts emerge. During 1934, the rainfall in the Epidemic Zone was the lowest in the Kurunegala and the Kegalla

TABLE V.

Rainfall during drought, April to September 1934.

(Briercliffe, 1935.)

Stations.	Number of years.	APRIL TO SEPTEMBER. Average.	1934 RAINFALL AND AVERAGE PERCENTAGE. Inches.	Percentage.	LOWEST TO 1938 AND AVERAGE PERCENTAGE. Inches.	Percentage.	JULY TO SEPTEMBER. Average.	1934 RAINFALL AND AVERAGE PERCENTAGE. Inches.	Percentage.	LOWEST TO 1938 AND AVERAGE PERCENTAGE. Inches.	Percentage
Kurunegala	48	37·45	14·63	39	23·35	62	12·98	1·99	15	3·63	28
Kegalla	63	57·89	32·50	56	22·98	40	23·55	5·04	21	7·90	34
Matale ...	62	33·73	22·52	67	16·92	50	14·23	3·70	26	3·87	27
Kandy ...	65	41·19	24·66	60	28·24	69	19·17	5·45	28	7·90	41
Padupola ...	64	152·77	83·46	55	81·65	54	79·44	26·56	33	44·22	56
Ratnapura	66	90·39	63·10	70	55·69	62	39·73	15·75	40	18·61	47
Kalutara ...	64	56·61	67·95	120	26·81	47	19·69	8·60	44	3·90	20

districts, but of considerably more importance is the fact that the rainfall for the 3 months actually preceding the epidemic was only one-half the average for the 6 months before the onset of sickness; again proceeding in a southerly direction although below normal, there is a gradual increase in the amount of precipitation during these 3 months.

This distribution in precipitation is reflected in the breeding of *A. culicifacies* throughout the area concerned. This is shown in Table VI.

TABLE VI.

Relative prevalence of anopheline larvæ, December 1934 to February 1935.

(Abstracted from Briercliffe, 1935.)

Area.	Number of anopheline larvæ identified.	Percentage of *A. culicifacies*.
Kurunegala ..	21,906	41·6
Kegalla ..	14,387	29·5
Kandy	15,963	28·0
Ratnapura	970	4·1
Kalutara	930	2·1

There is therefore a gradual decrease in vectorial prevalence southward from Kurunegala.

There is a further point to be considered. Malaria transmission is determined ultimately by the vector's individual longevity rather than by its abundance. A very large vectorial population having a length of life shorter than that necessary for the completion of the extra corporeal cycle would be comparatively harmless from the point of view of malaria transmission.

According to Russell and Rao (1942), with a low relative humidity and a high temperature, the longevity of *A. culicifacies* is about 10 days only. These two authors working with *A. culicifacies* in large out cages came to the conclusion that the maximum longevity of female *A. culicifacies* was 8 to 34 days under optimum conditions.

It will be noted that according to Gill (1935) the humidity in Kurunegala jumped up from 51 to over 70 per cent in the month of October 1934. This increase was coincident with the rainfall of that month and this increased precipitation was general for the whole of the Epidemic Zone. During the dry months of August and September, although prodigious numbers of *A. culicifacies* were being produced, the low relative humidity prevailing at the time would be inimical to longevity, hence the 'transmission index' would be comparatively low.

The rise in the relative humidity in October not only increased the longevity of the vector but also enhanced its metabolic activity which in turn would increase 'biting'.

Several spleen and parasite surveys have been carried out in Ceylon since Carter's original one in the early twenties. As a result of these surveys, Wickremesinghe (1945) reported 'The parasite rates have also been found to vary a great deal in the different regions and zero rates may occur in communities living both in healthy and severely malarious localities. The Intermediate Zone shows the greatest variations in the parasite rates and is the area with the largest number of parasite carriers'.

There is therefore a high level of infection in the zones to which Gill (1935) particularly refers, and with the increased abundance of the vector together with its increased propensity to bite in the month of October, because of the increase in the relative humidity in that month, the sudden onset of sickness is explained without recourse to the thesis of an 'epidemic of relapses'.

Rustomjee (1944) relates the malaria epidemics to the principal rivers in a form of a table. This is reproduced in Table VII.

It will be noted from this Table that the area which has a malaria season chiefly in the months October to January is the basin of the Deduru-oya which has its source in the hills of the Kandy and Kurunegala districts. These two districts always suffer severely from epidemic malaria. It will be seen that the south-west monsoonal influence is very much less in the catchment area of the Deduru-oya than it is in any of the other catchment areas, and that the north-east monsoonal influence is greatest. Map 1 shows that the Kandy and Kurunegala districts are mostly situated in the Intermediate Zone, this is also seen to be true for the catchment area of the Deduru-oya. The Intermediate Zone is contiguous with the Dry Zone.

Malaria in the Dry Zone is hyperendemic. The malaria season follows closely after the north-east monsoon. During these rains there is a marked increase in the areas of surface water, particularly in the form of pools in which *A. culicifacies* breed in prodigious numbers. However, the high north-east monsoonal influence in the Deduru-oya catchment area will tend normally to keep the river continually flushed, thus allowing no opportunity for *A. culicifacies* breeding. Outside the Intermediate Zone, as indeed in the northern parts of the zone itself, widespread malaria prevails at a time when there is little or no transmission in the actual basin of the Deduru-oya itself.

Even during normal years the precipitation from the south-west monsoon is meagre, consequently any failure of these rains leads immediately to pool formation in the Deduru-oya with the consequent increased breeding of the vector *A. culicifacies* leading to the malaria season in October to January. There could therefore appear to be an invasion southward of *A. culicifacies*, possibly when favoured breeding places are present from the Dry Zone to the Deduru-oya basin in the Intermediate Zone. The 1934-35 epidemic commenced with an autumnal peak due to the failure of the south-west monsoon. A south-west monsoonal drought in the Wet Zone therefore converts it into an imitation of the normal in the Deduru-oya basin. But whereas malaria in the latter is due to the invasion from the Dry Zone,

TABLE VII.

Principal rivers of Ceylon associated with malaria epidemics.

(From Rustomjee, 1944.)

Name.	Source.	Mouth.	Length (miles).	Area of watershed (square miles).	Malaria seasons.	Endemicity.	Monsoonal influence.
Mahaweli-ganga	Central Province mountains in Nuwara Eliya and Kandy districts.	South of Trincomalee.	206	2,550	Principally October to January; mid-year (May-June) rise present.	Upper basin healthy. Middle basin low to moderate. Lower basin (Dry Zone) hyperendemic.	South-west + North-east +
Dedura-oya ...	Hills of Kandy and Kurunegala districts.	North of Chilaw	87	975	Chiefly October to January: April to June rise also present.	Upper basin moderate to severe. Middle basin severe. Lower basin severe.	South-west North-east +
Maha-oya ...	Hills of Kegalla and Kandy districts (west of hills).	North of Negombo.	78	562	April to June and October to January.	Upper basin healthy to moderate. Middle basin moderate. Lower basin moderate.	South-west + North-east +
Kelani-ganga ...	Mountains of Kandy and Nuwara Eliya districts (west of main hills).	North of Colombo.	93	910	April to June essentially; October to January rise also present.	Upper basin healthy. Middle basin healthy to low. Lower basin healthy to low.	South-west + North-east +
Kalu-ganga ...	Adam's Peak Range.	Kalutara	70	1,025	April to June; October to January.	Upper basin healthy to low. Middle basin healthy to low. Lower basin healthy	South-west North-east +

The sign + denotes the influence of monsoons on the endemicity of malaria.

that in the Wet Zone is due to the same type of invasion from the Intermediate Zone.

There is a difference between the normal malaria season and epidemic malaria in the Deduru-oya basin, in that the normal season extends from October to January, whereas epidemic malaria usually commences with a spring peak of transmission. With the exception of the 1934-35 epidemic, spring peaks are characteristic of past epidemics. It would therefore appear that an enhanced breeding potential of the vector species during the months, which are normally followed by the malaria season, would produce an epidemic of unusually severe proportions. This is what happened in 1934.

It is of interest to note that the rainfall in 1933 was higher than normal, as in fact, it was during the first 3 months of 1934. '.....the rainfall for 1933 was above normal..... In January 1934 the rainfall was appreciably above normal; in February, on the whole, above normal; and in March above normal over nearly the whole area' (Briercliffe, 1935).

It will be seen from Table VII that the catchment area of the Deduru-oya is about 975 square miles. This area normally receives about 60 inches of rain from the north-east monsoon. This in effect amounts to approximately 62,400,000 tons of water falling on this catchment area during the period of the north-east monsoonal rains. Earlier in this paper it was stated that a natural forest floor is capable of absorbing water to the extent of about 40 tons to the acre. Obviously in its original state this catchment area would have unforested areas including the river with its tributaries, rock and other non-absorbent surfaces. As a general figure, it is not possible to be precise; allow that, of the 975 square miles, 700 square miles, which are equivalent to 248,000 acres, were covered with forest. This figure is not far from the truth when it is remembered that the first planting of tea took place in the Kandy Province as early as 1883 and that by 1887, 150,000 acres were already under this crop.

700 square miles of natural forest floor are capable, on the basis of 40 tons to the acre, of absorbing 25,000,000 tons, or 5,000,000,000 gallons of water. And this vast quantity of water can be absorbed from a 50 per cent normal north-east monsoon. In other words, the north-east monsoon can fail to the extent of about 50 per cent and yet sufficient rain will fall to satisfy the absorptive capacity of the forest floor.

Had the catchment area remained in its original primeval state, this colossal volume of water would have been stored up to be released in the form of seepage during the very months when the south-west monsoon rains were falling. Even during the 1934 epidemic year, the first 3 months of the south-west monsoon were not far below normal. It was the more or less complete drought during the months August and September that produced the conditions which led to the epidemic. In the present state of the deforested watershed, the vast quantity of water from the north-east monsoon and the first 3 months of the south-west monsoon was lost by rapid surface run-off. Little was stored in the soil. It is submitted that it is the vast deforestation of the watersheds of the Deduru-oya and the Maha-oya which has resulted in so marked a deterioration of the soil complex, which in turn has resulted in a lack of absorptive quality, which helped considerably

to producing those conditions favourable to the production of the vector species leading to the 1934-35 epidemic, particularly when the onset of that epidemic coincided with the normal malaria season in that area.

If the general malaria epidemics are taken in their entirety, the same picture with regard to the effect of deforestation is seen to exist. Widespread epidemic malaria since the beginning of the 20th century have occurred in 1906, 1911, 1918 (complicated by a pandemic of influenza), 1923, 1928, 1934, 1939 and 1943. In all these the basins of the rivers Deduru-oya, Maha-oya, Kalu-ganga, Kelani-ganga and the Mahaweli-ganga were the most severely affected, and particularly the districts of Kurunegala, Kandy, Matale and Kegalla. It will be seen from Map 3 that these areas are situated in those regions which have a high spleen rate and where there is the highest number of parasite carriers. To bring on epidemic malaria in these areas all that is required is an increase in the vector's prevalence and longevity.

Rustomjee (*loc. cit.*) has related mortality and rainfall to past epidemics for selected areas which include Kurunegala, Matale and Kandy as being the areas most seriously affected, and because of their association with rivers. Mortality figures are given because the morbidity figures for the years 1906 and 1911 are not available. Table VIII is taken from Rustomjee's (1944) report.

The significant facts which emerge from this Table are :—

1. In each case the epidemic is associated with deficient rainfall in the 3 months preceding the onset of sickness.

2. That where the rainfall during the first 3 months of the monsoon is high the epidemic is minimum as in 1911.

3. If the first 3 months' rainfall is low to be followed by good rains in the 3 months preceding the onset of sickness the epidemic is again of less severity.

4. The deficiency of the rainfall in the 3 months preceding the epidemics bear a direct relationship to the severity of the outbreak.

To take the 1906 epidemic as an example, the onset of mortality was generally in May, hence the rise in morbidity would have occurred in April or even March. These spring waves are associated with the north-east monsoon—November to March. There was a deficiency in the latter 3 months of this monsoon corresponding to the 3 months preceding the epidemic. The precipitation during the first 3 months of the monsoon was only slightly below normal. It has already been shown that the north-east monsoon can fail to the extent of 50 per cent and still give sufficient water to supply and satisfy the natural absorptive capacity of a primeval forest floor. The rains during these 3 months were therefore amply sufficient to supply these needs, but under the deforested conditions prevailing to-day, most of these rains are lost by surface run-off leading to excessive flow during the rains and greatly diminished flow after them. This is the rapid rise and fall referred to by most authors on epidemic malaria. A natural forest watershed would have stored a vast quantity of water during the first 3 months of the monsoon which would have been released in the form of seepage to keep a more continuous flow in the rivers during the critical months preceding the onset of sickness. The rains during the last 3 months would have augmented or replaced the water lost by seepage.

MAP 3.

(From Gill, 1935.)

Showing the spleen rate, 1922-23.

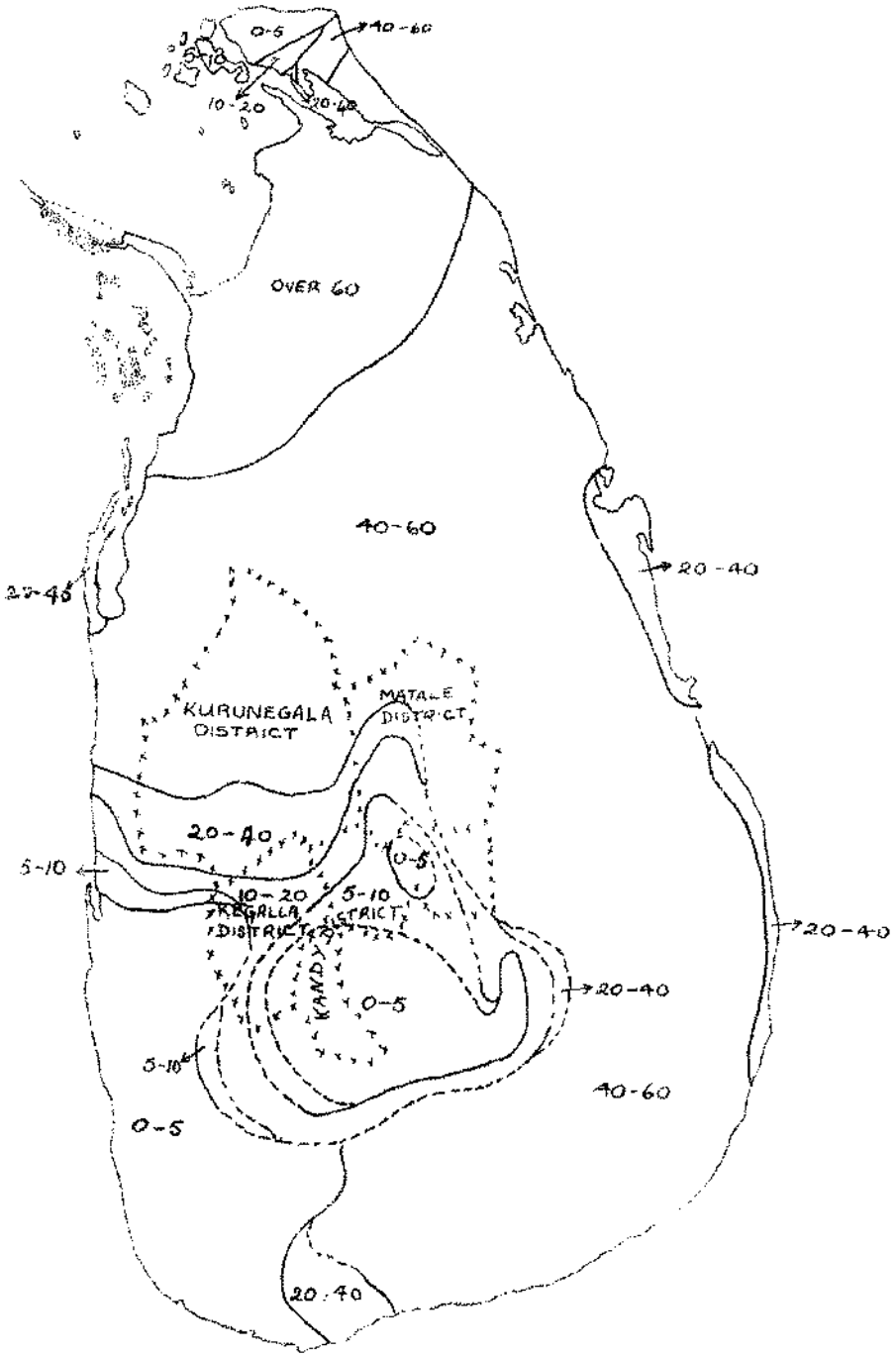


TABLE VIII.

Comparative analysis of rainfall and mortality in selected epidemics.

(From Rustomjee, 1944.)

Year.	District.	RAINFALL.				MORTALITY.				
		Number of months of deficient rainfall during the 6 months preceding the epidemic.	MEAN RAINFALL EXPRESSED AS A PERCENTAGE AVERAGE FOR 1911–1930.			Month of commencement of increased mortality.	Month of highest mortality.	Highest epidemic figure in any month of the epidemic.	Highest epidemic figure in January of the epidemic year.	Epidemic figure in January of year preceding epidemic.
			During 6 months preceding epidemic.	During the first 3 months.	During 3 months immediately preceding epidemic.					
1906	Kurunegala	5	74	80	58	May	Jul.	3·59	1·89	0·96
	Matale ...	6	65	77	42	,,	,,	4·00	1·37	0·80
	Kandy ...	5	67	71	69	,,	,,	1·86	1·20	1·01
	Kegalla ...	3	82	78	89	,,	,,	2·41	1·51	0·83
	Ratnapura ...	6	77	81	70	,,	Jun.	2·34	1·37	1·09
1911	Kurunegala	4	91	107	47	Jul.	Aug.	2·42	1·21	0·98
	Matale ...	4	83	98	53	Jun.	,,	2·15	0·96	0·68
	Kandy ...	4	88	106	48	May	Jul.	1·82	1·02	1·00
	Kegalla ...	5	94	109	59	,,	Aug.	5·25	0·75	0·94
	Ratnapura ...	6	71	83	49	,,	Jul.	2·04	1·24	1·10
1934	Kurunegala	6	46	64	16	Oct.	Jan.	7·86	0·79	1·01
	Matale ...	5	64	95	17	Nov.	,,	5·95	0·82	1·05
	Kandy ...	5	66	89	42	,,	,,	3·89	0·92	0·92
	Kegalla ...	4	66	89	34	,,	Feb.	8·25	0·85	0·94
	Ratnapura ...	3	64	85	34	,,	Jan.	2·86	0·87	0·78
1939	Kurunegala	5	47	41	85	May	Jul.	2·25	0·75	1·19
	Matale ...	6	54	51	61	,,	,,	1·95	0·63	0·91
	Kandy ...	6	57	57	58	,,	,,	1·51	0·89	0·31
	Kegalla ...	5	46	43	54	,,	Jun.	2·40	0·60	1·62
	Ratnapura ...	6	60	53	73	,,	May	1·71	0·66	1·16

The severity of this and the other epidemics are in direct proportion to the deviation from normal of the rainfall during the 3 months preceding the epidemics. It must therefore be conceded that had the watersheds been left as they were originally the epidemics would have been prevented altogether or that, because seepage to the rivers would have kept them more uniform with regard to flow and therefore to the production of *A. culicifacies*, the severity of the epidemics would have been in themselves more uniform and not related to the deviation from normal of the rainfall.

Gill (1935), referring to the 1934-35 epidemic, remarks: "It represents, in fact, merely the last of a series of epidemics that have occurred on the island at intervals of about 5 years with considerable regularity for the past *30 years (and it may be added to the last 60 years)*."* This would mean about the year 1875. Is it merely coincidence that tea cultivation started in 1867 and that by 1887 over 150,000 acres had been put under this crop in an area which suffers more severely and more constantly from epidemic malaria? Tea cultivation had been preceded by coffee which was first planted in 1827 and continued to about 1887. Soil erosion and general deterioration has been proceeding in this area for over 100 years.

In all the epidemics the districts of Kurunegala, Matale, Kandy and Kegalla are the ones most seriously affected. These are the districts associated with rivers whose catchment areas are situated on the lower foothills of the central montane tract and which have been heavily deforested. They are the districts which lie more or less in the Intermediate Zone where there is a constant ebb and flow of *A. culicifacies* breeding. In wet years the vector breeds in surface waters, particularly pools, following the north-east monsoon in the north; in drought years it invades the river basins. In all cases *A. culicifacies* prefers pools, whether these are created by excessive rains in one area or by deficient rain in the other.

It has been shown elsewhere that malaria first became established in the Dry Zone as a result of man's folly during war (Tyssul Jones, 1951). Has man not also contributed in a large measure to epidemic malaria in the Wet and Intermediate Zones?

SUMMARY.

1. Forested watersheds may or may not have an influence on rainfall, but the presence of a good forest cover certainly influences the flow of rivers.

2. Epidemic malaria in both the Wet and the Intermediate Zones of Ceylon is a direct result of increased breeding due to pool formation in the main rivers draining the Epidemic Zone.

3. Most of the montane tract has been deforested for the cultivation of tea and rubber.

4. Rubber and tea plantations have been no substitute for the primeval forests. Their cultivation has led to extensive soil erosion to a very marked degree, which has resulted in the loss of the natural absorptive capacity of the soil.

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* The present author's italics.

5. Owing to the loss of this absorptive quality there is a quick run-off of the rain water which leads to the marked ebb and flow of the rivers.

6. The 1934-35 epidemic has been discussed in some detail and it has been shown that this epidemic can be explained on climatological factors without recourse to the hypothesis of epidemic of relapses.

7. In all the epidemics there has been a marked deficiency in the rainfall in the 3 months preceding the onset of sickness.

8. Evidence is submitted to show that, were the forests left in the primeval state, sufficient rain water would have been held in the soil to tide over the deficiency of rainfall during these critical months.

9. It would appear that epidemic malaria in the Intermediate Zone is due to the invasion of the vector species into that zone from the Dry Zone to the north, whereas epidemic malaria in the Wet Zone is due to the movement of the vector from the Intermediate Zone.

10. Being essentially a pool breeder, the distribution of *A. culicifacies* in the three zones depends on rainfall under present conditions. A good north-east monsoon leads to its prevalence in surface pools in the Dry Zone, whereas a deficiency in precipitation is necessary for its prevalence in the rivers of the Epidemic Zone.

11. Kurunegala, Matale, Kandy and Kegalla are the districts most seriously affected, being associated with the rivers whose catchment areas are situated in the lower hills and which have been most severely deforested.

12. Epidemic malaria in the two zones of Ceylon probably started about 75 years ago, and would as a result correspond more or less to the commencement of the vast felling of the natural forests, which were Ceylon's heritage for centuries past.

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A BRIEF NOTE ON THE BREEDING HABITS OF *A. SUNDAICUS* IN THE CHILKA LAKE AREA.

BY

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[September 1, 1950.]

According to the observations of Senior White and Adhikari (1939), *A. sundaicus* in the Chilka Lake area breeds chiefly in the lake itself and interior to the shore line in waters subjected to admixture with saline water from the lake. The majority of inshore breeding places recorded were tanks, ponds and jheels with heavy aquatic vegetation and situated in the vicinity of the lake. They occasionally found larvæ of this species at the mouths of some streams in places where there was no current. Covell and Pritam Singh (1942) while making a survey of Chilka Lake area made similar observations and also stated that the inshore breeding places were such as were subjected to flooding by saline water from the lake.

Senior White *et al.* (1947) subsequently showed that saline water was not essential for their breeding and that the larvæ can also breed in fresh water in the presence of aquatic vegetation. As at no time were larvæ of this species found breeding in running water which was available in large quantities in the area, it appears that *A. sundaicus* is exclusively a still water breeder and running water is probably inimical to it. This was confirmed by an observation made near Kota Bommali (a small railway station in North Vizagapatam) where no *A. sundaicus* but large numbers of *A. stephensi* larvæ were found in a small nallah as long as the water in the nallah had a definite flow (Senior White *et al.*, *loc. cit.*). With the advent of dry weather when the water in the nallah was converted into a series of disconnected pools, *A. sundaicus* commenced to breed.

That the larvæ of *A. sundaicus* do not breed in running water and are found only in collections of standing water with abundance of aquatic vegetation was further confirmed by large-scale field experiments carried out near Humma, a railway station situated about 3 miles south of Chilka Lake. According to Senior White *et al.* (*loc. cit.*) fresh-water form of *A. sundaicus* is the prime vector of malaria in this area. Eight fresh-water tanks and a series of railway borrowpits were selected. Most of the depressions were deep enough to tap strong underground seepages. Two of the tanks were used by the people for drinking purposes while others were for irrigation

during the dry weather. In all these water collections, *A. sundaicus* larvæ were consistently found breeding.

The borrowpits were connected to one another by means of an open kutcha drain and led into a tank 2 miles away from the railway station. The pits gradually dried up but owing to the presence of seepages, most of the drains had clear running water during the major part of the year and had to be kept clear of vegetation by periodic attention. The irrigation tanks were also drained away at the close of the rains by opening the bunds at convenient places and making kutcha drains. The latter were connected to small irrigation channels running through the fields. All the channels finally led to a nallah over half a mile from the railway station. The main body of the water in the tanks was thus drained away but the seepages were strong enough to keep the drains full of running water till summer set in. The openings made in the bunds were closed before the onset of the monsoon to collect the water again in the tanks for the irrigation of the rice crop. This process was repeated annually for 3 years. The tanks were thus kept dry for prolonged periods whereby the vegetation occurring in them during the monsoon became scanty enough to obviate clean weeding and eliminate breeding of *A. sundaicus*. The tanks reserved for drinking purposes were kept free from vegetation by periodic cleaning through manual labour. During the 3 years when these experiments were being conducted, very few larvæ of *A. sundaicus* were found in any of the tanks or the drains. It is suggested that if observations carried out in other areas confirm that *sundaicus* breeding can be eliminated by converting stationary water into running water and keeping such water clear of vegetation, this would be a very cheap method of controlling the breeding of *A. sundaicus*.

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OBSERVATIONS ON PALUDRINE IN MALARIA.

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EARLY in 1948 an investigation was started at the Tata Main Hospital with a view to study the results of paludrine in the treatment of malaria and to discover a suitable dose of the drug. For the purpose of the study, to start with, two doses of paludrine were tried, viz. (*a*) paludrine 100 mg. 3 times a day for 2 days and (*b*) paludrine 100 mg. 3 times a day for 4 days. During the second half of the study, two more regimes were added, viz. (*c*) paludrine 200 mg. 3 times a day for 3 days and (*d*) paludrine 200 mg. 3 times a day for 4 days.

METHOD OF STUDY.

The diagnosis was made by examination of thick drop blood films stained with Simeon's stain. Blood smears were taken morning and evening from the time of commencement of treatment till no trophozoites could be seen in the peripheral blood. The dose of paludrine was administered by a nurse and the treatment started only if the full 24 hours' dose could be given on the first day. All the patients were in hospital under constant supervision. In the beginning, to check the accuracy of the results in each case, 2 blood slides were seen by 2 independent observers but later on as the results of the 2 observers were the same, double inspection was discontinued. All the cases admitted in the different wards of the Department of Medicine of the Tata Main Hospital were studied and in each ward the treatment was started in the beginning alternately on regimes A and B and towards the latter part of the study on C and D. Thus there was no selection of cases and difference in the number is due to the different numbers admitted to different wards.

The total number of cases surveyed were 427. The different treatments given to these cases were as given in Table I.

TABLE I.

	Treatment.			
	A.	B.	C.	D.
P. vivax ...	105	83	29	27
P. falciparum ...	45	91	16	31
Mixed ...	1	3	...	...
TOTAL ...	151	177	45	58

Results.—Results as analysed by the statistical methods are given in Tables II and III.

TABLE II.

Clearance of trophozoites from peripheral blood.

Infection.	Treatment.	Number of cases treated.	Average number of days taken.	Standard deviation.	Mode.
Fresh B.T. ...	A	97	1·40	0·60	1
	B	78	1·95	0·75	2
	C	26	1·38	0·57	1
	D	29	2·00	0·77	2
Fresh M.T. ...	A	40	1·35	0·57	1
	B	85	1·79	0·74	2
	C	15	1·27	0·43	1
	D	27	1·70	0·60	1
Mixed ...	A	1	2·00	...	2
	B	2	2·50	...	...
	C	1	3·00	...	...
Relapse B.T. ...	A	8	1·12	...	...
	B	5	1·20	...	...
	C	3	1·00	...	...
	D	4	2·00	...	...
Relapse M.T. ...	A	5	2·00	...	...
	B	6	2·17	...	...
	C	1	2·00	...	...
	D	4	1·50	...	...
Mixed ...	B	1	2·00	...	...

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

TABLE III.

Duration of pyrexia.

Infection.	Treatment.	Number of persons treated.	Average number of days.	Mode.	Standard deviation.
Fresh B.T.	A	97	2·20	2	...
	B	78	3·50	3	...
	C	26	2·80	3	...
	D	23	3·32	3	...
Fresh M.T.	A	40	2·45	3	...
	B	85	3·88	3	...
	C	15	2·87	3	...
	D	27	3·04	3	...
Fresh Mixed	A	1	4·00	...	...
	B	2	0·50	...	...
	C	1	4·00	...	...
Relapse B.T.	A	8	2·12	3	...
	B	5	2·80	3	...
	C	3	2·33	...	...
	D	4	3·25	...	...
Relapse M.T.	A	5	4·00	2	...
	B	6	2·83	...	...
	C	1	4·00	...	...
	D	4	3·25	...	...

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

Table IV gives the average duration of the clearance of trophozoites from the peripheral blood on treatments A, B, C and D. For *P. vivax* infection, the period in days on the four treatments was 1·38, 1·9, 1·34 and 1·67 days respectively; and for *P. falciparum* infection 1·42, 1·81, 1·31 and 1·67 days respectively.

TABLE IV.

Clearance of trophozoites from peripheral blood (in days) after commencement of treatment.

Treatment.	A.	B.	C.	D.
Benign tertian ...	1·38	1·90	1·34	1·67
Malignant tertian ...	1·42	1·81	1·31	1·67

As given in Table V, in 188 *P. vivax*-infected patients treated with 300 mg. paludrine daily (combined A and B treatments), the trophozoites on the average persisted for 1·6 days and in 136 *P. falciparum* infections for 1·8 days after the

commencement of treatment. On the other hand, with 600 mg. paludrine daily (C and D treatments combined), the average duration of the presence of the trophozoites was 1·6 days in 56 *P. vivax*-infected patients and 1·6 days in 47 *P. falciparum*-infected patients. Thus, there was no appreciable difference between the higher and lower dosage in *P. vivax* infection, although in *P. falciparum* infection, the peripheral blood trophozoites disappeared 0·3 days earlier on the higher dosage.

TABLE V.

Duration of clearance of trophozoites in peripheral blood and pyrexia (in days) on lower and higher dosage.

	Lower dosage (300 mg. daily).		Higher dosage (600 mg. daily).	
	B.T.	M.T.	B.T.	M.T.
Total	188	136	56	47
Trophozoites	1·6	1·8	1·6	1·5
Pyrexia	2·8	3·4	3·2	3·3
Pyrexia after clearance of trophozoites.	1·2	1·6	1·6	1·8

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

Table VI gives the period of pyrexia after the commencement of treatment which for *P. vivax* infection was 2·29, 3·46, 3·16 and 3·31 days and for *P. falciparum* infection 2·68, 3·81, 2·94 and 3·04 days on treatments A, B, C and D respectively.

TABLE VI.

Duration of pyrexia (in days) after commencement of treatment.

Total.	A.	B.	C.	D.
B.T. ...	2·29	3·46	2·16	3·31
M.T. ...	2·68	3·81	2·94	3·07

For lower dosage (300 mg. daily), the average duration of pyrexia after the commencement of paludrine was 2–8 days in 188 *P. vivax*-infected patients, and 3-4 days for 136 *P. falciparum*-infected patients; when compared with the higher dosage (600 mg. daily) tried in 56 *P. vivax*-infected patients it was 3·2 days and in

47 *P. falciparum*-infected patients it was 3·3 days. Thus, there was no appreciable difference in the duration of pyrexia in either *P. vivax* or *P. falciparum* infection in the higher or lower dosage.

In all the patients treated with paludrine, it was noted that trophozoites disappeared rapidly but pyrexia persisted. As given in Table V, the duration of pyrexia after disappearance of trophozoites in *P. vivax* infection, was 1·2 days with the lower dose and 1·6 days with the higher dose, and in *P. falciparum* infection it was 1·5 days with the lower dose and 1·8 days with the higher dose. Thus, there was no appreciable difference in the time lag of pyrexia after clearance of trophozoites between the higher and lower dose of paludrine.

Our results compare well with those of Fairley (1946) who found that with 300 mg. paludrine in *P. falciparum* infection, trophozoites disappeared rapidly in one day, but the temperature took 2-3 days to return to normal and in *P. vivax* infection the temperature did not remain normal for some 3–6 days.

TABLE VII.

Comparative study of clearance of trophozoites from the peripheral blood and duration of pyrexia on treatment with paludrine, quinine and mepacrine.

	B.T.		M.T.	
	Clearance of trophozoites.	Pyrexia.	Clearance of trophozoites.	Pyrexia.
Paludrine 300 mg. daily for 2 days ...	1·3	2·2	1·4	2·6
,, 300 ,, 4 days ...	1·9	3·4	1·8	3·8
,, 600 ,, 2 days ...	1·3	3·6	1·3	2·9
,, 600 ,, 4 days ...	2·0	3·3	1·6	3·0
Quinine gr. 30 daily for 3 days and Mepacrine 100 mg. 3 daily for 5 days	3·0	1·7	3·1	2·9
Mepacrine 600 mg. 3 daily for 2 days and ,, 300 ,, 5 days	2·9	1·9	3·1	2·6
Mepacrine 600 mg. 3 daily for 1 day and ,, 300 ,, 6 days	2·8	1·9	3·5	2·6
Mepacrine 600 mg. 3 daily for 1 day and ,, 300 ,, 6 days	2·9	2·1	3·3	2·8
Paludrine low dose (300 mg.) ...	1·6	2·8	1·8	3·4
Paludrine high dose (600 mg.) ...	1·6	3·2	1·5	3·3

Table VII which gives a comparison of our results of paludrine therapy, with those of Rogans (1944) in the same eastern part of India, with quinine gr. 10 three times a day with mepacrine, and different doses of mepacrine alone, ranging from 300 to 600 mg. daily, shows that in *P. vivax* infection the peripheral blood trophozoites disappeared on the average after 1·3–2 days with paludrine treatment, 2·8–2·9 days with mepacrine treatment and after 2 days with quinine combined with mepacrine; and in *P. falciparum* infection after 1·3–1·81 days with paludrine, 3·1–3·5 days with mepacrine and 3·1 days with quinine combined with mepacrine treatment. On the other hand, the duration of pyrexia after commencement of treatment with *P. vivax* infection was 2·9–3·6 days with paludrine; 1·9–2·1 days with mepacrine and 1·7 days with quinine combined with mepacrine treatment; and in *P. falciparum* infection 2·6–3·0 days with paludrine, 2·6–2·8 days with mepacrine and 2·9 days with quinine combined with mepacrine. It may, therefore, be concluded that on the average paludrine clears the peripheral blood of trophozoites quicker than mepacrine, or quinine combined with mepacrine, but period of pyrexia after the commencement of treatment is longest with paludrine than with mepacrine, or quinine combined with mepacrine.

SUMMARY AND CONCLUSION.

The study of 427 malaria patients treated with 4 different doses of paludrine is given. The clearance of peripheral blood of trophozoites was on the average 1·5–1·8 days and period of pyrexia 2·8–3·3 days. The higher dose of paludrine (600 mg.) and the lower dose of 300 mg. a day, gave similar results. Comparison is made with the effect of mepacrine, and mepacrine combined with quinine, from which it appears that paludrine clears the peripheral blood of trophozoites quicker than either mepacrine or quinine combined with mepacrine, but the period of pyrexia is longer in paludrine and shortest in quinine combined with mepacrine treatment.

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FIELD TRIALS OF D.D.T. AND B.H.C. IN ASSAM.

BY

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[March 2, 1951.]

TRIALS of D.D.T. (dichloro-diphenyl-trichlorethane) and B.H.C. (benzene hexachloride) against anophelines, and in particular against *A. minimus*, were carried out during 1950 in a small village of the Mikir foothills, in the Brahmaputra Valley of Assam. The trials started early in June and continued until cold weather in December reduced the mosquito catches below critical levels. The village consisted of 9 scattered houses, all of which were used in the trials; it was situated about half a mile from labour lines of a tea estate. Streams breeding *A. minimus* surrounded the village on three sides; nearby forest yielded *A. leucosphyrus* and ricefields *A. vagus*.

The area is very malarious. Parasite and spleen rates in children, aged 2 to 10 years, from the neighbouring estate were taken before and after the insecticide trials and are shown in Table I. The malaria incidence rate in the estate lines, in whose records are included the village inhabitants, was 570 per mille in 1950.

TABLE I.

Parasite and spleen rates in children.

Date.	Parasite rate.		Spleen rate.	
	Number examined.	Rate, per cent.	Number examined.	Rate, per cent.
April 1950 ...	31	42	53	58
November 1950 ...	46	72	46	61

The type of house.—Roofs are thatched and there are no ceilings. The walls, both outer and partition, consist of a mud-cowdung plaster applied over a framework of bamboos. Floors are of earth, similarly plastered, and raised about one foot above ground level. Test houses were repaired before the trials and doors made close-fitting so that the only entrance for mosquitoes was between wall-top and under-surface of the roof. The usual occupants remained in their houses during the trials and their co-operation, particularly in regard to care of floor sheets, was ensured by cash payments from time to time.

RECOVERY OF MOSQUITOES.

Floor sheets and exit-traps, affixed to openings on the east walls, as described by Thomson (1950), were used. Both traps and sheets were removed daily, soon after dawn, and dead mosquitoes counted and classified. Traps were not emptied of their living mosquitoes but were covered with green leaves and stored in a dark and cool hut for 24 hours. Although this necessitated duplicate traps, it avoided damaging insects through handling. Traps were labelled so that the same pair was always used on the same house. Most scrupulous care was taken to see that the traps were not contaminated with insecticide.

ENTOMOLOGICAL RECORDS.

Catches were recorded as :—

A. Culicines.

B. *A. minimus.*

A. leucosphyrus.

Other anophelines.

A record was also kept as to whether or not a blood meal had been taken.

The fate of recovered mosquitoes was recorded as :—

Dead on floor sheets.

Dead in trap at dawn.

Alive in trap at dawn.

Living in trap 24 hours after its removal.

During the 27 weeks of the trials, 2,364 anophelines and 4,555 culicines were recovered from sprayed houses. The anophelines included 1,254 *A. minimus.*

INSECTICIDES TESTED.

The dosages of D.D.T. and B.H.C. that were tested are given in Table II. Both were used as suspensions, the former as 'Geigy Malaria Spray' and the latter as 'Gammexane P.520'. The rate of application aimed at covering 1,000 square feet with one gallon of spray. It was necessary to apply the two highest dosages of D.D.T. in two applications, the second immediately after the first had dried.

TABLE II.

Dosages of D.D.T. and B.H.C.

Chemical (mg./sq. ft.).	Percentage of suspension at 1 gallon/1,000 sq.ft.
D.D.T. (*a*)	
45 ...	1
90 ...	2
135 ...	3
180 ...	4
225 ...	5
B.H.C. (*b*)	
10 ...	...
20 ...	...

(*a*) Dosage as 'Technical' D.D.T.
(*b*) Dosage as gamma isomer.

Spraying was done with a 'Geigy' stirrup pump and double nozzle. The suspension was kept thoroughly agitated during application.

COMPARISON.

Provision of satisfactory controls presented special difficulties. As reported by Thomson (1947), and subsequently by others, once D.D.T. has been used in a catching station, daytime catches, whether by pyrethrum and floor sheets or by torch and collecting tube, are likely to be unreliable as an indication whether mosquitoes are entering, and feeding, at night.

Daily catches, prior to spraying, may also yield deceptive information when dealing with species such as *A. minimus* whose breeding places are disturbed by heavy rains, or with *A. vagus* which is dependent on water-filled ricefields. Thus if spraying were carried out just before heavy rains reduced the *minimus* population,

a reduction in numbers, compared with the weeks before spraying, might be misinterpreted.

In the present trials it was decided to use village houses fitted with exit-traps. The usefulness of this method presupposes a dawn egress of the particular species under study. In the case of *A. minimus*, work by Thomson (1941) had suggested that comparatively few left the houses at dawn and he thought that for 1 or 2 leaving, 10 remained indoors. However, a considerable number were recovered from window traps and, over the 27 weeks of the trial, the following were recorded from two unsprayed comparison houses :—

A. minimus	...	207
A. leucosphyrus	...	25
Other anophelines	...	242
Culicines	...	1,508

Mortality in anophelines recovered from comparison houses.—In spite of a minimum of disturbance and great care in storage, a considerable mortality occurred in unfed female anophelines (Table III).

TABLE III.

Mortality in anophelines recovered from unsprayed houses.

Female anophelines.	Number in exit-trap.	24-hour mortality rate, per cent.
Unfed	114	82
Blood-fed	198	13
Both unfed and blood-fed ...	312	38

This mortality may be of importance in evaluating the effect of an insecticide in cases where unfed females form a large proportion of the total anophelines recovered. In such circumstances, it would be proper to correct the numbers surviving 24 hours by applying the comparison mortality rate prevailing for a particular species in a particular locality and season. Alternatively, results might be evaluated on blood-fed females only.

Combining the numbers of anophelines recovered from all sprayed houses, in the present trials, unfed females were to blood-fed as 1 : 5. The proportion from unsprayed houses was 1 : 1·6.

SPECIES PREVALENCE.

The chief vector of malaria in Assam is *A. minimus* and seasonal fluctuations in the population are very marked. It is found in largest numbers at the beginning and end of the rainy season; it is rare during the cold, dry season; and present in greatly reduced numbers during the months of heaviest rainfall, July to September, when streams are silt laden and in spate and are thus unsuitable for breeding. Table IV gives the proportion of *A. minimus* to total anophelines during the six months of the trials.

TABLE IV.

Proportions of A. minimus, *other anophelines and culicines by months.*

(From sprayed houses only.)

Month (1930).	Classification.	Number.	Proportion, per cent.
June ...	*A. minimus* ...	125	8
	Other anophelines ...	183	12
	Culicines ...	1,226	80
July ...	*A. minimus* ...	64	7
	Other anophelines ...	158	18
	Culicines ...	659	75
August ...	*A. minimus* ...	53	5
	Other anophelines ...	203	18
	Culicines ...	884	77
September ..	*A. minimus* ...	86	9
	Other anophelines ...	236	23
	Culicines ...	681	68
October ..	*A. minimus* ...	111	12
	Other anophelines ...	192	20
	Culicines ...	674	68
November ...	*A. minimus* ...	660	61
	Other anophelines ...	91	8
	Culicines ...	329	31

Though the general pattern remains, there may be considerable variations in particular years related, not to the monthly rainfall, but to the number of heavy showers and the time interval between them.

EFFECTS OF INSECTICIDES.

The numbers of anophelines dead at dawn, on the floor sheets and in the traps, plus the number dying during the next 24 hours, are expressed as the 24-hour mortality rate of the total recovered. These rates are shown in Table V for periods up to 5, 8 and 12 weeks after application of the insecticides. D.D.T., in a dosage of 45 mg. per square foot, was not tested beyond 8 weeks.

TABLE V.

24-hour mortality rates for periods of 5, 8 and 12 weeks.

(Total anophelines.)

Treatment.	Dosage per sq. foot (mg.).	24-HOUR MORTALITY RATES FOR PERIODS (PER CENT).		
		5 weeks.	8 weeks.	12 weeks.
B.H.C. (*a*) ...	10	84	75	52
" ...	20	100	100	98
D.D.T. (*b*) ...	15	85	76	...
" ...	90	87	78	74
" ...	135	84	85	85
" ...	180	63	56	77
" ...	225	60	63	60

(*a*) As gamma isomer.
(*b*) As 'Technical' D.D.T.

Culicines.—A summary of the 24-hour mortality rates for culicines is given in Table VI for periods of 8 and 12 weeks after spraying. That the trends are irregular may be due to the fact that the class, 'Culicines', includes genera whose susceptibility to insecticides may vary within wide limits. It will be noticed that there is little loss of lethal power between the 8th and 12th weeks.

TABLE VI.

24-hour mortality rates for periods of 8 and 12 weeks.

(Culicines.)

Treatment.	Dosage per sq. foot (mg.).	24-HOUR MORTALITY RATES FOR PERIODS (PER CENT).	
		8 weeks.	12 weeks.
B.H.C. (*a*)	10	71	70
,,	20	90	94
D.D.T. (*b*)	45	72	72
,,	90	92	91
,,	135	73	73
,,	180	79	81
,,	225	46	47

(*a*) As gamma isomer.
(*b*) As 'Technical' D.D.T.

LOSS OF LETHAL EFFECT.

The mortality rates show a steady decline as the time interval after application increases. This effect is not, however, apparent with B.H.C. at 20 mg. per square foot. In this particular case the duration of the trials was insufficient to show any significant loss of lethal power. The rapid loss of effect is shown in Table VII which gives 24-hour mortality rates by weeks after treatments with B.H.C. 10 mg. and D.D.T. 45 mg. per square foot.

TABLE VII.

24-hour mortality rate per cent by weeks.

Week after application.	B.H.C. 10 MG. PER SQ. FT.		D.D.T. 45 MG. PER SQ. FT.
	Anophelines.	*A. minimus.*	Anophelines.
1	100	100	94
2	84	100	89
3	94	97	87
4	80	84	80
5	55	50	68
6	36	25	29
7	37	45	21

A. leucosphyrus.—As very little is known of this malaria vector in Assam, Table VIII is included to show the fate of the few recovered.

TABLE VIII.

Details of A. leucosphyrus *recovered.*

Treatment (mg./sq. ft.).	Alive in trap at dawn.	Alive, 24 hours.
B.H.C.		
10	20	5
D.D.T.		
45	11	3
90	6	4
135	3	*Nil.*
225	1	*Nil.*
Comparison (unsprayed) ...	25	17
All sources	66	29

DISCUSSION.

The irritant factor.—Thomson (1950), working with *A. gambiae*, found that D.D.T. possessed the power of irritating this species so that it did not rest long enough to absorb a lethal dose of insecticide. He did not find evidence of a similar factor in B.H.C. Bertram (1950), from a short trial of D.D.T. and B.H.C., reported similar results for mosquitoes in Assam although he did not investigate an irritant factor specific for anophelines or for *A. minimus*.

Table IX gives, for each treatment, the proportion of recovered anophelines that were found, alive or dead, in the exit-traps. The large proportion so found suggests that an irritant factor is present in D.D.T., at all dosages tested, and in B.H.C. at 10 mg. per square foot. The effect is much less marked, although still present, with B.H.C. at 20 mg.

TABLE IX.

Proportions of anophelines taken in exit-traps.

(For period of 8 weeks after spraying.)

Treatment (mg./sq. ft.).	Total anophelines recovered, alive and dead, on sheets and trap.	Percentage of column 2 recovered from trap.
B.H.C.		
10 ..	559	83
20 ..	107	20
D.D.T.		
45 ...	282	95
90 ..	125	95
135 ...	82	82
180 ..	59	88
225 ...	92	87

The weekly proportions of total anophelines, and *A. minimus*, taken in traps is given in Table X for B.H.C. at 10 mg., and D.D.T. at 45 mg. per square foot.

TABLE X.

Weekly proportions of anophelines and A. minimus *in traps.*

(*Per cent.*)

Week after application.	B.H.C. 10 MG. PER SQ. FT.		D.D.T. 45 MG. PER SQ. FT.
	Anophelines.	*A. minimus.*	Anophelines.
1	46	15	90
2	80	89	93
3	89	78	100
4	80	79	95
5	100	95	100

The present trials strongly suggest that there is an irritant factor in both D.D.T. and B.H.C.; this factor affects *A. minimus* but not to a greater extent than the other anopheline species encountered.

D.D.T. at dosages of 180 mg. and 225 mg. per square foot.—Reference to Table V shows that for anophelines the optimum dosage of D.D.T., within the conditions of these trials, was 135 mg. per square foot and that the 24-hour mortality rates are significantly less with dosages of 180 mg. and 225 mg. per sq. ft. This is difficult to explain unless there can be assumed a repellent effect at the higher doses, an effect that can keep the insects flying restlessly until attracted by dawn light to the exit-traps. The possibility of such an effect has been discussed by Field (1950) and by Reid (1950).

With culicines, the optimum tested dosage was 180 mg. per square foot and at a dosage of 225 mg. there was a sharp fall in the 24-hour mortality rate, from 81 per cent to 47 per cent, in a period of 12 weeks after application.

Table V also shows that the total numbers recovered from the house treated with B.H.C. at 10 mg. exceeded the numbers from all other sprayed houses. Whilst the possibility of a threshold repellency in the latter remains, nevertheless a more likely explanation is that the house sprayed with B.H.C. at 10 mg. was, although apparently similar to the others, one specially attractive to anophelines. Such a possibility was suggested by Thomson (1941) who found, during his work in Assam, that some houses regularly yielded unusually large numbers of mosquitoes compared with others.

CHOICE OF A RESIDUAL INSECTICIDE FOR MALARIA CONTROL.

Before discussing the most suitable insecticide, and its dosage, consideration must be given to the duration of a lethal effect and the chance that this effect will be disturbed by human interference. It is common in Assam, and no doubt in other provinces, for occupants to re-plaster their houses with mud and cowdung. Though there are no regular times, it is usually done at certain festivals, *Holi* and *Durga*, and on the occasions of births or deaths in the house. The plaster dries with a smooth cement-like finish which almost certainly renders ineffective any insecticide previously applied to the walls.

Were it not for re-plastering, the insecticide of choice would be B.H.C. at a dosage of 20 mg. per square foot; at 12 weeks after application this treatment gave a 24-hour mortality rate of 98 per cent for anophelines. At 15 weeks, although this is not shown in Table V, the rate was still 97 per cent.

If local conditions indicate the desirability of spraying at shorter intervals, i.e. at 5 weeks, there is surprisingly little to choose between B.H.C. at 10 mg. and D.D.T. at 45 mg. and 90 mg. per square foot. All give a mortality rate of about 85 per cent and the cheapest will probably be chosen.

That a mortality rate of even 75 per cent may interrupt malaria transmission, unless the vector is present in far greater numbers than were found in the present trials, is suggested by the experience of many tea estates in Assam and by the following simple calculation which, admittedly only an approximation, takes account of the 24-hour mortality rate and the proportion of *A. minimus* in the anophelines recovered.

Of every 400 anophelines entering a sprayed house, only 100 survive beyond 24 hours; 50 of them will be *A. minimus*; of these 50, only 1 will have sporozoites in the salivary glands.

In the present trials, the average number of anophelines entering the B.H.C. 10 mg. house was 66·4 per house-week, and entering the D.D.T. 90 mg. house only 8 per house-week, and this in an intensely malarious locality.

SUMMARY.

1. An account is given of field trials of D.D.T. and B.H.C. in Assam.

2. The difficulties in providing adequate controls are discussed and reasons given for deciding on unsprayed houses fitted with exit-traps.

3. A high mortality rate was found amongst unfed female anophelines recovered from unsprayed houses.

4. It is necessary, in evaluating the effects of insecticides, to take into account seasonal fluctuations in the populations of important species.

5. A summary is given of the 24-hour mortality rates following application of insecticides.

6. D.D.T. in dosages of 180 mg. and 225 mg. was found to be less lethal than in smaller doses.

7. The choice of an insecticide for malaria control is considered to depend on whether a long duration of effect will be disturbed by the common practice of re-plastering house walls. If this is likely to occur, then an insecticide with an effective duration of 4 or 5 weeks may be used. For this period there is little to choose between B.H.C. at 10 mg. and D.D.T. at 45 mg. and 90 mg. per square foot, and relative costs can decide the choice. For spraying with intervals longer than 5 weeks between rounds, B.H.C. at 20 mg. of gamma isomer per square foot is recommended.

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NEW METHODS FOR MOUNTING MOSQUITO LARVÆ.

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THE preparation of good permanent mounts of mosquito larvæ is an essential preliminary to systematic research and to teaching and museum work in medical entomology. A satisfactory all-purpose preparation must possess the following characteristics :—

1. It should be permanent and not deteriorate with age.
2. It should be sufficiently transparent to allow all details to be seen, but not to a degree where visibility of unstained elements is lost.
3. Distortion of dimensions and fine structure should be minimal.
4. The method of preparation should be rapid and simple, and involve a minimum of handling of specimens.

The classical methods of the past have used mounting media based on xylol-balsam or chloral-gum formulæ, both of which are far from foolproof or possess severe disadvantages. Xylol-balsam mounts require lengthy processing and are frequently unsatisfactory, while chloral-gum mounts are hard to render permanent and do not keep in some climates. These methods have been reviewed at greater length by Wanamaker (1944) and Bhatia (1948), each of whom describes an improved mounting medium; the former, creosote-balsam and the latter, eucalyptus oil-pine resin. These media represent a distinct advance but, after a series of trials, the writer believes that the new methods described below give even better results.

The mounting medium used in these new methods consists of canada balsam dissolved in phenol (Phenol liq., B.P.). It is prepared by gently heating the canada balsam to drive off any solvent or water present, until a thread drawn off on a glass rod becomes brittle on cooling. The balsam is then allowed to cool to a thick viscous mass, when the phenol is added and stirred in, adding successive small amounts until a satisfactory consistency is obtained.

Using this medium, there are several alternative mounting methods, according to the condition of the specimens and the requirements of the preparation.

Method 1.—The first method is applicable to (*a*) specimens which have been stored in alcohol for a long time, or (*b*) pale larvæ reared on soft foods, or (*c*) where extreme transparency is not required or (*d*) where a rapid method is most desirable. The procedure is as follows:—

1. Take larvæ from preservative or fixative to 90 per cent alcohol for about 10 minutes.
2. Using fine needles, pierce the ventral surface of thorax and abdomen in several places.
3. Transfer to 95–100 per cent alcohol for 15 minutes.
4. Transfer to slide, arrange, cover with phenol-balsam, and place cover-slip in position.

Method 2.—The following method is sometimes useful but requires careful timing and is applicable only to live material. It is, moreover, only applicable to *some* species; with *A. sundaicus*, for instance, a very poor result is obtained. The procedure is as follows:—

1. Kill larvæ in a slow-acting fixative—a mixture of 90 per cent alcohol, glacial acetic acid, and formalin (1 : 1 : 1) is useful as it tends to reduce the troublesome flexion of the head produced by many fixatives. Fixation should be allowed to proceed to a point where the larvæ are dead but not rigid, for reasons described below.
2. Pierce with needles as in step 2 of Method 1.
3. Transfer to phenol for 15 minutes. It is essential at this stage that fixation should not have proceeded to the point where the contents of the body become solid; if this has occurred, the specimen will burst, but if not, much of the body content is extruded and the specimen becomes conveniently extended. Specimens may be left in the phenol for long periods without harm.
4. Mount as in step 4 of Method 1.

Mounts prepared by the two preceding methods suffer little distortion and clear rapidly. Except in the case of very dark larvæ or old specimens from alcohol, much detail of ventral structures can be seen by focusing through the body, and by using the second method, almost complete transparency can sometimes be obtained.

Method 3.—The third method is more lengthy and is applicable only to living or freshly killed specimens, but is extremely useful when very dark larvæ are to be mounted or when a very transparent mount is required, e.g. for taxonomic studies where ventral hairs are to be examined. It involves a preliminary maceration in a strong solution of chloral hydrate, followed by mounting in the phenol-balsam medium. The procedure is as follows:—

1. Kill the larvæ in hot water or other fixative and pierce with needles as in step 2 of Method 1.
2. Transfer to strong chloral hydrate solution (80 gm. in 20 c.c. of 30 per cent acetic acid) and leave until cleared. This takes several hours but specimens may be safely left in the solution for months if so desired.

The optimum period is probably around 3 to 5 days but only small differences are involved.

3. Wash in 90 per cent alcohol for 10 minutes.
4. Transfer to phenol for 10 minutes.
5. Transfer to slide—a pipette should be used for this operation as the specimen is very soft and easily crushed.
6. Arrange specimen and draw off surplus phenol with pipette and blotting paper; cover with a generous drop of phenol-balsam and place the coverslip in position. The amount of phenol taken over with the specimen should be as small as possible since, if it runs over the slide, the medium will do likewise, producing an untidy mount. Also, it is best to lower the coverslip from the tail of the larva towards the head to avoid spoiling the arrangement.

A very useful refinement of this method is to omit step 1 and place the living larva direct into the chloral hydrate. If this is done, not only is the larva cleared, but most, frequently all, of the gut content is extruded, allowing perfect visibility of the ventral surface. However, the chitinous skin must still be pierced before mounting and this must be done with extreme care as the specimen is now very soft and easily crushed. A satisfactory technique for this operation is as follows:—

1. Transfer individual larvæ to an excavated block or similar vessel half filled with the chloral hydrate solution and arrange with ventral surface uppermost.
2. Take a pair of fine sharp needles (No. 20, Tayler's entomological pins are useful) and thrust one back into the thorax from where it joins the head; thrust the other forward in a similar fashion from where the thorax joins the abdomen, until the points meet. By rubbing the points together both can be made to penetrate. If the thorax has been crushed at all during this operation, keep the first needle in position and gently prod the abdomen with the second until the internal pressure restores the normal shape.
3. Turn the larva on to its side and make a nick on the ventral surface at the anterior margin of the sixth or seventh abdominal segment.

These operations must of course be carried out under a dissecting microscope. Despite their apparent difficulty, the technique is quickly mastered and produces a most satisfying result.

A *mount prepared* by this third method is extremely transparent by virtue of the fact that it consists of little more than a larval skin, and all detail, both dorsal and ventral, is equally visible. It combines, in fact, the permanence of balsam with the clarity of a chloral-gum mount. There is no loss of pigmentation of the chitin though of course tissue colours are lost. Distortion is slight, usually no more than a slight flattening due to the soft nature of the specimen; this can be largely overcome by using plenty of mounting medium. The method is however more lengthy and requires more care than Methods 1 and 2 which are probably preferable for preparing large numbers of mounts for class work, etc.

Although the refractive index of phenol-balsam appears to be somewhat higher than that of Bhatia's medium and chloral-gum, the visibility of larval structures mounted in it is quite good. Bhatia's pine resin has also been tested in phenol solution and found to give a similar result, sometimes perhaps a little better. But until the keeping qualities of the resin are known better, the writer prefers to use canada balsam.

A disadvantage of the phenol-balsam medium is that it dries rather slowly although it should be noted that under Borneo conditions, Bhatia's medium is also slow to dry. However, mounts are quite firm enough for use after several days in the sun and if care is taken in storing them the disadvantage is slight. There is little shrinkage of the medium and sealing is unnecessary.

It should be noted that the chloral hydrate solution used for maceration is also a most useful collection medium for use in the field. Using a pipette or wire loop, larvæ can be transferred from scoop or pool direct to small phials of the chloral hydrate. Subsequent examination and identification is greatly facilitated by the clearing action of the solution and its low refractive index, while its viscous nature minimizes damage to delicate hairs, etc., during transport. The best specimens can then be used for permanent mounts later, although this requires the use of the more difficult technique for piercing the skin.

Care should be taken when using the phenol-balsam medium since phenol is a corrosive fluid and should not be allowed to come into contact with the skin.

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CAMPHOR OILS AS SOLVENTS FOR D.D.T. AND GAMMEXANE.*

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In 1948, large stocks of technical grade D.D.T. became available for health work in Formosa but solvents for D.D.T., such as xylene and kerosene, were expensive and difficult to obtain in quantity. This led to a search for a D.D.T. solvent among the oils manufactured locally and resulted in the finding that two of the oil by-products of the manufacture of camphor could be used satisfactorily. This paper is a report on some of the properties of these oils and on the possibility of their use for mosquito control work.

PROPERTIES OF CAMPHOR OILS.

The oils obtained from the distillation of the wood and bark of *Cinnamomum camphora* to obtain camphor are a series of highly complex organic compounds. The chemical structure of these oils are said to vary with different manufacturing processes; the authors have not studied this matter. In Formosa, the principal

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by-products of camphor manufacture are five substances: camphor tar, perfume oil, white camphor oil, blue camphor oil and brown camphor oil. The tar is a solid at room temperature, the blue oil has a high viscosity and the perfume oil is produced in small quantities and is quite expensive.

Studies have been confined to the other two oils. The white oil was obtained from the Taiwan Camphor Bureau and the brown oil from the Taiwan Pharmaceutical and Chemical Company. The latter oil contains less than one per cent safrol; both oils have a pleasant aromatic odour and both are available to the extent of about 500 tons per year. These oils were found to have the following physical properties:—

1. *Specific gravity:*

		Degrees centigrade.		
		15	*20*	*35*
White oil	...	0·880	0·878	0·868
Brown oil	...	0·972	0·968	0·961

2. *Solubility of technical grade D.D.T. in 100 grams of oil:*

			Degrees centigrade.	
			25	*30*
White oil	...	...	20 gm.	30 gm.
Brown oil	..	...	29 gm.	35 gm.

3. *Coefficient of viscosity:*

White oil, 0·0148 poise at 25°C.
Brown oil, 0·0299 poise at 26·5°C.

4. *Flash point:*

White oil, 59·5°C.
Brown oil, 93·5°C.

5. *Boiling points:*

		Degrees centigrade.	
Per cent boiling.		*White oil.*	*Brown oil.*
0–10	...	130·0–170·5	184·0–192·5
11–20	...	170·5–172·0	192·5–198·0
21–30	...	172·0–173·0	198·0–207·0
31–40	...	173·0–174·0	207·0–221·0
41–50	...	174·0–175·0	221·0–224·0
51–60	...	175·0–176·0	224·0–227·5
61–70	...	176·0–177·0	227·5–231·0
71–80	...	177·0–179·0	231·0–234·0
81–90	...	179·0–184·5	234·0–237·0
91–95	...	184·5–199·0	237·0–241·0

LABORATORY TESTS.

The larvicidal activity of white camphor oil and brown camphor oil (hereinafter referred to as W.C.O. and B.C.O., respectively), and of five per cent solutions of para-D.D.T. and of the pure gamma isomer of benzene hexachloride in these oils was determined in the laboratory.

1. Materials.

Mosquito larvæ.—Fourth instar larvæ of *Culex fatigans*, Wiedemann, were used. Larvæ were collected as needed from a natural breeding place near the laboratory and from these fourth instar larvæ of approximately the same size were selected for the tests.

D.D.T. oil solutions.—Technical grade D.D.T. was treated with alcohol and re-crystallized twice with acetone. Five per cent solutions of the para-D.D.T. so obtained were prepared with W.C.O. and B.C.O. Hereinafter, these solutions will be referred to as D.D.T.-W.C.O. and D.D.T.-B.C.O., respectively.

Benzene hexachloride oil solutions.—The pure gamma isomer of benzene hexachloride (Gammexane), manufactured by Imperial Chemical Industries, Limited, England, was prepared by the Department of Entomology of Cornell University, Ithaca, New York. Five per cent solutions were prepared with W.C.O. and B.C.O.; hereinafter, these solutions will be referred to as B.H.C.-W.C.O. and B.H.C.-B.C.O., respectively.

All materials were kept at room temperature, approximately 20°C.

2. Methods.

Shortage of glassware required the use of two sets of beakers, 250 c.c. and 500 c.c. in volume. These beakers were used in sets of 12 of the same size, because our incubator would not hold a larger number. Tap water was placed in the beakers, 100 c.c., in the 250 c.c. beakers and 200 c.c. in the 500 c.c. beakers, in order to have the depth of water the same in the two sizes. Ten larvæ were introduced into each beaker.

In five beakers the oil solvent was added to the water; in five beakers the oil solution was added; and the two remaining beakers served as untreated controls. The dosages employed were as follows:—

	Milligrams per square centimetre.	
	500 c.c. beakers.	*250 c.c. beakers.*
W.C.O. ...	0·077	0·115
B.C.O.	0·085	0·128
D.D.T. and B.H.C. solutions .	0·0044	0·0066

After adding the test materials, the beakers were placed immediately in an incubator set for the temperature desired. The temperature in the incubator did

not vary more than 0·5°C. from a given setting. Each set of beakers was used to test each material at 15, 25, 35 and 40°C. Each test was repeated and in certain instances was done for four times.

The time required for 50 per cent mortality and the mortality at the end of 6, 8, 12 and 24 hours were recorded.

3. Results.

Mortality in 24 hours.—All larvæ used in the untreated controls were alive at the end of 24 hours.

The data in Table I show that both oil solvents were quite toxic to mosquito larvæ. B.C.O. seems to be a bit more toxic than W.C.O.; this would be expected,

Table I.

Comparative toxicity of camphor oils and camphor oil solutions of D.D.T. and B.H.C. to Culex fatigans *larvæ after 24 hours' exposure at various temperatures and dosages.*

Substances tested.	Dosage, mg./cm.	Mean per cent mortality after 24 hours.			
		15°C.	25°C.	35°C.	40°C.
W.C.O.	0·115	54±1·8	56±4·5	89±2·9	80±3
	0·077	47±4	53±6·9	85±4	48±7·4
B.C.O.	0·128	70±5·4	70±2·1	86±1·3	81±4·7
	0·085	69±4	80±4	97±6	65±4·4
D.D.T.-W.C.O.	H	100	88±7·8	100	90±3
	L	70±2·9	88±3·3	100	69±3·1
D.D.T.-B.C.O.	H	100	100	100	100
	L	83±8·5	79±4·7	90±1·3	65±7·3
B.H.C.-W.C.O.	H	100	100	100	94±2·7
	L	100	100	100	82±6·8
B.H.C.-B.C.O.	H	100	100	100	100
	L	100	97±2·1	100	92±2·9

H = higher dosage (0·0066 mg./cm.). L = lower dosage (0·0044 mg./cm.).
W.C.O. = white camphor oil. B.C.O. = brown camphor oil.

especially at higher temperatures, because of the higher volatility of B.C.O. However, the difference in toxicity demonstrated may have been due to the slightly higher dosage of B.C.O. employed.

The D.D.T. and B.H.C. solutions were definitely more toxic than the solvents themselves, and the B.H.C. solutions were more toxic than the D.D.T. solutions, for both dosages and at all temperatures. The B.C.O. solutions seem a little more toxic than the W.C.O. solutions on an overall basis, but the difference is not great. The lower dose employed, 0·0044 mg. per square cm., was only slightly less effective than the higher dosage, for both chemicals in both solvents.

Fifty per cent mortality.—Table II and Charts 1 and 2 show the mean time required, in minutes, to produce 50 per cent mortality of test larvæ. These data show that B.H.C. solutions are more efficient than D.D.T. solutions at nearly all temperatures for the comparable doses. While there is a suggestion that mortality with a given dosage increases with temperature, this is not a consistent finding.

TABLE II.

Time required to produce 50 per cent mortality of Culex fatigans *larvæ exposed to camphor oil solutions of D.D.T. and B.H.C. at various temperatures.*

Substances tested.	Dosage.	Time in minutes.			
		15°C.	25°C.	35°C.	40°C.
D.D.T.-W.C.O.	H	265·7	230·5	104·3	117·9
	L	709·4	293·1	135·0	...
D.D.T.-B.C.O.	H	184·2	140·7	65·7	107·4
	L	517·1	178·1	146·8	...
B.H.C.-W.C.O.	H	67·4	96·8	77·2	42·3
	L	90·2	186·8	72·3	...
B.H.C.-B.C.O.	H	49·5	74·1	28·9	31·0
	L	65·1	157·8	123·1	...

H = higher dosage (0·0066 mg./cm.). L = lower dosage (0·0044 mg./cm.).
W.C.O. = white camphor oil. B.C.O. = brown camphor oil.

CHART I.

Time required to produce 50 per cent mortality of Culex fatigans *larvae when exposed, at various temperatures, to D.D.T. and the pure gamma isomer of benzene hexachloride, in brown and white camphor oils, applied at the rate of 0·0066 mg./sq. cm.*

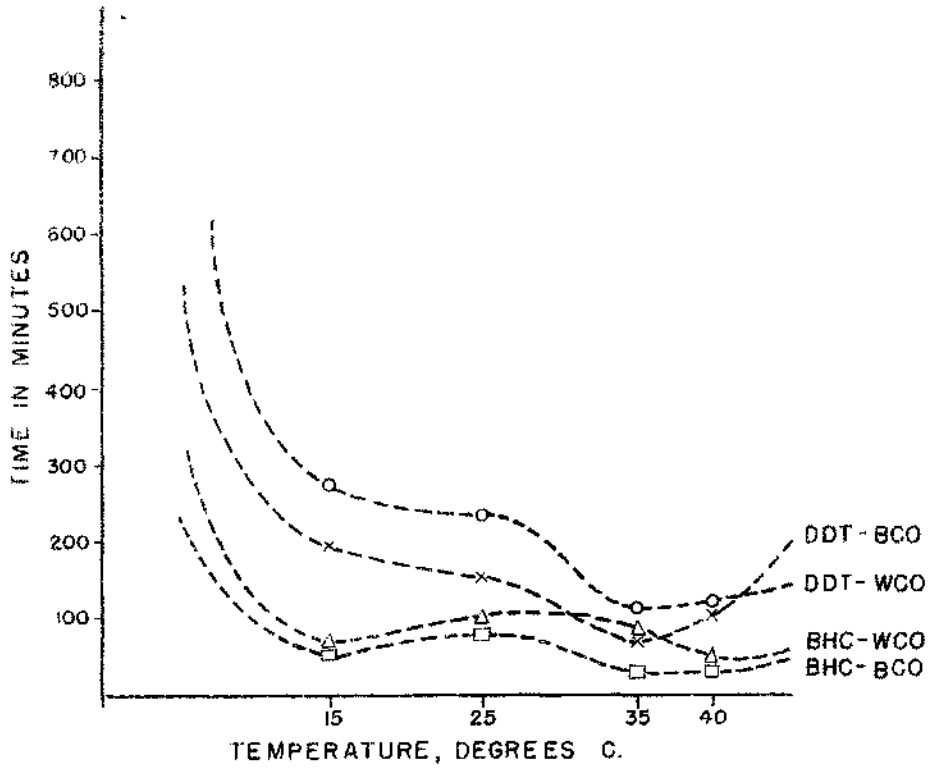
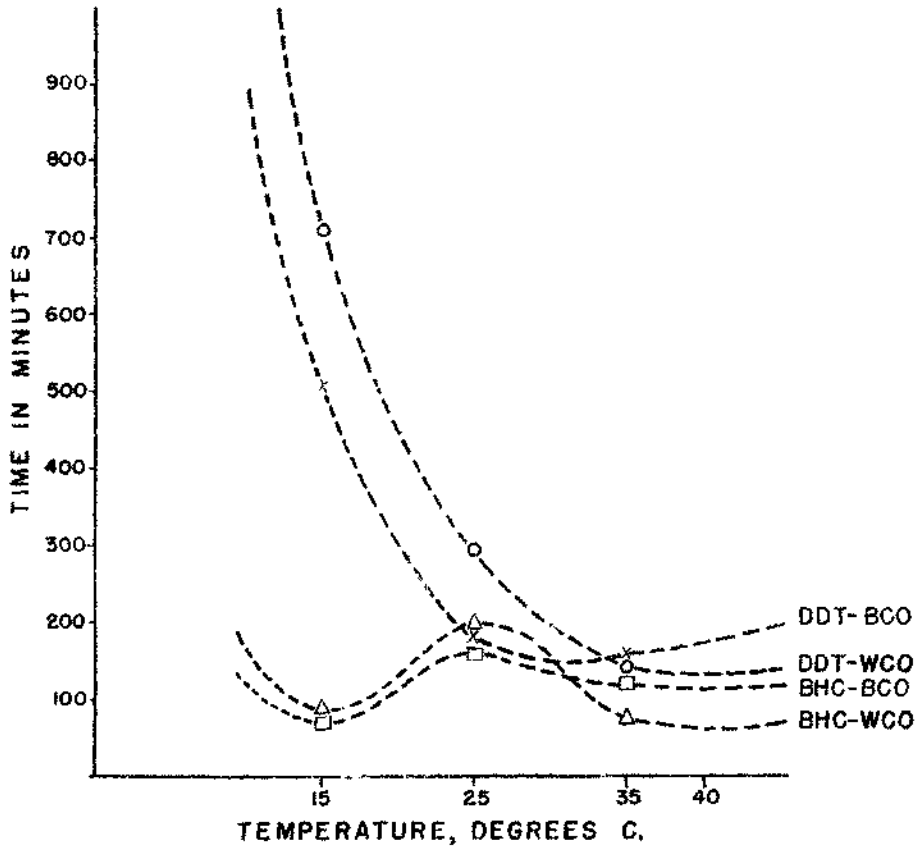


CHART 2.

Time required to produce 50 per cent mortality of Culex fatigans *larvæ when exposed, at various temperatures, to D.D.T. and the pure gamma isomer of benzene hexachloride, in brown and white camphor oils, applied at the rate of 0·0044 mg./sq. cm.*



DISCUSSION.

The common solvents for insecticides are derivatives of petroleum, such as xylene and kerosene, though cyclohexanone is sometimes used. Locally manufactured xylene (60 per cent) and kerosene are available in Formosa but the kerosene has a high sulphur content, a foul odour therefrom and low solubility for D.D.T. The cost of locally made D.D.T. solvents in Formosa, at the time of writing (March 1951), in terms of United States dollars per kilogram was: kerosene, \$0·08; xylene, \$0·24; brown camphor oil, \$0·16; and white camphor oil, \$0·18. Thus the

camphor oils, all things considered, compare favourably with other available solvents.

Either of the camphor oils studied can be used for making insecticide concentrates for use in the preparation of emulsions. The use of such emulsions for D.D.T. residual spraying and for D.D.T. anopheline larvicide programmes will be reported in another paper; but it may be noted here that the addition of 1 to 3 per cent Triton X-100 to the camphor oil concentrate produces excellent emulsions. The brown camphor oil is superior to white camphor oil in that it costs less, has a somewhat higher innate toxicity for larvæ and dissolves larger quantities of D.D.T.

This study seems to show that B.H.C. solutions are definitely more toxic to *Culex fatigans* larvæ than D.D.T. solutions, the results being similar to those obtained by Incho and Deonier (1950), who used No. 2 fuel oil as a solvent. They differ from the results of Kearns *et al.* (1945), who found that there was no significant difference between D.D.T. and B.H.C., regardless of the formulations used and the type of application.

While Formosa is noted for its camphor production, *Cinnamomum camphora* is also cultivated in Japan and in mainland China, so that camphor oils are presumably available in these countries. In India, camphor is being extracted from the plant *Ocimum kilimandscharicum* Guerke, on a more or less experimental basis to date. If an Indian camphor industry develops from the cultivation of this plant on a large scale, by-product camphor oils may be available for insecticide work.

SUMMARY.

In an effort to find a local substitute for kerosene or xylene as solvents for D.D.T. and benzene hexachloride (Gammexane), the authors studied two oil by-products of the manufacture of camphor in Formosa. These were white camphor oil and brown camphor oil. It was determined that these oils have inherent larvicidal properties. Both oils are good solvents of D.D.T. and the pure gamma isomer of benzene hexachloride. These solutions in minute quantities are quite toxic to larvæ of *Culex fatigans*.

The authors conclude that either oil might be used satisfactorily in mosquito control operations and that brown camphor oil is slightly superior to white camphor oil for this purpose.

ACKNOWLEDGMENT.

The authors wish to acknowledge their indebtedness to Dr. R. B. Watson of the Medicine and Public Health Division of The Rockefeller Foundation for his help in the preparation of this report.

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METHODS OF EVALUATING INSECTICIDES IN MALARIA CONTROL.

BY

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[February 27, 1951.]

INTRODUCTION.

PAMPANA (1948) has given an exhaustive review of the different methods of testing residual toxicity of insecticides in the laboratory, and has discussed various criteria which are in use for evaluating insecticides in malaria control in the field. Descriptions of the techniques have proved most helpful to malariologists, and as an attempt to standardize techniques at different centres, much useful information has been gained regarding the behaviour of mosquitoes to insecticides.

Since the publication of his paper, newer methods for testing residual toxicity under laboratory conditions have been devised which are claimed to be comparatively simple and can be easily improvised at places where adequate laboratory facilities do not exist. A description of the new techniques may be useful to workers in the field.

LABORATORY STUDIES.

Residual toxicity of larvicides.—Pal, McCaulay and Fay (1951) described a laboratory technique for testing larvicides, whereby mosquito larvæ are exposed to a treated water surface for a limited period of time, and removed with minimum disturbance of the surface film.

The basic device used for this technique is a 17 × 14 × 2½ inches white enamelled tray with a glass partition placed across it from side to side in such a manner that when the tray is filled with 6,000 c.c. of water, the surface is divided with a glass plate barrier extending about ½ inch below the water level (Plate I, fig. 1). The partition is so adjusted that the area of water surface on one side of the pan equals 104·5 sq. inches or about 1/60,000 of an acre.

The crevices at the end of the glass barrier are sealed with a solution of one part clear pine resin and four parts flexible collodion (this seal effectively prevents the spread around the glass barrier of a thin surface film of oil) and then covered

with a thin coat of paraffin to add rigidity to the joints and to reduce any creeping action of the surface film. The glass barrier is further supported at the ends by slotted wooden blocks. In position the lower edge of the barrier is about 1½ inches above the bottom of the tray, thus permitting free manipulation of a small wire net for introducing or removing larvæ from underneath the glass barrier.

A rectangular basket ½ inch deep, ¾ inch wide, and 2 inches long made of galvanized 16-mesh/inch wire screen is fitted with a stiff wire handle long enough to insert the basket to any point in the tray beneath the surface film.

About 6,000 c.c. of water is placed in the tray, and a calculated quantity of a test larvicide formulation is applied with a graduated pipette fitted with a rubber bulb on the measured surface of water.

Tests are made by introducing larvæ under the treated surface of the tray where they are left for an exposure period of 35 minutes. In the process of removal, any larva not under the water surface is disturbed with a dissecting needle inducing them to go to the bottom where they can be easily caught in the net basket. They are then washed in tap water and transferred to a beaker with food. They are kept under observation for 24 hours. After this holding period, the rate of mortality is recorded (*Appendix I*). Comparisons are handled in an identical manner except that no larvicide is used.

This technique is most useful where repeated tests have to be made for determination of progressive changes, in the residual effect of a larvicide and when mechanical agitation is to be avoided. Once the larvicide has been applied, the surface film is not disturbed till such time the residual effect appears to wear off; moreover, unlike other methods, this technique has the added advantage that the mosquito larvæ come into contact with the treated surface from below as would happen under natural conditions.

Residual toxicity of adulticides.—A special technique has also been developed to test the biological effectiveness of residual deposits of insecticides. Test panels (glass, wood or mud plaster, etc.) 6 × 6 inches are sprayed by means of a Devillbis atomiser using a standardized method of application.

The sprayed panels are placed between two wooden boards 18 × 8 inches in such a manner that a circular area of the panel, 5 inches in diameter, is available for exposure to test insects (Plate I, fig. 2). Another panel (unsprayed) is moved over the treated area so that the two panels are 0·5 cm. apart. A known number of insects (usually 25) are introduced onto the treated surface by means of a glass plunger-tube through a movable perforated square panel of ply-wood. The insects are able to move freely but remain in contact with the sprayed surface all the time. After a specified exposure (usually 15 minutes) the insects are transferred to a cage, 5 inches in diameter (or in an ordinary glass chimney). Both the knock-down time and 24-hour percentage mortality are recorded for each set of experiment (*Appendix II*).

This technique is fairly sensitive in detecting differences in knock-down and mortality rates for different deposits. It has the advantage of allowing the test insects to remain constantly in contact with the surface film for the desired exposure period. The treated surfaces are well protected from any extraneous mechanical

PLATE 1.

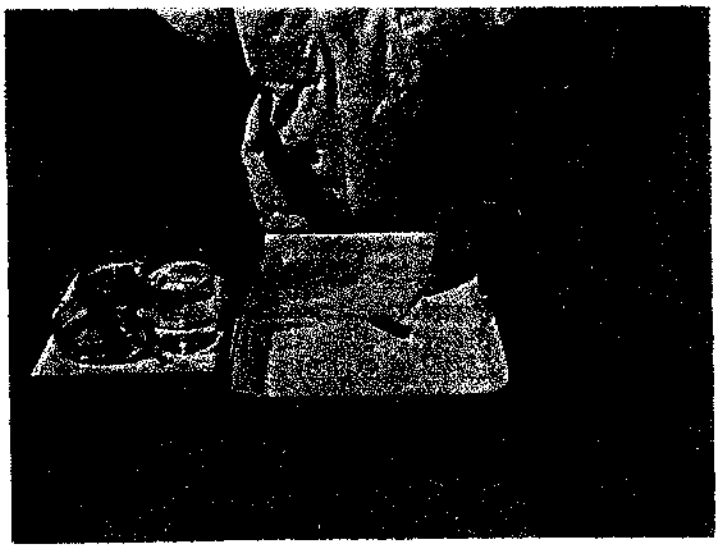

Fig. 1.—Equipment used in the barrier technique for larvicidal studies showing white enamelled tray, glass barrier with supporting wood blocks, transfer net, containers for washing and holding test larvæ, and microscope for film observation.

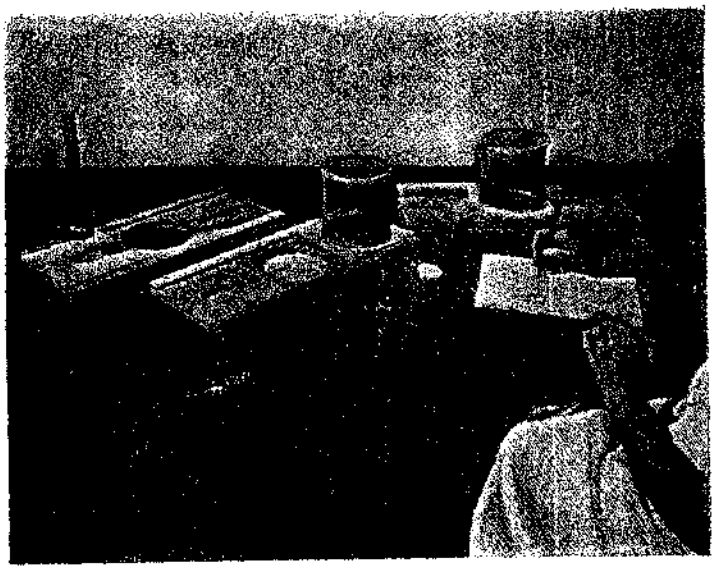

Fig. 2.—Apparatus used for testing the biological effectiveness of insecticidal deposits on sprayed panels showing procedure in placing insects on the treated panels, observing them during exposure period removing insects to holding cage for 24 hours' mortality.

disturbance other than the normal biological activity of the test insects. But this technique is not suitable for insecticides which have fumigant effect.

FIELD STUDIES.

Determination of mosquito density and interpretation of data.—The method frequently employed in assessing efficacy of an insecticide in malaria control in the field is to record densities of mosquitoes (particularly of the vector species) on the assumption that such densities are a measure of the total population of the vector species which plays an important part in the transmission of disease. If the density of the vector species begins increasing after a few weeks or months of spraying, or exceeds a supposed *critical density* (previously determined), this may mean waning of the insecticidal action and indicate the need of a second round of spray.

The usual procedure is to spray a village or a group of villages with an insecticide and study the effects of insecticide on the density of mosquitoes and compare it with that in an untreated village. Sometimes one or two catching stations within a sprayed village are left untreated for comparison purposes. Mosquito density is determined weekly either on a *per man-hour* basis or by spraying a weak solution of pyrethrum and making a *total collection* of the mosquito population present in any catching station.

Per se determination of densities is on theoretical grounds sound but in actual practice there are a number of difficulties. Firstly, considerable doubt exists as to the accuracy of the technique employed in obtaining such data. Density index as expressed *per man-hour* has serious drawbacks of personal factors. Total collections yield more accurate results in this respect. Secondly, determination of *critical density* of vector species remains highly problematical due to seasonal variations in the population of mosquitoes from year to year. Thirdly, the experimental villages or catching stations in a village generally do not behave similarly in regard to variations of mosquito population *with time*. The study of the effect of spraying depends on the assumption that conditions other than the experimental treatment are similar. This lack of comparability seriously impairs the validity of the techniques employed.

Accordingly, the following points are put forward for consideration :—

(1) Variations in the mosquito density in different catching stations in the same village generally show less variations than in two different villages, howsoever carefully these might have been selected. Comparison villages are, therefore, unlikely to serve the useful purpose one would expect for a purely entomological evaluation. But for malariometric studies, unsprayed areas are essential for proper understanding of the influences of the spraying on the incidence of the disease.

(2) Comparison areas form an essential feature of any operative programme, and it seems desirable to select a few villages, leave them untreated, and study the variations of mosquito population *with time*. The selection should as far as possible ensure comparable conditions in relation to their effects on mosquito population. The mosquito population should be observed for the season during which experiments are likely to be started after preliminary surveys. This requires a long

time to build up the necessary data by a competent investigating unit, and may not fall in the purview of the purely routine malaria control organizations.

(3) The selection of four or more catching stations in the same village may be comparatively more useful but both sprayed and unsprayed catching stations should be suitably planned with the considerations outlined above. As far as possible, the initial mosquito population should not be very different in the different catching stations.

(4) As the supposed *critical density* of mosquitoes is a questionable criterion, the following suggestion is put forward as an alternative for the interpretation of data :—

If sprayed and unsprayed catching stations have been carefully selected, the ratio of sprayed/unsprayed mosquito populations should give a useful index of the duration of effectiveness of insecticides. Since sprayed and unsprayed catching stations differ in one being sprayed with insecticide, apart from any '*carry over*' effect of spraying on the 'unsprayed' stations, this 'carry over' effect reduces mosquito population in the unsprayed station, and in spite of any reduction on this account, population in unsprayed catching stations can still be taken to measure the natural variations in population with time. On *prima facie* grounds it would appear that the ratio sprayed/unsprayed should be small and may increase slowly with time, but as the effectiveness of the insecticides becomes lower, the ratio would show an abrupt increase. When an insecticide becomes ineffective, population in sprayed catching stations multiplies quickly.

Other criteria.—Determination of mosquito density is an unsatisfactory criterion in the case of D.D.T. as has already been stated by Thomson (1947) and subsequently by others, because once a residual insecticide has been used in a catching station, daytime catches, whether by pyrethrum and floor sheet or by torch and collecting tube, are likely to be unreliable as an indication whether mosquitoes are entering and feeding at night. Moreover, mosquito densities may not show any reduction, yet malaria control may be achieved merely through the phenomenon of interception. Experience in the field has thus shown that for the proper evaluation of insecticides more than one criterion should be applied concurrently so that the results may, as far as possible, be correctly interpreted. Besides the determination of mosquito density, window trap catches and percentage survival of captured mosquitoes after 24–48 hours *, determination of residual toxicity of a given surface in premises by 'wall cage' test† (Simmons *et al.*, 1945) and chemical estimation of insecticides from scrapings of treated surfaces should be made. Such criteria may not give materially consistent results, probably due to the different physical effects they measure; but if any one of the methods suggests loss of insecticidal efficacy, it should be possible to confirm it on the basis of evidence presented by the additional observations whether or not the loss is clearly established or whether it should be considered as doubtful.

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* A slightly modified design of window trap used by Thomson (1948) has been found very satisfactory. The funnel is made from plastic material instead of mosquito netting; this allows smooth passage of mosquitoes.

† Wall cages made of cellophane paper have been found equally satisfactory.

It is admitted that the real test of efficacy of any method of combating malaria is mainly its effect on the incidence of the disease, but its determination is a lengthy process and it is often difficult to get complete and accurate data. It is necessary, therefore, to obtain *entomological data promptly* so that it may serve as a guide to the progress of work and indicate waning of the insecticidal action, if any, in which case subsequent round of application of insecticide may be ensured in time. The standardization of techniques for obtaining entomological data should prove helpful for correct evaluation of an insecticide in malaria control in the field.

SUMMARY.

For economy and efficiency it is desirable to subject insecticides to a preliminary evaluation in the laboratory and in small-scale field tests before recommendations are made for their large-scale use in malaria control programmes. Various methods for testing residual toxicity of insecticides in the laboratory and in the field have been discussed.

ACKNOWLEDGMENTS.

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APPENDIX I.

Mosquito larvicide date sheet.

Experiment No.....

Formulation..... Dosages..... Dates treated..... Type treatment.....

Type exposure..... Species..... Instar..... Number.....

Test number.	Date.	Pan number.	Age (days).	Time in.	Time out.	Experiment time.	Number down.	Remarks.	24-hour kill.	Remaining.		Per cent kill.
										Pupæ.	Larvæ.	

APPENDIX II.

Adulticide date sheet.

Experiment No. .....

Panel No. ..... Deposit ..... Date of spraying .....

Date of testing.	Age of deposit.	Knock-down in minutes.										24 hours mortality (after 15 minutes exposure).						Remarks.
												Dead.		Alive.		Percentage mortality.		
		15	30	45	60	75	90	105	120	150	180	♀	♂	♀	♂	♀	♂	

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FILARIASIS IN PORBANDAR, SAURASHTRA.*

BY

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[April 15, 1951.]

DESCRIPTION.

PORBANDAR is a busy port town on the Arabian Sea, situated 69° 24′ east longitude and 20° 48′ north latitude on the west coast of Saurashtra (Map), and has acquired great importance being the birth place of Mahatma Gandhi. Cargo and passenger boats ply between this and other ports on the Malabar Coast, East Africa and the Persian Gulf. The city consists of the old town and new residential and industrial areas. The former is near the jetty, and is about three-quarters square mile in area, with a population of 37,000 of whom about 3,000 are seamen. The new suburban area about one square mile in extent is approximately three-quarters of a mile away from the old town and has a population of nearly 28,000.

Average annual rainfall during the south-west monsoon period is about 25 inches and for the greater part of the year the place is reported to be humid.

HISTORY OF FILARIASIS.

A family of sailors from Malabar Coast, the head of which had elephantiasis, is reported to have settled in Porbandar about 30 years ago. Since then elephantiasis is said to be on the increase and markedly so during the last 5 years. The local people are inclined to associate the origin of the disease to these settlers and they also incriminate the boats and boatmen engaged in the coastal trade as the source of transmission of filariasis.

PLAN OF SURVEY.

Observations were made by house-to-house visits in representative areas of the various wards. All persons there were examined between 8 and 11 p.m.

* A filariasis survey of Porbandar was carried out in November/December 1949 at the request of the Government of Saurashtra.

Detailed investigations on symptoms and duration of the disease were made. A fair amount of co-operation was forthcoming though it was difficult to examine for any genital manifestations.

MAP.

Blood from a pricked finger was obtained from 2,999 persons and made into thick smears. They were stained the following day with aqueous methylene blue solution for 2 hours (15 mg. to a litre of water) or for 4 minutes in J.S.B. solution I only (Jaswant Singh and Bhattacharji, 1944).

INCIDENCE OF DISEASE.

Clinically the manifestations of the signs and symptoms of the disease processes were found to be protean in nature. In addition to fever, lymphadenitis and elephantoid conditions of various parts of the body, other peculiar manifestations were observed. The latter consisted of pain or painful lumps in the right groin or in the right side of abdomen which yielded to antifilarial treatment. Microscopic examinations revealed only *bancroftian* infections.

An analysis of the survey data by age-groups as set out in Table I shows a microfilarial rate of 10·1 per cent, disease rate of 6·8 per cent, and the resultant endemicity rate of 16·9 per cent. Microfilariæ were detected in the peripheral blood of 17 out of 205 persons with elephantoid conditions. Average infestation was 16·6 microfilariæ per 20 c.mm.

TABLE I.

Results of filaria survey of age-groups.

Group (years).	Numbers examined.	Number positive for		Infection rate, per cent.	Disease rate, per cent.	Endemicity, per cent.
		Microfilaria.	Disease.			
Up to 5 ...	63	2	0	3·17	...	3·17
6–10 ...	396	36	2	9·10	0·50	9·60
11–15 ...	476	48	12	10·08	2·52	12·60
16–20 ...	453	43	17	9·49	3·75	13·24
21–25 ...	350	34	12	9·70	3·40	13·10
26–30 ...	301	23	28	7·60	9·30	16·90
31–35 ...	207	22	21	10·62	10·10	20·72
36–40 ...	231	22	23	9·50	9·90	19·40
41–45 ...	144	15	24	10·40	16·60	27·00
46–50 ...	159	17	9	11·30	6·00	17·30
Above 50 ...	218	25	33	11·40	15·10	26·50

Details of the various parts of the body affected by elephantiasis are shown in Table II.

TABLE II.

Details of parts affected, i.e. elephantoid conditions in the population examined.

Part or parts affected.	Numbers.
Either upper extremity	16
Both upper extremities	5
Either lower extremity	103
Both lower extremities	56
Either upper and either lower extremities.	9
Left upper and both lower extremities	2
All extremities	2
Genitalia	11
Both lower extremities and genitalia ...	1
TOTAL	205

The youngest persons showing infections were a boy of 3 years having microfilariæ in the blood and a girl of 7 years with established disease.

ENTOMOLOGICAL OBSERVATIONS.

Breeding places.—Most of the houses in the old town have soakage pits which are locally known as 'Bonkhal'. These get clogged and do not function for more than a year or two depending on the quality and quantity of the sullage they receive. Most of them were exposed or loosely covered with big slabs of stones. In many cases the pipes leading into the soakage pits were found leaking and numerous little pools had formed. In the new town and industrial area, intense culicine breeding was noted in the numerous cess-pits, small kutcha drains, and in some wells which were not in use. Thus ideal conditions for mosquito breeding and adult resting places especially for *C. fatigans* exist in close proximity to the houses.

Adult mosquito catches.—Adult mosquitoes caught from houses were identified as *A. subpictus*, *A. vagus*, *C. fatigans*, *C. vishnuii* and *Aedes ægypti*.

Adult *A. subpictus*, *C. fatigans* and *Aedes ægypti* were also collected from the country boats in the port. Culicine breeding was also noted in the water receptacle kept in one of them.

Dissections.—945 *C. fatigans* caught from houses were dissected and 138 of them were found to contain infective filarial forms, giving an infectivity rate of 14·6 per cent.

Precipitin tests of 26 blood meals from *C. fatigans* caught from houses were carried out at the Malaria Institute of India, Delhi. Of these, 19 (73 per cent) gave a positive reaction for human blood. The transmission was thus significantly intense.

On an examination of 63 apparently healthy boatmen who had recently arrived in the port, 23 had *Mf. bancrofti* in their blood. Passive transmission of disease vectors by boats is presumably an important factor. From the history of filariasis in Porbandar given earlier, it can be assumed that filariasis was imported probably by the coastal traffic. This was shown to occur in water-borne traffic in South India (Raghavan and Krishnan, 1949). This aspect requires further investigation in this area, and bears particular importance with special reference to the importation of yellow fever vectors from the African ports.

SUMMARY.

1. Porbandar is a highly endemic filarial town with a low rainfall.

2. *W. bancrofti* infection only is prevalent, the sole vector of which is *C. fatigans* Wied., 1828.

3. Filarial disease manifestations were protean in their types.

4. Results of precipitin tests of blood meals of mosquitoes caught from houses yielded a high percentage of positive reaction against human blood pointing to high potentiality of active transmission of the disease.

5. Soakage pits in close vicinity of human habitations provide ideal breeding grounds for culicine mosquitoes. These pits have their limited utility, even in comparatively dry regions with fairly absorbent soil. Only properly laid out drainage system is the ideal permanent antimosquito measure.

6. Danger of passive transmission of filarial infection and infected vectors by coastal traffic is apprehended.

7. Passive transmission of yellow fever vectors by boats from Africa has to be seriously taken into consideration.

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THE HUMIDITY PREFERENCES OF MOSQUITOES.

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INTRODUCTORY.

It is well recognized that climate exerts considerable influence on the incidence and distribution of malaria. As this disease is transmitted by an insect highly susceptible to climatic factors considerable research is needed in the climatology of malaria, too little being known about the effect of these environmentals in the epidemiology of the disease (Russell *et al.*, 1946).

Climate in relation to insects differs from that in relation to man. Insects live in tiny niches and each insect species have their own preferential niches and micro-climate, which vary with the locality as well as the season in any one locality. The climatic conditions in such situations differ from those in a Stevenson's screen, from which the meteorologist collects his data regarding temperature and humidity. Malariologists are interested in the collation of micro-climatic conditions in the environments where insects breed and rest, and not with climatic conditions recorded in a Stevenson's screen primarily for a human population. As Russell *et al.* (*loc. cit.*) has pointed out, it is necessary to improve methods of collecting information about climate in connection with surveys and control work.

In recent years, increasing attention has been paid to the relationship between mosquitoes and their physical environment, and attempts have been made to interpret their behaviour in terms of simple factors such as temperature, humidity and light. Under natural conditions in the field, the interpretation of behaviour is extremely difficult and necessarily inconclusive, because so many factors vary at the same time. For complete analysis of behaviour it is necessary to dissociate

these environmental factors, and to study the reactions of the insect to one variable factor at a time. This can only be done in a laboratory where one climatic factor alone can be studied with all other climatic factors kept constant.

In this paper, reactions of mosquitoes to humidity at constant temperature and light have been studied in detail.

APPARATUS AND TECHNIQUE.

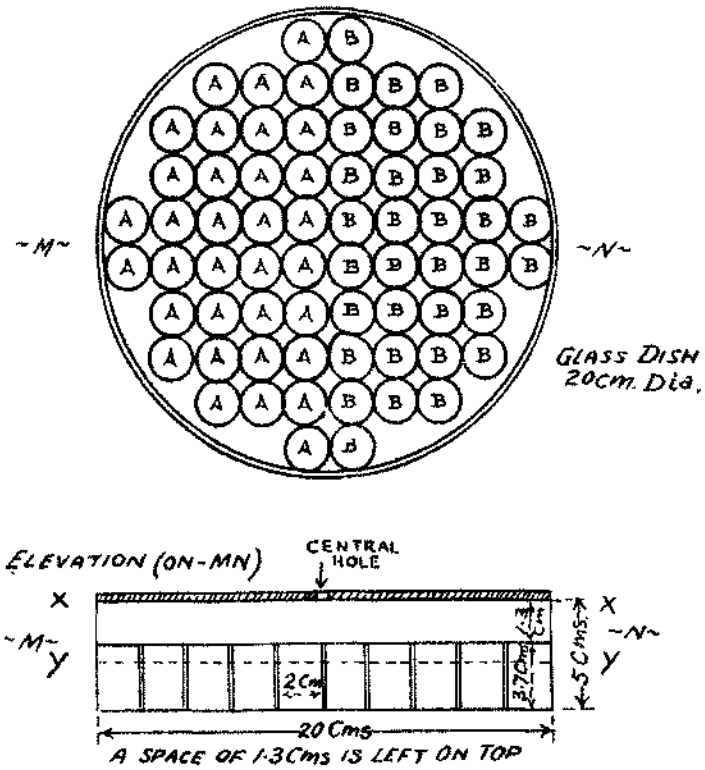
The control of humidity was effected by methods described by Buxton (1931), and Buxton and Mellanby (1934) using sulphuric acid-and-water mixtures, whilst the apparatus used for the experiments is a choice chamber described by Gunn and Kennedy (1936), and Thomson (1938), with improvements designed by Dakshinamurty (1948) to obtain more accurate results, on a pattern permitting the results to be examined by statistical technique (Dakshinamurty, 1949).

Apparatus.—It consists of a cylindrical glass dish of 20 cm. internal diameter and 5 cm. deep. It is provided with about 70 thin glass capsules, each 3·7 cm. deep, 2 cm. in diameter to cover the entire floor of the glass dish. Sulphuric acid and water R.H. mixtures* are poured into these capsules to a height of 3 cm. so that those in one-half of the chamber contain one R.H. mixture, and those in the other half, another R.H. mixture. On the top of these capsules rests a perforated circular piece of paper, which forms a floor for the mosquitoes. Three micro-hygrometers (Dakshinamurty and Sharma, 1949), one in the centre and one on either side, are placed on the paper floor of the insect chamber. A circular glass slab about 22 cm. in diameter, with a central hole which is corked, is placed on the top of the chamber. It forms the roof of a space 1·3 cm. deep in which the mosquitoes move about. This is fixed to the chamber with molten paraffin wax, so as to make the enclosed space airtight. The apparatus is so designed as to give an enclosed space with a steep gradient of humidity, so that the two halves of the chamber approximate to the two humidity mixtures under study. This is achieved in the vertical plane by just leaving sufficient height to enable the mosquitoes to move about, at the same time restricting horizontal movements of water vapour. Again, in the horizontal plane by arranging batteries of capsules closely approximating to the two halves of the floor, give the mosquitoes a common floor but two humidity atmospheres to choose. To enable equilibrium to be attained with the two humidity mixtures in the chamber, the apparatus is generally set overnight as described above, twelve hours before the experiments are performed. After the experiment, the humidity gradient is checked up from the readings of the three micro-hygrometers. Temperature in the chamber will necessarily be the same on both sides and is recorded before proceeding with the experiment. To illuminate both halves of the chamber equally, that is to make light a constant factor, the chamber during the experiment is placed in an illuminated compartment consisting of a wooden box 2 × 2 × 1¾ feet darkened from inside, and fitted with a ground glass top and sliding darkened glass shutter on one side. It is illuminated through the ground glass top by a 'day-light-bulb' to approximate light in quality to that of day light. Readings are taken by sliding the glass shutter (Figs. 1 and 2).

* The notation R.H. is used to represent relative humidity throughout the paper.

Technique.—The experiments are made with freshly hatched laboratory bred specimens of *A. stephensi* ♀♀ and *C. fatigans* ♀♀. Ten mosquitoes are introduced into the chamber, after setting the apparatus overnight as described above,

FIG. 1.



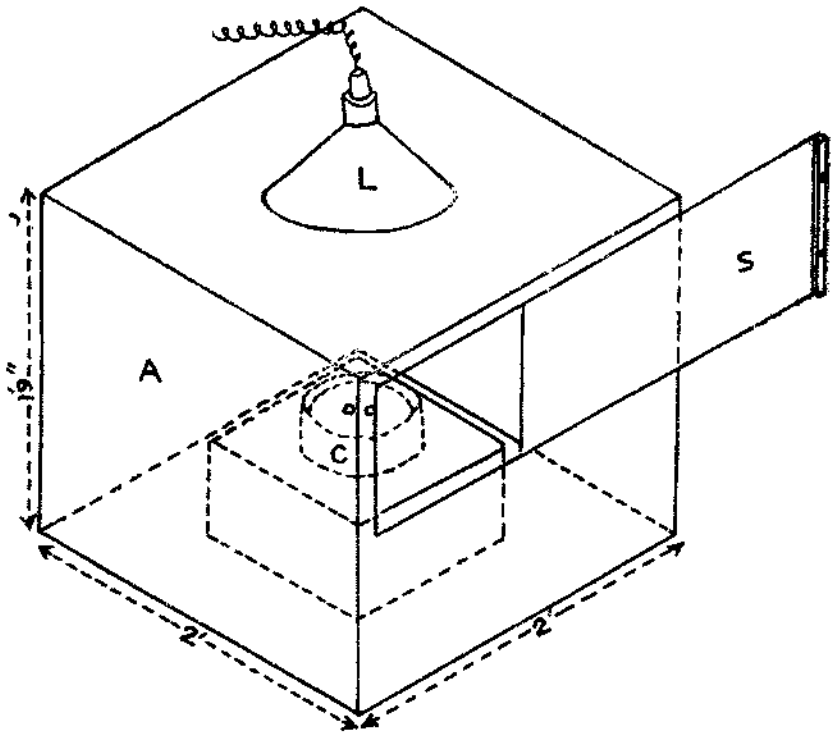
APPARATUS FOR THE STUDY OF HUMIDITY GRADIENT EXPERIMENTS.

AA. CAPSULES CONTAINING ONE R.H. MIXTURE.
BB. CAPSULES CONTAINING ANOTHER R.H. MIXTURE.
X. GLASS SLAB WITH A CENTRAL HOLE FOR THE INTRODUCTION OF MOSQUITOES.
Y. PERFORATED CIRCULAR PIECE OF PAPER WHICH FORMS THE FLOOR ON WHICH THE MOSQUITOES MOVE.

through the central hole in the glass slab which is immediately recorked. The chamber is now placed in the illuminated wooden compartment. After a five

minutes' interval to permit them to settle down, the number of mosquitoes on either half of the chamber are counted every minute, viewing through the sliding shutter.

FIG. 2.



ILLUMINATED DARK COMPARTMENT (A), SHOWING LIGHT (L), CHOICE CHAMBER (C), KEPT IN A DARK BOX WITH SLIDING GLASS SHUTTER (S).

EXPERIMENTS.

With both *A. stephensi* and *C. fatigans*, experiments were carried out at temperatures above 30°C. and below 30°C. Humidity gradients offered to the mosquitoes in the former case were 20–60, 40–60, 40–80, 60–80 and 80–100 per cent relative humidity and in the latter case, 20–60, 40–60, 60–80 and 80–100 per cent R.H. Temperature though varying slightly for different experiments, which depended on the room temperature on different days, was however constant for each experiment generally taking about half an hour. At temperatures above 30°C., ten readings were taken for each experiment, whereas at temperatures below 30°C.,

thirty readings were recorded, as at higher temperatures the insects are more active.

Above 30°C., both *A. stephensi* and *C. fatigans* preferred in all cases higher humidities to the lower ones, when the choice was in the range 20–80 per cent R.H., but when humidity gradient of 80–100 per cent R.H. was offered, they preferred 80 per cent R.H. (Tables I and II).

Below 30°C., *C. fatigans* did not exert any choice for humidity, whatever be the humidity gradient offered. *A. stephensi* exerted choice only when the gradient offered was 80–100 per cent R.H., when it preferred 80 per cent R.H. to 100 per cent R.H. (Table III).

STATISTICAL ANALYSIS.

1. *Humidity choice above 30°C. for* A. stephensi (*Table I*) *and* C. fatigans (*Table II*).—The 't' test was applied in each case, the 't' values have been calculated for each treatment, and the results are summarized below for the different gradients of humidity (series A, B, C, D and E).

Series A.—In series A (20–60 per cent R.H.), in most cases 't' values are significant at 1 per cent level of 'P'.

Series B.—In series B (40–60 per cent R.H.), in case of both *A. stephensi* and *C. fatigans* the choice for the higher humidity is not markedly significant as in series A, while in a few cases the choice is for the lower humidity. It is possible that this difference of behaviour in these series may be due to a less steep gradient with 20 per cent R.H. difference in series B, as against 40 per cent R.H. difference in series A. Further, the lower limit in series A (20 per cent R.H.) is much more uncomfortable than the one in series B (40 per cent R.H.).

Series C.—In series C (40–80 per cent R.H.), in most cases the choice for the higher humidity is significant at 1 per cent level of 'P'. This may be due to the optimum of 80 per cent R.H. presented at one end.

Series D.—In series D (60–80 per cent R.H.), in the majority of the experiments choice is significant for the higher R.H. at 1 per cent level of 'P'. Comparing series D with series B, the significance at 1 per cent level of 'P' for the higher humidity is more marked in D than in B. This may be explained, as in the series D the higher humidity is at 80 per cent which is perhaps nearer the optimum, while in series B it is 60 per cent which is short of the optimum.

Series E.—In series E (80–100 per cent R.H.), the choice for the lower humidity in case of both *A. stephensi* and *C. fatigans* in almost all the experiments is significant at 1 per cent level of 'P'. The conclusion appears to be that 100 per cent R.H. above 30°C. is definitely uncomfortable for the mosquitoes.

2. *Humidity choice below 30°C. for* A. stephensi *and* C. fatigans.—(*a*) *A. stephensi* with humidity gradients of 20–60, 40–60 and 60–80 per cent did not exert any choice for humidity; either the mosquitoes remained inactive, or they preferred indiscriminately the lower or the higher R.H., in other words the choice was not

statistically significant. But in experiments with 80–100 per cent R.H. they preferred 80 per cent R.H., though here also *in most* of the experiments the mosquitoes were inactive (Table III).

(*b*) *C. fatigans* did not exert any humidity choice below 30°C., whatever be the humidity gradient offered, as in almost all the experiments the mosquitoes remained inactive.

Above 30°C., the optimum relative humidity choice of both *A. stephensi* and *C. fatigans* appears to be 80 per cent R.H. In the range 20–80 per cent R.H. they exerted a significant choice in every case for the higher humidity, and again in the range 80–100 per cent R.H., choice was for 80 per cent R.H.

DISCUSSION.

(1) *Physiological considerations.*—Apart from the importance of water as a constituent of the tissues, it plays a significant part in the reaction of the insect to extremes of temperatures in its environment. When exposed to high temperatures, the insect may be killed by the temperature itself, or by desiccation consequent on evaporation (Mellanby, 1932). This explains the behaviour of *A. stephensi* and *C. fatigans* at temperatures higher than 30°C. in preferring higher humidity to lower ones in the range 20–80 per cent R.H. Both *A. stephensi* and *C. fatigans* always preferred higher humidity when the choice was between 20–60, 40–60, 40–80 and 60–80 per cent R.H. It seems probable as contended by certain investigators, that low humidity or high evaporating power causes the body fluids of the insect to dry out, and renders them too concentrated for health (Metcalf and Flint, 1932). This may be yet another reason why mosquitoes always preferred higher humidities to the lower ones. When the choice was between 80 and 100 per cent R.H., both *A. stephensi* and *C. fatigans* behaved in the same way, and preferred 80 per cent R.H. to 100 per cent R.H. This behaviour can be explained by presuming that insects have to lose their water of metabolism through their tracheal system, and this cannot be done in an atmosphere saturated with water vapour.

Below 30°C., *A. stephensi* did not seem to exert any choice for humidity when 20–60, 40–60 and 60–80 per cent R.H. were offered, but with 80–100 per cent R.H. it preferred 80 per cent R.H., while in the case of *C. fatigans* there was no choice for any humidity whatever the humidity gradient offered.

A. stephensi also seems to react to humidity in a different way than *A. minimus* where Thomson (1941) found that when exposed to different gradients of relative humidity between 50 and 100 per cent on the humidity scale, the behaviour of *A. minimus* at all stages was quite erratic. There was never any marked humidity preference either by day or at night, and he concluded that it was unlikely that this factor plays any major part in influencing the choice of resting place, or the movements in and out of houses.

Thomson, 1938 (*loc. cit.*) also elicited the humidity choice of *C. fatigans* at 25°C. He found that the choice was always for a higher humidity where the range of humidity difference is 40 per cent and that they avoided humidities higher than 95 per cent. In the present work it was possible to elicit humidity choice by *C. fatigans* with 20 per cent R.H. difference on the two sides in this apparatus. These results are

also at variance with his findings in that: firstly, in these experiments *C. fatigans* showed no choice for any humidity below 30°C. and, secondly, it avoided 100 per cent R.H. when the choice present was 80–100 per cent R.H. at temperatures above 30°C. It appears that for insects choice of humidity depends on temperature, and as to how best at certain temperature they can maintain their water balance. In the case of mosquitoes at temperatures above 30°C. when the humidity choice is between any two gradients from 20–80 per cent R.H., the mosquitoes choose the higher humidity because, in the higher humidity, they can avoid desiccation as well as they can lose some water of metabolism if necessary for proper water balance. However, when the choice is between 80 and 100 per cent R.H. they choose 80 per cent, as in saturated air they cannot lose any water of metabolism. For the insect 'water loss' and 'water conservation' are both necessary, and a balance between the two has to be struck for its proper 'water balance'.

(2) *Epidemiological considerations.*—These experiments in the laboratory have elicited an optimum R.H. of about 80 per cent at temperatures above 30°C. which is not in variance with the conclusions derived by Gill (1938) on epidemiological grounds in the Punjab. He found that with *suitable* temperature a mean monthly relative humidity of *not less than* 63 per cent was suitable for transmission of malaria.

Pal (1945) while considering the bionomics of *A. culicifacies* observed that since adult mosquitoes spend the *major* portion of their life inside houses it is obviously the indoor micro-climate which affects them more than that recorded outside.

SUMMARY AND CONCLUSIONS.

1. The reactions of *A. stephensi* ♀♀ and *C. fatigans* ♀♀ to different humidity gradients were studied in an improvised humidity choice chamber at constant temperature and light.

2. Five humidity gradients were used for study, viz. (A) 20–60, (B) 40–60, (C) 60–80, (D) 40–80 and (E) 80–100 per cent R.H. and at temperatures above and below 30°C.

3. At temperatures above 30°C., both *A. stephensi* and *C. fatigans* preferred the higher humidities to the lower ones in series A to D, while in series E they preferred the lower one.

4. At temperatures below 30°C., *A. stephensi* showed no choice when 20–60, 40–60 and 60–80 per cent R.H. were offered, but in case of 80–100 per cent R.H. they preferred 80 per cent R.H. *C. fatigans* did not exert any choice in any series.

5. It appears that for insects choice of humidity depends on temperature, and as to how best at certain temperature they can maintain their water balance. In the case of mosquitoes at temperatures above 30°C. when the humidity choice is between any two gradients from 20 to 80 per cent R.H., the mosquitoes choose the higher humidity where they can avoid desiccation as well as lose water of metabolism if necessary for proper water balance. However, when the choice is between 80 and 100 per cent R.H. they choose 80 per cent R.H., as in saturated air they cannot lose any water of metabolism.

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TABLE I.

Humidity preferences of A. stephensi (♀ ♀) *at temperatures above 30°C.*

(A) HUMIDITY CHOICE OFFERED 20-60 PER CENT R.H.

Temperature	36·5°C.	36°C.	35·5°C.	35·5°C.	38°C.	37°C.	38°C.	38°C.	38°C.	38°C.	39°C.
Mean choice for 20 per cent R.H.	0·6	3·2	3·2	3·8	3·1	3·5	3·8	3·8	2·6	4·1	3·9
Standard deviation ..	0·52	1·02	1·02	0·63	0·99	0·84	0·63	1·02	0·84	1·43	0·99
't' ..	26·73†	5·57†	5·37†	6·01†	6·06†	5·64†	6·01†	3·71†	9·04†	1·98	3·50†

(B) HUMIDITY CHOICE OFFERED 40-60 PER CENT R.H.

Temperature	35°C.	31°C.	31°C.	33°C.	31°C.	32°C.	32°C.	32°C.	32°C.
Mean choice for 40 per cent R.H.	2·8	4·1	3·1	4·0	5·0	3·9	3·9	5·0	4·5
Standard deviation ..	0·91	0·56	0·73	1·05	1·66	0·73	0·73	1·37	0·70
't' ..	7·63†	5·07†	8·22†	3·01‡	1·71	4·76†	4·76†	2·07	2·25‡

(C) HUMIDITY CHOICE OFFERED 40-80 PER CENT R.H.

Temperature	36·5°C.	35·5°C.	35·5°C.	36°C.	37°C.	38°C.	38°C.	38°C.	38°C.	35·5°C.	33°C.	35·5°C.	33°C.
Mean choice for 40 per cent R.H.	1·8	4·2	4·1	3·9	4·0	3·8	3·8	3·5	4·1	3·4	4·2	2·7	2·9
Standard deviation ..	0·69	1·54	0·73	0·97	0·66	1·03	1·03	1·07	1·36	0·96	1·31	0·82	0·87
't' ..	14·65†	1·64	3·89†	3·58†	4·78†	3·67†	3·67†	4·42†	2·09	5·26†	1·92	8·86†	7·62†

(D) HUMIDITY CHOICE OFFERED 60-80 PER CENT R.H.

Temperature	31°C.	33°C.	33°C.	32°C.	30·5°C.	32°C.	31·5°C.	31·5°C.	31°C.	31°C.
Mean choice for 60 per cent R.H.	5·9	4·3	3·3	4·0	3·9	6·1	3·9	3·9	4·0	3·9
Standard deviation ..	1·27	0·67	0·48	0·94	0·73	0·99	0·56	0·56	0·81	0·56
't' ..	2·23‡	3·30†	11·19†	3·36†	4·76†	3·51*	6·20†	6·20†	3·90†	6·20†

(E) HUMIDITY CHOICE OFFERED 80-100 PER CENT R.H.

Temperature	31°C.	32°C.	32·5°C.	32°C.	31°C.	31°C.	32°C.	32°C.	32°C.	31°C.
Mean choice for 100 per cent R.H.	2·5	3·1	3·9	4·6	2·7	2·8	3·6	3·8	3·6	4·1
Standard deviation ..	0·51	0·73	0·87	1·28	0·67	1·31	0·84	0·63	1·17	1·19
't' ..	15·49†	8·22†	3·99†	0·98	10·84†	5·30†	5·26†	6·01†	3·78†	2·38‡

* Significant choice for 60 per cent R.H.
‡ Significant at 5 per cent level of 'P'.
† Significant at 1 per cent level of 'P'.
R.H. = relative humidity.

TABLE II.

Humidity preferences of C. fatigans (♀ ♀) *at temperatures above 30°C.*

(A) HUMIDITY CHOICE OFFERED 20–60 PER CENT R.H.

Temperature	35°C.	35·5°C.	35·5°C.	34°C.	35°C.	36°C.	35°C.	36°C.	35°C.	36°C.	35°C.	33°C.	35°C.
Mean humidity choice for 20 per cent R.H.	3·0	2·1	3·3	3·5	3·4	3·1	3·4	4·2	3·0	4·3	3·9	5·0	3·4
Standard deviation ..	1·24	0·73	0·94	0·86	1·26	1·19	1·26	0·91	0·81	1·35	0·73	1·41	1·07
't' ..	5·09†	12·55†	5·71†	5·58†	4·00†	5·08†	4·00†	2·77‡	7·80†	1·63	4·76†	2·24	4·72†

(B) HUMIDITY CHOICE OFFERED 40–60 PER CENT R.H.

Temperature	35·5°C.	33°C.	32°C.	31°C.	31°C.	31°C.	31·5°C.	32°C.	32°C.	32°C.	31·5°C.	32°C.
Mean humidity choice for 40 per cent R.H.	3·4	1·5	5·3	4·3	6·9	2·3	4·0	5·8	3·9	5·5	4·7	3·8
Standard deviation ..	1·26	0·70	0·82	0·94	0·56	0·48	0·66	0·91	0·56	0·70	0·82	0·78
't' ..	4·01†	15·80†	1·15	2·35‡	10·72*	17·77†	4·78†	2·77*	6·20†	2·25*	1·15	4·86†

(C) HUMIDITY CHOICE OFFERED 40–80 PER CENT R.H.

Temperature	35°C.	35°C.	35·5°C.	35·5°C.	35·5°C.	35·5°C.	35°C.	33°C.	36°C.	38°C.	38°C.	38°C.	38°C.	38°C.
Mean humidity choice for 40 per cent R.H.	2·8	6·1§	2·6	3·8	4·5§	3·6	3·6	2·3	4·2§	3·5	3·1	5·3	3·7	3·8
Standard deviation ..	1·22	1·04	1·07	1·03	1·11	1·25	0·96	0·81	1·32	0·84	1·15	1·07	0·94	0·78
't' ..	5·69†	1·90	7·08†	3·67†	2·98‡	3·53†	4·60†	10·53†	5·64†	5·64†	5·22†	1·47	4·37†	4·88†

(D) HUMIDITY CHOICE OFFERED 60–80 PER CENT R.H.

Temperature	35·5°C.	35°C.	35°C.	36°C.	33°C.	32°C.	32°C.	30·5°C.	33°C.	33°C.	31°C.	31·5°C.	31·5°C.
Mean humidity choice for 60 per cent R.H.	4·8§	2·6	5·3	3·5	4·9§	3·9¶	4·5	3·3	4·1	4·0	3·6	3·5	2·4
Standard deviation ..	0·87	1·07	1·33	0·84	0·94	1·31	1·58	1·05	1·10	0·94	0·69	0·84	0·96
't' ..	2·66‡	7·67†	0·71	5·64†	2·11	5·54†	1·00	5·11†	2·58‡	3·36†	6·41†	5·64†	8·55†

(E) HUMIDITY CHOICE OFFERED 80–100 PER CENT R.H.

Temperature	33°C.	32°C.	33°C.	32°C.	32·5°C.	33°C.	33°C.	32·5°C.	32°C.	32°C.
Mean humidity choice for 100 per cent R.H.	3·8	3·8	3·8	3·7	3·8	3·7	3·6	3·7§	4·0	3·5
Standard deviation ..	0·78	0·63	0·78	0·67	0·63	0·67	0·41	0·64	1·05	0·70
't' ..	4·86†	6·01†	4·86†	6·13†	6·01†	6·13†	9·24†	9·29†	3·01‡	6·77†

* Significant choice for 40 per cent R.H. instead of 60 per cent R.H.
‡ Significant at 5 per cent level of 'P'.
† Significant at 1 per cent level of 'P'.
§ Number of mosquitoes in these experiments is 11.
¶ Number of mosquitoes in this experiment is 12.

TABLE III.

Humidity preferences of A. stephensi (♀ ♀) *at temperatures below 30°C.*

	HUMIDITY CHOICE OFFERED 80–100 PER CENT R.H.									
Temperature ..	28°C.	28°C.	28°C.	28°C.	28°C.	26°C.	24°C.	24°C.	24°C.	24°C.
Mean choice for 100 per cent R.H.	2·2	4·4	3·4	3·0	3·9	3·8	2·7	4·8	1·3	2·9
Standard deviation ..	0·67	0·84	0·84	0·81	1·19	1·03	2·40	1·54	0·48	0·87
't' ..	12·53†	2·25‡	6·01†	7·80†	2·92‡	2·96‡	1·71	0·41	24·35†	7·62†

† Significant choice at 1 per cent level of 'P'. ‡Significant choice at 5 per cent level of 'P'.

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THE TEMPERATURE PREFERENCES OF MOSQUITOES.

BY

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INTRODUCTORY.

It is well known that temperature exercises a profound influence upon insects in diverse ways. The extremes of temperature limit insect activities both in space and time. Their rates of metabolism, and consequently those of growth reproduction and general behaviour, are largely controlled by temperature. If temperature observations are to be translated into conclusions of practical value, too exclusive reliance must not be placed upon the usual standard meteorological readings in the field. The latter are not taken with the frequency necessary for entomological purposes, and furthermore, duration of specific temperatures, especially when the latter are maxima or minima, is of prime importance. The full value of meteorological data cannot at present be estimated for the reason that they require to be correlated with the *micro-climatic* conditions of the immediate environment under which a given insect species lives. Little attempt has been made towards correlation of this kind and consequently their utility and limitations are as yet unexplored (Imms, 1931).

For complete analysis of behaviour it is necessary to dissociate these environmental factors, and to study the reaction of the insect to one variable factor at a time. This can only be done in the laboratory where one climatic factor alone can be studied with all the other climatic factors kept constant. In the present paper, reactions of mosquitoes to gradients of temperature at constant humidity and light have been discussed in detail.

APPARATUS AND TECHNIQUE.

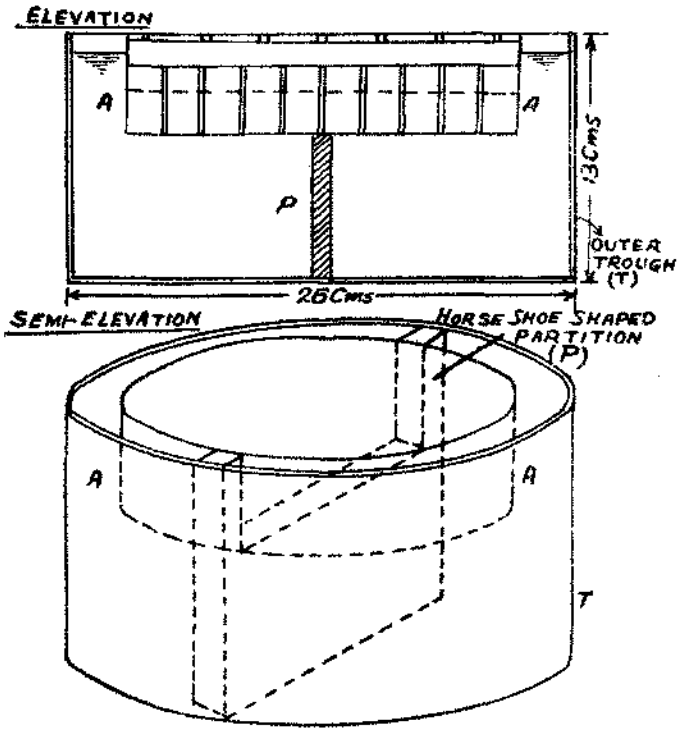
To arrange a gradient of temperature without at the same time creating a gradient in relative humidity and saturation deficiency was thought impossible

(Thomson, 1938). This initial difficulty of designing an apparatus with a temperature gradient and without a gradient of humidity has been solved by Dakshinamurty (1948) who elicited the temperature preferences of *Musca domestica.*

APPARATUS.

The apparatus in general outline is similar to the one used for recording the humidity preferences of mosquitoes (Dakshinamurty and Sharma, 1951). The glass chamber having a suitable lay-out of capsules containing sulphuric acid and water mixtures for the control of humidity (Buxton, 1931 ; Buxton and Mellanby,

FIG. 1.



Apparatus for the study of temperature gradient experiments. On either side of the horse-shoe partition P, water is led through the outer chamber, T; one side 30°C. and the other side 20°C. or 25°C. to produce the gradients 20–25°C. and 25–30°C.

1934) rests on a horse-shoe wooden partition separating the two halves of the water basin where water at thermostatically controlled temperatures of 20°C., 25°C., and 30°C. is circulated from water baths to give the required temperature gradients of 20°–25°C. and 25°–30°C. (Figure 1). The temperature control was therefore from the

outer-jackets, the humidity control from the inner chamber, and the light control is from above by a daylight bulb. To obtain the same humidity on both sides with a gradient of temperature, to start with the apparatus is laid with a virtual *falling* gradient of humidity parallel with a *falling* gradient of temperature, when the apparatus attains equilibrium, the desired requirements are attained. Buxton (1932) and Mellanby (1935) have shown that when assessing the duration of life and water-loss during starvation in several insects, humidity is better expressed as saturation deficiency than relative humidity, as the saturation deficiency figures are directly proportional to the drying power of the air. In these experiments, therefore, saturation deficiency (S.D.) was kept constant on both sides of the apparatus. The relative humidity mixtures to be used for this purpose are primarily known from humidity and temperature nomogram (Hicks and Majid, 1937). With temperature gradient of 20°–25°C., relative humidity mixtures of 60 and 70 per cent would give 7 mm. S.D. on both sides. Similarly, with temperature gradient of 25°–30°C. and relative humidity mixtures of 60 and 70 per cent, S.D. would be 9·5 mm. on the two sides.

TECHNIQUE.

The experiments were done with freshly hatched laboratory-bred specimens of *A. stephensi* ♀ ♀ and *C. fatigans* ♀ ♀. When the desired temperature gradient is achieved, ten mosquitoes are introduced into the chamber through the central hole of the glass slab forming the roof of the chamber, which is immediately recorked. After a five minutes' interval to permit them to settle down, the number of mosquitoes on either half of the chamber on the false perforated paper floor are counted every minute till 30 readings are recorded.

Experiments.—Both with *A. stephensi* and *C. fatigans*, experiments were done at the temperature gradients of 20°–25°C. and 25°–30°C. Few control experiments were also done with same temperature and S.D. on both sides of the chamber, to determine the choice by chance for one or the other side.

Statistical analysis.—The 't' test was applied in each case, and 't' values have been calculated for each treatment and the results are summarized in Tables I and II for the different gradients of temperature.

Temperature gradient of 20°–25°C.—In the case of *A. stephensi*, the choice for 25°C. is highly significant. The 't' values of 9 experiments out of the 10 performed are significant at 1 per cent level of 'P' and in one experiment at 5 per cent level of 'P'. With *C. fatigans*, however, out of the 10 experiments performed, choice for 25°C. is significant at 1 per cent level of 'P' in 1 experiment only, and in the other 9 experiments the choice is not significant.

Temperature gradient of 25°–30°C.—With *A. stephensi*, in all the 10 experiments choice for 25°C. is highly significant at 1 per cent level of 'P'. With *C. fatigans* out of the 7 experiments, in 5 experiments mean choice for 30°C. was nil and in the other two choice for 25°C. was significant at 1 per cent level of 'P'.

With the same temperature and S.D. on both sides of the chamber, *A. stephensi* and *C. fatigans* in most of the experiments did not exert significant choice for any one side, and in very few cases the choice was significant for one or the other side as would be expected in choice by chance.

TABLE 1.

Temperature preferences of A. stephensi *at constant saturation deficiency.*

TEMPERATURE GRADIENT : 20°–25°C.										
Mean choice for 20°C.	1·9	2·4	1·4	1·7	4·4	1·9	3·2	2·2	0·9	2·1
't' value ...	21·04†	12·22†	21·95†	19·74†	2·88‡	21·61†	10·90†	16·39†	41·93†	16·08†
TEMPERATURE GRADIENT : 25°–30°C.										
Mean choice for 30°C.	2·06	1·8	3·4	2·7	2·1	1·7	1·2	3·0	1·9	2·9
't' value ...	15·17†	20·69†	11·30†	12·09†	19·97†	20·8†	33·20†	13·90†	15·55†	10·73†
SAME TEMPERATURE AND SATURATION DEFICIENCY ON BOTH SIDES.										
Mean choice for one side	...	5·00	5·10	5·50	5·40	5·10	...	...	...	...
't' value ...	..	0·00	0·54	2·29‡	2·18	0·52	...	...	...	...

† Significant at 1 per cent level of 'P'.
‡ Significant at 5 per cent level of 'P'

TABLE II.

Temperature preferences of C. fatigans *at constant saturation deficiency.*

TEMPERATURE GRADIENT : 20°–25°C.										
Mean choice for 20°C.	3·6	5·1	5·1	4·4	4·7	4·7	4·6	4·3	4·5	4·4
't' value ...	15·40†	0·52	0·56	1·31	1·69	2·05	1·91	2·09	1·93	2·07
TEMPERATURE GRADIENT : 25°–30°C.										
Mean choice for 30°C.	...	...	6·0	6·8	6·0	6·0	6·0	6·0	6·3	...
't' value ...	...	...	...	32·17†	...	...	...	...	53·22†	...
SAME TEMPERATURE AND SATURATION DEFICIENCY ON BOTH SIDES.										
Mean choice for one side	...	...	...	4·2	4·5	6·8	5·5	4·6	...	...
't' value ...	...	...	...	3·83†	1·61	6·11†	1·79	1·77	...	...

† Significant at 1 per cent level of 'P'.

DISCUSSION.

The effect of temperature on mosquitoes has to be considered from two more or less distinct points of view: the effect on mosquitoes of a gradient in temperature, when the mosquitoes can show a preference; and the effect on the mosquitoes of a constant environmental temperature, where no choice is possible (Bates, 1949). The former has been undertaken in these studies. Such work was previously considered impossible because of the problem of producing a temperature gradient while maintaining a constant humidity. If temperature plays a part in the habitat orientation of mosquitoes, its effect should be demonstrated with temperature gradients.

These laboratory studies definitely indicate an optimum preference for 25°C. in the case of *A. stephensi* with the temperature gradients of 20°–25°C. and 25°–30°C., its choice for 25°C. was always highly significant. *C. fatigans* with 20°–25°C. temperature gradients exerted a significant choice for 25°C. only in one experiment. Unlike *A. stephensi*, therefore, *C. fatigans* does not seem to exert any choice when the temperature gradient is 20°–25°C. When, however, the choice was between 25°–30°C., *Culex fatigans* too exerted a very significant choice for 25°C.

Avoidance of higher temperature by the mosquitoes was very significant. A mosquito on approaching the hotter side would immediately turn away. This avoidance of high temperature which is shown by all insects is clearly an example of 'phobotaxis'; as soon as the insect comes in contact with an unfavourable temperature it makes a reflex avoiding reaction and turns away (Wigglesworth, 1934).

Findings of Thomson (1941) with *A. minimus* were also similar, who recorded that at all stages, including gravid females, the main feature of the behaviour was the avoidance of high temperature, the response being distinct above 25°C. in hungry females and above 30°C. in blood-fed ones.

These results are also in conformity with Thomson (1938) who in the case of *C. fatigans* found that newly emerged females showed avoidance of high temperature below 30°C. but not below 25°C.

SUMMARY AND CONCLUSIONS.

1. The reactions of *A. stephensi* ♀♀ and *C. fatigans* ♀♀ to different temperature gradients were studied at constant saturation deficiency and light.

2. Temperature gradients of 20°–25°C. and 25°–30°C. were used for these laboratory studies.

3. *A. stephensi* exerted a significant choice for 25°C. with temperature gradients of 20°–25°C. and 25°–30°C.

4. *C. fatigans* exerted a significant choice for 25°C. with the temperature gradient of 25°–30°C., but when the gradient was 20°–25°C., the choice in most of the experiments was not significant.

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FIVE YEARS' OBSERVATION ON THE INCIDENCE OF BLOOD PROTOZOA IN HOUSE SPARROWS (*PASSER DOMESTICUS* LINNAEUS) AND IN PIGEONS (*COLUMBA LIVIA* GMELIN) IN DELHI.

BY

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[May 11, 1951.]

HÆMOSPORIDIA, occurring naturally in the sparrows and pigeons obtained in Delhi and its environs, have been used as a routine for the preparation of teaching material at the Malaria Institute of India during the past 12 years. In the present report, only data on the incidence of hæmosporidial infections and their infectivity to mosquitoes collected during the last 5 years (1946–50) have been analysed.

METHODS.

Our routine practice has been to procure birds in the market periodically and examine each on 3 consecutive days before declaring any bird negative. In each case, air dried thin blood films stained by J.S.B. stain (Jaswant Singh and Bhattacharji, 1944) were examined for a minimum of 15 minutes per slide before a negative diagnosis was made. Some of the parasite-negative sparrows were inoculated with blood from parasite-positive sparrows to determine the state of premunition.

Only sparrows showing large numbers of gametocytes of *Plasmodium relictum* were used for mosquito feeding, and invariably the presence of exflagellation of the microgametocytes was determined. The mosquitoes fed on the sparrows were *Culex fatigans* Weidman, *Culex lutzia* Theobald and *Anopheles subpictus* Grassi.

The routine adopted for feeding mosquitoes was to keep the infected birds in cages overnight with the mosquitoes. Rarely when this method of feeding was unsuccessful, forced feeding of mosquitoes was resorted to on defeathered birds. The fed mosquitoes were kept under optimum conditions of temperature (25°C. to 30°C.) and humidity (70 to 90 per cent). 10 per cent of each lot of mosquitoes fed were dissected during the extrinsic incubation period. Of these, some were dissected a few hours after feeding to demonstrate the early stages of development and others later to demonstrate oöcysts and sporozoites.

DATA AND ANALYSIS.

A total of 869 house sparrows and 214 pigeons were examined of which 294 sparrows and 46 pigeons showed hæmosporidia in the blood. In the sparrows, the infections consisted of *P. relictum* and *hæmoproteus*, and in pigeons *Hæmoproteus columbæ* only. Out of the 551 sparrows that were negative, 194 were inoculated intravenously with blood from positive ones. Only 66 of these became positive within 3 to 11 days after inoculation, and the remainder after showing negative results during a period of 30 days' observation were discarded.

Of the 294 sparrows that revealed natural protozoal infection, 235 showed *P. relictum* only, 38 *hæmoproteus*, and 21 mixed infections of *P. relictum* and *hæmoproteus*.

A total of 7,516 *C. fatigans* and 24 *C. lutzia* were successfully fed on sparrows infected with *P. relictum*. Sporozoites developed in 6,566 *C. fatigans*. Twenty *C. lutzia* were dissected during the early extrinsic incubation period and all showed oöcysts in the gut. Unfortunately, due to short numbers, further development of the parasite in this species was not followed up. On one occasion an isolated specimen of *C. fatigans* showed trypanosomes as well as sporozoites in its salivary glands, as reported by Jaswant Singh *et al.* (1950). Several attempts to feed *A. subpictus* on infected sparrows, as practised with *C. fatigans*, were entirely unsuccessful. Out of a total of 500, only 75 mosquitoes fed on defeathered birds during the attempts of forced feeding, but no development of the parasite was noticed in any. The details of the blood examination and results of feeding have been analysed in the Table for the different months of the year.

DISCUSSION.

It will be seen that out of 869 sparrows examined, 29·5 per cent were found to harbour *P. relictum* and 7·1 per cent *hæmoproteus* gametocytes. This high prevalence of *P. relictum* infection in sparrows in nature is similar to some extent to the findings of other workers (Hunninen and Young, 1950; Micks, 1949; Sergent and Sergent, 1948; Haiba, 1948) in other countries. The data presented in this report also indicate that 66 per cent of the 194 apparently healthy birds remained refractory to inoculation of blood containing *P. relictum* which in all probability is an indication that these birds at the time of examination may have been in the latent phase of homologous infection. It is difficult to determine therefore from a short-term examination of these birds whether they were altogether free from plasmodial infections. Wenyon (1926) came to such a conclusion in the case of *hæmoproteus* infection.

TABLE.

Results of natural protozoal infection in wild caught Indian house sparrows and domestic pigeons and the experimental mosquito transmission during different months of the year between 1946 and 1950.

Months.	Sparrows positive for blood protozoa.		Sparrows positive for *P. relictum* (percentage).	Sparrows positive for *hæmoproteus* (percentage).	Sparrows positive for mixed infection, *P. relictum* and *hæmoproteus* (percentage).	Results of inoculation of the blood forms of *P. relictum* to birds that showed initial negative results.		Results of feeding *C. fatigans* on sparrows showing natural *P. relictum* infection.		Pigeons positive for *Hæmoproteus columbæ*.	
	Number examined.	Percentage positive.				Number inoculated.	Percentage of birds that became positive.	Number fed.	Percentage infected.	Number examined.	Percentage positive
Jan. ...	22	18·2	18·2	0·0	0·0	15	6·6	...	...	10	1·0
Feb. ...	75	62·2	58·6	1·4	2·6	...	...	302	90	85	16·4
Mar. ...	209	33·4	18·2	8·1	7·1	49	42·0	1,981	84	16	25·75*
Apr. ...	24	37·5	37·5	0·0	0·0	...	...	...	...	6	66·6
May ...	89	20·2	19·1	0·0	1·1	23	43·6	363	88	...	...†
Jun. ...	21	4·7	4·7	0·0	0·0	...	...	...	...	15	0·0
Jul. ...	153	31·4	20·3	10·5	0·6	42	23·8	557	86	39	38·4
Aug. ...	107	32·7	30·9	0·9	0·9	31	19·3	614	90	21	19·0
Sep. ...	60	23·3	18·3	3·3	1·7	22	59·1	404	85	11	36·4
Oct. ...	76	43·4	43·4	0·0	0·0	...	...	275	84	11	0·0
Nov. ...	33	45·5	42·5	3·0	0·0	12	41·6	2,920	80	...	...
Dec. ...	...	...	...	...	...	...	...	...	...	...	...

* *A. subpictus* fed on defeathered sparrows but without any development of parasites.

† 47 *C. fatigans* fed on pigeons. No development of parasite occurred.

During October to February, the plasmodial infection rate in sparrows was 47·1 per cent, between March and April 26·6 per cent, May and June 17·3 per cent and between July to September it was 24·4 per cent. The latent infection rates obtained after subinoculation tests also indicate a more or less parallel fluctuation, in that the percentages of sparrows that did not take up the infection were 77·7, 57·1, 56·5 and 69·5 respectively during the corresponding periods. These findings indicate that the highest percentages of sparrows show *P. relictum* infections during the cold weather and least during the summer season. A similar low rate of natural plasmodial infection in birds during summer months has been recorded by Hunninen and Young (1950) in Columbia and by Hart (1949) at Manning, both in South Carolina (U.S.A.). The findings of Micks (1949), however, are at variance in that in English sparrows caught mostly from Maryland he found maximum infection rate during the summer months.

Hæmoproteus infection in sparrows is not uncommon. *H. orzivorœ* occurs in Java sparrows (Anschnts, 1909 : 1910) as quoted by Wenyon (1926). This type of parasite has also been reported in sparrows from various other parts of the world such as Sicily, Algeria, Baghdad (Sergent and Sergent, 1948) and Italy (Haiba, 1948). Infections in the sparrows of various geographical regions probably belong to different species. Sergent and Sergent (1948) have given the distinct name *H. wenyoni* to those seen in Sicily, Algeria and Baghdad on account of their marked irregularity in outline and the small oval size of the young gametocytes. On morphological variations, Haiba (1948) has named the hæmoproteus seen in black ducks as *H. hermani*. The authors' own experience shows that the hæmoproteus gametocytes occurring in Delhi sparrows are not identical with *H. columbæ* commonly seen in pigeons but to what species this actually belongs is difficult to say until a detailed comparative study has been made with other forms so far described. Regarding its incidence during different periods of the year, the percentages of infection seen were 2·13, 7·0, 0·9 and 6·9 for the periods October to February, March and April, May and June, and July to September respectively. A similar finding has been observed with *H. columbæ* in pigeons, the figures being 15·8, 36·3, 3·0 and 32·4 per cent respectively for the different seasons mentioned above. Though the highest percentage of infection observed in the case of hæmoproteus both in sparrows and pigeons was during the presummer period or spring, the incidence was in these cases also as in plasmodial infections in the sparrows; the lowest during the very hot months of May and June.

Hunninen and Young (1950) succeeded in infecting *A. quadrimaculatus* readily with *P. relictum* of wild caught English sparrows. Though *A. subpictus* did not show any susceptibility to this species of parasite in our hands, the possibility that some other species of anophelines in India may also be susceptible to avian malaria cannot be ruled out.

SUMMARY.

The blood of 869 house sparrows (*Passer domesticus* Linnaeus) and 214 domestic pigeons (*Columba livia* Gmelin) of Delhi were examined between 1946 and 1950. *P. relictum* was found in 29·5 per cent and hæmoproteus gametocytes in 7·1 per cent of the sparrows examined. The only protozoa found in pigeons was *Hæmoproteus columbæ* and the same was found in 21·5 per cent of the birds

examined. The incidence of both plasmodial and hæmoproteus infections in the birds was the minimum during the summer season. Mosquito feeding experiments on sparrows with *P. relictum* gave a result of 87·3 per cent sporozoite infection in *C. fatigans*. *C. lutzia* was found susceptible to *P. relictum* but *A. subpictus* was refractory to this infection.

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FIELD STUDIES ON THE COMPARATIVE EFFECTIVENESS OF D.D.T. AND B.H.C. AGAINST MOSQUITOES WHEN APPLIED SEPARATELY AND IN COMBINATION.

BY

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INTRODUCTION.

FIELD studies on the comparative effectiveness of D.D.T. and B.H.C. were carried out during 1950 in a group of seven villages near Palwal, District Gurgaon, Punjab (I), about 36 miles south of Delhi. The experiments were started in July 1950, and were continued until December, when due to the severe cold weather the mosquito population was reduced to low levels.

This area is known to be malarious, and epidemiology of the disease in this district is similar to the rural areas of Delhi State where *A. culicifacies* is the vector. No antimalaria measures had been taken for combating the disease in this district and the spleen rates in the villages ranged from 34 to 54 per cent.

Majority of the houses are poorly built and are 'kutcha' with mud-plastered walls and thatched roofs. The ceiling is generally about 10 feet high and floors are plastered in the same manner as walls. Majority of the houses are without windows, but have one or two entrances with or without doors. The number of houses and total population in each village is shown in Table I.

INSECTICIDES TESTED.

The formulations and dosages of D.D.T. and B.H.C. individually and in combination that were tested are also given in Table I. D.D.T. was used both as an emulsion and suspension, the former as D.D.T.-aromex-soap emulsion prepared at the Institute according to the formula described by Puri and Pal (1947) and the latter as 'Geigy malaria spray' * 50 per cent water-wettable powder. B.H.C. was used as suspension 'Gammexane P. 520' † containing 6·5 per cent gamma isomer. D.D.T. and B.H.C. in combination was used as suspension; 2·5 per cent D.D.T. suspension and 0·5 per cent B.H.C. gamma isomer suspension prepared from 'Geigy malaria spray' and 'Gammexane P. 520' respectively were mixed in equal parts.

Surfaces of all houses in the villages were measured and the quantity of formulation required for each house or group of houses was sprayed in particular areas. The spraying equipment consisted of double-barrel stirrup pump fitted with an oil-resisting hose and a brass lance. The nozzle used had an orifice of 3/64th inch diameter with an output of about 600 c.c. per minute in a cone-shaped spray. The spraying was done by experienced workers and under the direct supervision of one of the authors. All structures whether human dwellings or cattle-sheds were treated. Inner surface of walls and as much of the low roofs as could be easily reached were sprayed.

ENTOMOLOGICAL RECORDS.

Experience in the field showed that for the proper evaluation of insecticides more than one criteria should be applied concurrently so that the results may, as far as possible, be correctly interpreted (Pal, 1951*a*). During the present investigation the following data were recorded.

A. *Density of mosquitoes per week.*—By making a total catch of mosquitoes in catching stations after spraying a weak solution of pyrethrum and collecting the dead mosquitoes on floor sheets. In each village a number of catching stations both sprayed and unsprayed were established for this purpose. The unsprayed catching station served as comparison.

Mosquito catches were recorded as:—(*a*) culicine, (*b*) *A. culicifacies* and (*c*) other anophelines. A record was also kept as to whether or not blood meal had been taken, the condition of the ovaries and age of mosquitoes.

B. *Window trap collections and percentage survival of trapped mosquitoes after 24 hours.*—A slightly modified design of window traps used by Thomson (1948) was found more satisfactory. The funnel in these traps was made from plastic material instead of mosquito netting (Fig. 1); this allows smooth passage of mosquitoes.

C. *Chemical estimation of insecticides in scrapings from treated surfaces.*—This was possible only in the case of D.D.T. Chemical estimation was made by the method described by Alessandrini (1948).

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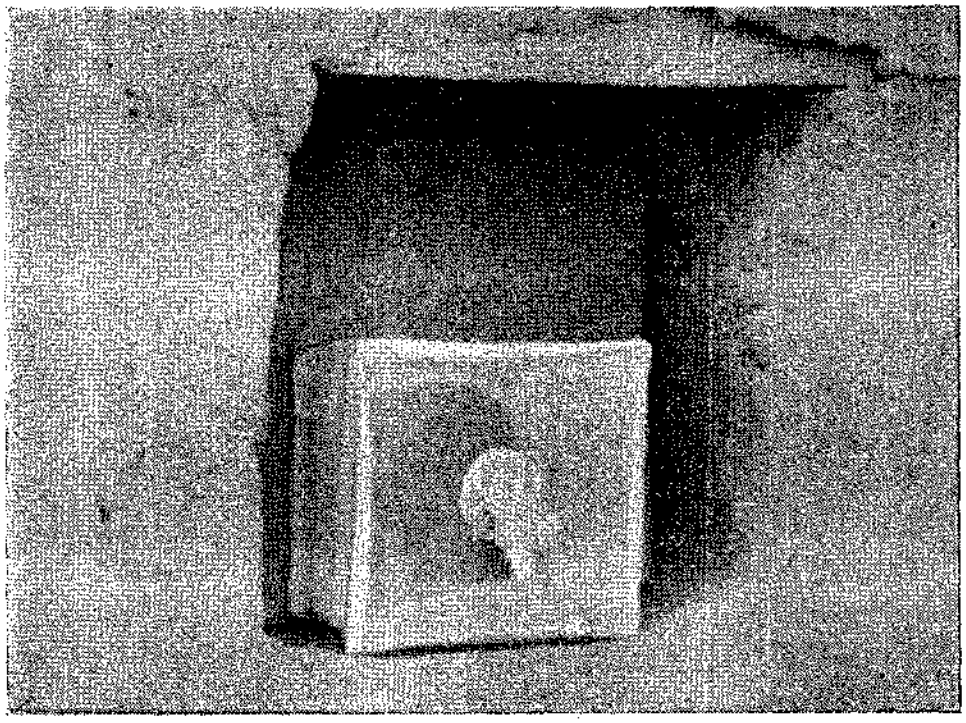
* Supplied by Geigy Insecticides Ltd., Bombay.

† Supplied by Imperial Chemical Industries (India) Ltd., Calcutta.



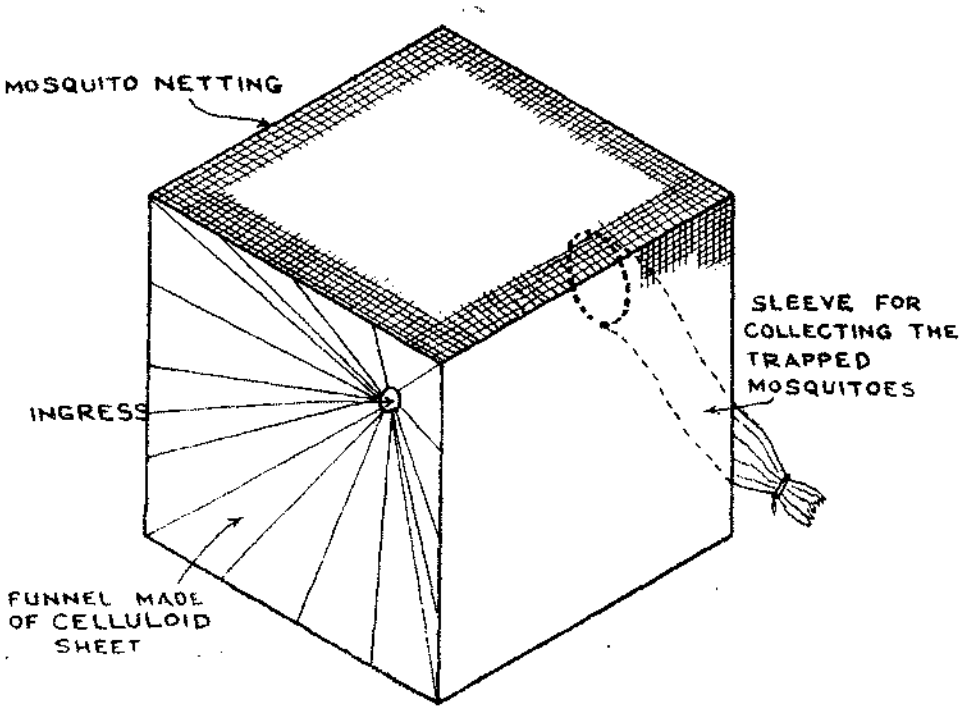


FIG. 1.—Showing the modified type of window trap cage: (top) cage fixed to a window of a catchingstation, (bottom) design of a cage showing the celluloid funnel.

MALARIOMETRIC RECORDS.

A. Spleen and parasite rates in children 2–10 years.

B. Infant parasite rate every month.

C. Blood slides of all fever cases in each village every week throughout the period of observation (as these villages are not served by any regular dispensary).

Similar records were also maintained in the untreated village for purposes of comparison.

STUDIES ON D.D.T.

(*a*) *Emulsion* versus *suspension*.—Mosquito density, survival rate of trapped mosquitoes and results of D.D.T. estimation from the scrapings are given in Tables II, III and IV.

It would appear from the data presented that irrespective of the dosage employed, D.D.T. suspension was not only comparatively more efficacious in keeping down the density of mosquitoes in the catching stations, but was also effective for a longer period (two weeks or more). Chemical estimation of D.D.T. from the scrapings also showed that recovery of D.D.T. was slightly better in the case of suspension than emulsion and D.D.T. could be detected for a longer period on surfaces treated with suspension.

A large number of workers (Barlow and Hadaway, 1948; Sundararaman and Peffly, 1949; Bishopp, 1949) have noticed the superiority of D.D.T. suspension over emulsion on mud-plastered surfaces. It has been stated that emulsions and solutions are easily absorbed into the inner layers of porous surfaces like mud plaster and are thus not available on the surface. Water-wettable powders of D.D.T. have other advantages: they are easy to handle and less bulky to transport, but it may be mentioned here that the choice of formulation of D.D.T. for extended malaria control programme will largely be determined by economic conditions. Most of the wettable powders marketed in this country do not conform to the standard specifications laid down by W.H.O. Expert Committee on Insecticides (W.H.O./Insecticides/11, 9th November, 1950). D.D.T. emulsion has therefore been used in preference. Moreover, personnel of malaria control organizations can locally prepare D.D.T. emulsion themselves at a comparatively cheaper cost. It would seem likely that D.D.T. water-wettable powders may replace other formulations of D.D.T. if good quality products become available at competitive costs. Recent studies on D.D.T. formulations have shown that there is considerable amount of deterioration of D.D.T. in solutions when held in storage. This may be an additional factor in favour of water-wettable powders.

(*b*) *Choice of dosage 50 mg.* versus *200 mg. D.D.T./sq. ft.*—Massive dosages of D.D.T. when applied on mud-plastered walls whether as suspension or emulsion do not show a proportionate increase in the duration of residual effectiveness. Analysis of the data collected has shown that contrary to the common belief, D.D.T. deposits deteriorate rather rapidly under tropical conditions, particularly in rural areas. After 12 weeks of application, no D.D.T. could be detected even in the scrapings removed from houses treated with 200 mg./sq. ft. The results of D.D.T. estimation may be open to criticism, because it is extremely difficult to deposit

precise amounts of D.D.T. on wall surface by field equipment and there may be considerable variations in the amount deposited. Alessandrini's method of chemical estimation of D.D.T. has been stated to give erratic results, particularly when the samples are contaminated by straw and cowdung. But nevertheless the results of chemical estimation of D.D.T. from scrapings closely correspond to the biological results discussed above. The progressive deterioration of D.D.T. deposits in residual effectiveness when applied on mud-plastered walls may be due to a variety of factors, i.e. mechanical removal, masking effect by dust and dirt, nature and composition of mud, absorption into inner layers of the surface, crystal habit, volatilization, chemical disintegration due to organic and inorganic contamination, and the prevailing temperature and moisture conditions.

It is likely that when massive doses of D.D.T. are deposited on mud-plastered walls, not only D.D.T. flakes off easily but it also forms conglomeration of crystals which are not satisfactory from the viewpoint of toxicity.

Moreover, the choice of dosage will depend not only upon the duration of lethal effect of the deposit, but also whether this effect is disturbed by human interference. It is common practice in this part of the world, particularly in rural areas, to replaster the houses with mud and cowdung frequently, thereby rendering ineffective any insecticide deposit present on the walls. Moreover, labour is cheap and only 30 per cent of the total cost of residual spraying is appropriated under this head, 70 per cent being spent on the insecticidal formulation. In the American continent and some of the European countries, it is the usual practice to apply 200 mg. D.D.T. per sq. ft. and this has been found to remain effective for over six months. The most expensive item in residual spraying of houses in these countries is the cost of labour, the ratio may probably be just the reverse, i.e. 70 per cent on labour and 30 per cent on material.

In actual practice under conditions obtaining in India, it may be feasible to spray D.D.T. at the rate of 50 mg./sq. ft. during the first round and, after six weeks or so, a second round at the rate of 50 mg. or even less depending on the duration of the transmission season, formulation employed, nature of the surface sprayed, and the prevalent climatic conditions.

(*c*) *Excito-repellent effect.*—Window trap collections indicate that there is a definite repellent effect of D.D.T. deposits on local mosquitoes, as the number of mosquitoes collected in the traps is more than that in the sprayed catching station. But the percentage survival rate of the captured mosquitoes is almost nil for the first few weeks, indicating that in spite of the repellent effect, the mosquitoes pick up a lethal dose and eventually die. In subsequent weeks, although a greater percentage of mosquitoes survive for 24 hours, yet there is no transmission of the disease (*see* malariometric data, Tables VII and VIII) due to the phenomenon of interception. In fact, complete malaria control may be established entirely through the process of interception, in which without significant actual reduction in the density of the vector species, the longevity of adult mosquito is effectively reduced to below that required for the incubation period of the parasite in the mosquito. Unlike *A. gambiæ* and *A. melas* in Africa (Thomson, 1947*a*, 1947*b*, 1949, 1950), complete control of *A. culicifacies* may be achieved by indoor residual spraying of D.D.T., and this has been fully substantiated by the results achieved in India during the past few years.

STUDIES ON B.H.C.

B.H.C. has not been used so far in extensive malaria control programmes in this country, firstly, because D.D.T. had captured the market before B.H.C. was introduced, secondly, D.D.T. effectively checked the incidence of malaria and gave full satisfaction to the public health authorities and, thirdly, D.D.T. was comparatively economical to use. Vishwanathan *et al.* (1949) tried B.H.C. on experimental basis in the villages of Bombay State where *A. fluviatilis* and *A. culicifacies* are vectors and stated that D.D.T. had a slightly more marked residual action than B.H.C. Formulations of D.D.T. and B.H.C. tried were, however, not similar; the former was used as emulsion and the latter as suspension.

The entomological data collected during the present investigations are briefly summarized in Table V. The mosquito density remained very low throughout the period of observation, and the mosquitoes collected from sprayed rooms and window traps did not survive for 24 hours after the collection, during the first six weeks of spray. Under the local conditions, there is surprisingly little to choose between B.H.C. 10 mg. gamma isomer and D.D.T. 50 mg. per sq. ft. Both give full protection for about 6 weeks.

It appears that B.H.C. also exerts a slight repellent effect on local species of mosquitoes for the first few weeks, but the trapped mosquitoes in window traps do not survive when kept under observation, indicating that they pick up a lethal dose and eventually die. It was observed that in catching stations sprayed with B.H.C. the survival rate of trapped mosquitoes in window traps was nil for a period of 6 weeks after application.

STUDIES ON D.D.T. AND B.H.C. COMBINED SPRAY.

D.D.T. and B.H.C. have so far been applied separately in malaria control programmes. In order to find out how these two insecticides would behave in a combined spray, experiments were carried out in the laboratory and in the field. Preliminary experiments in the laboratory indicated that D.D.T. and B.H.C. in combination gave a longer residual effect than either D.D.T. or B.H.C. alone applied at the same dosage. The synergistic action of D.D.T. and B.H.C. in combined spray was quite obvious and this has been reported in a preliminary note (Pal, 1951*b*).

Field data on the effectiveness of D.D.T. and B.H.C. combined spray is given in Table VI. D.D.T. and B.H.C. in combined spray when applied at the rate of 25 mg. D.D.T. and 5 mg. gamma isomer per sq. ft. was effective for at least 9 weeks after which there was a sudden decrease in the density of mosquitoes due to incessant heavy rains, thereby hampering further observations. It would be evident from the data presented that D.D.T. and B.H.C. combined spray was not only effective for a longer period but was also more efficacious in keeping down the density of the mosquito population in the treated village. Throughout the period of observation the density of mosquitoes was very low in the village treated with the combined spray and, as would be evident from the malariometric data discussed later in the paper, there were comparatively very few cases of malaria in this village. Observations on the excito-repellent effect of combined sprays

and the survival rate of trapped mosquitoes are not available at present and will be investigated further during the subsequent malaria season.

MALARIOMETRIC DATA.

(*a*) *Spleen and parasite rates.*—The spleen and parasite rates of children recorded in July and November 1950, before and after spraying (Table VII), showed that there was a substantial rise in spleen and parasite rates, in November 1950, in the comparison village. Villages treated with 200 mg. D.D.T./sq. ft. and B.H.C. and D.D.T. combined spray (25 mg. D.D.T. + 5 mg. B.H.C. gamma isomer per sq. ft.) registered a significant fall in the spleen rate. There was practically no change in the other treated villages though there was a marked decrease in parasite rates. Monthly infant parasite rates were also recorded, but as the number of infants in these villages was very small, much information regarding the exact duration of transmission season in this area could not be gained by this criterion. It was, however, clear that during the period of observation there was very little transmission going on in the treated villages, but in the comparison village 7 infants out of 8 showed malaria parasites in their blood in September 1950.

(*b*) *Malaria morbidity rates.*—To study the effect of insecticidal spraying on the incidence of malaria or to be more accurate on the incidence of fresh malaria infections in the villages not attended by any regular dispensary, was very difficult. Blood slides of all fever cases in the villages were, however, collected once a week for this purpose. In Table VIII are shown the malaria morbidity rates (per 1,000 of the population) from fever cases found positive for malaria parasites; this obviously included all those cases which may have relapsed. The weekly distribution of malaria morbidity is comparatively a more significant index than the rate itself, as a village with a total population of 120 even with a single case would show a higher per 1,000 rate than a village with a population of, say, about 400. It was significant that in the comparison village malaria cases were encountered throughout the period of observation, but in the treated villages malaria cases were sporadic and were probably relapse cases.

From morbidity rates alone it is not possible to say which of the insecticidal sprays was best. However, in the case of Atohan Village, treated with D.D.T. and B.H.C. combined spray, malaria morbidity was confined to only the 11th and 12th weeks after spraying, indicating that a single application of D.D.T. and B.H.C. combined spray at 25 mg. D.D.T. and 5 mg. B.H.C. gamma isomer/sq. ft. was effective almost throughout the malaria transmission season.

SUMMARY AND CONCLUSIONS.

1. Field trials on the comparative effectiveness of D.D.T. and B.H.C. against mosquitoes when applied separately and in combination are discussed. Studies were conducted in villages near Palwal, District Gurgaon, Punjab (I).

2. For proper evaluation of insecticides, more than one criteria have been applied concurrently so that results, as far as possible, be correctly interpreted. Density of mosquitoes, window trap collections and percentage survival of trapped

mosquitoes after 24 hours, chemical estimation of insecticides from scrapings of treated surfaces, and malariometric data have been collected.

3. Irrespective of the dosage employed (50–200 mg. D.D.T. per sq. ft.), D.D.T. suspension was more effective than emulsion on mud-plastered walls.

4. Massive doses of D.D.T. when applied on mud-plastered walls whether as suspension or emulsion do not seem to show proportional increase in the duration of residual effect. After 12 weeks of application no D.D.T. could be detected even in the scrapings removed from the houses treated with 200 mg. D.D.T. per sq. ft.

5. Under local conditions there is little to choose between D.D.T. 50 mg. (applied as suspension) and B.H.C. 10 mg. gamma isomer per sq. ft. Both give protection for about 6 weeks. Relative costs can decide the choice.

6. Both D.D.T. and B.H.C. exert a slight repellent effect on local species of mosquitoes but the insects pick up a lethal dose and eventually die. Control of malaria is effected by reduction in the density of mosquitoes and by the phenomenon of interception.

7. The synergistic action of D.D.T. and B.H.C. in combined spray (25 mg. D.D.T. + 5 mg. B.H.C. gamma isomer/sq. ft.) was obvious. D.D.T. and B.H.C. combined spray was not only effective for a longer period but was also more efficacious in keeping down the density of mosquito population than either of them applied separately.

8. Choice of insecticides for malaria control is considered to depend upon the duration of the residual effectiveness and whether this effect is disturbed by human interference depending upon the habits and practices of inhabitants. Repeated application of D.D.T. and B.H.C. in combination at a dosage of 25 mg. D.D.T. and 5 mg. B.H.C. gamma isomer per sq. ft. at an interval of about 8 weeks is recommended.

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TABLE I.

Particulars of the experimental villages. Formulation and dosages of insecticides employed.

Villages.	Number of houses.	Population.	Total area sprayed. sq. ft.	Insecticide and formulation used.	Dosage. mg. sq. ft.	Quantity sprayed. c.c. sq. ft.
Miranpur	49	313	58,998	2·5 per cent D.D.T. emulsion	50	2
Kakrali	34	204	36,006	Do.	200	8
Meghpur	24	120	18,575	2·5 per cent D.D.T. suspension.	50	2
Dholagarh	26	101	12,492	Do.	200	8
Bahraula	78	429	55,071	0·5 per cent B.H.C. gamma isomer suspension.	10	2
Atohan ...	85	387	47,207	D.D.T. (2·5 per cent) and B.H.C. (0·5 per cent gamma isomer) combined suspension.	D.D.T. 25 B.H.C. 5 gamma isomer.	2
Khusrupur	102	511	Comparison village.	...	...	..

TABLE II.

Weekly percentage density of mosquitoes as compared to density at the start of the experiment (before spray) and ratio of mosquito catch from sprayed and unsprayed (sprayed/unsprayed) catching stations.

Week.	50 MG. D.D.T. PER SQ. FT. (EMULSION).		200 MG. D.D.T. PER SQ. FT. (EMULSION).		50 MG. D.D.T. PER SQ. FT. (SUSPENSION).		200 MG. D.D.T. PER SQ. FT. (SUSPENSION).		COMPARISON.
	Percentage density.	Ratio of S/Us.	Percentage density.	Ratio of S/Us.	Percentage density.	Ratio of S/Us.	Percentage density.	Ratio of S/Us.	Percentage density.
0	100·0	1·03	100·0	1·02	100·0	1·08	100·0	1·11	100·0
1	3·5	0·69	1·4	0·31	1·4	0·46	0·0	0·13	100·0
2	34·0	0·77	0·5	0·007	27·4	0·43	1·1	0·14	117·4
3	36·6	1·34	4·5	0·04	30·3	0·45	1·0	0·19	234·6
4	55·6	1·45	5·6	0·05	31·2	0·72	1·2	0·16	22·5
5	46·8	1·20	5·9	0·06	30·5	0·77	1·8	0·28	192·0
6	37·3	0·64	4·2	0·03	47·3	0·73	4·1	0·32	216·5
	Resprayed.				Resprayed.				
7	12·3	0·16	46·8	0·41	3·9	0·06	4·9	0·00	14·0
8	12·3	0·36	24·6	0·65	0·0	0·00	0·0	0·52	40·6
9	29·8	0·47	19·5	0·34	18·4	0·36	3·7	0·06	140·8
10	16·8	0·77	0·8	0·02	0·0	0·00	0·0	0·10	11·8

S = Sprayed. Us = Unsprayed.

TABLE III.

24 hours' percentage survival of mosquitoes collected from sprayed rooms and window traps.

Week.	50 MG. D.D.T./SQ. FT. (EMULSION).			200 MG. D.D.T./SQ. FT. (EMULSION).			50 MG. D.D.T./SQ. FT. (SUSPENSION).			200 MG. D.D.T./SQ. FT. (SUSPENSION).		
	Average 15 minutes' daily collection from room.	Average daily window trap collection.	Percentage survival.	Average 15 minutes' daily collection from room.	Average daily window trap collection.	Percentage survival.	Average daily 15 minutes' collection from room.	Average daily window trap collection.	Percentage survival.	Average 15 minutes' daily collection from room.	Average daily window trap collection.	Percentage survival.
1	23·8	0·0	0·0	0·3	0·0	0·0	9·2	00·0	00·0	21·1	...	0·0
2	19·4	1·4	0·0	0·7	0·0	0·0	22·8	12·2	00·0	1·1	0·5	0·0
3	33·8	6·1	0·0	0·7	1·0	0·0	10·5	11·8	34·2	12·7	1·7	0·0
4	32·5	4·1	0·3	0·7	1·0	4·2	17·8	11·7	48·8	24·0	0·3	17·1
5	28·0	4·4	34·1	0·3	0·0	0·0	17·7	18·7	23·4	12·2	0·0	4·0
6	16·2	4·2	17·5	0·3	1·7	0·0	33·4	13·4	26·0	17·7	0·0	4·8
	Resprayed.						Resprayed.					
7	2·7	3·4	0·0	0·7	2·0	2·8	6·0	6·2	0·0	7·3	1·3	2·5
8	4·2	6·8	0·5	1·1	0·7	7·1	0·8	18·5	4·5	3·0	0·0	4·1
9	11·4	3·1	10·0	0·1	0·5	7·1	1·0	8·2	1·1	5·1	0·0	2·8
10	5·1	5·5	7·8	0·5	1·7	23·5	1·2	2·5	12·4	0·5	0·0	4·7

TABLE IV.

Chemical estimation of D.D.T. in scrapings removed from surfaces treated with D.D.T.

Week.	50 mg. D.D.T./sq. ft. (suspension).	200 mg. D.D.T./sq. ft. (suspension).	50 mg. D.D.T./sq. ft. (emulsion).	200 mg. D.D.T./sq. ft. (emulsion).
	Mg. D.D.T.	Mg. D.D.T.	Mg. D.D.T.	Mg. D.D.T.
1	27·5	127·5	26·6	133·2
2	20·1	49·6	14·4	41·0
3	21·6	47·5	11·5	30·9
4	11·5	34·5	0·0	30·9
5	10·8	33·8	0·0	29·5
6	7·2	32·4	0·0	6·5
	Resprayed.		Resprayed.	
7	28·5	25·2	25·2	4·3
8	28·0	13·6	16·5	3·6
9	25·0	7·2	7·9	0·0
10	14·4	7·2	0·0	0·0
11	12·9	0·0	0·0	0·0
12	3·6	0·0	0·0	0·0
13	0·0	0·0	0·0	0·0

TABLE V.

Weekly percentage density of mosquitoes as compared to the density at the start of the experiment (before spray), ratio of mosquito catch from sprayed and unsprayed catching stations and 24 hours' percentage survival of mosquitoes collected from sprayed room and window trap of village sprayed with B.H.C. 10 mg. gamma isomer/sq. ft.

Week.	Percentage density.	Ratio of S/Us.	Percentage survival.
0	100·0	0·91	...
1	0·1	0·005	0·0
2	2·5	0·08	0·0
3	2·6	0·07	0·0
4	2·6	0·09	0·0
5	2·0	0·09	0·0
6	4·7	0·13	0·0
7	6·1	0·20	2·8
8	3·7	0·13	0·0
9	1·5	0·09	12·0
10	1·0	0·05	0·0

S = Sprayed. Us = Unsprayed.

TABLE VI.

Weekly percentage density of mosquitoes as compared to the density at the start of the experiment (before spray) and ratio of mosquito catch from sprayed and unsprayed catching stations of the village sprayed with D.D.T. plus B.H.C. (25 mg. D.D.T. and 0·5 mg. B.H.C. gamma isomer/sq. ft.).

Week.	Percentage.	Ratio of S/Us.	Week.	Percentage.	Ratio of S/Us.
0	100·0	0·60	6	2·2	0·06
1	0·0	0·00	7	4·4	0·18
2	0·6	0·01	8	9·3	0·34
3	0·1	0·00	9	9·0	0·36
4	0·1	0·00	10	6·4	0·33
5	1·1	0·05			

S = Sprayed. Us = Unsprayed.

TABLE VII.

Spleen and parasite rates of children recorded in July and November 1950.

Villages.	Insecticide used.	Spleen rate, per cent.		Parasite rate, per cent.	
		July 1950.	Nov. 1950.	July 1950.	Nov. 1950.
Mirzapur ...	50 mg. D.D.T./sq. ft. (emulsion).	38·0	38·0	34·0	20·5
Kakrali ...	200 mg. D.D.T./sq. ft. (emulsion).	34·0	37·0	21·0	18·5
Meghpur ...	50 mg. D.D.T./sq. ft. (suspension).	36·0	38·0	28·0	15·0
Dholagarh ...	200 mg. D.D.T./sq. ft. (suspension).	45·0	23·5	27·0	23·5
Dahvada .	B.H.C. 10 mg. gamma isomer/sq. ft. (suspension).	34·0	40·0	17·0	5·7
Atohan	B.H.C. 5 mg. gamma isomer + D.D.T. 25 mg./sq. ft. (suspension).	54·0	35·0	19·5	10·0
Khusrupur (comparison).	... ...	46·5	58·0	30·0	45·0

TABLE VIII.

Malaria morbidity rate per 1,000 of population from August to November 1950.

Villages	Insecticide used.	Week.															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Miranpur ..	50 mg. D.D.T./sq. ft. (emulsion).	..	..	..	..	..	..	..	..	3	..	..	..	3	..	..	..
Kakrali ..	200 mg. D.D.T./sq. ft. (emulsion).	..	..	5	..	10	..	..	..	10	..	5	..	..	..	..	..
Meglpur ..	50 mg. D.D.T./sq. ft. (suspension).	..	8	8	8	..	..	..	..	8	..	..	..	25	..	..	..
Dholagarh ..	200 mg. D.D.T. sq. ft. (suspension).	..	..	20	10	10	..	..	..	20	..	..	..	..	..	..	..
Bahraula ..	10 mg. B.H.C. gamma isomer/sq. ft. (suspension).	..	5	..	..	12	..	..	..	2	..	..	..	..	..	..	..
Atohan ..	B.H.C. 5 mg. gamma isomer + D.D.T. 25 mg./sq. ft. (suspension).	..	..	..	..	..	..	..	..	..	..	3	5	..	..	..	..
Khusrupur (comparison).	....	..	6	4	6	4	..	4	4	4	..	8	8	..	..	..	..

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A NOTE ON TUBERCULOSIS IN A LABORATORY MONKEY COLONY.

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[June 1, 1951.]

TUBERCULOSIS amongst monkeys in captivity has been reported by a number of workers including the recent work of Kennard (1941) and Habel (1947) who recorded observations on a large number of autopsies done on monkeys maintained in laboratories for experimental purposes. The only report from India, to the authors' knowledge, about tuberculosis amongst monkeys kept in laboratories for experimental purposes is that of Krishnan (1936). He observed a fatal outbreak of tuberculosis amongst monkeys kept in the Malaria Department of the All-India Institute of Hygiene and Public Health, Calcutta. This was attributed to the infected guinea-pigs that were kept in the same room along with the monkeys.

Tuberculous lesions were observed to be varying from the size of a pin-point to complete consolidation of whole lung in 95 (41·0 per cent) monkeys out of a total of 232 on whom autopsies were performed at the Malaria Institute of India. Some of the monkeys had died during captivity without experimentation while others died during the course of investigations.

HISTOPATHOLOGICAL FINDINGS.

Lungs.—Showed multiple raised greyish white nodules of the size varying from a pinhead to a split pea. Histologically, these nodules showed areas of caseation, epithelioid cell proliferation and multi-nucleated giant cells. Many smaller tubercles consisting mostly of epithelioid cells and lymphocytes were also seen with hardly any caseation and with or without a central giant cell. The tubercles were not only present in the pleural surface, but were also seen scattered throughout the lung.

Spleen.—Was found riddled with similar tubercles presenting the typical almond toffee spleen on cut section. Histologically, areas of caseation with giant cell systems were seen.

Liver.—Showed similar tubercles and histologically it had the typical structure of caseating tuberculosis.

Kidneys, mesentery and pleura showed tubercles similar in appearance and histology.

Mesenteric lymph nodes also showed areas of caseation with giant cell systems.

Post-mortem examination in two others showed similar lesions in lungs and spleen.

BACTERIOLOGICAL FINDINGS.

Smears made from the caseating matter in some of the nodules showed acid-fast bacilli morphologically resembling tubercle bacilli. The organism was isolated and cultures showed the typical characters of human type of tubercle bacillus. This was confirmed by animal inoculation tests.

In view of these findings it became imperative that necessary measures be taken for the prevention of the spread of the disease amongst the healthy monkeys in the animal house. This led to a routine tuberculin testing of the entire stock. Post-mortem examination of the ones that show positive reactions has been started.

The incidence of the disease among the newly received monkeys and those in captivity for varying periods will be published in a subsequent report along with the technique of tuberculin test adopted in this Institute.

ACKNOWLEDGMENT.

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A REVIEW OF IMMUNITY AND ENDEMICITY IN MALARIA AND A DISCUSSION ON THEIR RELATIONSHIP WITH MALARIA CONTROL.

BY

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[June 23, 1951.]

INTRODUCTORY.

WILSON, GARNHAM AND SWELLENGREBEL (1950) have reviewed some of the records from highly malarious parts of the world and defined the true nature of 'hyperendemicity', the conditions essential to its occurrence and the degree of its association with inefficiency, suffering or mortality. They have then discussed to what extent malaria control was necessary or desirable for people normally living under 'hyperendemic' conditions and what effect reducing or eliminating malaria transmission is likely to have on such people. While emphasizing that the significance of 'hyperendemicity' lies in the development of immunity, they distinguish fully developed immunity in 'hyperendemicity' from high endemicity characterized by incomplete immunity. In the former type of malaria they believe that there are at least thirty chances of infection for an individual every year well distributed practically all through the year. Infants of this area experience severe attacks of malaria but after 18 months they are considered out of danger and suffer from no increase in the parasitic load despite the high seasonal transmission rate. This tolerance is accompanied by a capacity to contend rapidly and efficiently with the parasites of the locality concerned and their adverse effects. The authors sound a note of caution against embarking, without thought, upon unrestricted measures of control in 'hyperendemic' areas in tropical Africa lest by reducing or eliminating infection, the benefits of premunition should be discarded, and in times of any temporary setback to the continuance of malaria control, such loss of immunity should be attended with a severe epidemic. They, however, recommend that a carefully conducted scheme of malaria control was necessary in such a region in order truly to assess the need, desirability and economic possibility of malaria control under such conditions. In areas with incompletely developed immunity they do not question the need for vigorous malaria control measures.

Macdonald (1950*a*) refers to the prevalence of much discussion at the present time on the nature of immunity associated with 'hyperendemicity', whether it is purely dependent on the load of infection which the people carry and is simple acquired immunity or if there is a strong racial element in it, the immunity being acquired much more readily in some races than in others though the load of infection

may be lighter. He has elaborated a fascinating theory dealing with the relationship between inoculation rates and infection rates in those diseases in which superinfection can occur. By the application of this theory it is claimed that the load of initial infection to which different groups of people are exposed can be assessed which could then be compared with the degree of immunity obtained.

In this paper a few data collected from the published and unpublished records of the writer's work in India which have a bearing on the views and theory propounded in the two papers mentioned above are presented and the need, desirability and feasibility of malaria control measures under conditions of "hyperendemicity" are discussed in the light of available knowledge on the mechanism of immunity in the individual and the community separately reviewed by the writer (Viswanathan, 1951). The chances of a set-back to the continuance of malaria control schemes and the possibility of adverse effects on the community by such interruption are discussed in the light of experience gained on large scale rural malaria control schemes in underdeveloped countries.

DIFFERENCES IN HOST-PARASITE RELATIONSHIP IN THE SAME LOCALITY.

The following records of spleen and parasite rates in the Jeypore Hill tracts (formerly of the Madras Presidency but now attached to Orissa State) and the Gudem Agency tracts of Madras State compiled by the writer between 1928 and 1930 show the differences in host-parasite relationship in the various aboriginal types.

Spleen and parasite rates in Jeypore and Gudem Agency Hill tracts (Madras and Orissa States).

Groups.		Age (years).	Spleen rate, per cent.*	Average enlarged spleen.†	Parasite rate, per cent.*	Average positive parasite count.‡	Remarks on adult morbidity.
1	Gadabas	2–5	80·0	1·6	80·0	2,140	Practically no sickness due to malaria.
	Porjas	6–9	71·0	1·4	76·0	1,440	
	Khonds	15–	28·0	Not measured.	32·0	152	
2	Dhombs	2–5	84·0	2·0	72·0	680	Sickness due to malaria not uncommon.
		6–9	82·0	1·8	70·0	620	
	Malas	15–	46·0	Not measured.	46·0	124	

* The differences in childhood spleen and parasite rates are not significant but those in adults are.

† Just palpable spleens (within or at the costal margin in standing position) are weighted as 0 and other enlarged spleens as 1, 2, 3, etc., in terms of finger-breadths. The total enlargement is divided by the number of enlarged spleens including palpable spleens.

‡ Sinton's (1924) fowl-cell method.

In these tracts the vector is *A. fluviatilis* and its natural infection rates may reach as high as 5 per cent. Transmission may take place all through the year. The actual rainy months of July, August and September show the least transmission as the heavy rainfall washes away the larvæ from the streams and channels, the natural habitat of the vector, but transmission does not necessarily cease altogether during this period. In fact, if there is a prolonged cessation of rain during the monsoon months very active transmission may occur. During dry weather, mosquito densities are greatly diminished but during no month of the year can one assume a total absence of transmission. To the immigrant population, these areas spell disaster no matter what period of the year they enter into them and blackwater fever is not uncommon. *P. falciparum* is the most common species of plasmodium.

Perry (1914) has stated that the indigenous adults of these tracts exhibit a splendid physique and through centuries they have retained their ethnological identity. The more primitive hill tribes (group 1 in the above table), who are earlier arrivals by several centuries, do not have large degrees of splenic enlargement but their childhood parasitic prevalence is of greater intensity than in the hill tribes who have arrived later. In the latter group there is a greater tendency for adults to exhibit malaria morbidity.

The writer's data given above closely fit in with Perry's observations. In this area, therefore, although the inoculation rates are the same, two primitive groups of communities react differently. In one group there is a greater degree of tolerance to the effects of parasitic invasion even in childhood and an almost complete freedom in adult life from sickness due to malaria. In the other group children suffer to a far greater extent and adults perceptibly, the parasite threshold or pyrogenic level is of far smaller magnitude and the degree of splenomegaly larger in all age groups. In the case of immigrants the pyrogenic level is lower still, the degree of splenomegaly larger and it is often the rule among them for fever to set in even before parasites can be detected in peripheral circulation by ordinary methods of microscopic examination.

Similar differences in adult spleen and parasite rates between different hill tribes are also noticed in the Dangs District in Bombay State. Here *fluviatilis* and, possibly, *culicifacies* also are vectors but the natural infection rates are low though the densities of adult Anopheles prevalence are very high. Transmission is distinctly seasonal from October to March. If the rainfall is fitful during the monsoon months of July to September, transmission may also occur then. In the more primitive Bhils, adult spleen rate is about 52 per cent and adult parasite rate is about 25 per cent. In the Konkans, who are more recent arrivals, the spleen and parasite rates among adults are 80 and 42 per cent respectively. Adults of both groups suffer noticeably from malaria. The childhood spleen and parasite rates are about 90 and 65 per cent respectively and exhibit no significant differences between the two groups. Parasites were not enumerated. *Falciparum* is the prominent plasmodium met with.

Sarkar (1932) has shown that while the children of Chittagong hill tribes show a progressive diminution in spleen rates from about 80 per cent in the 2nd year to about 35 per cent in the 10th year, children in Jalpaiguri Duars tea gardens show

a reduction from over 80 per cent in the 2nd year to only about 60 per cent in the 10th year.

In Kanara District in Bombay State, the writer has examined about 14 negroes whose ancestors migrated from Africa into the West Coast of India more than five hundred years ago. They are locally called 'Siddis'. Both children and adults exhibit a high degree of parasitæmia and adults had nearly as big spleens as the local inhabitants. Both *P. falciparum* and *P. vivax* were found to occur among these people.

MALARIA ENDEMICITY.

While the above data show the prevalence of true 'hyperendemicity' in certain tribes in the Jeypore and Gudem Agency tracts and in Chittagong tribes, there is no evidence of 'hyperendemicity' as defined by Wilson *et al.* (*loc. cit.*) prevailing elsewhere in India although they have stated that such conditions prevail in quite wide regions in India. Even in the former area, one group of communities exhibits true 'hyperendemic' conditions but another exhibits incompletely developed immunity. Endemicity connotes the malariousness of a place or community and the spleen rate, according to the League of Nations' Malaria Commission (1940), appears to give, in a broad and general sense, its best measure. In the example here quoted as well as in a few others, as for instance, in Southern Indo-China as reported by Farinaud and Prost (1939), the same place presumably having the same inoculation rate manifests different reactions among its indigenous residents. The African Conference on Malaria (1950) as well as the Expert Committee on Malaria of the World Health Organization, at its fourth session (1951), appears to have suggested the name 'holoendemic' to denote conditions of true 'hyperendemicity' and placed a lower limit of 75 per cent for the constant childhood spleen rate. In India childhood spleen rates of over 75 per cent are found in areas where transmission is not always perennial and hence parasite rates show seasonal fluctuations and in communities exhibiting considerable adult spleen and sickness rates. Although the definition of 'holoendemicity' also prescribes a low spleen rate in adults, its limits are not specified presumably because they are not definable. Hence there will be confusion in the determination of 'holoendemic' areas. While, thus, truly 'hyperendemic' communities as first visualized by Christophers (1924) and endorsed by Boyd (1949) and Wilson *et al.* (*loc. cit.*) will have a lower limit of constant spleen rates among children, not all those whose childhood spleen rates exceed this limit can be deemed to reflect truly 'hyperendemic' conditions. If by endemicity one merely wishes to connote the degree of risk of inoculation in a locality, existing and widely adopted classification into healthy, moderately endemic, highly endemic and hyperendemic areas denoted by childhood spleen rates of less than 10, 10–24, 25–50 and over 50 per cent respectively will quite serve the purpose. For Indian conditions, the range of inoculation risk and/or season of transmission more or less uniformly and progressively increases from one class to another. The class exhibiting 25–50 per cent spleen rates is epidemiologically very distinct from the class having a spleen rate of 10–24 per cent. In both classes malaria is seasonal but while in the latter the inoculation rate is low in the former class, the maximum inoculation rate during the season may be as high as in the areas having a more prolonged transmission every year mainly on account of an increase

in the numerical prevalence of the vector. Taking Bombay State alone into consideration, very extensive areas have a spleen rate of 25–50 per cent (*vide* Map of malaria distribution in Bombay State, Viswanathan, 1950). Hence the grouping 'mesoendemic' proposed to include spleen rates 10–50 per cent will be far too wide. A subdivision of the group hitherto classified as 'hyperendemic' (with spleen rates over 50 per cent into two classes, one with childhood spleen rate over 75 per cent and the other between 50 and 75 per cent) may be useful in indicating differences in the period of transmission, though inoculation rates may not vary very widely. Such a regional distribution has in fact been made in Kanara District, though limits of spleen rates were not specified (Viswanathan, 1950). If a new classification is needed, the following is suggested :—

(1) Hypoendemic—childhood spleen rate below 10 per cent.

(2) Mesoendemic—10–24 per cent.

(3) Highly endemic—25–49 per cent.

(4) Hyperendemic 50–74 per cent.

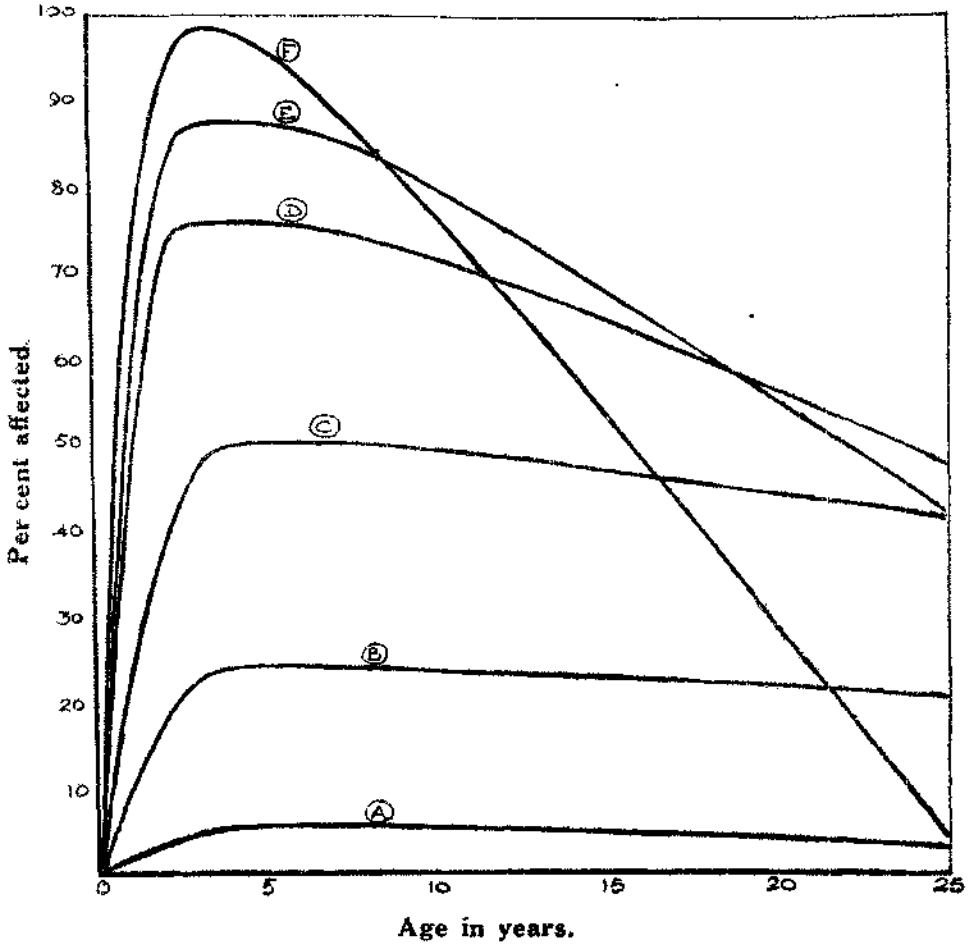
(5) Holoendemic—75 per cent and over associated with high adult spleen rate.

(6) Superendemic—75 per cent and over; long established communities acquire an almost complete freedom from adult sickness due to malaria and adult spleen rate is low.

In classes 2 and 3, transmission is seasonal. In class 2, inoculation rate is low and season of transmission, too, is short, usually less than 3 months. In class 3, inoculation rate is higher than in class 2 and may be the same as in class 4, but the period of transmission is less than 6 months. In class 4, the season of transmission is at least 6 months but usually not more than 9 months. Areas may fall into the third or fourth division by the combined effects of inoculation rate and period of transmission. Thus in some areas even with a 4 or 5 months season of transmission a condition of hyperendemicity (class 4) may be established due to higher inoculation rate. In class 5, the inoculation rate may be the same as in classes 3 and 4 or higher but the period of transmission is more prolonged lasting for at least 9 months in the year. In such areas adult spleen rates may still be high and there may be a considerable adult sickness. But some communities who have been exposed for centuries to the same high inoculation rate practically all through the year, develop an extreme degree of tolerance to the effects of parasitic invasion even in childhood and an almost total absence from sickness due to malaria in adult life. Such communities represent a small minority and may co-exist with other communities exhibiting lesser degree of immunity. They may be designated into the sixth class 'superendemic'. Degrees of endemicity then may be deemed as defining, objectively, the extent of risk of inoculation and the period of its operation every year. While these lend themselves to coarser groupings, the subjective effects of such infection vary from individual to individual too finely to lend themselves to precise grouping.

Boyd's (*loc. cit.*) diagram illustrating the concept of incidence of acute malaria infections with transmission at different endemic levels in stable indigenous populations would need to be modified as below.

FIG. 1.



Boyd's diagram (modified by Viswanathan) to illustrate the concept of acute malaria infections with transmission at different endemic levels in stable indigenous populations.

A. *Hypoendemic:* A person may escape infection till adolescence is reached or even all through life.

B. *Mesoendemic:* Maximum incidence commences in childhood and adolescence. Adult life may be reached before acquiring infection. Inoculation rate low. Transmission seasonal.

C. *Highly endemic:* Late in childhood a very large proportion infected. Adult sickness also considerable, though less than in childhood.

Inoculation rate high but confined to less than 6 months. Good recovery rate in the off season.

D. *Hyperendemic:* By late infancy or early childhood practically entire community infected. Adult sickness mild but still important. Inoculation rate high and season may last at least 6 months, usually 6 to 9 months.

E. *Holoendemic:* By late infancy entire community infected; inoculation rate high and season not less than 9 months. Adult sickness still present.

F. *Superendemic:* Same as E but inoculation rate higher. Communities exposed to infection for several generations exhibit little sickness but almost cent per cent infection before second year of life. Practically no adult sickness.

Epidemic phenomenon is not included in the above diagram. It may be superimposed in groups A and B only but not in others. When it occurs all age groups are equally involved though so far as mortality is concerned only extreme age groups are involved in epidemics in the sub-tropical regions as they are of short duration (Gill, 1914), but in tropical epidemics of longer duration all age groups will show increase in mortality (Viswanathan, 1936), the extreme age groups being involved in the early phases of the epidemic and the other groups in later phases.

RACIAL IMMUNITY.

Viswanathan (*loc. cit.*) has reviewed the mechanism of immunity in the vertebrate host against malaria. The rôle played by race is also discussed in that review. The fact that young children during the stage of acute infection exhibit a high degree of parasite prevalence shows that the resistance is not against parasitic invasion but only against its effects. The fact that groups exposed to infections for a greater number of generations exhibit a greater degree of such tolerance to the effects of infection indicates a pathological process of natural selection through death of less tolerant persons and survivors propagating hosts more and more resistant to the effects of infection. The groups exhibiting truly 'hyperendemic' conditions show not only a cent per cent infection rate in late infancy and early childhood but also higher positive parasite counts. The application of Macdonald's (*loc. cit.*) theory and formulæ would doubtless show more demonstrably the greater parasite load although so far there appears to be no record of a lower parasitic load in infancy and childhood being associated with an almost complete freedom from adult sickness due to malaria. Another relevant factor for consideration is the development of the 'hyperendemicity' in areas where *falciparum* is the predominant plasmodium. The pathological processes in *falciparum* infection are far more degenerative and necrotic than in infections with other species. Antiparasitic immunity which depends primarily upon hyperplasia of the lymphoid macrophage system is therefore less easy to develop against *falciparum* infections. The survivors on the other hand may pile up, through a process of natural selection in several generations, inherited resistance against the effects of parasitic invasion. This immunity is humoral, passive and transmitted. Thus, racial immunity is acquired. It is, however, of two parts. The first is passive and inherited and not antiparasitic but only against their effects. The second is active and acquired during the stage of immune infestation and related to a heavier parasitic load. As the manifestation of clinical symptoms due to the effects of the parasites favours a longer contact of the antigen with the tissues and consequent stimulation of antiparasitic immunity, it is postulated that the so-called racial immunity primarily manifested by inherited immunity in infancy and childhood against parasitic effects acts as a bar to the production of antiparasitic immunity. That is why one finds a far heavier parasitic load in early life in such communities during the stage of acute infestation.

CONGENITAL IMMUNITY: SOME APPLICATION OF MACDONALD'S THEORY AND FORMULA.

Boyd (*loc. cit.*) states that susceptibility at birth is universal. Macdonald (*loc. cit.*) concludes from the curves fitted in accordance with his theory that as they

were concave to the time axis and there was no trace of any convexity early in life, there is no evidence of any transmitted passive immunity even of a transitory nature. Barber *et al.* (1936, 1937) and Clark (1937) have recorded in a highly endemic or a hyperendemic area a relative low parasitic rate in the first 2 months of life and suggested the transference either through the placenta or during lactation of some degree of passive immunity. Viswanathan (1941) records that infants below 2 months in the hyperendemic areas of Assam show some evidence of inherited immunity which breaks down completely at the height of transmission. The parasite rates in this age group were nil in February, March, April and May (against 25·3 per cent in all infants), 2·2 per cent in June, July and August (against 32 per cent in all infants), 13·6 per cent in September, October and November (against 32 per cent in all infants) and 11·4 per cent in December and January (against 25 per cent in all infants). Expressed as an index of infections per cent of infant months of exposure in order to allow for the different periods of exposure in the different age groups, it was found that while in the months of September, October, November, December and January the youngest age group (below 2 months) showed the largest infection indices, in other months of the year they showed the least. In calculating these indices, loading the denominator with the full age of the older infants tends to lower the infection index in seasonal malaria, since in part of the age of the infant there is no exposure. In such cases older infants may show no higher proportion positive than the younger infants as both may be subject to the same period of exposure (Macdonald, *loc. cit.*) quoting the data of Russell (1938). The figures recorded by Viswanathan (*loc. cit.*) include examinations of different age groups at different periods of the year. Macdonald's (*loc. cit.*) formula is applied below to his cumulative data :—

	Below 2 months.	2-3 months.	4-5 months.	6 months.
Number examined ..	550	496	173	478
Number positive ...	34	74	56	229
Proportion ..	0·062	0·149	0·324	0·479

Group 1. Median period of exposure (actuals) less 10 days to allow for intrinsic incubation period is 25 days.

$$t = 25 ; \qquad x = 0{\cdot}062.$$

$$1-e^{-0{\cdot}005t} = 0{\cdot}116 ; \qquad L = \frac{0{\cdot}062}{0{\cdot}116} = 0{\cdot}53.$$

Group 2. $t = 90 ;$ $\qquad x = 0{\cdot}149.$

$$1-e^{-0{\cdot}005t} = 0{\cdot}36 \qquad L = \frac{0{\cdot}149}{0{\cdot}36} = 0{\cdot}41.$$

Group 3. $t = 150$; $x = 0{\cdot}324$.

$$1-e^{-0{\cdot}005t} = 0{\cdot}53; \qquad L = \frac{0{\cdot}324}{0{\cdot}53} = 0{\cdot}61.$$

Group 4. $t = 270$; $x = 0{\cdot}479$.

$$1-e^{-0{\cdot}005t} = 0{\cdot}74; \qquad L = \frac{0{\cdot}479}{0{\cdot}74} = 0{\cdot}65.$$

The mean value of $L = 0{\cdot}55$, and hence the inoculation rate is 0·00275. The parasite rate in children between 2 and 10 years in the area is 46 per cent or 0·46 expressed as a proportion. Thus, Macdonald's theory 'matches life' in this instance as well.

It is not always that observations on each age group of infants are available for different periods in the year. Let us therefore apply Macdonald's formula to only one of Viswanathan's data at the height of the season.

	Below 2 months.	2-3 months.	4-5 months.	At least 6 months.
Number examined ...	61	73	31	121
Number positive ...	11	22	13	66
Proportion ...	0·18	0·30	0·42	0·55

Group 1. $L = \frac{0{\cdot}18}{0{\cdot}116} = 1{\cdot}55.$

Group 2. $L = \frac{0{\cdot}30}{0{\cdot}36} = 0{\cdot}83.$

Group 3. $L = \frac{0{\cdot}42}{0{\cdot}53} = 0{\cdot}79.$

Group 4. $L = \frac{0{\cdot}55}{0{\cdot}74} = 0{\cdot}74.$

The mean value of $L = 0{\cdot}98$. This would give an inoculation rate of 0·0049. If this mean inoculation rate is maintained through most of the year, the ultimate parasite rate will be 100 per cent. Macdonald's factor h/r will be unity and the area will just merit, according to him, being called hyperendemic. The stabilized parasite rate is however only 46 per cent which shows that transmission is either seasonal or subject to wide fluctuations during the year. In the areas under reference the latter is the case. Rice and Mohan (1936) have shown that malaria transmission may and does occur even in the cold weather in north-east Assam, and Viswanathan (*loc. cit.*) while agreeing with them refers to the low quantum

of infection during the cold weather. Altogether, however, transmission occurs for as long every year as in most 'hyperendemic' tracts of the world and the mean effective inoculation rate is about half the maximum inoculation rate at the height of transmission. In the field application of Macdonald's theory and formula, suitable adjustments should be made for seasonal transmission as stressed by him and by implication, for fluctuations in transmission, even if it is more or less perennial, in order to assess the limit of parasite rate achieved. For this purpose, cumulative data compiled at different periods of the year from different age groups of infants appear to give a better picture of the mean inoculation rate and the stabilized parasite rate before the manifestation of the mechanism of immunity. Such cumulative data can only be used, however, if transmission is more or less perennial. The curve A (Fig. 2) fitted to the observations recorded at the height of transmission is in general accordance with Macdonald's theory and shows no evidence of any passive inherited immunity in the earliest age group. This curve, however, will 'match life' only if the inoculation rate prevailing at the time of collection of data operates all through the year. Where it does not, as in this instance, the formula cannot be applied without adjustment except to assess the seasonal inoculation rate. The curve B of the cumulative series (Fig. 2) is distinctly convex to the age axis in the earlier phase. This is indicative of inherited passive immunity. This passive immunity is transient and ineffective after 2 months of age. Even below 2 months, the immunity completely broke down during the height of transmission. Viswanathan (*loc. cit.*) has suggested that during the period of gestation of infants who are below 2 months of age at the height of transmission their mothers were not exposed to infection at its height and hence there was not the same stimulus of fresh infections to increase the quantum of humoral immunity and the infants did not have the benefit of inheriting this specially fortified passive immunity.

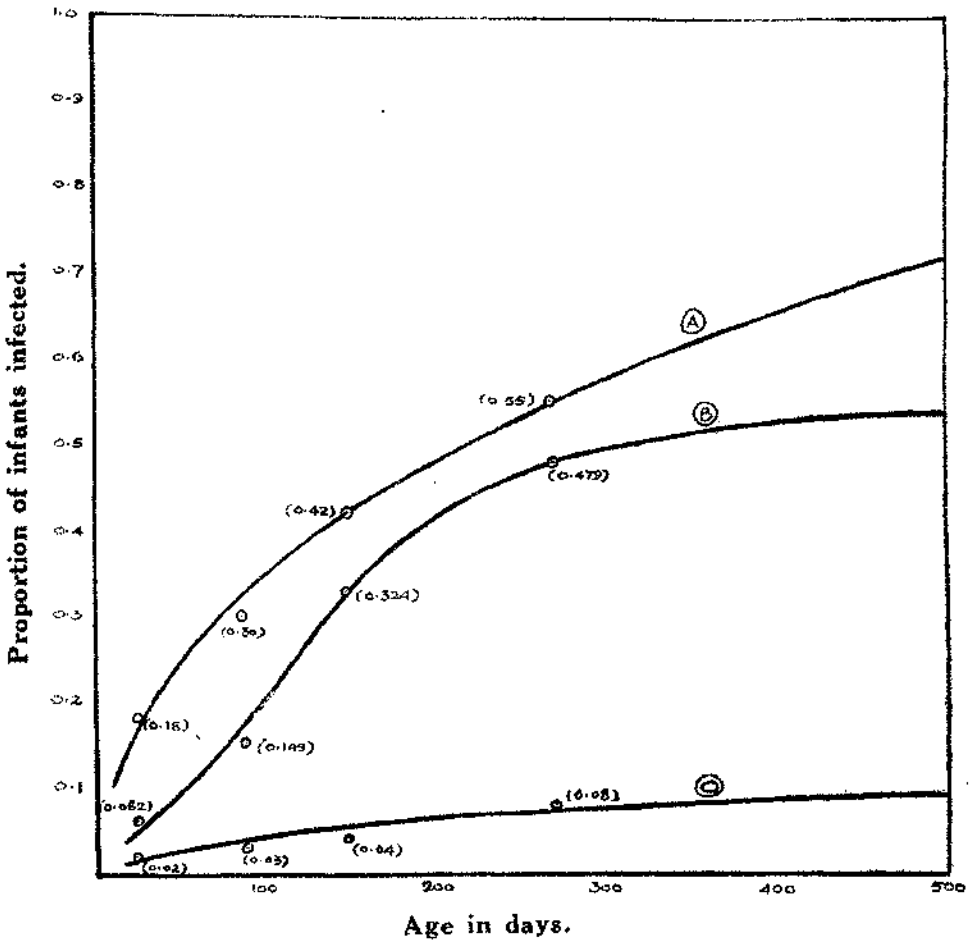
In the writer's experience of *A. minimus* density, the inoculation rate at the height of the season may be computed as 3·28 (density per room) × 0·027 (sporozoite rate) × 0·5 (biting index) × 0·20 (reciprocal of the density of man per room), which comes to 0·008 against 0·005 deduced from Macdonald's formula applied to data collected at the height of transmission.

Macdonald has applied his theory only to places where malaria is more or less perennial. Where malaria is seasonal and age distribution graphs discontinuous, he says that it should be possible to analyse them when this discontinuity is borne in mind and allowed for. Parasite rates in older children attain a level which corresponds to the same formula as in places where malaria is perennial and their interpretation when they are well below 90 (or 100) per cent should be simple.

Viswanathan's (1945) cumulative data regarding infant parasite rates in Kanara District, where, in the bulk of the areas, malaria is seasonal, are shown in Fig. 2 (curve C). While it is not like Macdonald's theoretical characteristic, illustrated by Fig. 2 (curve A), it is not like Fig. 2 (curve B) either. The curve is almost a straight line. The values of L from the cumulative data for each group are 0·18, 0·09, 0·07 and 0·11, with a mean of 0·11 which gives a mean inoculation rate so low as 0·0006. The stabilized childhood parasite rate in this area is a little over 25 per cent. As most part of the area has a season of transmission of about 6 months and the rest for about 9 months, the cumulative series including seasonal

and non-seasonal examination does not give a true picture of the inoculation rate which, though seasonal, does not much fluctuate within the season. In the area having seasonal malaria, the proportion of infants below 6 months showing infection

FIG. 2.



INFANT PARASITE RATES.

Application of Macdonald's formula to Viswanathan's data.

Curve A : Seasonal data ... } Perennial malaria
Curve B : Cumulative data ... } in north-east Assam.
Curve C : Cumulative data of seasonal malaria in Kanara District.

at the heigh of transmission is 0·083 which gives a value of L equal to 0·23. In the infants at least 6 months old, the proportion is 0·305 which gives a value of 0·41 for L. The mean value of L is thus 0·32, which, if malaria was perennial,

would betoken an inoculation rate of 0·0016. In the writer's experience, nocturnal *fluviatilis* density in the area is about 0·417 per room. The proportion showing sporozoites is 0·072. The number of people per room is about 4. The inoculation rate is thus $0{\cdot}417 \times 0{\cdot}072 \times 0{\cdot}5 \times 0{\cdot}25 = 0{\cdot}00375$. Without going into calculations for discontinuous age graphs in seasonal malaria, the above data show that Macdonald's formula gives an inoculation rate of 0·0016 operating all through the year to produce the stabilized parasite rate in the community. As, however, the season is only for 6 months, the mean inoculation rate during the season should be at least twice or 0·0032 which closely fits in with the writer's experience of infective Anopheles density and other relevant factors in the area. Thus, while in perennial malaria with fluctuating inoculation rates, Macdonald's formula may be applied to cumulative data of infant parasite rates in different age groups taken at different periods of the year, in seasonal malaria it should be applied to data during the season but the theoretical perennial inoculation rate thus deduced should be multiplied by twelve and divided by the number of months of transmission. The validity of such a simple hypothesis, however, requires to be further tested.

The transient antiparasitic immunity shown by infants below 2 months cannot be actively acquired due to stimulation by maternal antigen escaping into fœtal circulation through the placenta. In the first place, the reticulo-endothelial system of the infant is not so well developed as to produce effective antiparasitic immunity which is established in later age periods only after prolonged host-parasite contact. Nor can it be an actively acquired humoral response. In any case, there are comparatively few records of congenital malaria despite the widespread prevalence of the disease. This immunity is therefore definitely inherited and humoral. While it is not manifested in very wide regions in India with seasonal malaria, even in perennial malaria, it is effective only within a certain range of inoculation rate. At higher inoculation rates, it is ineffectual. Thus, to produce it, malaria should be perennial and the seasonal inoculation rate should exceed a certain limit. When this rate exceeds a further higher limit, as during the height of transmission in Upper Assam and presumably all through the year in certain countries like Africa, the mechanism breaks down. This transient immunity does not seem materially to affect the application of Macdonald's formula, except that to add to the many other factors such as greater degree of variations in number of days of exposure in the extremely young age group of infants (below 2 months), the factor of transient inherited immunity shows the need for caution in estimating the ultimate value of L from the proportion of infants of this age group only showing infection.

'COMMENSALISM' AND 'HYPERENDEMIC' MALARIA.

Christophers (1949) describes hyperendemic malaria as a condition of the most perfect balance of infection and immunity almost amounting to commensalism and occurring in certain aboriginal races. His own data in Singhbhum showed no racial element in the development of this immunity. It has been suggested to be possibly due to a pathological process of natural selection in so far as resisting parasite effects are concerned. Parasitic load in infancy is still most heavy; in fact, the higher the adult immunity, the greater the parasitic load in infants. While they acquire antiparasitic immunity during adulthood, this is not shown to be better

than in more recent settlers. But the essential fact is that there is in 'hyperendemic' malaria an active mechanism of antiparasitic immunity after the stage of immune infestation. The parasitic load rapidly declines. But cellular hyperplasia is not of the same intensity as in less seasoned communities. This is due to the prevalence of an abundant humoral factor, possibly inherited and by natural selection, which combined with moderate cellular activity, exhibits as much antiparasitic defence as large-scale cellular activity with adequate humoral factor which is present among more recent settlers. Thus, while, so far as the host is concerned, there is the best attempt at adjustment through generations of sacrifice, from the parasite point of view the adjustment is only partial. In that its effects are resisted and hence cellular activity is less strongly fostered against the parasite is an evidence of adjustment. But later in life, parasites are destroyed in large numbers and the adjustment breaks. In fact, 'commensalism' which is a fixed attribute and should remain true on sub-inoculation is as far away as ever.

ECONOMIC IMPORTANCE OF 'HYPERENDEMIC' MALARIA.

Macdonald (1950*b*) has, with a wealth of reasoning and argument, combated the view put forward by Wilson *et al.* (*loc. cit.*) that malaria is not of economic importance to the adult communities in 'hyperendemic' areas. Even in the oft-quoted classical example, Christophers (*loc. cit.*) by no means denies adult sickness. Only their relative freedom shines in contrast with the fate of the new-comer and the mechanism of development of immunity through stages of acute and immune infestations is graphically described. In all hyperendemic areas in the worst malarious tracts in India known to the writer, in Assam, Orissa, Madras and Bombay, malaria is an appreciable public health problem even among adults.

Viswanathan (1949) has shown the effects of malaria on vital statistics in Kanara District. His conclusions are :—

(1) Crude malaria death rate is 7·3 per mille (based on house to house canvass among 7,000 people against a registered rate of 4·6 in this area and over 2 per mille for the whole district).

(2) Out of 208 births, 40 died during the first year as actually verified, which gives an infant mortality rate of 192 per 1,000 live births and among them 6 died of malaria.

(3) Among parasite positive infants, 6·7 per cent died of malaria since and 11·1 per cent from all causes and among parasite negative infants, 1·7 per cent died of malaria since and 13·3 per cent from all causes before the end of the first year. Thus, although malaria was responsible for a considerable degree of infant mortality those infants who survived the primary infection showed no greater hazard of mortality from other causes within the first year itself.

(4) The population of malarious tract of the district shows a steady decline from decade to decade, the regression formula being $Y = 2{\cdot}2124 - 0{\cdot}0321\ (x - \bar{x})$ while for the non-malarious part $y = 2{\cdot}42617$ plus $0{\cdot}012\ (x - \bar{x})$ and for the rest of Bombay State the formula is $y = 4{\cdot}17646$ plus $0{\cdot}024\ (x - \bar{x})$ y being expressed in logarithmic values.

(5) The mean birth rate in Kanara District (31·08) is about 5·29 less than in the rest of the State.

(6) The mean death rate (26·22) is 1·32 higher.

(7) That in the malarious tracts the excess of death rates over birth rates can only account for 25 per cent of the observed decrease but the rest of the decline is due to excess of emigration which, affecting as it does the more sturdy people, further dilutes the physical stock of the people left behind.

(8) Malaria accounts for about 300,000 labour days lost every year in a total population of about 450,000 and on this account several hundreds of acres of the most fertile food-growing lands remain fallow.

(9) Altogether the psychology of the people was one of utter pessimism, in themselves, as well as the administration and resignation to a fatalistic view of life.

In the Assam tea gardens, too, the importance of malaria is well recognized and under the leadership of Ramsay, Rice and others, organized malaria control has been successfully undertaken. In the Dangs tract in Bombay, in the Agency tracts of Madras and Orissa, in Wynaad in the Nilgiris, Madras, in Coorg, in the Malnad of Mysore, in the Uttar Pradesh 'terai' region and in the Gangetic Delta in Bengal which represent some of the highly endemic, hyperendemic and holoendemic areas (according to the classification suggested by the writer), the economic importance of malaria has well been recognized and large-scale control measures instituted.

There is one other important consideration. Even assuming that the indigenous inhabitants with their values of life and its comforts restricted in the eyes of their more civilized brethren can very well afford to carry the light burden of malaria among adults, that their infants who die of malaria are not an economic wastage, a view, however, which is opposed to all considerations of humanity, that such a community cannot economically afford malaria control measures even with the advent of D.D.T. and that if control is first instituted and later interrupted the loss of immunity arising out of cessation of exposure to infection would bring about a very severe epidemic, a view which is later combated, such 'hyperendemic' areas are often the most fertile for agriculture including food crops, tea and spices and forests yielding the most valuable timber. These tracts are therefore subject to a considerable degree of exploitation by their more civilized brethren especially at the present period of greatly enhanced population growth and shortage of food. The indigenous inhabitants will not therefore be left in any rigid state of isolation. Educational and medical relief facilities are being provided in a greater degree and in the writer's opinion the ethnological identity maintained in the past runs the risk of being considerably shaken. Malaria control measures will therefore be called for vigorously in the interest not only of the intruding exploiter but of the indigenous residents whose hard won immunity through generations and early infantile sacrifice would be of no avail against heterologous strains of plasmodia introduced by the immigrants.

RESULTS ACHIEVED IN LARGE-SCALE MALARIA CONTROL MEASURES.

Swellengrebel (1950) has stated that the danger of embarking lightly upon unrestricted malaria control in hyperendemic communities has only been voiced by persons who have not had experience of large-scale malaria control measures.

The writer has the privilege of being placed in charge of malaria control schemes directly protecting 5 million population and indirectly about 9 million. He can therefore wholeheartedly endorse the implication behind Swellengrebel's statement that only those who have undertaken extensive malaria control can appreciate what a powerful sanction public opinion can be. D.D.T. indoor residual spray has given the public health staff, whose services in compulsory inoculations were hitherto sullenly suffered, if not actively resisted, a status quite unprecedented and such a status paves the way for institution, in due course, of other public health measures. While the possibility of a set-back to the continuance of malaria control resulting in adverse effects is discussed in a later section, there is, in the opinion of the writer, no warrant for any fear of a set-back. The results enumerated below are far too spectacular for the people to put up with any discontinuance of the measures of control.

During 1950 it is estimated that in the 9 districts in which malaria control measures are in progress in Bombay State, as many as a million cases of malaria have been saved in a total population of 9 million. Although these figures are based on clinical diagnosis and are therefore uncertain, they are not only the only data available for reckoning morbidity but it is on their basis that provision for treatment of the malarial sick has to be provided by the State. The total cost of control measures is about 3 million rupees.

In Kanara District which consists of 'highly endemic', 'hyperendemic' and 'holoendemic' areas and a narrow coastal belt of 'hypoendemic' areas (according to the classification suggested by the writer), control measures have been in progress for 5 years now. The birth rate has now increased by about 4 per mille and the death rate decreased by about 10 per mille, giving a net addition of about 14 per mille in survival rate. The present survival rate in Kanara District is about 19 per mille as against 5 in the pre-D.D.T. period. Whatever the opinion of the students of demography on such population growth, so far as this district is concerned this is the only way of full exploitation of the very rich natural resources in forests and agriculture. It was stated earlier that by careful house to house canvass the malaria death rate was found to be 7·3 per mille against a recorded rate of a little over 2 per mille. The decrease in death rate is now shown to be 10 per mille. In the absence of any other special efforts in the locality such a decrease in total death rate would show that firstly malaria causes at least half as many deaths due to its indirect effects as due directly to the disease itself and, secondly, that this indirect mortality can also be brought under control by the control of malaria. The present recorded death rate due to malaria is still 0·6 per mille. But no special canvass has yet been made to check its validity. Except in unsprayed stables the density of *fluviatilis*, the vector, is practically nil in the district. Infant parasite rates have been nil at the usual height of transmission as against 20 to 30 per cent in the past. Childhood spleen and parasite rates have dropped from over 50 per cent to less than 5 per cent. Nearly 200,000 cases of malaria are saved every year in a total population of 450,000.

Both in this and in other districts the collateral benefit of complete freedom from human plague has been secured. In all districts other than Kanara, cattle-sheds are also sprayed. Even cattle are reported to be more healthy after such spray. On that account farmers in non-malarious villages not having the benefit of the scheme are clamorous for an extension of the scheme.

CHANGE IN MALARIAL PREVALENCE DUE TO LACK OF PREMUNITION.

A very important point raised by Wilson *et al.* (*loc. cit.*) is the change likely to be brought about in adults by the withdrawal of premunition as a result of successful malaria control. Walton (1949) reports severity of symptoms and parasitic infestation under such conditions. It is difficult to reconcile this as much with our observed data though they do not relate to true 'hyperendemic' communities as with theoretical concepts. The first manifestation in true 'hyperendemic' areas is immunity against parasite effects as stated earlier and as evidenced by relatively lesser sickness rate among infants even in the stage of acute infestation coupled with a far *higher* parasitic load than among more recent settlers. This immunity against parasite effects is a heritage of ages, acquired by a pathological process of natural selection and passively transmitted from generation to generation as a humoral factor. Surely it cannot be lost due merely to absence of fresh infections in the community in one generation. It is true that its quantum is fortified in each generation by the stimulus of fresh infections. Such fortification may not be available after successful malaria control. Hence the passive immunity may be weaker in inheritance. Likewise, there will be no further natural selection in the absence of infection. These effects will take a few generations to manifest fully. But then, if peace is won and maintained, why need an arsenal ? What will happen if peace is not maintained will be dealt with later. Specific immunity is inevitably a function of infection with definite quantitative relationship but the one is needed with suitable fortification merely to fight the other and there is no loss in discarding one if the other can be successfully eliminated. Nowhere in Kanara District or anywhere else in India has there been a single record of greater severity of symptoms among the few people who suffer from what is termed 'residual' malaria after large-scale control measures are established. Even assuming without conceding that there may be a few such instances in truly 'hyperendemic' areas, they should be looked upon as martyrs. A community which has learned the lesson of martyrdom through countless generations of sacrifice to the enemy can for a little while easily afford to continue to be harassed by an inevitably transient 'guerrilla warfare'. With large-scale malaria control the parasite is fast losing its age-long battle with an unprecedented speed and the fate of the 'guerrillas' is inevitably sealed.

POSSIBLE EFFECTS OF DISCONTINUANCE OF MALARIA CONTROL.

So far as adults are concerned the absence of fortification of their acquired immunity by the forces of premunition may render them liable to more perceptible morbidity. In Gill's (*loc. cit.*) view adults of sub-tropical climates exposed to regional epidemics once every ten years or so are able to exhibit immunity even after such a long interval with the result that while they have a high morbidity they suffer from no increase in mortality. It is, therefore, safe to assume that there will be no immediate increase in adult mortality in former 'hyperendemic' areas after institution of control measures and their later discontinuance. It will take quite a few years before immunity wears out in

such a highly 'seeded' population. Young infants would continue to exhibit immunity though in a lesser degree with the result that they will probably retrogress to the same immunity status as that of the later settlers in the former hyperendemic area, that is, the pyrogenic threshold and parasitic load will be lowered. This will be a gradual process and in the meanwhile there will naturally be more perceptible sickness even in infants. That would only lead to a fresh impetus for resumption of malaria control. Wherever democratic forms of Government are in vogue the care of the underdog receives attention not only far more than in the past but also more than that of the more forward sections of the community. Hence, while there will be no catastrophic calamities by reason of suspension of malaria control measures, the supervening incidence will certainly be of high enough order to merit adequate attention. Another vital difference between 'hyperendemic' areas and 'epidemic' areas is that while in the former areas mosquito densities are more or less stable, in the latter areas during epidemic periods the density increases several times as in the Ceylon epidemic of 1935 and in the ceded districts of Madras (Viswanathan, *loc. cit.*) and the infection rates are far higher. There is no warrant to assume that the vector in hyperendemic areas which had reached stability in its inoculation rate will after suspension of operations make up for their reduction during the control phase and either assume much higher densities than in the pre-control period or exhibit higher natural infection rates. Mosquito density is essentially a matter of ecology and indoor residual spray of an insecticide will only reduce its numbers and when the spray is stopped the mosquito will build up no more than its original seasonal density. As for infection the reservoir is now no doubt of a far smaller magnitude due to the control phase but in the experience of malaria transmission in India even a small reservoir of infection is adequate to set the cycle of transmission in full swing so long as the life of the vector and its habits are fostered by ecological conditions. The writer has not yet mustered sufficient courage to suspend D.D.T. application in Kanara District after 6 years of continuous spray even though the prevalence of vector species is reduced to a very low point (but not yet eradicated) and the reservoir of infection too is extremely small. The bulk of infectors in nature, viz. infants and children, are completely free from infection. If and when some circumstance arises placing a bar for the continuance of the control measures there will be an opportunity to study the effects of fresh transmission after its suspension for several years.

MASS PSYCHOLOGY IN EXTENSIVE RURAL MALARIA CONTROL.

Judging from mass psychology, however, there seems no warrant whatever to expect and much less to hope for any such set-back even for a scientific purpose. Viswanathan (*loc. cit.*) has recorded an instance where a city medical practitioner wished to have possible association between some cases of conjunctivitis and the D.D.T. spray carried out earlier properly investigated. During the investigation the people were so afraid of a possible set-back that they denied the occurrence of even a single case of conjunctivitis and attributed some unworthy electioneering motive to a sincere medical man who never himself claimed the association but merely wished to have it investigated. The pace of progress may no doubt be

halted due to want of funds but closure of the scheme in an area when it has already shown results would never be countenanced. D.D.T. scheme is altogether one of the most popular among all activities of the State and it has also been shown to be within the economic competence of underdeveloped countries (*per capita* annual cost not exceeding 8 annas or about 10 cents or 9 pence). In the writer's opinion there is no need for entertaining any fear that malaria control measures may receive a set-back in such areas. On the other hand, a possible view that malaria control was not necessary in such areas may help a close-fisted administrator find additional arguments for not embarking upon any such scheme and providing funds for them. Although Wilson *et al.* (*loc. cit.*) have only raised the need for caution solely in truly 'hyperendemic' (or 'superendemic' according to the writer's classification) areas, there is ever the risk of administrators being tempted to sound a similar note even in communities with less developed immunity. The purpose of this paper is mainly to dispel any such tendency.

SUMMARY.

The review of 'hyperendemic' malaria by Wilson *et al.* and Macdonald's theory and formula linking inoculation and infection rates in a disease like malaria where superinfections can occur are discussed in the light of some available data in India. Boyd's concept of malaria infections at different levels of endemicity is slightly modified and a provisional classification of endemicity modifying the recommendations of the Expert Malaria Committee on the World Health Organization (4th session) is suggested. The characteristics of immunity exhibited by certain primitive communities are discussed in terms of racial characteristics or parasitic load of infection and number of generations of exposure. Macdonald's formula has been applied to perennial and seasonal malaria in India. The economic importance of malaria in 'hyperendemic' tracts is considered as well as the need for and feasibility of its control discussed. Results of large-scale malaria control measures and the mass psychology behind them are advanced as sufficient arguments against any set-back to malaria control measures. During the control phase there is no warrant to expect any change in the type of malarial prevalence and in the most unlikely event of discontinuance of control conditions will arise no worse than those existing prior to its institution and these will only be built up gradually without any catastrophic calamity. Malaria is a 'preventable' disease, it has been prevented; and it should be prevented at all costs, in all areas and in all communities.

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A REVIEW OF IMMUNITY IN THE VERTEBRATE HOST AGAINST MALARIA.

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INTRODUCTION.

PLASMODIAL inoculation, patent infection and symptoms of malaria are all intimately mixed up with the status of immunity in the vertebrate host. In this paper, available knowledge on this behalf has been compiled chiefly from the chapter on immunity by Taliaferro in Boyd's *Malariology* (1949) and the earlier excellent monograph by Taliaferro and Mulligan (1937), the symposium on human malaria (several authors, 1941), Culbertson's (1941) book on immunity in animal parasites, Sinton's (1939*a*; 1939*b*) review and Macgraith's (1948) book on the 'Pathological Processes of Malaria'.

NATURAL OR INNATE RESISTANCE.

Natural or innate resistance is exhibited by the host independent of any prior stimulation by the parasite, its products or like antigens from other sources. The phenomenon is non-specific and involves both cellular and humoral factors.

Skin and mucous membrane.—As the sporozoite is injected beneath the skin, resistance usually, exerted by epithelial cells and mucous discharges against the invasion of parasites, is not available.

Host parasite specificity.—Taliaferro and Cannon (1934) have been able to infect the howler monkey with *P. falciparum*, and Rodhain (1939) infected the chimpanzee with *vivax*. Ciuca *et al.* (1938) have infected man with *P. knowlesi* and *inui*, and Rodhain (1940) has infected man with *P. rhodhaini* from the chimpanzee, although doubts are cast against its separate specific entity. The bird malarial parasites have a far wider range of hosts than those of men and monkeys. Barring casual experimental infections, however, where the parasite of one natural host has been made to infect a different host, natural or innate immunity accounts for much of the host parasite specificity which occurs in nature, probably lasts throughout life and may increase with age (Taliaferro, *loc. cit.*).

In the immunological sense, host parasite specificity involves non-specific factors. The tissues are made non-suitable for the parasite to gain a foothold, metabolism of the parasite is greatly hindered and there is also a direct phagocytosis

of the parasite. The spleen plays a vital rôle in resistance. Krishnan, Smith and Lal (1933 : 1934) have shown that after extirpation of spleen, resistance of monkeys was lowered and malarial infections rendered more severe. This innate resistance manifested by the spleen is greatly enhanced, as will be shown later, in acquired resistance.

James and Shute (1926) consider that biochemical differences in blood (alkalinity, lecithin, protein or phosphorus content) may account for differences in innate resistance.

Age.—As regards the first infection, there is apparently no difference due to age as such except that, in the absence of any transmitted inherited resistance, the extremely young infants appear to be more susceptible to infection presumably because of their reticulo-endothelial system not having sufficiently developed. Boyd (1949) refers to infants being more attractive to mosquitoes. In the Karoo highlands of Sumatra, Schüffner (1919) found a higher evidence of malaria in young unmarried adults and attributed it to their habit of courting outdoors at dusk and consequent special liability to acquire outdoor infections. Rao (1951) has recorded an infant parasite rate of 70 per cent in Laghman District in the eastern province of Afghanistan. The rate in children (2-9) was only 30·9 per cent which he attributes to acquired specific immunity. As reference is also made by him to the habit of people sleeping outdoors during the malaria season and as the vector species, *culicifacies* and *superpictus*, are recorded in houses during day-time in abundant numbers, it is possible that the higher rate of infection among infants is due to their greater exposure to infection inside houses than in the case of adults sleeping outdoors. It is not otherwise possible to explain the differential parasite rates in infants and children since the very low natural infection rates in the vector species is not conducive to the establishment of a high-grade immunity in childhood. Most workers report that severity of infections varies inversely with age in avian malaria (Taliaferro, *loc. cit.*) but in man one cannot distinguish between the effects of innate and acquired immunity at various age groups.

Sex.—Sex *per se* plays no part in innate resistance. During surveys boys and girls below 5 years of age manifest no difference in spleen or parasite rates in India. Older girls, however, show smaller spleen rates than boys presumably because of their being better clothed and offering less exposed surfaces for mosquitoes to bite. Schüffner (1919) cites an unpublished study by Swellengrebel and Swellengrebel (1918) in the Rhio Archipelago, where, among the fishing community, adult males spending their nights fishing at sea are less exposed to malarial infection and consequently show a smaller spleen rate than adult women and children.

Individual variations in susceptibility.—True innate refractoriness to infection has not been undeniably demonstrated in a host to which the parasite is naturally specific. Variations occur in individuals and acquired resistance due to prior infections often fails to be elicited on account of lapse of time. The dose of inoculum and the virulence of the strain play a significant part. Mild, sub-clinical infections may be missed and computed as refractoriness. Thus, deficiencies in parasite due to dosage, technique (in artificial infections), etc., may be misinterpreted as refractoriness on the part of the host.

According to the Ayurvedic concept of cosmogenesis (primordial matter called *Prakriti*, consisting of three *gunas : Satva, Rajas* and *Tamas*) and the *tridosha* theory

of diseases (*Pitta*, *Vata* and *Sleshma*) the *paitic* individual is more susceptible to malarial infection. The concept is essentially humoral and manifestations of disease depend on the preponderance of a particular *dosha*. One wonders if the discovery by Shortt *et al.* (1948 : 1951) of the pre-erythrocytic phase of development of simian and human plasmodia in the hepatic parenchyma could be correlated with the above Ayurvedic concept of susceptibility to malaria of the *paitic* individual.

Race.—Making allowances for factors of individual variations, the negro race appears to exhibit a higher innate resistance especially against *P. vivax*. Evidence is yet incomplete if any genetic characteristic is involved in a speedier acquisition of immunity by certain races. Boyd (*loc. cit.*) is of the opinion that racial immunity does not exist and, while conceding the need for further study, he thinks that the reaction of different races may only vary in degree but cannot be fundamentally distinct. Wilson *et al.* (1950) also consider that the evidence is yet incomplete if the characteristics of 'hyperendemicity' (as originally defined by Christophers, 1924, and supported by Boyd, *loc. cit.*) are wholly dependent on circumstances of infection (and therefore acquired) or whether only some races are capable of developing it. 'Hyperendemicity' as defined by Christophers is always associated with a high parasitic load in infants. Those communities who show a high degree of resistance in adult life show a relatively higher parasitic load in infancy. Despite such a higher load these infants are relatively less ill. Viswanathan (1951) has shown that the resistance exhibited by such communities is higher against the effects of parasitic invasion in communities who have lived for a very much longer period of years (generations) and who exhibit a higher parasitic load. This is presumably due to a pathological process of natural selection and the survivors exhibiting a higher degree of innate resistance against the effects of parasitic invasion. Sinton and Harbhagwan (1935) consider that this increased innate resistance may be transmitted as a mendelian genetic characteristic.

Innate resistance during further development of the sporozoites.—Sporozoites that are injected in the skin need not directly be introduced in the blood, since Boyd and Stratman-Thomas (1934) have established infection by introducing sporozoites into the fluid of a blister. Direct capillary penetration is certainly fortuitous. This, therefore, allows for a limited degree of local defensive mechanism against the sporozoites. Shute (1937) has shown that at least 50 sporozoites are necessary for establishing infection in man and for the normal incubation period about 2,000 sporozoites of *vivax* are necessary. Fairley (1945) has shown that *vivax* and *falciparum* sporozoites reach the general circulation very soon after their introduction beneath the skin and remain in the peripheral blood for half an hour and Raffaele (1937) has shown that the parasites reach the peripheral blood again in the trophozoite stage from the 7th to the 9th day. This period of incubation from the time of introduction of sporozoites to the appearance of trophozoites in the peripheral blood has never been reduced however much the dose of the inoculum is increased. Subject to this minimum, the period of intrinsic incubation has been shown by Boyd (1940) to be inversely proportional to the number of sporozoites injected. In this connection mention has to be made of the tendency in some *vivax* infections to have a long incubation period or at all events long-term relapse (Hackett, 1937).

It is not clear if the innate immunity manifested by a host which is not specific to a particular species of plasmodium is exercised against the sporozoite, the

pre-erythrocytic stage or very early and sub-patent blood infection. If on sub-inoculation into a specific host within the normal period of peripheral circulation of sporozoites in a non-specific host previously injected with them plasmodial infection is established, obviously the innate immunity in host parasite specificity is not exercised against sporozoites.

On the whole, although the malarial parasite is a blood parasite it is found free in the blood only for very short periods of time and it is essentially a tissue parasite involving both the blood cells and the somatic tissue cells. The forces of resistance both innate and specifically acquired after antigenic stimulation are therefore primarily exerted in the tissues.

Blood invasion.—According to Ross and Thomson (1910) the pyrogenic level of *vivax* is from 200 to 500 per c.mm. and of *falciparum* from 600 to 1,500. Kitchen (1941) found amongst negroes that on the day on which fever appeared about 71,000 *falciparum* parasites were present in the peripheral blood per c.mm. Altogether, there is a good deal of variation in the pyrogenic level from host to host and it is also largely dependent upon the status of the host with reference to previous infections and their duration. But that will take us on to the phase of specific acquired immunity dealt with later.

Host parasite adjustment.—It is difficult to classify if the relation between plasmodia and their hosts is an example of good, indifferent or poor adjustment. On the one hand, *falciparum* infections in man and *knowlesi* infection in monkeys are liable to have a very virulent course with disastrous results to the host and therefore ultimately to the parasite itself. Again, although at every schizogony a large number of merozoites are liberated in the blood stream, only a few succeed in invading fresh blood corpuscles. Taliaferro (*loc. cit.*) found that, in bird malaria, 10 out of 15·5 merozoites in each schizogonic cycle failed to develop into mature schizonts. There is thus an innate resistance against unrestricted multiplication of parasites in the host which is manifested both by failure of many merozoites to enter fresh R.B.C. and by removal of parasitized cells. If the parasite still multiplies it is not because there is no natural immunity but because it is not adequate. On the other hand, Stauber (1939) has been able to establish blood infection with a single trophozoite. Again, Maegraith (*loc. cit.*) has shown that in the New Guinea strain of *falciparum* there is close agreement between the actual numbers and the theoretical computation of the increase in parasites for 2 or 3 cycles. During the logarithmic phase most merozoites enter red blood corpuscles. During this phase there is apparently very little natural resistance against *falciparum* in the early stages. In all malarial infections, however, the density curve flattens out after certain periods of time, which vary with different plasmodia. This represents the phase of development of specific immunity and the host parasite adjustment under such conditions is dealt with later.

PATHOLOGY OF MALARIA.

The pathological processes that take place in malarial infections according to Maegraith (*loc. cit.*) are briefly (*a*) primary anoxæmia, (*b*) tissue anoxia due to local circulatory changes, (*c*) focal degenerative and necrotic changes in acute infections and, later, (*d*) hyperplasia of the lymphoid macrophage system, (*e*) production of

antibodies stimulated by plasmodia themselves or their metabolites such as lytic and pyruvic acids, and (*f*) later selective phagocytosis of parasites and parasitized red cells by splenic chords of Billroth.

The local changes in tissue circulation may be either due to (*a*) constriction of the efferent extremities (Kinsley's view), (*b*) mechanical obstruction by mobilized macrophages (Mackenzie's view), (*c*) passive congestion due to swelling and proliferation of endothelial cells and occasionally thrombosis or plugging of small vessels with large masses of parasitized R.B.C., free parasites, phagocytes and pigment, and (*d*) in later stages by circulation of sludged blood formed of agglutinated R.B.C.

The splenic arterial blood is richer in parasites and on that account the spleen is said to act as a filter. Due to the slowing of circulation of blood through the sinuses and the exposure of the parasitized cells to macrophages, the bulk of phagocytosis is concentrated in spleen and liver and to a lesser extent in the bone marrow.

Taliaferro and Mulligan (*loc. cit.*) found that the enlargement of the spleen in acute infection was due mainly to congestion and hyperæmia and if there is continued malaria infection hyperæmia is replaced by hyperplasia of cellular tissue. Menon (1939) considers that factors other than congestion and hyperæmia are involved in the enlargement of spleen in *knowlesi* infection such as cellular infiltration and swelling of degenerated pulp cells.

At the time of crisis in *cynomolgi* and *brasilianum* infections, parasitized R.B.C. appear to accumulate in the Billroth chords of the spleen where they adhere to macrophages. By far the most important source of new macrophages is the lymphocyte according to Taliaferro (*loc. cit.*).

The clinical features of malarial paroxysms are essentially those of a reaction to non-specific pyrogenic agent such as typhoid vaccine. There is no definite evidence to support the view that a specific malarial 'toxin' or metabolite is involved although some authors claim that the toxic effects are due to some such agent. The hypothesis that an anaphylactoid reaction is involved is not confirmed. According to Maegraith (*loc. cit.*), most of the humoral phenomena upon which this theory is based can be explained on the grounds of production of increasing quantities of circulating hormones such as adrenalin due to acute anoxia of the adrenals. This anoxia is due to local vasoconstriction resulting from hypothalmic stimulation by some non-specific agents liberated at the time of sporulation of the parasite. In any case the febrile paroxysms further affect and retard local circulation and cause prolonged contact of parasitized cells and the products of sporulation with the somatic tissue cells and stimulates hyperplasia of the cells and the formation of specific antibodies.

Nature of the antigen.—There is comparatively little known regarding the nature of malaria antigen involved in acquired immunity. In precipitin and complement-fixation tests, serologic activity increases with the concentration of parasites. Hence the parasite itself is deemed to be the antigen but there is no proof that the serologic tests deal with the antigens which stimulate acquired immunity to infection. Zuckerman (1945) showed that highest opsonin titre was obtained *in vitro* after hyperimmunization with large doses of parasitized erythrocytes in the case of *gallinaceum* and *lophurae*. Her work further showed that the

antigen present in the parasite was also present in normal red cells. Taliaferro and Cannon (1936) found like Zuckerman (*loc. cit.*) that, in primate malaria (*brasilianum*) too, both normal and infected cells are avidly phagocytosed. But Eaton (1938) found that anti-*knowlesi* agglutinins act solely on red cells infected with large parasites. Such parasitized cells were, however, actively agglutinated even after thorough washing and consequent removal of all soluble, readily diffusible antigens on the surface of the cells. Eaton suggested that with the growth of the parasite the red cell is damaged and becomes permeable to antibody or parasitic antigen or both.

ACQUIRED IMMUNITY.

Acquired immunity is manifested by the host as a result of specific antigenic stimulation by the parasite or its products.

Taliaferro (*loc. cit.*) and Thomson (1933) have maintained that immunity in malaria is not different from that in other protozoan and bacterial diseases and involves fundamentally the same mechanism. That the reactivity of acquired immunity is specific is shown by its restricted efficacy against homologous infections. The specificity is largely, and may be entirely, due to the appearance of antibodies according to Boyd (1940). The great stimulation of the lymphocytes and macrophages in the spleen at a time when acquired immunity is operative tends to minimize the importance of specific immunity mechanism. But such an increase in non-specific factors is not only associated with an antigen antibody reaction but some believe that the increased cells themselves produce the antibodies.

Immunity in relation to the course of infection.—A patent parasitæmia is the sum of two opposing factors, infection and resistance. The latter is at first non-specific or innate. The period of struggle represents intrinsic incubation (pre-patent period) and by the time innate resistance is overcome and parasitæmia becomes patent, acquired resistance becomes operative in which the mechanism of innate resistance is greatly increased aided by specific reactivity of cells and fluids against the homologous parasite.

Immunity against the sporozoite and pre-erythrocytic stages.—Recent discovery by Shortt and Garnham (1948) and Shortt *et al.* (1951) of the pre-erythrocytic stage in *cynomolgi, vivax* and *falciparum* makes it necessary to extend all observations on acquired immunity hitherto made against sporozoites and trophozoites in blood to the pre-erythrocytic stage of development. Boyd, Stratman-Thomas and Kitchen (1936) suggested that the sporozoite may be antigenically different from the trophozoite and that the immunity of the host may be mainly directed against the trophozoite. Russell, Mulligan and Mohan (1941) have demonstrated the agglutinogenic properties of sporozoites of *gallinaceum* inactivated by exposure to ultra-violet radiation. The agglutinating titre thus obtained was higher than that of serum of fowls with acute or chronic blood-induced infections. Mulligan *et al.* (1941) have also shown that fowls subjected to repeated injection of inactivated sporozoites of *gallinaceum* showing an agglutination titre of 1/16,000 or less were as susceptible to pathogenic effects of infection of homologous strain as normal fowls and suffer 50 per cent mortality, but those whose titre was raised to 1/32,000 or higher developed only mild infections with no mortality. Huff and Coulston (1946)

found that (*a*) chickens are extremely susceptible to both erythrocytic and pre-erythrocytic stages of *gallinaceum*, (*b*) geese, duck and fowls allow pre-erythrocytic development abundantly but erythrocytic development is patent but transient in the goose, only sub-patent in the duck and not demonstrable in the guinea-fowl, and (*c*) canaries are insusceptible both to pre-erythrocytic and erythrocytic development. The same authors have also shown that immune pigeons and chickens inoculated intracutaneously with sporozoites of *gallinaceum* and *relictum* allow pre-erythrocytic but not erythrocytic development. The effective antigens in the sporozoite, tissue and blood stages appear therefore to be different.

Immunity against erythrocytic stages.—The further course of infection may be (*a*) acute and regular rise in parasites in erythrocytes, (*b*) peak density of parasites, (*c*) parasite decline either slowly or rapidly enough to be designated as crisis, (*d*) chronic infection, (*e*) parasitic, latent (or sub-patent) period, (*f*) parasitic relapse, and finally (*g*) radical cure. In very severe infections death may occur at stage (*b*). When the forces of innate resistance are at play the number of parasites decreases during the period of intersegmentation but the peak at the time of sporulation increases progressively. The forces of acquired resistance slowly gather force during this phase and when they begin to be operative parasitic decline commences with progressively decreasing peaks.

Further course of infection depends on the virulence of the parasite, dose of inoculation and the immunity status of the host. Dose and virulence exert a combined effect and within limits one factor may compensate for the other. While differences in virulences between species are well understood (*falciparum* in man and *knowlesi* in monkeys being known to be virulent species), some strains of the same species may be more virulent than others. Again *malariæ* tends to establish itself as a developed or chronic infection while *falciparum* more readily leads to death of the host or radical cure of the infection after only a few relapses. *Vivax* tends to have a number of relapses with periods of latency in between and some *vivax* strains have a tendency for long-term relapses. In addition to parasitic factors the host factor plays a dominant part in determining the further course of infection. A person with a high status of immunity will more readily and speedily be radically cured of a homologous infection. Latent infections can be proved either by sub-inoculation and establishment of patent infection in a susceptible host or by refractoriness to superinfection with homologous strain. This refractoriness has been variously termed infection immunity, concomitant immunity or premunition (other names are omitted being less often in vogue). The acquired immunity that persists after radical cure is called true immunity or residual immunity and its duration varies with different species and strains and for each strain depends on the duration of infection with that strain which has, since, been radically overcome. Each successive reinfection with homologous strain results in progressively milder infections. Too largely or too closely spaced superinfections may, however, decrease the potency of immune serum or immunity to superinfection (Taliaferro, 1949). Again treatment modifies the development of immunity by reducing the amount of antigen absorbed. In the vast majority of cases of human malaria, treatment which, in recent times, is increasingly adopted early after infection tends to lower the quantum of immunity. Sinton (1939*a*: 1939*b*) has shown that the immunity which develops after treatment depends both

upon the amount and duration of antigenic stimulation. When the antigenic stimulation is slight and of short duration there is no detectable immunity. When the stimulus is larger and prolonged, considerable immunity is acquired. With low dosage of antigen but its prolonged stimulation a higher degree of immunity is acquired than with larger but transient antigenic stimulation.

Relapses.—During parasitic relapse there is a wearing out of the homologous immunity. There is no evidence to show if the immunity is lost against the exo-erythrocytic stages which are responsible for relapses in avian malaria (and by inference in human and simian malaria) or against trophozoites. So long as protection against trophozoites is maintained relapses can be effectively checked.

Specificity of acquired immunity.—Acquired immunity is specific against the homologous strain. Some strains may however be immunologically similar. Mulligan and Sinton (1933) and Sinton and Harbhagwan (*loc. cit.*) have shown a partial heterologous immunity between some strains of *P. knowlesi* which is strengthened, as in homologous immunity, by multiple superinfections. Partial heterologous immunity is acquired in some strains of *vivax* but not in others and is more readily acquired in *ovale* infections than in *vivax* or *falciparum*. These inequalities may be related to treatment and consequently the amount of antigen absorbed (Boyd, 1940). Heterologous immunity to different species does not prevail in man and monkeys but some avian species manifest heterologous immunity. *Gallinaceum* confers a heterologous immunity against *lophurae* but not *vice versa* (Taliaferro and Taliaferro, 1945). Such non-reciprocal cross-immunity may be due to quantitative differences in the same antigen according to Redmond (1939) or differences between the specificity of the antiserums as suggested by Landsteiner (1945) in the case of azoprotein antigens and their antiserums.

Sinton, Hutton and Shute (1939) and Sinton (1940) have recorded the establishment of a higher degree of immunity in sporozoite-induced *ovale* infections than in blood-induced infections. Sinton attributes it to the greater aggressiveness of sporozoites and consequent greater antigenic efficiency. On the other hand, Taliaferro and Taliaferro (1945) relate the degree of immunity to the quantity of antigen absorbed in *gallinaceum* and *lophurae* infections induced in either way.

Cellular mechanism.—The rôle of macrophages in the phagocytosis of free parasites, parasitized cells, pigment and other debris has been understood for long. Cannon and Taliaferro (1931) and Taliaferro and Cannon (1934) found that during innate immunity in *cathemerium* and *brasilianum* infections death of the parasite is correlated with a sluggish phagocytosis of all stages of parasites by macrophages chiefly of spleen, liver and bone marrow. During parasitic crisis there is agglutination of parasites in the Billroth chords of the spleen. Very soon they are phagocytosed due to the superimposition of acquired immunity during the crisis on the previously manifested innate immunity. Superinfection during latency rapidly mobilizes (within an hour) the above phenomena of splenic infiltration, opsonization and phagocytosis in the spleen, the liver and the bone marrow. The above authors believe that the limitation of phagocytosis to these organs and occasionally the adrenals is a question of opportunity, for only in these organs is the blood flow sluggish at times and the orientation of the macrophages is most suitable for removal of material from the blood. On this account Taliaferro (1934) suggested that the so-called

general immunity in many blood infections may actually be a local immunity in strategically placed organs. The functional macrophages are the Kupffer's cells of the liver, the reticular cells in Billroth chord of the spleen and similar reticular cells of the bone marrow. The greatest number of new microphages in acquired immunity come not from a homoplastic development of the fixed and free macrophages (mitotic proliferation of the cells of the reticulo-endothelial system) but heteroplastically from blood agranulocytes (lymphocytes and monocytes) as shown by Taliaferro and Mulligan (1937) in their study of *cynomolgi* and *knowlesi*. They also suggested the term 'the lymphoid-macrophage system' to bring together macrophages and all their precursors. The cellular changes in peripheral blood such as increased monocytes and cells intermediate in structure, leukopenia, anæmia and macrophages with or without malarial pigment only inadequately reflect the bulk of the cellular changes in the tissue.

Humoral mechanism.—Indefinite as the nature of the antigen in malaria may be, the parasite liberates many antigens which stimulate antibody production. Only some of them like opsonins and agglutinins are functional in acquired immunity. Others like precipitins and complement-fixing antibodies are important only in serologic diagnosis. Agglutinins are strain specific but the latter antibodies are only genus specific. The increase in plasma-globulin concentration in the late stages of acute malaria is cited as an evidence for formation of humoral antibodies as many immune bodies are generally included within *gamma* globulins. While there are several negative records as regards protective antibodies due to the inadequacy of immune sera, Coggeshall and Kumm (1940) unequivocally demonstrated the presence of antibodies in the serum of *rhesus* monkeys with developed infections which protected normal monkeys from establishment of infection when the parasite was simultaneously injected. These results have since been corroborated by others. Zuckerman (*loc. cit.*) has demonstrated the presence of opsonins *in vitro* during superinfection of *gallinaceum* and *lophurae*. The greatly increased rate of cellular phagocytosis in acquired immunity is most reasonably ascribed to the presence of opsonins. Eaton (*loc. cit.*), Jaswant Singh and Harwant Singh (1940) and Ray, Mukherjee and Roy (1941) have demonstrated *in vitro* the presence of agglutinins increasing in titre as the infection subsides and after superinfection with *knowlesi*. Clumping of red cells in capillaries of living tissues recorded by a number of earlier observers may be due to the formation of a fibrin-like substance around the cells making them sticky and phagocytable (Kinsley *et al.*, 1941 ; Kinsley and Bloch, 1942). This effect is however non-specific and can be demonstrated in trauma. Brown and Broom (1935) suggest that phagocytosis depends upon reduction in the electrical charge of cell surface which occurs both in parasitized and non-parasitized cells but such reduction was related directly to the appearance of humoral antibodies in the plasma. The reduction in electrical charge of some parasitized cells has been observed by others including Krishnan, Chopra and Mukherjee (1935).

Krishnan, Ghosh and Bose (1936) found an inverse relationship between cholesterol content and acquired immunity in *knowlesi* infections. Probably lipoid changes affect the behaviour of microphages. The variations in cholesterol content found by Kehar (1937) during the febrile period are probably associated with by-products of segmentation rather than immunity.

Barber *et al.* (1936 : 1937) and Clark (1937) have noted a low parasite count in the first 2 months of the life of infants born of malarious mothers. Viswanathan (1941) has also recorded a similar phenomenon which however does not hold good during the height of malaria transmission. There is thus some evidence of passive transmission of humoral immunity from mother to offspring through the placenta.

Combined cellular and humoral mechanism.—Mulligan, Somerville and Swaminath (1940) conclude that the mechanism of defence against malaria involves the interaction of both cellular and humoral agencies and is probably fully effective when a *full* measure of one of these factors operates in the presence of an *adequate* measure of the other. They found that administration of large doses of homologous immune sera was very effective in controlling *knowlesi* infections in *rhesus* monkeys in which the lymphoid-macrophage system had been adequately stimulated by previous infection with *cynomolgi*. Dikshit (1941) has confirmed the above hypothesis by studying the effects of replacement of blood of an immunized monkey with normal non-immune blood and that of a normal monkey with homologous immune blood. Even after replacement of 80 per cent of blood, the immunized monkey retained its protective power. The normal monkey acquired immunity when 70 per cent of its blood was replaced with immune blood.

Antiparasitic effects of immunity.—Taliaferro and Taliaferro (1944) conclude that (1) innate immunity is associated with marked death of parasites (about 66 per cent) and a sporadic variation in asexual reproduction (up to 15 per cent), (2) acquired immunity brings about a heightened death of the parasite (more than 90 per cent)—this is by a combined cellulo-humoral mechanism—and a marked lowering of asexual reproduction both by delayed cell division and reduction in the number of merozoites and (3) parasiticidal factors continue to operate after the crisis and gradually cause the infection to disappear unless they are temporarily reduced in which case relapses of varying severity occur.

Antitoxic effects of immunity.—No true toxin of the malarial parasite has yet been demonstrated. The symptoms arising out of parasitic invasion need not even be attributed to antigen-antibody reaction. They may simply be a reaction to a non-specific pyrogenic agent. The antitoxic effect is often assumed on account of the higher pyrogenic threshold in immune hosts. Until however proof is forthcoming for the presence of an antigenic toxin in the red cell and parasitic debris including malaria pigment liberated into the blood at the time of plasmodial segmentation, antitoxic immunity is an assumption. Taliaferro (1934) suggested that in some helminth infection antiparasitic factors of immunity may produce lowered parasitic metabolism and consequently fewer effects of its infection. A similar phenomenon, he concludes (1949), may occur in malaria.

Host parasite adjustment under different field manifestations of acquired immunity—hypoendemicity.—Viswanathan (1951) has suggested a classification of endemicity slightly modifying the proposal made at the African Conference on Malaria held under the auspices of the World Health Organization Expert Malaria Committee. In the first of his groups—hypoendemic—a person may escape infection till adolescence is reached due to lack of contact with an infective mosquito and not because of any immunity factor. His reaction to infection is as in a primary infection without any prior immunity status. The forces of natural

innate immunity come into play at first and later of acquired immunity. Superinfections and reinfections are uncommon. While intrinsic factors are not unfavourable for the parasite, extrinsic factors are most unfavourable. Further course of infection depends on the virulence of strain and species and the dose of sporozoite inoculation. Ultimately full recovery is the rule, though initially the host suffers from acute clinical symptoms. On the whole there is no question of host parasite adjustment.

Mesoendemicity.—In this group although adult life may be reached without acquiring infection, maximum prevalence is in childhood and adolescence. Transmission is seasonal and of low grade. Chances of full recovery occur during the non-season and hence acquired immunity may be completely or partially worn out. In this group too both children and adults suffer acute symptoms. Host parasite adjustment does not develop to any degree mainly by reason of limitation of infection to a season.

Epidemicity.—This condition can only be superimposed on the above two groups because of lack of host parasite adjustment, and wearing out of whatever immunity was acquired during an earlier epidemic. During the epidemic phase the parasite is aggressive with acute clinical symptoms betokening a low-grade resistance against the effects of infection. Multiplication of parasites too goes on unchecked until acquired immunity gathers force. Gill (1914) has shown that adults exhibit no mortality because of the resistance acquired in earlier epidemics. This however occurs only in regions where the duration of epidemics is limited to a few months. Viswanathan (1936) has recorded that in epidemics of longer duration adults also exhibit increased mortality in later waves. The small quantum of immunity is of no avail against multiplicity of strains and species occurring in epidemics of longer duration. It is under such conditions that epidemics cause greatest havoc as, for instance, the Ceylon epidemic in 1935.

In epidemic malaria of short or long duration natural innate immunity is not adequate to prevent the multiplication of parasites sufficiently rapidly to produce clinical symptoms ending in death in a considerable proportion of the extreme age groups. Adults exhibit resistance to the extent of suffering no increase in mortality, but their acquired immunity (in the previous epidemic) is not adequate either to lower the pyrogenic threshold or mitigate clinical symptoms. In longer epidemics there is a further breakdown and increased mortality also results. Thus in epidemic malaria the parasite has had the best deal and exercised greatest aggression and the host could exhibit no adjustment. The parasite has however overshot its mark and by the end of the epidemic the host has developed an abundant immunity against the parasite as well as its effects. If within the next 2 or 3 years, even if as favourable facilities for mosquito life are created as during the epidemic period, a second epidemic does not result on account of this dual manifestation of immunity by the host. Here too it is not a case of host parasite adjustment but the host dominating over the parasite. In due course with the aggregation of non-immunes (mainly of new births) the communal immunity wears down and there is a partial host parasite adjustment depending on the degree of transmission in the inter-epidemic period, fresh infections being able to gain a foothold due to the wearing down of host immunity but in its turn adding to the residual immunity. So long as there is this state of balance, no

further epidemics can result. But if infection subsides due to natural causes or even if it remains of a low grade, further dilution of communal immunity sets the stage for another epidemic when conditions favourable for mosquito life are established.

High endemicity.—Transmission is seasonal in this group but during the season it is of a high grade. By late childhood, practically the entire community is infected. The stage of immune infestation is reached late in childhood but the immunity is only partial both against parasites and their effects. Due to such partial immunity adults suffer from parasitic effects though not to the same extent as young children. There is a good recovery during the non-season and wearing out of immunity. Hence when the season begins infections 'take' produce symptoms and in due course engender immunity. The immunity in the community is high enough to prevent epidemics. But exacerbations do occur due to variations in inoculation rates.

Hyperendemicity.—Transmission usually occurs for about 9 months in the year. Inoculation rate is high. By late infancy entire community is affected. In late childhood immune infestation is reached. Acquired immunity both against parasites and their effects occurs in late childhood and even more in adults. But adults have a high spleen rate and also suffer from clinical symptoms. Pyrogenic threshold is higher than in previous groups.

Holoendemicity.—Transmission is perennial. Adult sickness is mild but not absent. Best host parasite adjustment occurs in the age group 5–9 during the stage of immune infestation. Earlier, host suffers ill effects. In later age group parasite gets destroyed but anti-parasitic immunity is not complete. Hence there is some persistence of parasites. Immunity against parasitic effects too is not absolute. Hence symptoms occur but such symptoms retard circulation of antigen in spleen, liver and bone marrow and stimulate defensive antibody formation. Vast areas in India formerly considered 'hyperendemic' really come under this group and in all age groups there is definite sickness. Adults exhibit a fairly high spleen rate.

Superendemicity.—Transmission is perennial. Inoculation rate same as in last group. Communities which have settled only for a few centuries exhibit the same features as in the last group. But communities who have lived locally for more than a thousand years exhibit features of a natural selection by a pathological process. Greatly fortified humoral immunity is inherited by infants almost fully effective against parasitic effects but of no avail against parasite multiplication. Adults suffer practically not at all. Infants and children have the heaviest parasite load and the parasite threshold is the highest. Splenic hyperplasia is of a smaller order in children. Adult spleen rate is low. In the stage of 'acute infestation' (0–5) there is the best evidence of host parasite adjustment. Parasites multiply freely but host suffers little damage. The absence of symptoms retards specific antibody formation on account of the rapid circulation of parasite antigen in spleen, liver, etc. If there is fever there will be stasis, larger contact of antigen with the defensive tissues and greater stimulation of antibody formation. Later in life the cumulative effect of inherited and acquired immunity brings about the most effective check to parasite multiplication. Thus in later life there is no parasite adjustment at all. The state of near commensalism exists only in extreme young age below 5. It cannot be called however commensalism as it is not a fixed

characteristic, and sub-inoculation of these parasites in other susceptible hosts will provoke considerable adverse effects. Altogether while in this group the herd immunity is most effective in all ages, during infancy and early childhood it is mainly inherited and humoral and manifested primarily against parasite effects but not against parasite multiplication. This inherited immunity is won after repeated sacrifice over countless generations.

Interdependence of immunity against parasites and their effects.—Acquired immunity against parasites will naturally be of avail against their effects as well for there is stimulation of both humoral and cellular factors and direct destruction of parasites is the most certain defensive mechanism. Acquired humoral immunity is always associated with acquired cellular immunity and the cumulative effect is primarily manifested against parasite multiplication. If the defensive mechanism fails against parasite multiplication at any stage, parasite effects are manifested in the host sooner or later. The parasite, *per se*, is the main victim of the defensive mechanism. In well-seeded communities, however, after countless generations of exposure to heavy and perennial infection, infantile sacrifice, adult sickness, etc., the survivors build up a very high degree of humoral immunity which is transmitted to their offspring. There is also a natural selection by a pathological process for the survivors represent the more capable individuals to develop defensive antibodies and this capacity is transmitted as a mendelian characteristic as previously stated. The inherited humoral immunity being almost fully effective against parasite effects protects the hosts from febrile symptoms. Not only does the inherited humoral factor have no effect by itself in preventing parasite multiplication but by affording protection from febrile symptoms it retards the acquisition of defensive immunity since the antigen is allowed to pass through the main defensive tissues like the spleen quickly, whereas if fever were present the resultant stasis will establish a much longer period of contact of parasite antigen with the tissues and greater antibody formation. To that extent an abundant inherited humoral immunity is least conducive to further acquisition of active immunity. Such abundant humoral immunity is manifested in India only by the most primitive hill tribes. In all other areas hitherto classified as hyperendemic the inherited humoral immunity does not completely prevent sickness in infancy and childhood. Later in life among these primitive hill tribes the acquired defensive mechanism both cellular and humoral though not perhaps of the same order as in communities inheriting a lesser degree of humoral immunity is adequate to establish the most effective prevention of parasite multiplication. In one case there are an *abundance* of *inherited humoral factor* and *an adequate actively acquired cellular and humoral factor*. In the other case there are *adequate inherited humoral factor* and abundant *actively acquired cellular and humoral factor*. Prevention of parasite multiplication is more effective in the former combination.

SUMMARY.

The characteristics of innate natural immunity are reviewed with reference to age, sex, race, host parasite specificity, individual variations, stage of infection and host parasite adjustment. The pathology of malaria is very briefly described. Acquired immunity with respect to course of infection, stage of the parasite, species and strain of the parasite, etc., is reviewed and the cellulo-humoral mechanism is

described. Immunity against the parasite itself and its effects are distinguished and their field manifestations under different conditions of endemicity discussed. A hypothesis is put forward regarding the interdependence of the two types of immunity manifestation and their effect on the host-parasite adjustment.

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Indian Journal of Malariology, **5**, 2, *June 1951*.

ABSTRACT.*

MALARIA IN ARAKAN.

BY

MAJOR G. S. DHALIWAL, I.A.M.C.

[July 26, 1950.]

THE author was posted to Arakan in the early part of 1944 as Officer Commanding, 38 Indian Antimalaria Unit with Headquarters at Tumbru Ghat in Arakan. Its sub-sections were spread over the surrounding area, at one time up to Taungbazar across the Mayu Hills. This Unit had been in Arakan since August 1943.

Malaria was the commonest disease of this area and was responsible for 40 to 60 per cent of patients admitted to the hospital. *P. vivax* was the commonest type of malaria parasite forming 46 to 64 per cent of the total number of blood slides examined. *P. falciparum* formed 36 to 56 per cent and *P. malariæ* was the least common type. The period of highest malaria transmission is from July onwards. *A. sundaicus*, *A. minimus* and *A. leucosphyrus* were incriminated as vectors. Several new species were recorded from this area (*A. jamesi*, *A. karwari*, *A. kochi*, *A. leucosphyrus*, *A. maculatus*, *A. minimus* and *A. philippinensis*).

Antilarval measures such as flushing, removal of vegetation, herbage packing, oiling with malariol, paris green dusting and D.D.T. were employed to deal with the extensive brackish lagoons which were the most characteristic feature of this area. Pyrethrum spraying was extensively employed in the Units and civilian habitations. D.D.T. was not available for large-scale residual spraying till about the end of 1944. Usual personal and prophylactic measures were also enforced.

As a result malaria and NYD fever admission ratios in the area under control were brought down from 77 per thousand per month in November 1943 to 9·8 per thousand per month in November 1944.

J. S.

* The original manuscript (14 typed script pages, 2 maps, 8 photographs, 3 charts and 2 figures) 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*.

Indian Journal of Malariology, **5**, 2, *June 1951*.

REVIEWS.

INTRODUCTION TO MALARIA PROBLEM IN INDIA.

Introduction to Malaria Problem in India. By LIEUT.-COLONEL B. L. RAINA, A.M.C. Published by New Book Co., Ltd., 188-90, Hornby Road, Fort, Bombay. 1951. Price Rs. 7 As. 12 (cloth bound), Rs. 6 As. 12 (stiff paper bound).

IT is a handy book, written in easy style summarizing the public health problem of malaria, and there are ten chapters dealing with history, causation, spread and control of the disease in India.

The author has done well in commencing the book with a brief history of malaria from Vedic times to the present day. The details including Sanskrit texts on malaria in ancient times have been made available in one booklet for the first time.

The appendices include the recommendations of the Health Survey and Development Committee on Malaria, and suggestions on Provincial Malaria Organization, Malaria Control Ordinances, etc.

The value of the book lies in the combination of concise, straightforward and practical approach to the problems, illustrated with excellent colour reproduction of malaria parasite of man, a malaria map of India, Pakistan and Burma, sketches and illustrations, etc. It is a handy book which will be useful to all those interested in the general knowledge of malaria problems in India and Pakistan.

JASWANT SINGH.

OBITUARY.

EMILE BRUMPT.

It is with profound regret that we have to announce the death of Professor Emile Brumpt at the age of 74 years. He was one of the greatest parasitologists of his time, and commanded highest esteem and universal fame.

Just prior to his death, Dr. Brumpt had been informed of the forthcoming award to him of The Theobald Smith Medal at the ensuing annual meeting of the American Society and the American Academy of Tropical Medicine.

Professor Brumpt has left us an imperishable monument: the 6th Edition of his '*PRECIS OF PARASITOLOGY*'.

We wish to convey our great admiration and deep sympathy to his family, and friends all over the world.

J. S.

ALBERTO MISSIROLI.

It is also with deep regret that we have to record the death on July 18, 1951, of Professor Alberto Missiroli, the world-renowned malariologist and the Director of the Antimalaria Experimental Station, Rome. He was 68 years old but very active and fit in April when last interviewed by the Editor.

He was the founder of the *Revista di Parasitologia* and had worked in collaboration with The Rockefeller Foundation, the World Health Organization expert committees, and other international bodies.

The world in general and Italy in particular has lost in him a most courteous and respected worker. We convey our heartfelt sympathy to the bereaved family, his colleagues and friends.

J. S.

Indian Journal of Malariology, 5, 3, September 1951.

STUDIES ON THE BIONOMICS OF *ANOPHELES AQUASALIS* CURRY, 1932.

Part I.*

BY

R. A. SENIOR WHITE.

(*Entomologist, Malaria Division, Trinidad, B.W.I.*)

[April 12, 1951.]

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SECTION I.

INTRODUCTORY.

AT the time when Ross' discovery stimulated fresh interest in the systematics of Anopheles, the known species which were placed in the Subgenus Nyssorhynchus of Blanchard, 1902, were three: *albimanus* Wiedemann, 1821; *argyritarsis* Robineau-Desvoidy, 1827; and *albitarsis* Arribalzaga, 1878. But as malaria investigations in the Neotropics commenced, other species in the subgenus were discovered, and by 1905 Goeldi had distinguished his '*tarsimaculatus*', separable from *albimanus* on the colour of the 4th palpal segment. There is much confusion attached to Goeldi's name (Causey, Deane and Deane, 1945) and its validity still awaits the decision of the International Commission on Zoological Nomenclature. But through work carried out from 1922 onwards, the '*tarsimaculatus*-group' of the subgenus (as it is now styled) has been shown to consist of twelve* species, many of them only separable on the male terminalia. Table I shows the distribution of the group in the order of date of description, over its general limits between 30°S. and 25°N. latitude. Whilst south of 5°N. there are enormous areas of the sub-continent awaiting far more detailed collecting, from Venezuela northwards a great deal of work has been done, and it is clear that north of 15°N., though *A. strodei* extends into Southern Mexico and *A. aquasalis* through the Lesser Antilles, *A. albimanus* is the only species of the group that extends to the margins of the Nearctic Region.

The species with which the studies subsequently to be detailed are concerned is *A. aquasalis* Curry. According to the map given by Gabaldon in 'Boyd' (1949) its distribution extends from coastal Brazil in the region of 23°S., around the north coast of South America to coastal Ecuador in 2°S.†, along the Atlantic coast of Central America as far as 10°N. in Costa Rica, and through the chain of the

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* *A. rondoni* Neiva and Pinto, 1922, has some black on the third hind tarsal, and was a new discovery and not part of the 'split-up' of '*tarsimaculatus*'. The author has accepted Lane's (1949) sinking of *clarki* Komp in *noroestensis* Galvao and Lane. Causey, *in litt.*, informed the author that *goeldii* is a synonym of *nunez-tovari*, and the author has the former accordingly in Table I.

† Guppy (1906) describes the abrupt transition from a region of drought and semi-sterility to a mangrove zone at 3°30′–3°20′S. latitude. This mangrove area extends around the Gulf of Guayaquil, but north of it the desert coastal region recommences and extends through 2°50′ to the Equator. A detailed survey for *aquasalis* from the Equator to the Ecuador-Peru frontier would be illuminating.

Lesser Antilles, to where its recent discovery (Senior White, 1950) in St. Kitts (17°N.) marks the limit of its northward distribution; as van der Kuyp's (1949) survey of both the Dutch and French portions of St. Martin (18°N.) yielded no species of Anopheles. It is not found in Puerto Rico in the same latitude. What is on record regarding its capacity as a malaria vector is set forth in Table II. Owing to its not being distinguished as a species until 1932 all earlier dissection work has to be neglected, as the '*tarsimaculatus*' of earlier authors may refer to so many species*. It is probable that the classic epidemiological studies of Boyd *et al.* (1926) in Brazil were in the main concerned with *aquasalis* (and Boyd, 1949, Table 104, refers his 109 dissections to that species), but it is now impossible to be sure what species they or any of the earlier neotropical workers on the mainland were dealing with. The same dubiety applies to the bionomical work. Judging from her localities map, the '*tarsimaculatus*' referred to by Beattie (1932) in her paper on physico-chemical factors in relation to anopheline breeding in Trinidad was a *mèlange* of *aquasalis* and *oswaldoi*, and this must be borne in mind when the results in Sections IV and VI of this series of papers are compared with hers. The pioneer anopheline survey work of de Verteuil (1931, 1933) in Trinidad was, from the locality covered, certainly almost entirely concerned with *aquasalis*, though it was not until his second paper that he became aware of Curry's division of '*tarsimaculatus*' into *aquasalis* and *aquacoelestis* (*oswaldoi* Perryassu). For this reason the dissections given and quoted in his paper of 1931 are included in Table II. De Verteuil discovered that 68 per cent of tropical sea-water (approximately 24 per thousand in the notation of Marine Biology) was the limit of salinity tolerance in *aquasalis*, which he also discovered would breed as successfully in fresh water. It will be seen that his limiting value agrees with that of Gabaldon *et al.* (1940) and that found by the author (Section IV), but the author's rain's optimum value, 10 per cent of sea-water, is lower than that found by de Verteuil, 20–45 per cent. De Verteuil was much impressed with the long-range flights he found to occur soon after the onset of the rainy season, a phenomenon which also impressed Shannon (*vide infra*) but for which the author has obtained no evidence.

After 1933, malaria investigation in Trinidad languished until work was recommenced by the Rockefeller Foundation in 1941. This is summarized in the monograph by Downs, Gillette and Shannon (1944), but only half a page is devoted to the biology of *aquasalis*. Rather more is to be gathered from the Annual Reports of the Malaria Division from 1941 to 1946, and from the weekly diary of Shannon as submitted to the Foundation up to his death early in 1945. Rather than attempt to summarize these in this section, they will be referred to in the individual sections which record the author's own work, where they can be discussed *vis-à-vis* the present results, in a more connected fashion.

The only other recent general work on *aquasalis* is in the monograph by Deane, Causey and Deane (1948), on the anophelines of north-east and Amazonian Brazil. The principal points noted by these authors are: †

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* This should make all younger workers in the Orient realize their debt to Sir Rickard Christophers, whose paper of 1916 placed the Indian species on a sufficiently sure basis to render their biological and dissection work valid to this day. *Per contra*, as Lane remarks in Boyd (1949), in the neotropical fauna 'it will take some time before systematic stability can be achieved'.

† The author is indebted to Miss Beaubrun, his Systematist Technician, for the translation from the Portuguese.

(*i*) *Geographical distribution.*—It is common around estuarine sand and mud-banks, but rare where the coast is without large rivers. Exceptions to a distribution generally restricted to a few kilometres from the coast occur (*a*) where there are saline areas inland as in Gloria do Goita (Pernambuco), where it is found 250 km. from the coast and 750 m. above sea level (Pinotti, 1951), (*b*) under the special conditions of the Amazon Delta, where the strong fresh-water flow of the main river inhibits establishment of saline conditions, whereas the weaker flow of the Rio Para is subject to tidal influence, and it therefore occurs up to 140 km. from the coast at Belem and Ponta de Pedras.

(*ii*) *Seasonal variation.*—In the wet season (March-April), at the mouth of the Rio Jaguaribe, a density of 36·0 per house was found, which fell to zero in October, the dry season. Around the city of Belem, during the dry season, its breeding places are restricted to those under tidal influence. An interesting point is noted in connection with the house captures just quoted, in the wet season almost all the specimens were collected high up on the walls, whereas in the dry season the majority were captured near the floors. (Observations with a tier of Assmann psychrometers would have yielded very interesting data on micro-climatic preferences.) Deane and Damasceno (1948) have amplified this part of Deane, Causey and Deane's (1948) paper.

(*iii*) *Larval breeding places.*—These also exhibit seasonal variation. In the wet season breeding occurs in almost any collection of water, other than that in active movement, in sunlight or partial shade, but in the dry season breeding is limited to permanent collections of water, such as deep pools. As regards salinity preference, these authors' Table 13 gives only figures for 44 positive breeding places distributed as under :

NaCl* per thousand.	Times found.	NaCl per thousand.	Times found.	NaCl per thousand.	Times found.	NaCl per thousand.	Times found.
0·0	2	1·0	0	6·6	4	18·4	1
0·2	1	1·3	0	11·6	6	19·9	1
0·3	1	1·7	1	13·3	5	21·6	1
0·7	1	3·3	6	14·9	2	23·2	0
0·8	1	5·0	6	16·6	4	24·9	1

Comparison of these figures with the very much longer series from Trinidad given in Table VI indicates a salinity limit of 71 per cent of pure sea-water, which is very close to the limiting values found by de Verteuil (*loc. cit.*) and Gabaldon *et al.* (*loc. cit.*). Compared with those found by the author, the optimum, 3·3 to 13·3 per thousand, is more like the author's dry than wet season value. The Brazilian data were collected over 3 years, but in the light of the seasonal

* Deane, Causey and Deane's Table 13 is given in terms of Cl+ion. The values have been multiplied by 1·66 to bring them into terms of NaCl.

habitat differences emphasized by the previous authors, it seems strange that they do not specify the seasons during which the titrations were carried out.

The previous authors also carried out experiments on egg and larval salinity tolerances. Eggs hatched even in solutions of 150 per thousand (equivalent to over 4 times that of sea-water), and matured and emerged in solutions of 30 per thousand, which is equivalent to 86 per cent of sea-water. As also found by the author (Section IV) pupation and emergence was higher between 5 and 15 per thousand than in fresh water. Using newly hatched larvæ, racial differences in tolerance were found. In material from Fortaleza (Ceara State) complete development was obtained in concentrations of up to 30 per thousand and again it was found that more pupation occurred in up to 20 per thousand than in fresh water. But in the race from Sao Luiz de Maranhao (Maranhao State) the tolerance to NaCl was not so great. Complete development was not obtained above 20 per thousand, and better development occurred in fresh water. Whether these differences in tolerance are locally racial, or the result of experimenting with dry and wet season forms, is not discussed. In an experiment in which eggs were subjected to a gradually increasing salinity due to evaporation from undiluted sea-water, 11 larvæ surviving from 500 eggs succeeded in producing 6 pupæ up to a concentration of 58 per thousand, but the few later survivors all succumbed by the time 73 per thousand (more than double that of sea-water) was reached. Two oviposition preference experiments with caged specimens of the Ceara race yielded 1048 eggs, distributed as under :

Salinity per thousand ...	Fresh	10	20	50	100
Percentage ...	35·2	41·7	13·0	9·0	0

As was to be expected the eggs laid in 50 per thousand remained white, and did not hatch even after being transferred to fresh water, although the embryo was apparently well developed. The experiment would have been more informative had narrower steps from fresh to full sea-water of 35 per thousand been tried out.

The life-cycle found in another series of experiments was : Egg, 2 days : Larva, 8–35 days (90 per cent, 11–22 days) : pupa 1½–2 days. This gives a minimal developmental time of 11½ days and a maximal time of 39 days. The very great difference in the time of larval development is very instructive in the light of the author's own work set forth in Section V. The previous authors note that nearly all the pupæ were formed during the afternoon.

(*iv*) *Adult habits.*—Along the north-east coast of Brazil, as noted in (*ii*) above, house captures were made at the rate of 2·4 per man-hour, at a time when outside catching, man or horse bait, was only yielding at the rate of 0·3 per man-hour; *per contra*, in coastal Para, during the wet season, though house resting specimens were quite numerous, a very much larger number was always found outside. In the drier months, practically none was found in houses, whilst the outside catch was very much reduced. It has been shown in Section VIII that this is what also occurs in Trinidad.

In the dry and wind-swept state of Ceara, *aquasalis* seems to enter houses much more frequently than in other states of Brazil. It is to be noted that the region has a sparse and xerophytic vegetation (Figs. 14–16 of Soper and Wilson, 1943),

whereas in other provinces the coastal bush is more or less dense, and 'the forest possibly provides sufficient protection for the mosquitoes to shelter and feed out of doors'. This is the condition existing everywhere that the author has studied in Trinidad. Climate and its effect on vegetation may have a very great bearing on the vectorial capacity of the species as set out in Table II.

(v) *Food preferences.*—Outside catches in two towns in Ceara State showed an almost equal preference for man and horse or donkey. Two further experiments were made in the same area using a range of baits, both in the open and indoors. Both experiments were carried out on the same night, during the wet season, i.e. at the time of maximal prevalence. Compared with the numbers which at least the outdoor experiment would attract in Trinidad, the catches were very small.

	Open: 18.00–20.00 hours.		Indoors: 01.30–03.30 hours.	
	Number caught.	Percentage of total.	Number caught.	Percentage of total.
Man ...	22	51	51	55
Goat ...	1	2	5	5
Horse ...	13	30	27	30
Pig ...	3	7	Not used	
Dog ...	4	9	8	9
Fowl ...	0	0	2	2
	43		93	

In Belem, *aquasalis*, though biting man readily, shows a decided preference for horse, in the ratio 1 : 5. It is very noteworthy that in these experiments there is no mention of bovine bait. Dr. Causey informed the author that no cow was used in this experiment because none was found in the particular locality (Beirada) where it was carried out. Apparently the ox is rare in some parts of coastal Brazil, another very important point as regards intensity of transmission.

(*vi*) *Biting hours.*—In Ceara State, using human bait in a house, *aquasalis* was found to bite all day, but the intensity was greatest in the late hours of the night, decreasing towards morning. Actual biting was seen at 10.00 hrs. and again at 15.00 hrs. No parallel day observations have been made in a house in Trinidad, where house densities by day are too low to make this worth doing.

In Belem, in thirty 24-hr. catches in the open, with animal bait, between May and August, biting occurred all night, but the major attack was at dusk.

(*vii*) *Length of stay in houses.*—Staining experiments in Ceara showed that less than 10 per cent of *aquasalis* remained in the house over a period of 24 hours.

By using acid fuchsin and methylene blue stains it was found that, after 24 hours, specimens had moved between two houses 40 metres apart. This is an interesting proof of the multiple feeding referred to in Section VIII of the present paper, as *aquasalis* does not often appear to enter houses simply to rest, though some specimens which have fed out of doors do subsequently enter houses (Table XXXII).

(*viii*) *Vectorial capacity.*—This is best studied in Table II, but in Deane *et al.'s* paper it is stated that (*a*) when *darlingi* and *aquasalis* occur together, the former is usually the more important species. During the *gambiæ*-epidemic in Brazil, at Cumbe (Ceara State), *gambiæ* was found infected to the extent of 5·6 per cent, whereas *aquasalis* showed a total infection rate of 1·5 per cent only.

A. aquasalis is stated to cause annual epidemics in some marshy coastal areas, as in Ceara State. The authors conclude by stating that the species is only a serious vector when abundant, owing to its habit of frequently biting outside houses, and, by attacking animals other than man, it has less opportunity of acquiring and of transmitting malaria. The authors incidentally mention that in Belem, where *W. bancrofti* is endemic, a specimen was found with micro-filariæ in the thorax, which may have been of this species.

In Table II are tabulated all the dissections that the author has been able to find in the literature that refer, with certainty, to *aquasalis*. Earlier dissections of '*tarsimaculatus*' may refer to almost any of the species listed in Table I, and on the advice of Dr. R. B. Hill are not included. The table tacitly shows the many countries from which there are no dissection records. The most notable *lacuna* is the type locality, Panama, but Venezuela forms another glaring gap in the records. Both countries possess large malaria organizations.

Study of Table II shows *aquasalis* as a quite efficient vector all round the coast of Brazil from the Tropic of Capricorn northwards. The French Guiana dissections are insufficiently numerous to dogmatize, but they were all negative. The recorded dissections from British Guiana indicate its apparent innocuousness, though Dr. Giglioli informs *in litt.* that there are districts in the north-west of the Colony, near the Venezuelan frontier, where there is no embanking against the sea, in which there is an appreciable amount of malaria that he considers to be probably due to *aquasalis*, but apparently no dissections from these districts have been carried out.

In Trinidad, if only the 1941–43 dissections are considered, the sporozoite rate is in the second place of decimals. Yet in that island it causes, as Downs, Gillette and Shannon (1944) have confirmed earlier work to show, malaria which is considered to be of economic importance.

The very malarious island of Tobago, where the species is now under eradication, has never had any dissection work carried out in it, and *aquasalis*, rather than the other two local species, is blamed purely on analogy with Trinidad work.

The whole island-chain of the Lesser Antilles is malarious, but it is only in the southernmost of the Windward group that any dissection work has been carried out. From Dominica northwards (Senior White, 1950) the problem of malaria

causation is complicated by the simultaneous presence of *A. albimanus*, and until the relative local vectorial status of *aquasalis* and *albimanus* in each of the islands in which both species occur has been evaluated by adequate dissection work, malaria control in these islands must be based on the 'hit or miss' method of residual D.D.T. on houses in which, as it will presently be shown by the author's work in Trinidad and that of Muirhead-Thomson in Jamaica, that no more than a part of the local malaria is contracted. Very considerable dissection work will be required before the relative parts played by *darlingi*, *albimanus* and *aquasalis* in malaria causation in Venezuela, and by *albimanus* and *aquasalis* in Panama (and presumably Costa Rica also) can be evaluated.

Summarizing this section: it is known that *aquasalis* differs from its close relative *oswaldoi* in its capacity to breed in saline water, and has a range extending 5° of latitude further from the Equator both northward and southward. *A. aquasalis* shows local differences in its tolerance of high salt concentrations, which are in part seasonal. During favourable seasons for production, the only restrictions in its breeding water is a concentration exceeding about 24 per thousand, and water in definite movement. As an adult it prefers to rest by day outside houses, but where the exterior terrain is inhospitable, it tends *p.c.* to remain indoors, though it is not there static throughout the gonotrophic cycle. In some localities man is equally preferred to domestic animals, in others man is much less sought. Its capacity as a vector is seen to differ greatly in those few parts of its distributional range where a reasonably adequate amount of dissection has been carried out. The need for much further dissection work has been emphasized.

SECTION II.

RACES IN *A. AQUASALIS*.

Many of those who have worked on *aquasalis* have apparently experienced difficulties with its systematic definition. Root and Andrews (1938) dealing with the problem of malaria transmission in Grenada state: '*tarsimaculatus* of the Lesser Antilles is a sort of local race of the species, differing in habits from the Continental races, but not differentiated as to structure'.

Komp (1941) was the first competent systematist who attempted to divide *aquasalis*. The separatory characters for his *emilianus* (type locality Brazil, Para State, Belem) were based entirely on egg differences, no constant separatory characters being found in the larva, adult or male genitalia.

In the same year, Rozeboom (1941) gave a valuable summary of what was then known on its varying ecology in the Caribbean Region, quoting its original describer, Curry, as stating that it is not attracted to man in Panama, where, in former years, the appearance of great numbers in the cities was not followed by a rise in the malaria rate, and comparing this with the proven vectorial status of the species in Grenada (Section I). This author did not then suggest that these variations in ecology and vectorial capacity were related to any systematic differences, but in his subsequent paper (Rozeboom, 1942) he stated that there is strong evidence that *aquasalis* is composed of at least two sub-species. After again recounting local biological differences in *aquasalis* in various countries of the Caribbean Region,

he goes on to state that in British Guiana and Trinidad the eggs were much more variable in their morphology than those he had described from Panama.

Russell, Rozeboom and Stone (1943) retained Komp's species as valid, but Deane, Causey and Deane (1946) sank it in *aquasalis*. Komp's name, however, remains available for one of the races into which, as will be shown presently, the *aquasalis rassenkreise* must be divided.

Shannon's Trinidad diaries clearly indicate the amount of difficulty that he encountered with the systematics of *aquasalis** So early in his career in Trinidad as the diary of November 17–22, 1941, he noted that *aquasalis* from Sangre Grande (wet forest zone) = ? the coastal form, = ? *rangeli*. By March 1–7, 1942, he had written that 'possibly we shall reach the point of classifying the "*tarsimaculatus*" spp. by geographical locality instead of morphological species'. On June 7–13, 1942, he comments on the St. Lucia race, 'hind tarsi 2/5th to ½ black'. On October 5–10, 1942, he records, 'Gabaldon thinks that the Trinidad race differs from the Venezuelan, both as eggs and larvæ, and is similar to *emilianus*, which he regards as a subspecies'. With this Shannon expresses disagreement, stating 'our race is not the same as *emilianus*', and goes on to say 'if Gabaldon is right, there is a "variety" of *aquasalis* in every country in which the species occurs, a very complicated matter'. On October 18–24, 1942, he notes, 'Komp considers the Trinidad form different from Panama; it is not *emilianus*, but intermediate'. In the Annual Report of the Malaria Division for 1941, Shannon stated that between the saline and fresh water forms (of Trinidad) there were neither egg nor male genital differences.

In the diary of November 13–19, 1944, Shannon quotes Galvao along the lines of the opinion of Causey given to and quoted by the author in the 1947 Report of the Division, that Nyssorhynchus is a recent genus as yet constitutionally unstable. Corbett (1933), arguing from the Malayan Rhopalocera, gave as his opinion:

> A comparatively young species will be characterized by (*i*) much individual variation, (*ii*) no well-differentiated geographical races, (*iii*) a number of closely allied species in the genus. On the other hand a species which has entered the final phases would exhibit (*i*) little individual variation, (*ii*) well-differentiated geographical races, (*iii*) few or no similar species in the genus.

He goes on to state, with examples, that 'young species' have an approximately equal sex ratio, whereas 'old species' have a low female sex ratio.

By these criteria, the genus Nyssorhynchus confirms Causey's opinion as to its recent evolution. As regards *aquasalis*, the difficulties, just recounted, that various workers have experienced with its systematics are eloquent as to its individual variation, and except by the use of the maxillary index (*vide infra*), geographical races are exceedingly difficult to distinguish and to define; whilst Table I clearly indicates the number of closely allied species in at least the *tarsimaculatus* series

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* It was not until 1949 that it became certain that the closely allied *rangeli* was not described from Trinidad, British West Indies, but from a town of the same name in Colombia. It seems certain that *rangeli* does not exist in Trinidad, B.W.I. (Komp, *in litt.*).

of the genus. As regards sex ratio, in Section IV (iii) the author adduces evidence that female production exceeds male, a very anomalous finding.

Finally Komp (1948) again summarizes to that date differences within the area of *aquasalis* distribution in respect of house-haunting habits and vectorial capacity. The latter are succinctly summarized in Table II.

Personally, the author feels that none of the workers quoted above have sufficiently appreciated the evolution of a widespread species into crypto-subspecies separated genetically, the evidence for which is so lucidly set forth by Huxley (1942), whose fundamentally important book the author has endeavoured (Senior White, 1948) to amplify in respect of the lessons it holds for malaria workers. Shannon (*sup.*) came nearest the truth, probably there is what he styled a ' variety ', i.e. a subspecies, often not definitely distinguishable, but vaguely unstable in the accepted systematic characters, in every country of its distribution.

Quite early in his career in Trinidad, therefore, the author realized the need to investigate systematic differences in *aquasalis*, as reading for the preparation of Table II of the present paper had so clearly indicated its varying capacity, throughout its great range of distribution, as a malaria vector. It was obvious that a separatory character or characters more tangible than the amount of white scaling on the fourth palpal segment, or the measurement of the extent of black scaling on the second hind tarsus, both characters which are also affected by scale rubbing of individual specimens, were required. Differences in male genitalia in *aquasalis* there appear to be none (Downs, 1950 : Shannon's diary notes quoted above), and are *per se* nearly useless to the biologist, in the main concerned with the female sex of any mosquito. The author therefore fell back on the maxillary index of Roubaud, which has afforded such valuable data in both the Palearctic and the Oriental Anopheles. The maxillary index is a purely statistical concept, and the differences it reveals are not applicable to the determination of individual specimens *. The biologist, whatever his particular line of enquiry, is much more concerned with the matter of populations and their differences than with the exact identification of individual specimens, however important these may be to the pure systematist.

In his work on the Maxillary Index of the Indian Anophelines (Senior White, 1937) the author followed Trensz in choosing 200 counts of individual maxillæ (though as far as possible by pairs from the same specimen) as the number required to give an index statistically significant to a whole number, the S.E. of 200 counts being stated to be 0·3. Examination of Trensz' original paper by Armitage and Miss Harrison at the London School of Hygiene reveals that Trensz worked on ' fiduciary limits ', and his limit of confidence is considered ' fantastically high '. Further they find that Trensz' table on p. 207 giving numbers to be counted for any required degree of significance is invalid, as it neglects variations in dispersion. Thus the doing of 200 counts, though it adds to the microscopical labour, results in a higher limit of accuracy than a whole number, and they informed the author

* None the less they are more certain (inasmuch as the differences are numerical and so subject-able to statistical analysis for validity) than ' dark spot at tip of wing diffuse ', ' dark spot at tip of wing distinct ', which have to serve for the separation of *A. punctimacula* (a vector) and *A. apicimacula*; whilst the latter is only separable from *A. intermedius* on the male genitalia.

that variations found between the author's long series of 200 counts in *aquasalis* are significantly different by gradations of 0·29 in the index, and not by 1·0 as the author believed when doing his Indian work on similar numbers.

As also studied in the author's work on the indices of the Indian species, there are certain preliminary points to settle before instituting comparisons between the indices of this single species as found in different countries. These are : (*a*) can the indices of bred and of caught specimens be validly compared, (*b*) is there temporal variation in the index in any particular locality, (*c*) is the index affected by the species of host animal attacked ?

(*a*) Two sets of data are available on this question : a comparison of bred material from El Socorro and adult caught material from Curepe, these two Trinidad localities being situated within three miles of one another, and of bred material from Belem and adults caught at Maracanan, these two Brazilian localities lying within about 40 miles of one another, both (Section I) being under the tidal influence of the Rio Para. In respect of the Curepe material seven counts are available and so instituting a χ^2 comparison with the El Socorro bred material one totalled array is seven-fold that of the other. The results show :—

Numbers of teeth ...	13	14	15	16	17	18	19	20	21	22
Bred	2	0	0	2	0	0	2	5	26	58
Caught	0	0	0	0	4	4	11	36	123	274

Numbers of teeth	23	24	25	26	27	28	29	Maxillary index
Bred ...	44	39	16	5	1	0	0	22·61
Caught ...	386	321	162	58	19	1	1	23·14

The difference is nearly twice the significant level for 200 counts. The Brazilian material shows :—

Numbers of teeth ...	18	19	20	21	22	23	24	25	Maxillary index
Bred	5	23	49	57	36	26	4	0	20·95
Caught ...	1	18	30	48	50	37	15	1	21·52

The difference again is nearly twice the significant level for 200 counts, though here the dispersion is over the same range, whereas the dispersion in the Trinidad material is widely different. It is therefore concluded that bred and caught adults from the same locality differ significantly in their maxillary indices, and therefore the El Socorro and Belem results are excluded from Table III.

(*b*) *Temporal variation.*—This has already been shown (Senior White, 1937) to occur in *A. subpictus* in Calcutta. In the case of *A. aquasalis*, material

from Moruga, on the south coast of the island, was chosen, as this was the only locality from which it had been possible to obtain material prior to the application of D.D.T. to the village. The locality is isolated from the greater part of the local *aquasalis* breeding area by the forested Trinity Hills. *Gene* flow is possible along the coastline both eastward and westward, though along this stretch of coast the hills descend sharply to the sea, and except at Moruga, behind which there is low land for a mile or more before the hills are reached, the only breeding possible is in sand-blocked stream mouths. D.D.T. was first applied one month after the author's first count. A second count was made nineteen months later. The results were :—

Numbers of teeth ...	14	15	16	17	18	19	20	21
viii. 47. Pre-D.D.T. ...	1	1	3	4	5	11	29	27
iii. 49. Post-D.D.T. ...	0	0	0	0	0	0	4	11

Numbers of teeth ...	22	23	24	25	26	27	Maxillary index
viii. 47. Pre-D.D.T. ...	34	50	22	12	0	1	21·72
iii. 49. Post-D.D.T. ...	47	52	49	20	13	4	23·32

The difference is highly significant. It suggests that D.D.T., by killing the human-feeding house entrants, has radically raised the local index, and has led to the evolution of a primarily zoophilic race, conformable in index to that of Trinidad *aquasalis* generally. This point obviously requires further investigation, but there are no more isolated non-D.D.T. contaminated areas in the island. There still, however, remained the possibility of temporal fluctuation and not D.D.T. being the cause of the variance. The analysis of this at Moruga was undertaken by Mr. Simmonds, who requested counts of 100 maxillæ derived from 50 specimens, averaging for each insect, rather than by furnishing him counts of individual maxillæ. The results were :—

Month	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.
Maxillary index ...	22·72	22·82	23·33	22·84	22·82	23·12	22·88

Month	Mar.	Apr.	May	Jun.	Jul.	Mean
Maxillary index ...	22·90	23·15	23·20	23·24	22·76	22·98

The variance ratio (months) = 2·48, p = 1 per cent, least significant difference between means = 0·504 teeth.

Variation evidently takes the form of a temporal fluctuation rather than an orderly seasonal variation related to climate. The limits of the variation, August

22·72, October 23·33, nowise approach the pre-D.D.T. count of August 1947, but the March 1950 count, 22·90, is not significantly below that of March 1949.

But throughout 1949-50 it was obvious that the *aquasalis* population of Moruga was struggling to maintain itself. Notes in Shannon's diaries speak of the pullulating production of the Moruga River, and the extremely high densities encountered. But, during the making of these monthly collections captures have been very small. Had Mr. Simmonds asked for 100 instead of 50 specimens, there were months in which it would have been impossible to satisfy the demand without sending the technician who went down there each month out for more days than could be spared. Even to collect fifty, trap captures (the traps were in very bad condition and were being left to rot) had to be supplemented by outdoor captures on animal bait and the collectors themselves, and in February only 46 specimens could be captured in a three-day stay.

Obviously therefore significant differences between counts from a single locality can be expected in some cases to obscure true geographical differences, and in others, to simulate them where they do not truly exist. In Table III, just significant variations are therefore suspect, but the table shows that as between individual countries, the differences are, when significant, generally of a higher order of magnitude.

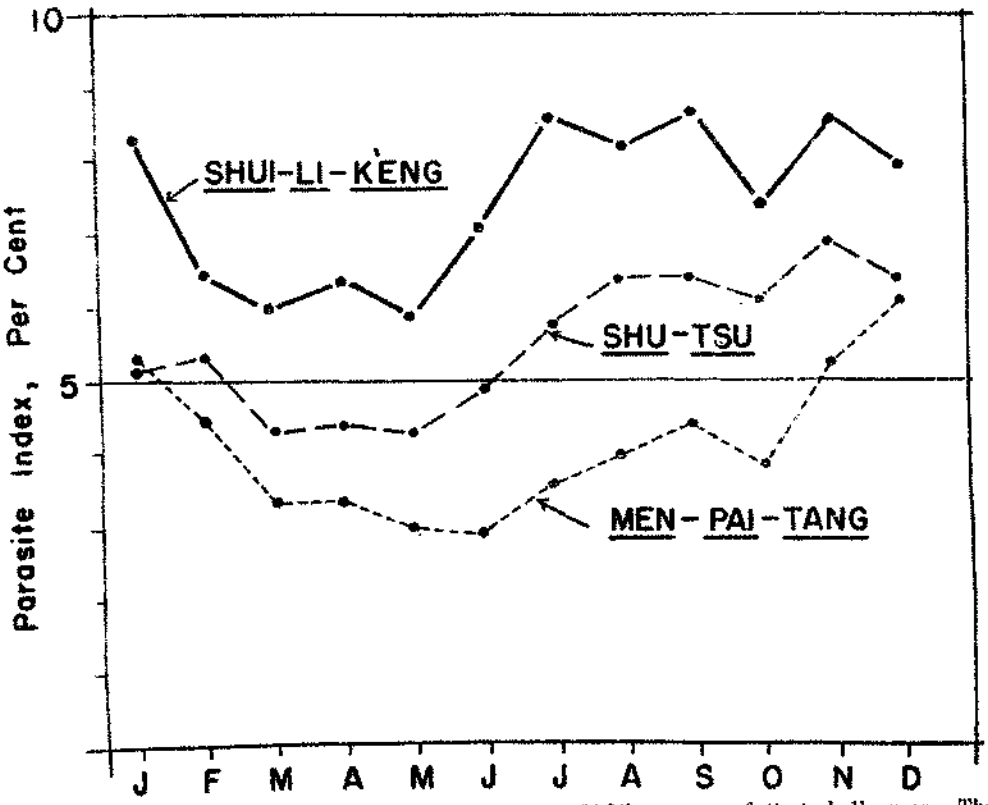
(c) The possibility of variation caused by differences in the host attacked was considered by analysing seven series of 200 maxillæ each of captures on various baits at Curepe, captures on man and horse being made both in traps and in the open. No particular months were covered by this work. The results found were :—

Bait.	Maxillary index found.	Dispersion.
Man in open	22·98	17–27
Ox in dawn trap ...	23·00	19–26
Goat in dawn trap ...	23·12	20–27
Man in dawn trap ...	23·17	18–29
Pig in dawn trap ...	23·17	19–27
Horse in open ...	23·22	20–27
Horse in dawn trap ...	23·32	20–27
Average ...	23·14	17–29

Mr. Simmonds finds that the variance ratio between hosts is not significant, and it is, therefore, concluded that the host species does not affect the maxillary index (M.I.) of that part of a local population drawn to attack it.

It is now possible to proceed to the consideration of geographical variations in the maxillary index. These are set forth in Table III in order of geographical

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	44	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.

p. 164), which in the neotropics seem only to exist in the sub-genus Kerteszia, the point is well made, but it may well be, and is here maintained as showing, that differences in the maxillary index may not only be indicative of food preferences, and thus vectorial capacity, caused by ability or otherwise to pierce the skin of various animals, but also be the external indications of a deeper difference in genetical make-up affecting the entire behaviour of a species or subspecies and clearly indicative of the existence of 'races' within a species, whether or not such be considered by the systematists worthy of sub-specific rank and nomenclature.

Robinson concludes his paper by remarking 'it would also be interesting to know whether or not the maxillary index is inherited in a Mendelian manner'. The author is in full concurrence with this statement, which however could only be investigated in a stenogamic species which can be cage-bred by individual pairs over a long series of generations. *Aedes aegypti* is the only stenogamic species known to the author in which individual pairs will mate. So far as is known to the author, the maxillary index of this species has never been studied. Robinson's last point, therefore, can more easily be investigated in the first instance in the comfort and with the facilities available in European or American Institutes than with the makeshift contrivances available, and more urgent problems which present themselves to the worker in the tropics.

The author expresses his deepest gratitude to Mr. Armitage and Miss Harrison of the London School of Hygiene and to Mr. N. H. Simmonds of the Imperial College of Tropical Agriculture for all the trouble and labour they have undertaken in the course of this part of the present investigation. The thousands of maxillary mounts used in this work have all been made by his technicians, Mr. K. Ahloy and Mr. P. Lee.

SECTION III.

DISTRIBUTION IN TRINIDAD.

The general topography of the island is shown in Map 1 of Downs, Gillette and Shannon (1944). The geological history of the island reveals that it is an aggregation of two formations, and its history since at least the Cretaceous, as tabulated in Senior White (1950), is that the Northern Range, itself an extension of the Venezuelan Cordillera, has since that epoch been unsubmerged, whereas the Southern, and much the largest portion, of the Island has been submerged through many subsequent epochs, and the island only assumed its present size and shape in the Upper Pliocene.

The plain extending across the island at the foot of the Northern Range is stated to cover the former lower course of the Orinoco before the sinking of a section of the Cordillera, which, leaving the four 'Bocas' between the three island summits of Chacachacare, Huevos and Monos, now separates the Paria Peninsula of Venezuela from Trinidad, and, with an uplift south of the Range, forced the Orinoco to debouch into the shallow Gulf of Paria. All the detailed investigations set out in this paper have been made in this plain which, bounded west and east by the Caroni and Nariva Swamps, has only an elevation of under 200 ft. at the water shed of its drainage to the Gulf and to the Atlantic.

Map 4 of Downs, Gillette and Shannon (1944) shows the general distribution of *A. aquasalis* throughout the island, and indicates that the species is only absent from a central area of the island covering the eastern edge of St. George County and the western half of St. Andrew County. All this area falls within the 91–100 inches rainfall belt and is more or less heavily forested, though much of it has been opened for cocoa plantations. There is no point in the island that is a full twenty miles from one or other of the four coasts, and the map shows that there are *aquasalis* records to almost that distance from the nearest of these. Its absence is therefore determined by high rainfall and forest, and not by distance from salt water. But, as Table XXXIII shows, *aquasalis* density rapidly falls off as distance from the sea increases. There is no evidence that the breeding in the central districts of the island is of any public health importance whatever.

Water in noticeable movement is completely inhibitory to *aquasalis* breeding. When larvæ are found in a stream or channel it is invariably the case that they are restricted to pockets of ' dead ' water.

In Tobago, breeding is said to have occurred up to the 500 ft. contour.

SECTION IV.

GENERAL BREEDING ECOLOGY.

(1) EGG-PRODUCTION.

Hocking and McInnes (1948) working with *gambiæ* and *funestus* state that the mean egg number laid appears to be appreciably lower in the wet than in the dry season. This point was therefore examined for *aquasalis* by counting the number of eggs in 25 mature ovaries each month over a year. The results were as under :

A. aquasalis. Egg counts in both ovaries at 82 hours *p.c.*

Month 1949.	Rainfall in inches, at Curepe.	Average content.
Feb. ...	2·45	153·7
Mar. ...	0·14	134·2
Apr. ...	0·17	117·4
May ...	2·65	144·4
Jun. ...	10·28	159·9
Jul. ...	8·09	**110·6**
Aug. ...	13·02	134·9
Sep. ...	6·94	142·6
Oct. ...	7·42	**103·4**
Nov. ...	7·66	**173·2**
Dec. ...	8·66	**174·1**
MEAN ...	...	140·8

S.D. = 23·4 *S.E.* = ±7·3.

Significant differences from the mean are in heavy type. During the very rapid build-up of the population after the onset of the rains, and in October, egg production was significantly below the mean. At the onset of the cool weather,

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 parasitaemia.				
	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. malariae ...	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

Marshes [Section VI (ii)], and the Cressbeds [Section VI (iv)], but the combined results seem best discussed in this section, dealing with physico-chemical factors generally. So little *aquasalis* breeding (and that chiefly of larvæ doubtfully distinguishable from *oswaldoi*) was found during the ricefield surveys [Section VI (iii)] that the relationship between larval production and saline ammonia in rice is not here considered. Both in the Glaudon Marshes and in the Cressbeds, it is certain that the only species concerned was *aquasalis*.

The author's work in Ceylon and in India (Senior White, 1926, 1928) showed that a natural concentration of saline ammonia exceeding 1 p.p.m. was inhibitory to the breeding of several vector Anopheles, but not to the harmless *A. subpictus*, which showed a much higher ammonia tolerance than the various vector species.

Working with *A. tarsimaculatus* (*s.l.*) in Trinidad, Beattie (1932) arrived at the conclusion that ammonia nitrogen had some effect upon oviposition, but no effect upon larval growth. The figures in her paper show generally saline ammonia at concentrations less than 1 p.p.m., though in her ricefield 'D' they went as high as 1·8, but this field produced no larvæ at six examinations. In her general larval surveys only her pond '4', in February, had an ammonia content exceeding 1 p.p.m., and in that month alone it produced no larvæ.

Beattie's results were subjected to statistical analysis by Buxton (1934), and their validity confirmed, though he was cautious regarding the acceptance of her conclusion that ammonia exerted its effect by repelling the ovipositing female.

The results, as regards larval findings, of 239 analyses for saline ammonia are set out in Table X. These show larvæ present up to the single, exceptional, value of $1{\cdot}05 \times 10^5$ (= 10 mg. per litre), of saline ammonia. The percentage of larval positive to total samplings, and of number of larvæ per positive finding, at each step in the tabulations exhibit no regularity whatever. It is therefore concluded that saline ammonia concentration plays no part in regulating either the oviposition or the larval life of *A. aquasalis*.

SECTION V.

DURATION OF A COMPLETE GENERATION.

The author's own laboratory rearings, certainly not under optimum conditions and with less close time-observations than could be desired, show:

	Hours.		
	Minimum.	Maximum.	Average.
(*a*) Emergence to first (unfertilized) feed	7	50	32·5
(*b*) Feed to oviposition (captured hungry specimens) ...	35	83	59·0
(*c*) Oviposition to hatching	48	72	56·0
(*d*) Larval duration	140	576	357·0
(*e*) Pupal duration	36	48	42·0
TOTAL ...	274	829	546·5
(As days) ...	11·5	34·5	22·7

These figures agree well enough with those from the experiments of Deane, Causey and Deane (1948), 11·5 to 39 days. But they are very far from elucidating the duration of complete generation, i.e. from one oviposition to the next.

So far as the observations on mating described in Section VII (ix) go, they indicate that, with *aquasalis* in nature, mating precedes the first blood meal, but whether a nectar meal (Section VIII) is a prerequisite to the mating flight is not certain. This throws doubt on the natural duration of period (*a*). As regards period (*b*) the figures tabulated above were obtained from females captured whilst attacking. Dissections of such indicate that over 90 per cent have been fertilized, but the data in Table XXXV show that hungry females in ovarian Stage I are less common than those in ovarian Stage II, and thus the captured specimens used experimentally may well have been in Ovary II, i.e. the maturation period of their ovaries would be shorter than in *nulliparæ* with ovary in Stage I. Table XXXIV further shows that in this species females frequently seem to require more than one blood meal during a single gonotrophic cycle, and hungry females with ovaries in Stage III are not infrequently encountered and may still more shorten the apparent cycle.

The phenomenon of not taking enough blood to mature the ovaries during a whole night's attraction to an ox-bait trap was examined by a series of dissections at 82 hours *p.c.* (assuming midnight as mean hour of feeding), dissecting 50 specimens on each occasion, save during the exceptional drought at the end of May 1949, when only 42 specimens were taken in the trap in four days. The results are set out in Table XXXVI. They show that on an average 24 per cent of *aquasalis* captured under the above circumstances have failed to attain ovary Stage V after 82 hours. In 19 series of such dissections, 100 per cent Stage V was never found. On an average, therefore, 24 per cent of an *aquasalis* population must feed at least twice during one gonotrophic cycle in order to mature the ovaries. This figure should be compared with the percentage of peaks shown in Table XXXVII to occur at shorter intervals than the minimum developmental period. But with trap captures it is impossible to know how many of these were feeding for the first or for a subsequent time in their current gonotrophic cycle.

It then occurred to the author that there might be a relationship between total night's captures and numbers apparently full-fed, on the supposition that with very great numbers in the trap the bait animal might be so restless as to interfere with undisturbed full feeding. Much time was spent in dividing trap captures into 'empty', 'half-fed' and 'full-fed', on the external appearances of the abdomens. Plotting the results, no trend was found indicating a relationship between the two sets of figures, and why so many specimens given all night in contact with a bait animal to feed remain insufficiently engorged to complete ovarian maturation, is still unknown.

Periods (*a*) and (*b*) are really comprised of the following substages :—

(*i*) Hardening of trophi and (?) flight for nectar.

(*ii*) Mating flight and (?) dispersal flight.

(*iii*) Finding first blood meal.

(*iv*) Maturation of the ovary from Stages I to V.

The duration of substage (*iv*) is further complicated by the refeeding habit above referred to. As periods (*a*) and (*b*) are therefore (in the absence of a natural cage-colony) indeterminate and indeterminable, it has been necessary to fall back on measurement of the total length of a generation deduced from field observations.

Ribbands (1949) has recently set forth such an alternative method of measurement of duration, based upon the average of 100 daily dippings in a breeding place transect, the captures (other than pupæ, retained for laboratory emergence for check upon species) being returned after counting by instars each day. By this method, working with *A. minimus* with a 26 per cent admixture of two other species of the *fluviatilis* group*, Ribbands found an average cycle for periods (*a*) to (*e*) inclusive of 14 days, or 2 to 5 days less than the period estimated by the experiments of Muirhead-Thomson (1940) conducted in as nearly natural conditions as possible by caging *minimus* larvæ in natural breeding places.

This very valuable method was therefore used in the study of *aquasalis*. Just as Ribbands was compelled to work with a mixture of larvæ of the *fluviatilis* group, so the author was (in his first transect) compelled to risk a small but unknown admixture of *A. oswaldoi* (even microscopically only separable with some doubt from *aquasalis* as a Stage IV larva); but the venue of the experiments had to be changed, from the onset of the rains, to an area where, owing to a salinity generally exceeding 200×10^5, there was no fear of the captures including *A. neomaculipalpus*† which commences to become common in fresh water after the start of the rains.

In the author's own experiments, a transect was staked out foot by foot over 100 ft. The technician was provided with 4 sections of a standard kerosene oil tin (covering 81 sq. in.) one of which he placed between each of four successive stakes.

The area within a section was dipped out (including 'muddying'), the larvæ classified and counted by instars, and then returned to the area enclosed by that section of tin. The tin in Foot 1 was then moved to Foot 5, and so forward. Pupæ were retained for laboratory breeding out.

Prior to the onset of the rains the venue of the experiments was an acre of Bordenal Savannah on lease to the Division, since Shannon used it for certain experiments in 1942–1944. As now used, it consisted of an uncultivated riceplot, water in which was (beside rainfall, very sparse save on 8 days) due to seepage from an irrigation channel running nearby. The flora consisted principally of *Cyperus surinamensis*, *Fimbristylis meliacea*, *Eclipta alba* and *Sphenoclea zeylanica*. There was no direct overflow from the channel, flow in which was usually too swift for any local neotropical Anopheles breeding. Temperatures in the pools and

* By naked eye or hand-lens determination no closer separation is possible. Determination must rest, under these circumstances, on the very conspicuous abdominal tergal plates of this group. The specific distinctions require a 1/6th objective.

† *A. oswaldoi* and *albitarsis* were, by this change, also automatically excluded as contaminants. The author did not, pre-rains, find an area, easily accessible for daily work, with a salinity inhibitory to other species, that was not also unfavourable to *aquasalis*. But *A. neomaculipalpus* is too uncommon pre-rains to contaminate the results, which in the fresh water pre-rains transect can safely be accepted as referring only to *aquasalis*, which was the sole species bred out.

puddles in the transect varied from 78° to 96°F., as against 61° to 93°F., along the grassy river bank used by Ribbands in Assam.

The more saline transect, used from mid-June, was in the reed-covered marshes immediately east of the canalized Malik River about 3 miles east of Port of Spain. The transect closely approached, but did not enter the outermost Avicennia scrub of the Laventille mangrove area. The flora in this region of the transect consisted of *Cyperus articulatus, Eleocharis mutata*, and patches of *Paspalum vaginatum*. A few clumps of the fern *Acrostichum aureum* grow on drier earth-banks where apparently attempts at rice-cultivation have been made and failed through too high salinity. In this locality there is a clear line of demarcation between the reeds and the lowest of the now cultivated ricelands. Observations show that the rice-reed frontier is that of the limit of spring tides. Owing to tidal movement and periodical heavy rains run-off, there must be some dispersal of larvæ, but the transect was located nearly at tide-limit, and among this thick growth of reeds tidal force must be very small. It is shown below, however, to have affected the results obtained. Unavoidably, a good deal of trampling of the reeds by the collectors occurred in a narrow belt on one side of the line of stakes marking the transect, and though the reeds crushed down by the insertions of the kerosene tin sections tended to re-erect themselves, none the less the transect had to be shifted a few feet north or south periodically. Six measurements of salinity were made across the transect once weekly, or more frequently when rain flooding had occurred.

They have averaged (pp. 10^5):

Jun.	14	...	689	Aug.	2	...	126	Sep.	13	...	976
	21	...	255		9	...	86		20	...	135
	28	...	401		16	...	32		26	...	236
Jul.	5	...	1,155		17	...	33	Oct.	4	..	115
	7	...	1,540		19	...	36		11	...	28
	12	...	296		23	...	34		18	...	17
	19	...	143		30	...	275		26	...	170
	26	...	543	Sep.	7	...	69				
									Average		321

Temperatures across this saline transect varied from 74·0° to 97·7°F.

There was therefore originally little fear of any of the larvæ counted and returned being of any other species than *aquasalis*, as salinity tolerance measurements with *neomaculipalpus* made during the three general survey periods described in the general study of *aquasalis* distribution showed that in 119 findings of *neomaculipalpus* breeding, yielding 688 larvæ, 90 per cent occurred at salinities of less than 50 pp. 10^5, and none in salinities higher than 150 pp. 10^5. But when, as in August–October, salinity in certain weeks fell to values as suitable for *neomaculipalpus* as for *aquasalis*, the author naturally became anxious, but again no other species than *aquasalis* was bred out from pupæ. Now if the data for these two species as found in the three general surveys (tabulated for *aquasalis*

in Table VI), *with salinity values inhibitory to* neomaculipalpus *omitted*, are treated by the formula for the '*Coefficient of association*' of Mozley (1936), this was found to work out at 0·69 for the rains and 0·74 for the post-rains survey only. According to Mozley this indicates 'an important difference in the requirements of the two species'. It is apparent, therefore, that as between *aquasalis* and *neomaculipalpus* there are biological differences other than salinity tolerances, and whatever these differences may be, they seem to inhibit the latter species from the reedy area of this transect, even when the water in it is as fresh as any that exists on the Caroni Plain, and *on salinity alone* appears to be quite suitable for its breeding.

A. oswaldoi and *A. albitarsis* are strictly species of fresh water. All the pupal emergences again proved to be of *aquasalis*. As noted in Section IV (iii) pupal production was considerably higher than that found in the fresh water transect. From mid-June to end-October, 6,221 larvæ and pupæ were collected, of which 9·4 per cent were pupæ. But the sex ratio was unusual: of 537 pupæ only 33·3 per cent were those of males. There was no differential sex mortality. No reason can be given for this discrepancy. Of 162 pupæ bred from a fresh water area in 1949, 47 per cent were male, a normal ratio.

In the two transects during the 184 days between May 1 and October 31, it was possible to chart fifteen series of peaks running in sequence from Stage I to pupæ. These peaks were distributed as under (Chart 1):

Duration in days ...	4	5	6	7	8	9	10	11	12	13	14
Number ...	1	1	6	3	1	2	3	1	0	0	1

and between them occupied 124 days, giving an average developmental period of 8¼ days from Stage I to * pupa. This figure may be compared with the laboratory breeding figures given at the commencement of this section, when the same periods, (*d*) and (*e*), extended over an average of 16¼ days, with minimum of 7·7 and maximum of 26·0 days. This clearly indicates the adverse effect of breeding under laboratory conditions, which may result in a developmental time twice as long as found in nature by Ribbands' method. The 60-day difference between the total number of days observed and the number of days occupied by the fifteen peaks, suggests that periods (*a*) to (*c*) of a generation cycle together average 4 days. Chart 1 clearly shows how generations overlap, and between July 23 and August 6 it is possible that two generations, one developing more rapidly than the other, culminated in one joint pupal peak after 14 days. Peaks of individual instars not related to sequences are seen to be frequent, and not uncommonly peaks of two instars occurred on the same day. Such 'out of sequence' peaks are indicated by dotted squares on the chart. They are not understood.

If the above calculation is correct, then a complete generation in *aquasalis* occupies an average of 12 days. This figure should be compared with the density

* The above figures and the chart, with the original protocols, have been analysed by Ribbands, using the method of best fits explained in his paper. This method involves the use of the 'mode' and not the 'mean' of the series, and indicates eleven days as the total duration from hatching to emergence, or nearly three days longer than that indicated, as above, by the mean. The difference, which is of importance, will be considered in a subsequent paper.

fluctuations considered in Section VII (xiii), and is a further proof of the amount of multiple feeding in one gonotrophic cycle that occurs in this species.

Duration of individual larval instars.—Considering only the saline transect, the percentages over 5½ months of the totals of the various instars were : I, 4·9 ; II, 11·5 ; III, 25·2 ; IV, 49·0 and pupa 9·4. This is very different from the percentages found by Ribbands, which were : I, 36·5 ; II, 30·2 ; III, 18·1 ; IV, 11·1 and pupa 4·2. Ribbands assumes that rate of development is rectilinear, and charts accordingly. It seems that in *aquasalis* such is not the case. If the instar percentages are a guide to their individual duration, it would appear that Stage IV occupies half the total larval developmental period. If this is so, the return of larvæ after counting each day results in most of those in Stage IV being counted twice, the apparent percentage of that stage rising accordingly.

Bates (1941), considering data from *atroparvus, maculipennis* and *marteri*, shows that there are considerable differences between the number of days occupied by each instar in these three species, and arrives at a theoretical general ratio, assuming continuous breeding and no mortality, of I, 16 ; II, 20 ; III, 24 and IV, 40. Deducting pupal percentages, as given above for *aquasalis*, figures for that species work out at I, 5 ; II, 13 ; III, 28 and IV, 54. These figures suggest that in *aquasalis* Stage IV occupies longer than Marston Bates' calculation for the three European Anopheles *sensu stricto*, and very much longer than for the three Myzomyias studied by Ribbands, but the error introduced by counting Stage IV twice had still to be discounted.

An attempt was next made to evaluate this error. In the transect area two ponds, each 10 ft. × 4 ft., were isolated by surrounding areas of reed with side walls of sacking. This permitted of fairly free water movement through the ponds, but held back any larvæ that, in the transect, were liable to be drifted out by flow from higher ground above or by tidal movements. At the start, both ponds were rendered larva-free : Pond A, by petrolization at the rate of 1·5 c.c. per sq. ft., Pond B, by daily dipping out until it remained free of all breeding for 48 hours. There was the danger that petrolization in A killed out predators in addition : equally a few predators may have been thrown out with the larvæ in B*. In Pond A, all Stage IV larvæ and pupæ were removed as found, in Pond B, no stage was removed. Daily dipping continued for two months. The results were as below, and up to Stage III the figures are remarkably similar :—

Pond.	Larvæ numbers.				Pupæ.	Total.	Larvæ as percentages.				Pupæ.
	I.	II.	III.	IV.			I.	II.	III.	IV.	
A ...	794	465	323	163	13	1,758	45·2	26·4	18·4	9·3	0·7
B ...	790	519	362	251	48	1,970	40·1	26·4	18·4	12·7	2·4

* During this work it was discovered that Lathrocerus species are voracious predators on both larvæ and pupæ, and these species were common in the reeds. A study of the bionomics of these two Hemiptera has been started. Both species are, however, extremely cannibalistic.

It is obvious that the daily removal of Stage IV and pupæ causes a big decrease in the numbers in these two instars, which the author considers proves that in daily dipping and returning work, as done by Ribbands and by himself, many Stage IV and pupæ (if not removed) get counted on two consecutive days, resulting in a 137 and 357 per cent increase in the numbers in these stages over those found by daily removal counts.

Most striking, also, is the complete reversal in the stage percentages in the ponds as compared with the transect. This indicates how many of the younger *aquasalis* larvæ are washed away by water movement, though why this is not masked by 'in-drifters' to an equal extent is obscure. The fact remains that the instar percentages approximate to those found by Ribbands. Now his experiment was also conducted in a transect where there was water movement. The conclusion to be drawn is that whilst *A. minimus* can keep place against slow water movement, *A. aquasalis* cannot.

Peaks of the various instars occurred in the ponds at such widely varying intervals that it is concluded that they are related to random oviposition in such small enclosed areas, and indicate nothing.

SECTION VI.

DETAILED BREEDING AREA STUDIES.

The natural plant succession on the Caroni Valley Plain appears to have been (*a*) the coastal mangrove zone; (*b*) beds of salt tolerant grasses and reeds*, chiefly *Paspalum vaginatum*, *Eleocharis mutata* and *Cyperus articulatus*; (*c*) dry land forest gradually increasing in height and density as the high rainfall belt of the centre of the island was entered (Beard, 1944). But with the opening up of the plain for cultivation, the dry land forest lying behind the reed belt has long since virtually disappeared. After the arrival of (East) Indian immigrants in the 1860's, the lower patches of the non-saline lands adjacent to the reeds have largely been opened up for rice cultivation, a process which is being increased by Government reclamation and irrigation schemes centered upon a dam in the Caroni River some twelve miles above its mouth, i.e. just above the limit of tidal influence, and by the regulation of the Tacarigua River descending from the Northern Range north of the area commanded by the Caroni Dam. Other patches of riceland have been recovered by embanking against salt water along the lower reaches of the Caroni, down-stream of the dam, up to the fringes of the mangrove, which areas are inundation-irrigated by temporary dams erected by the cultivators along the plains lengths of the St. Joseph and St. Juan rivers, which also descend from the Northern Range into the Caroni. This flood irrigation, together with hill-foot springs conducted in channels, also serves for the intensive vegetable cultivation below the hill-foot carried out by Indians for the Port of Spain market, and renders the whole plain a vast *aquasalis* breeding ground.

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* Professor Baker considers that these reed belts may be a fairly permanent succession to mangrove deforestation and not a natural climax.

Intermingled with the riceland in the west, but further east in almost pure stand, are the sugarcane lands that extend up to the original rain forest or its replacement by cacao plantations.

Three of these major vegetational types were taken for study: (*i*) the mangrove, (*ii*) the reeds, (*iii*) the ricelands. In addition, (*iv*) the watercress cultivation centered upon a hill-foot seepage belt situated above the reeds, and (*v*) the upper reaches of the Maracas River, down to the hill-foot near St. Joseph, were subject to long-term survey. The latter was studied since there is a certain amount of presumably *aquasalis*-caused malaria in this and other similar valleys, the causation of which was attributed by Shannon not to local breeding, but to mass influx of *aquasalis* from the Caroni Plain. This phenomenon is considered in Section VII (xvi). The canelands require no detailed consideration. They are deep drained to render the land surface dry, and in the drains themselves very little breeding has been found.

These five types of breeding areas will now be separately considered.

(i) THE MANGROVE.

The zonation of the plants of this formation is there much less clear than has been described by Chapman (1939, 1940) in Jamaica, by Davis (1940) in Florida, or by Muirhead-Thomson (1945) in West Africa.

The normal landward succession of the western shores of the Atlantic is stated to be :—

Rhizophora mangle (red mangrove)—inundated by all tides, but not tolerating an average flooding of over 2 ft., though it can tolerate persistent submergence of 1 to 2 inches above the plumule.

Avicennia nitida (black mangrove)—flooded by the majority of ordinary tides, and by spring tides and some neaps. It will not establish itself when the average water depth exceeds 6 inches.

Laguncularia racemosa (white mangrove) is regulated by the same average water depth as the preceding species, but is normally only flooded by the higher spring tides.

Conocarpus erectus (button mangrove) is normally washed only by abnormal or extremely high equinoctial spring tides. Unlike the two preceding species, it has no pneumatophores.

Ground vegetation (Salicornia) does not appear until the Laguncularia zone is reached, and is not seen in the Laventille area. Muirhead-Thomson makes no reference to species other than Rhizophora and Avicennia in his West African paper.

In Trinidad, in localities where there has been no interference with natural conditions (as on the south coast of the Cedros Peninsula), these zonations are succeeded by the 'swamp fern', *Acrostichum aureum*, and finally by the 'gru-gru' palm, *Acromia aculeata*, the spines of which render penetration of the mangrove within them in places impossible. Along many parts of the coast line,

owing to sand dunes, the zonation is reversed, the mangroves lying inland of the palm and the fern being laved by the tides entering by upstream inlets, but on the coast at the edge of the Caroni Plain the Rhizophora facies extends into the sea. The Laventille swamp, in which the studies to be detailed were made, has been so subjected to human interference in the form of abortive drainage channels, ill-considered fellings, etc., that the zonation is far from clear. Muirhead-Thomson, inspecting this mangrove with the author, is of the opinion that all the growth is secondary, so naturally, no clear succession can be made out.

Islands of *Avicennia* as described by Muirhead-Thomson (*loc. cit.*) are found among the Rhizophora, and though *Laguncularia* exists, it is everywhere intermingled with the *Avicennia*, and searches for a pure-stand of the former were unavailing, a fact verbally confirmed to the author by Professor Baker of the Imperial College. *Conocarpus* has not been encountered by the author (it is common along the fringes of the Oropuche Swamp south of San Fernando). *Acromia aculeata* does not appear to be anywhere in actual contact with the Laventille area mangroves, though there is some of it in the neglected coconut plantation referred to in Section VII, less than a mile inland from the present eastern *Avicennia* face. *Acrostichum aureum*, its spotty growth on old bunds clearly indicating its hygrophobia, is only represented by a few plants as referred to in Section V.

The study of the mangrove area was attempted in pure-stand quadrats of 25 × 25 feet. Four areas were chosen, of Rhizophora, Avicennia, one erroneously believed to be of pure Laguncularia, though when flowering it was found to contain that species intermingled with Avicennia, and an area from which all vegetation has disappeared, save for isolated clumps of Avicennia and young plants of Rhizophora, though old stumps indicate that it was formerly in mangrove, and from the shape and extent of the clearing, it is considered to be artificial, but various local naturalists confirm that they have seen areas of mangrove completely die out from natural causes. When first found in 1947 this area, of some acres in extent, was covered with *Paspalum vaginatum*, but by 1950 this had entirely disappeared, and the area was an open stretch of usually submerged mud, with isolated Avicennia bushes and a scattered re-growth of young Rhizophora. At this juncture further human interference supervened. de Verteuil (1933, Plate IV) figures a canal south of the railway and immediately north of the mangrove fringe. In 1947-48, this canal had degenerated into a shallow, partially blocked ditch, over which hill-foot run-off through the suburban area along the Eastern Main Road in Success Village passed hardly diverted into the mangrove. At the moment when these quadrats were brought into regular examination the Public Works Department proceeded to re-excavate this canal to a depth of over six feet. In so far as the P.W.D. proceeded with this work, this re-excavation cut off the hill-foot from the mangroves, and restricted the water within them to tide entering from another derelict west to east channel, 'Chancellor's Cut', lying further south. But thanks to the non-completion of the re-excavation, a long pool remained just north of the supposed Laguncularia quadrat, just within the mangrove face, subjected both to hill-foot run-off and to tidal influence from the south. No quadrat could be demarcated within it, as its extent varied from week to week, but it was simultaneously put under study, as containing a mixture of water of sea and land run-off origin.

Breeding in crab-holes was searched for, as *A. albimanus* is recorded as affecting this type of habitat. Neither within, nor in the sunlit Paspalum-covered mud outside the mangroves was any such breeding of *aquasalis* found, though Deinocerites species and a Culex were commonly found in crab-holes in both areas. The results of these examinations are set forth in Tables XI to XV.

So few detailed and continued studies of breeding in mangrove have been carried out (the author knows of none from the Oriental Region) that it is felt that the individual results are worth presentation in full, as they may be of value to ecologists other than those interested in malaria only. For this reason salinities are presented in the normal 'parts per thousand' notation of marine workers, rather than the parts per 100,000 notation peculiar to the water analyst working for the medical profession.

Comparing these local secondary growths with mangrove elsewhere, Muirhead-Thomson was struck with the amount of muddy silt in suspension, which is contrary to his experience of virgin mangrove in West Africa. Salt is supposed to precipitate silt, why it does not do so here would be an interesting subject of investigation to water chemists. The quadrats were designedly chosen as close as possible to the mangrove face in that area of the Laventille Swamp which the broad surveys of 1947-48 had alone showed as breeding Anopheles. Muirhead-Thomson (*loc. cit.*) has also shown that it is mangrove areas in the vicinity of tidal limits which are most productive of breeding. Table XI therefore commences the series with the study of a pool almost at the forest edge near the north-east angle of the swamp, affected by fresh water run-off from the land proper. The apparent higher salinity in this pool than that in the nearby quadrat is caused by the absence of examinations taken during weeks when it formed a continuous sheet of water with the mixed stand quadrat set forth in Table XII. Neither present any particular features of interest. The density of the pool per positive (wet) examination (per dip figures are throughout impossible owing to examinations with puddles only involving picking larvæ off the surface with a pipette direct) is high, but the pupal percentage is the lowest of all the five areas examined. The quadrat shows a lower density but a high pupal percentage.

The pure Avicennia quadrat (Table XIII) was between one-quarter and one-half a mile nearer the sea-face than the two areas just considered. The re-excavated canal reached up to it, and diverted hill-foot run-off. Its average salinity presents no striking differences from them, it shows the highest per examination larval density, but a much lower percentage of pupation than is shown for the area generally in Table VII.

The Rhizophora quadrat was at exactly the same distance from the sea-face as the Avicennia but situate about 100 yards south of it, as soon as one could pass into an area of pure-stand Rhizophora. Generally, as the known ecology of the species calls for, the water stood deeper on it, and for eleven more weeks of the year, than with Avicennia further inland. Its per examination density is half that of the adjacent Avicennia quadrat, but its pupal percentage is double. It is evident that in this Laventille Swamp, the Rhizophora areas have to be treated seriously, and not dismissed as harmless, as in West Africa (Table XIV).

The quadrat (Table XV) that alone produced *Ruppia maritima* was the best part of a mile nearer the sea-face than the previous pair. When first found in 1950 it was a stretch of bare mud from which the Paspalum cover of 1947-48 had disappeared, with isolated bushes of fairly old Avicennia and Rhizophora seedlings, rather widely spaced, as stated earlier. Its salinity was at intervals, in spite of tidal incursions, very low, and it had returned to a very low value in the last fortnight of the year. No sign of springs could be seen but such appear the most likely explanation of this very fresh water. *Ruppia maritima* was first noticed on February 22, and was then thought to be the first signs of the regrowth of the Paspalum, for the disappearance of which between 1948 and 1950 no suggestion can be offered. The growth of Ruppia was killed off above ground by the dry season, and only recommenced with the rains in the third week in June, but it was not present in January. Whilst the plants were young and short it will be seen that they were periodically submerged by the tides, but by August the plant had grown so that it was always in contact with the surface, and usually the tips formed floating mats (it is never emergent other than the flower stalks). The plant occurs in isolated patches, sometimes on bare mud, sometimes around mangrove plants. Its growth is almost sharply defined by the outermost bushes of the mixed Rhizophora-Avicennia forest surrounding this peculiar area, suggesting that shade is inimical to it. The Rev. Father Graf of St. Mary's College, Port of Spain, informs that the plant has long been known to him from some of the drainage channels through the Caroni Marsh south of that river in the Bejucal area. These canals exhibit violent fluctuations in salinity according to whether they are carrying off flood water from the higher lands to the east, or the tide is pressing up them.

On June 13, when the Ruppia was very short and sparse, very large numbers of *Trichocorixa verticalis* were noticed swimming over the bare mud. Three weeks later the shoots were seen to be covered with a brown deposit, which proved to be closely apposed eggs of the Hemipteron, and in that week the adults were not in evidence. Microscopic examination of the shoots showed discoloured spots, with hyphæ of a fungus emerging from them. These spots appear to be the sites of attachment of the Trichocorixa eggs, though they may originate from punctures made for feeding by the nymphs and adults. In either case, the effect on the Ruppia is adverse, the plant turning brown, though some of the discoloration may be due to silt in suspension being deposited on the shoots roughened by the eggs or the punctures. There appears to be a relationship between 'browning' and multiplicity of Trichocorixa eggs, but the whole subject of the relationship between Ruppia and Trichocorixa obviously calls for an intensive piece of ecological investigation for which no personal opportunity or suitably trained staff to undertake has been available. None the less, the relationship is malariologically important. *A. aquasalis* larvæ in this denuded area are not entirely but largely confined to the Ruppia mats, and in the author's opinion the plant represents a definite malaria hazard, and it will be necessary to study the history of the growth of Ruppia in this patch over many years. Whether the Hemipteron is of value in restraining the growth of Ruppia is a matter for detailed investigation. Apart from the transient appearance of Spirogyra and Cladophora in the early spring, the only other plant commonly found (except diatoms) was the chlorophyceous *Prasiola ? crispa*. This is a North American alga and was hitherto unknown from the neotropics, though a chance-found reference in an account of the work of the

R.R.S. 'Discovery II'd.' records it as occurring in the South Sandwich Islands between Cape Horn and the Graham's Land Peninsula of Antarctica! This genus of alga also occurs [Sub-section (iii)] on mud in ricefields where there is no more than a mere trace of salinity, and if only one species is found locally, its tolerance of changes in salinity is immense. It is apparently an acceptable larval pabulum.

A tabulation of the salinities in the five areas studied, week by week (which is not reproduced as the actual data are all in Tables XI to XV), whilst generally showing parallel fluctuations of the same trend, reveals also some striking differences, and it is concluded that there is nothing in the nature of a uniform ebb and flow of salt-water through these mangroves, either from the sea-face south of Port of Spain, or northward from tidal changes in Chancellor's Cut, entering the mangroves a mile or more further south of the areas examined. In the absence of close-placed detailed levelling the movement of the tidal streams cannot be deduced from the salinity changes, even though each single day's samples were all taken within two to three hours of one another.

As regards *aquasalis* production, as was to be expected from the results set out from the first 1947-48 surveys in Section IV (ii), this is seen to be the highest at the lower salinity values, but it has to be remembered that larvæ may have been washed into values where they had not originally bred.

Salinity per thousand.	1–10	11–20	21–30	31–40	41–50	51–60	61–70
Times found	58	51	35	15	10	1	2
Times with larvæ	15	42	23	8	2	1	1
Percentage of total findings ...	88	82	66	53	20	100	50
Total larvæ	652	642	470	52	5	2	2
Larvæ per positive find ...	13	15	20	7	2	2	2

The Government of Trinidad has on more than one occasion contemplated or even commenced work on the recovery of these mangroves for an extension of the City of Port of Spain, up to the eastern boundary of which they extend. This year's work is eloquent of the danger to health that further interference with them would evoke, unless the engineers are placed directly under the control of the malariologists in all their procedures. Gilroy (1948) has set forth from his own large-scale recovery work in West Africa methods by which mangrove can be reclaimed without engendering an increased malaria hazard, and a prerequisite to any further plans by the local Government for the recovery of the Laventille area mangrove must be a study of Gilroy's book by the engineers charged with the preparation and carrying out of any scheme, always, it may again be emphasized, in continuous consultation with the Health Department of this colony.

Promiscuous fellings (largely unauthorized) in this mangrove were suspected of increasing the malaria hazard. Accordingly, from June to December, twelve general surveys were made, each including an equal number of dips in areas the

fieldmen classed as being in 'shade' or in 'sunlight'. The results were very similar, save that in shade pupæ formed 7·1 per cent of a catch of 381, whilst in sunlight pupæ formed 11·7 per cent of a catch of 393. The total per dip density averaged 0·07 only, which clearly brings out the generally low productivity of even secondary mixed mangrove.

(II) THE OPEN MARSHES.

These were studied in the vicinity of Glaudon Village, on the south bank of the Caroni, about 2 miles east of the mangrove face. Between the village and the marshes is a strip of slightly higher land, surrounded by a low embankment, which formed part of another defunct reclamation scheme, but which still serves to exclude salt-water and permits of the cultivation of a small area of rain-fed rice which, with some coconuts, is the *raison d'être* of the village. On the east a deep drain separates the marshes from sugarcane lands. Looking southwards, the marsh, covering some 12-13 sq. miles, extends beyond the horizon, a solitary tree here and there denoting higher spots of land. The Caroni River here is brackish, and the tables show that the soil of the swamps contains much salt, which dissolves into the surface water as soon as the rains cease off. The usual black glutinous mud of salt swamps renders movement difficult, but examinations were made some yards south of the old embankment, and it is hoped are typical of the marsh generally.

In this marsh seven quadrats, each 9′ × 9′, were chosen and examined fortnightly from February 1948 to January 1949. At the time of selection there was no reason to suspect that this natural marsh flora, apparently a stable formation, would show seasonal changes, but it was later found that *Eleocharis* shows a definite tendency to invade *Cyperus* areas, and, therefore, to study breeding within a pure stand it is necessary to tabulate as one area results from different quadrats at different times of the year. The quadrats were designedly kept small, so that the author's then restricted staff could dip them out thoroughly, whilst simultaneously collecting so far as possible all other macrofauna found in them, in order to study any possible relationships between larval production and possible predators, but, owing to their usually much greater agility, sampling of the latter was only qualitative. Larvæ were returned after counting and staging, but pupæ were retained for breeding out to make certain (as proved to be the case), that there was no other anopheline in the marsh fauna than *aquasalis*. *A. oswaldoi*, *albitarsis* and *neomaculipalpus* were taken in nearby adult searches, but these fresh water species must have originated from within the salt-water exclusion embankment above referred to.

Glaudon Village has a neglected and almost disused earth well, liable to dry out completely in the dry season, about 7 ft. in diameter, with nearly vertical sides and an earth curb, grass covered. This well is within the area protected by the afore-described embankment. Save that it may contain a higher concentration of salt, breeding in this well is fairly representative of crop irrigation wells covered by the extensive surveys of 1947-48, and though not directly germane to the study of the marsh, is included for consideration with other areas studied.

The results are set forth in Tables XVI to XX for the four dominant species of the marsh flora and for the village well. Though the swamp appears to be of uniform level, this is seen actually not to be so, though the west-east traverse

of the quadrats did not exceed ¼ mile. The *Cyperus articulatus* and *Eleocharis mutata* quadrats were continually dry over six examinations from mid-March to the end of May. The *Paspalum vaginatum* quadrat was dry over two examinations in the second half of March, and another two in the first half of May. Between these two dry periods it had water on April 13. After December 22, the *Cyperus* quadrat again dried, though none of the others did so, and the summary subjoined shows that the *Nelumbo amazonica* quadrat carried water, and of a greater average depth than the others, throughout the twelve months. In descending order of elevation (though not of linear distribution), therefore, is found *Cyperus*, *Eleocharis*, *Paspalum* and *Nelumbo*. In the prolonged drought of 1949 the whole marsh dried off, as indicated by the complete absence of adults in Glaudon Village throughout April and May of that year (Table XXI).

GLAUDON MARSHES.

Summary and averages of 12 months fortnightly surveys.

Plant.	Number of wet observations.	Average depth water, inches.	pp. 10^5.			Average larvæ and pupæ per 100 dips.			
			Average temperature, °C.	Average salinity.	Average XH_3	Stages I-II.	Stages III-IV.	Pupæ.	Pupæ percentage.
Cyperus ...	18	5	27·3	493	0·082	100	81	4	2·3
Eleocharis ...	20	6	27·4	684	0·065	87	37	1	1·0
Paspalum ...	22	5½	28·2	997	0·080	59	58	2	1·4
Nelumbo ...	26	9	28·1	955	0·024	6	2	0	0·0
Village well	25	...	27·0	261	0·096	141	45	3	1·6

The more low lying the quadrat, therefore, the higher the salinity. Water underlying the tall reeds is on an average nearly a degree cooler than under the open tillering *Paspalum* and the procumbent *Nelumbo*.

The anopheline output of any area is best judged by pupal numbers. Using this criterion with reference to Tables XVI to XX :—

Cyperus.—After reduction to nearly fresh water by July 22, there was a rise in salinity not associated with decreased depth in August, followed by nearly fresh water again until the equinoctial tides at the end of September greatly raised the salinity, except over the neaps of mid-October, in spite of a continuation of rainy weather. Pupæ were present just before the quadrat dried off in March, and through eight weeks from the end of June to early August. Thereafter, save on September 16, pupæ were not found for the rest of the year.

Eleocharis.—This area closely followed *Cyperus* in regard to salinity changes, but the rise in early August was over one fortnight only, and the reduction between

the two increases of August 5 and September 30 was not so marked. Pupæ were found prior to drying off, but not again until the last weeks of October and November, thereafter ceasing.

Paspalum.—Except for the very marked drop between the two sudden rises attributed to the equinoctial tides salinity showed less marked fluctuations here than in the two more elevated quadrats. There was an outburst of pupation prior to the spring dry-off, and this recommenced as soon as the area was submerged by mid-June. Pupal production was then sporadic to mid-September, after which none were found.

Nelumbo.—Showed the two equinoctial salinity rises, but, like *Eleocharis*, with a less marked fall between them. There were no pupæ found throughout the year, which in the light of the very low larval density was to be expected.

Village well.—The old embankment and its own earth plinth preclude the invasion of surface water save under exceptional rain flooding, which only occurred in 1949, and not at all during the 1948 surveys. There was, however, a transient rise in salinity on August 19, which otherwise only showed in the elevated *Cyperus* quadrat, suggesting a wave of near-surface percolation of saline water, if this be possible. Pupal production occurred between June and August, and also at the end of December, when none of the marsh quadrats was yielding pupæ.

Predators.—The following possible insect predators of larvæ were found:

DIPTERA.

Ephydra sp.
Pentaneura sp.
Tabanus sp.

COLEOPTERA.

Berosus geayi
B. truncatipennis
Lissorhopterus foveolatus
Laccophilus ovatus
Hydrocus sp.
Tropidosternus ovatus
Hippodamia sp.
Hydrocanthus debilis
Suphicellus nigerrimus
Paracymus armatus
Enochrus melanthus
Phaenonotum dubium

ODONATA.*

Anax amazili
Orthemis ferruginea
Erythrodiplax fervida
Ishnura ramburii

RHYNCHOTA.

Tenagobia signata
T. socialis
Tricoriza verticalis
Mesovelia sp.
Buenoa sp.
Microvelia robusta
M. longipes
Microvelia sp.
Lathrocerus micantula
L. anura
Delphacodes sp.
Ofthea sp.
Pelocoris femoratus
Cyclorrhinus sp.
Tenagogus guerini

* Only nymphs are tabulated: adults caught around the quadrats may have bred in the saline reeds or in the fresh water across the old bund.

But probably none of the insects equal the fish *Lebistes reticulatus*, and a few *Polycentrus schomburgkii*, as larval predators. Fish, however, were not very numerous. As regards larval output, it was quite unexpected to find that *Cyperus* exceeded the other marsh quadrats both in its total pre-imaginal and its pupal output. There would thus appear to be an inverse relationship between average water depth and larval output. The figures in the tables show saline ammonia frequently at what are accepted as inhibitory values for vector anophelines, in the light of the work of both Beattie (1932) locally, and the author in Ceylon and India. But in that sub-continent the author has shown (Senior White, 1928) that the wholly zoophilous *A. subpictus* is not inhibited from breeding at concentrations which are inhibitory to even the primarily zoophilous *A. culicifacies*. *A. aquasalis* is, in Trinidad, a feebler vector than the latter, and appears to tolerate ammonia as well as the former. The correlation in these marsh surveys between NH_3 concentration and larval production is $r = 0{\cdot}17$, which is negligible.

The use of small single quadrats to represent vast breeding areas is obviously unsatisfactory, but was enjoined by the numbers of field staff then available. As has been pointed out by Shannon (1942) *Nyssorhynchus* group larvæ are never found at the per dip densities of the species of the Old World. Their total numbers are enormous, but this caused by the catholicity of their breeding, rendering a low 'per dip' density the equivalent of a 'per area' output far exceeding most of the species of at least the Orient. In connection with these larval surveys an outdoor adult resting catch was made during each larval survey in a patch of *Acroceras* sp. grass close to Glaudon Village. These catches were continued over 1949, and are set out in Table XXI.

Comparing 20 fortnightly adult catches between March 29, 1948 and January 20, 1949, with the simultaneous total pre-adult densities in the quadrats a correlation of $r = 0{\cdot}65$ was found, remarkably high considering the minute area larval-sampled and the fact that there is much other suitable adult resting scrub around the village, which forms the sole close-by mammalian blood supply for all the marsh output within flight range. Yet the high female densities shown in Table XXI only result, on an examination carried out by Dr. Gillette in March 1948, in a spleen rate (including P.D.I.'s) of 10·9, and a nil parasite rate. By Indian methods of splenometry, the rate would have been 4·3 only. The explanation lies in the amount of animal deviation (Section VIII) that here occurs. The man : ox + horse ratio at Glaudon is 2 : 1, and Table XXXVIII shows that of 97 positive stomach smears from outdoor captures of *aquasalis* around this village (which is not D.D.T'd) during 1948 only one was positive for human blood. Animal diversion here is therefore nearly complete, and so long as the villagers maintain their bovine animal husbandry, they require no other form of malaria protection.

Comparison of breeding among reeds in Glaudon and Malik marshes.—The observations in the transect in the Malik marshes described in Section V indicated a similar degree of breeding density to that in the marshes at Glaudon. The flora of the Malik marshes is in the main *Eleocharis mutata*, with an admixture of *Cyperus articulatus*, therefore Glaudon Tables XVI and XVII have been taken for comparison.

But it is seen that, on the basis of pupal findings, the adult production of the Malik marshes is very much higher than those at Glaudon. In the absence of measurements of densities of larvivorous fish and other possible predators, no explanation for the difference in anopheline productivity can be advanced. The

Area.	Dips.	Larvæ and pupæ.	Density per dip.	Percentage of pupæ.
Glaudon ...	925	541	0·6	1·7
Malik ...	14,200	6,221	0·4	9·3

average salinity in the two Glaudon quadrats was 567, as against an average of 321 in the Malik transect. On the findings set forth in Section IV (ii) the Malik transect was on an average more favourable to *aquasalis* production than the two Glaudon quadrats, but this is not supported by the larval results from these two small areas.

(iii) RICEFIELDS.

Prior to the arrival of (East) Indian immigrants, there appears to have been no rice growing in Trinidad. At first, apparently, the Indian who had finished his indentures and settled on the land grew only sufficient rice for his own needs. But the enormous increase of population of late years, and the rising price of British Guiana rice, caused the Indian to produce increasingly for market, and the demand arose that Government construct irrigation schemes. One such is in being, with headworks about twelve miles above the mouth of the Caroni River, from which a main left bank feeder channel commands 5,000 acres (including areas still under reclamation) of the Caroni Plain landward of the saline marshes described in Sub-section (ii). The Caroni River runs in a flood plain, with low banks, and there is no great impounded stretch on the river above the barrage to store water against seasons of short precipitation. There is no right bank channel, but the Tacarigua River, descending from the Northern Range to join the Caroni about a mile below the barrage, has been canalized for half a mile in its lower length and fitted with a flood-gate, water held up by which is passed into subsidiary channels serving some 300 acres of riceland in the Freeman Road area, in which Beattie made many of her ricefield observations of 1932 (her fig. 2, 1X). A little further east, there is inundation irrigation from an indefinite stream passing through the Streatham Lodge area (Beattie's fig. 2, 2X). This stream has a much less constant flow, being in its natural course with no flood-gate, and, as the tables show, these fields appear to be generally short of water, even during the rainy season.

South of the Caroni Plain, among the sugarcane lands, there are two other types of riceland: (*a*) Shallow ravines divided into fields by low earth embankments, the course of the original stream being lost in the fields. Such ravine streams have very short catchments, and their water supply is precariously dependent on day-to-day precipitation. The strong seepages which feed, and render so dangerous, similar ravine fields in the Indian hills, are absent. (*b*) Flat areas, such as are common on the plains of Peninsular India, with low embankments to individual field-squares, water depending solely on the conservation of immediate local precipitation.

Six fields were chosen for weekly study :—

(*i-ii*) Inundation fields in Streatham Lodge area.

(*iii-iv*) Canal irrigation fields in Freeman Road area.

(*v*) A terraced ravine near Carapichaima.

(*vi*) A flat plain field, in the same vicinity as (*v*).

The results are set out in Tables XXII to XXVII. It will be seen that the inundation fields only carried a steady and reasonable depth of water for 5 to 6 weeks in July-August. For most of the remainder of the growing season the rice grew among shallow puddles only, and was found quite surface dry on several weekly visits, though water was then found at a few inches only below ground. Transplanting to harvest occupied 17 to 18 weeks. The irrigation fields carried an adequate water supply, except on two occasions when the embankment of the canalized river breached, up to about a month before harvest, when the main gate was opened and the fields were deliberately dried off. In one field harvest was carried out at 19 weeks, in the other, at 16 weeks.

The ravine field is seen to have had a most precarious water supply, but the growth appeared good, and harvesting occurred in 17 weeks from transplanting. The flat rain-fed field carried an adequate supply of water throughout the season. Harvesting was carried out at 18 weeks from transplanting.

In all the areas studied, nurseries appeared to be communal rather than individual, the growers apparently purchasing seedlings from the nurseries of those whose lands are favoured with an early water supply. The plants are usually quite tall at transplanting, up to 12 inches height, and individual seedlings (not clumps, as in India) are set at nearly 12-inch spacing. Vacancies were not filled in : the author gathered that the seedlings were in too short supply, though this is not usually the case.

Saline ammonia fluctuated violently, again serving no more than to indicate that with these neotropical species it possesses none of the regulatory effect of this factor on the clean water vectors of the Orient.

The pH of the water was frequently acid, in the flat field (*vi*) nearly always highly so, unless algal growths were filling the water with dissolved oxygen, as observable from the remarks columns of the tables.

Owing to the impossibility of distinguishing young larvæ of *aquasalis* from those of *oswaldoi* the species of Anopheles breeding in the fields was much less certain than one could desire, but the results may be tabulated as under :—

Month.	Times found.				
	aquasalis.	*oswaldoi.*	*tarsimaculatus*-group.	*albitarsis.*	*neomacculipalpus.*
Jun. ...	2	0	1	0	3
Jul. ...	6	1	6	9	3
Aug. ...	2	0	4	8	4
Sep. ...	0	0	3	7	2
Oct. ...	0	1	5	11	2
Nov. ...	1	2	0	2	2

Of the total larvæ surviving transport for laboratory examination there were found :—

Species.	Number determined.	Percentage of total.
aquasalis	41	2
oswaldoi	12	0·5
tarsimaculatus-group ...	292	15
albitarsis	1,343	67
neomaculipalpus ...	301	16

These small findings indicate that the much the largest proportion of the rice-fields production is of *albitarsis*. Not only were definitely identified *aquasalis* larvæ rare, but in the rice its breeding was virtually confined to June–August (the November record is 1 larva). This is in accordance with Beattie's (1932) findings. The author had hoped to be able to correlate the species being produced with the percentage of incident light falling on the water compared with that measured simultaneously above the growing plant, but the photometer went out of action at the end of August, just as the rice was really commencing to shade the water. Much further study will therefore be required before it can be decided whether the autumnal absence of *aquasalis* in ricefields is the result of reduction of light incidence, or due to some chemical change in the water itself, to which neither the ammonia nor the pH values afford any clue. The oxygen values, as indicated by the pH, apparently depend upon algal growth, and in shallow puddles there should be free interchange with atmospheric oxygen, so the regulatory factor for *aquasalis* breeding would appear to be either a light reduction effect, or in the nature of inhibition to ovipositing females either by tall rice, or changes in the water which the saline ammonia and pH analyses do not indicate. Nothing is yet known of the ovipositing habits of any of the species found in this work.

Beattie (1932), working in the Streatham Lodge area, found that *tarsimaculatus* larvæ were far more frequently found in the canals than in the fields themselves. This is entirely contrary to the author's experience in 1950. The main canal was examined every week in which it contained water up to grass level. When the gate was open or the bank breached the flow was swift, over bottom mud, and obviously unsuited to *aquasalis* breeding. Not a single larva of any species was found. Ammonia and pH values were so similar to that in fields which the canal fed that they have not been considered worth separate tabulation. Searches in subsidiary channels both in the inundation and the canal areas in September yielded only 24 larvæ in 36 stations, composed of *tarsimaculatus*-group 11 and *albitarsis* 8.

Now Trap D2 is located close to the two inundation fields tabulated in Tables XXII and XXIII. It showed (Table XXXIII) high *aquasalis* catches between

week ending June 24 and week ending August 12 (weeks 25–32), that is at the time that there was some breeding of this species in the actual rice, but the provenance of these adults may have equally well been in pools generally, so that the trap catches are no more than a general reflection of the generally highest production of *aquasalis* which occurs in this vicinity in the first two months of the wet season.

One must conclude that, on the Caroni Plain, rice presents no special *aquasalis* hazard, rather than it is far more a source of *albitarsis* than of *aquasalis*.

Further studies of ricefield breeding will be undertaken in 1951, using ricefields adjacent to the almost purely *aquasalis* area east of the Laventille mangrove.

(IV) WATERCRESS CULTIVATION.

The principal locus of cress cultivation is an area situated about 3 miles east of Port of Spain, full of springs, arising below the hill-foot of the Northern Range above the saline reed belts, east of the coastal mangrove. There are other areas of this cress cultivation in the Diego Martin Valley west of Port of Spain, and in the Santa Cruz Valley, drained by the San Juan River. Cress is cultivated in elongate pits about 10 feet broad. Irrigation is provided through earth channels from the springs, the water circulating through the beds, which are connected in parallel to the feeding channels and in series from bed to bed at slightly lower elevations. The soil of the beds is a palpaceous silt, over which free water is held at ½–2 inches depth. Stagnation is contra-indicated, but, except at the inlet and outlet openings of the individual pits, movement is imperceptible. The plant is sown simply by broadcasting broken-up root masses from the previous crop, which thereupon root themselves. A crop is mature in six to eight weeks, and is manured at least once during its growth by the broadcasting among the plants of handfuls of well-rotted animal manure,—a procedure which any Health Department of the Orient would certainly prohibit for a crop which is eaten raw.

During the hot, dry weather, the crop is shaded by palm fronds stuck among it, and during the last three weeks of growth is sprinkled by hand. During this season the leaves tend to be covered with brown spots, which appear to be burns due to the water drops acting as lenses. Some of the beds are beneath the shade of coconut palms, and these require neither artificial shading nor sprinkling. Beds in all stages of growth are found side by side, and the clearest exposition is gained by tabulating and averaging the records of all beds examined of the same age in weeks, rather than by selecting for record individual beds throughout a single crop cycle. Observations were carried out between February and April in the hot dry weather and August to November during the rains. The spring and main feeding channel to one set of beds are tabulated in the series set out in Table XXVIII, in which minimal and maximal as well as average values are shown. The spring and channel are seen to be fairly highly charged with CO_2, the residual pH value being neutral. So long as the water is flowing the actual beds are just on the acid side of neutrality, the residual pH value being faintly alkaline. Surface temperatures fall steadily from planting to maturity, doubtless owing to increasing plant shade. After reaping there is an abrupt rise. Beds over 8 weeks old were only found during the dry season, when owing to a glut and fall in prices some beds were left unreaped. At this age the crop appears to be no longer marketable and is useful

only for resowing. During this season water was scarce, and was not run through such beds, accounting for the rise in temperature.

Two sharp rises in saline ammonia between weeks 1–3 and in the 6th week are doubtless related to manuring. The rise after reaping is either due to root decay, or to the trampling during reaping bringing to the surface subjacent organic matter. Salinity is very low.

Anopheline production is very small throughout, though there are slight increases apparently connected with manuring. The paucity of larvæ and pupæ is almost certainly due to the very large population of *Lebistes reticulatus*, with occasional *Rivulus hartii*, *Aequidens pulchra* and *Hoplias malabaricus* that occur in the beds. In the spring and the feeding channel the larger fish are more numerous, mainly *Rivulus hartii*, with a few *Polycentrus schomburgkii*, *Cichlosoma bimaculatum*, *Tetragonopterus maculatus* and *Aequidens pulchra*. Lebistes is also common in the feeding channel, but only in the spring is anopheline production noticeably greater than in the beds. The apparent larval increase in the reaped beds is possibly artificial. During the growth-period larvæ are well concealed among the plant. After reaping, they naturally concentrate against the bed margins, and are then more easily dipped out. An abandoned bed (the few found were under coconut shade) is no more than a small area of fresh water marsh. It becomes overgrown with *Cyperus* spp., *Fimbristylis miliacea*, *Eclipta alba*, *Fuerena umbellata*, *Eleocharis caribbea*, etc., and the table shows that such beds are more productive of larvæ than beds in cultivation. The pH of these abandoned beds suggest that ground water enters them direct, as well as by the usually blocked feeding channel. One was found with a pH of 4·0 (4·6).

The population of other, possibly larval-predaceous, insects found was not large. The species found include:

COLEOPTERA.	RHYNCHOTA.	ODONATA.*
Sulphisellus nigrinus	*Pelocoris femoratus*	*Anax amazili*
Tropisternus lævis	*Lathrocerus anura*	*Orthemis ferruginea*
T. collorris	*L. micantula*	*Enallagra civila*
Megadytes sp.	*Tenagobia signata*	
Berosus geayi		
Enochrus sp.		
Paracymus sp.		
Derallus altus		
Laccophilus ovatus		

but as regulatory of anopheline breeding, these must be negligible as compared with the fish population.

* Only nymphs are considered. Adults around the beds may have bred in their fresh water, or in the saline Malik area marshes not far off.

The water-fern Azolla was generally present in the beds, but never tended to form a complete cover between the cress plants. Algal growth in the beds showed interesting but unexplained seasonal changes. In the dry season Spirogyra was generally present, and was seen in conjugation far more frequently than ever seen elsewhere. An alga near Oedogonium, usually covered with Naviculid diatoms, and at least two species of Oscillatoria were found.

After the onset of the rains, Spirogyra disappeared from the beds, and the (?) Oedogonium was the only filamentous Chlorophyceous alga found.

The crystal-clear feeding channel usually had a bottom growth of Spirogyra, and frequently large floating Cyanophyceous plaques, which are removed by the cultivators as impeding flow. It contained a good deal of *Nelumbo* (not determined, as it never flowered). The spring was always covered with a convolvulaceous plant, with *Nelumbo* and *Cyperaceæ*.

There are several species of molluscs in the beds, those determined being *Planorbis terversianus* D'Orb.: *Haplotrema* (*Zophos*) sp. (very rare): *Physa rivalis* M. & R. (very rare): *Ampullaria glauca* Linn. (common): *Marisa cornuarietes* Linn. (rare): *Homalonyx unguis* Ferussac (var.) (rare): *Paludestrina crystallina* Pfr. (common): but this snail population is noteworthy for the enormous numbers of *Planorbis terversianus*, which is a definite pest of the cress plant. The cultivators spoon out this snail with some regularity, as the heaps on the bed margins show, but they fail entirely to control its numbers. As a matter of biological interest, at each visit to the beds the staff hand-collected all the *Planorbis* within their reach from the edges of the pits, and graded the shells through a template with holes of ¼–2 inches diameter. Very small specimens are not found wandering free over the cress roots, but gather on the undersides of the leaves, and those obtained came up in the larval dippers, and thus specimens of under ¼ inch may be more numerous than the figures show. The results are given in Table XXIX. Two points emerge from this table: (*a*) that there are large seasonal differences in the percentages in the classes 1–1½ inches diameter, (*b*) that there is always a very great reduction in numbers when class 1½ inches is passed. These changes are not considered to have been caused by any conscious or unconscious selection by the cultivators when hand picking.

This snail is not found in any numbers in the spring or in the feeding channel, indicating its definite association with cress, though the author has seen a Planorbis in ricefields, species not determined.

In the general collecting in the beds an interesting find was one full and two empty puparia of the dipteron *Sepedon macropus* Wlk., which is new to the Trinidad fauna. Some flies of this family are said to be parasitic in molluscs, but the life history of this apparently rare species is quite unknown. The species is not in the Natural History Museum, South Kensington, and the author is indebted to Dr. Steyskal of Gross Ile, Michigan, for the identification.

(V) THE MARACAS VALLEY.

The headwaters forming this stream arise on the southern slopes of El Tucuche (3 072 feet), the second highest mountain of the Northern Range. Survey work

was carried out around the sites of the dawn traps C6 to C11 which were located up this valley in 1948, all of which are below the 500 feet but above the 100 feet contour. The valley is steep sided, sloping abruptly up from the stream banks, the road following the stream on a shelf-trace. The stream is normally crystal-clear, over rocks and pebbles. In dry weather the channel contracts, and relict pools, nearly if not absolutely stagnant, as there is probably in and out flow beneath the pebbles, are formed. Near the site of Trap C7 (La Baja) there are a few marginal swampy areas, probably seepage-caused. From August to November some of these were, in 1950, cultivated for the first time in rice. Frequent short side streams enter the main stream from the hill-sides. In a temperate climate the whole picture would be that of a trout-stream; 'riffles' and small over-falls doubtless render the water highly oxygenated. No signs of pollution, such as might be caused by effluents from the septic tanks of the frequent rural bungalows built on flatter spots above the road, have been seen. The hill slopes are largely under cacao. The results are tabulated for the survey as a whole in Table XXX, in which January and February 1951 results are placed in monthly order ahead of the 1950 results, the survey having been commenced in March 1950. The rainfall figures apposed are those of Ortinola estate at the head of the valley. A comparison of these with Table IV shows that much more rain fell there than in the corresponding months at I.C.T.A.

It will be seen that breeding in the river, pools and swamps was found only in the months of short rainfall from March to May. Pool breeding was recommencing early in January, when after the first day's work around site C6 very heavy rain supervened and again rendered the whole valley sterile to February. Distribution of breeding in the side streams appears to be contingent on light showers. They are short hill-side torrents that dry off very rapidly, and for flushing are regulated by purely local precipitation. An interesting point was that in several months when no anopheline breeding was found larvæ of a *Uranotænia* near *calosomata* Dyar and Knab, and of an undetermined Culex were found, though one would not expect larvæ of the culicines to be able to exist in water too swift for an anopheline mosquito. The origin of the adult *aquasalis* population of this and similar valleys is considered in Section VII (xvi).

(VI) THE 'NELSON' BAMBOO PLANTATION.

It seems best to include the detailed study of this area in the section dealing with detailed breeding places, though the area actually contains no breeding at all. But it possesses a special adult population the origin of which, as will be shown, must be related to some particular set of breeding conditions. So many references to particular aspects of this population will be made in the following section that the area is best described beforehand.

The Nelson Bamboos appear to have been planted by a Paper Manufacturing Company either during or shortly after World War I. The set-up included a pulp factory, but the project failed owing to the natural regeneration of the cut plants being very slow. The land originally planted* stretched from the north bank

* Much of this plantation has now been cleared, either for industrial development around the old factory, now converted to glass manufacture, or for cultivation by the I.C.T.A. A fair-sized block remained up to 1950, in which the observations recorded were mostly made.

of the Caroni (which here is embanked to prevent flooding) for about 2 miles northward to the Eastern Main Road, covering some 2 square miles. The plantation was deep drained (though of late years there has been no maintenance), and is still invariably surface dry. After periods of heavy rain the drains fill up in places, but only culicines (chiefly *Limatus* sp.) have been found breeding in them during numerous larval searches.

Nevertheless, the bamboos contain a very large population of *aquasalis*, the study of which has been very instructive, for, as will appear, this population is a naturally selected part of what seems to be the normal *aquasalis* population of the Caroni Plain. Though within the bamboos there may be a few small rodents, there is no large number of mammals, and no birds are noticeable. In other words, the *aquasalis* population is purely a sheltering one. It is invariably avid for blood, and attacks as soon as a supply is presented to it, even around midday. This attack was measured on four occasions in December and January by putting out parties of field men to catch from one another. The 'sitting party' remained in one spot between 09·00 and 16·00 hours. The 'moving party', over the same period of the day, moved 200 paces between each catching halt of 15 minutes' duration. Thus the second party steadily 'drew on' the mosquitoes resting within a fixed attraction range of a previously unsatisfied fresh population at each halt. The results, in catches per man-hour, were :—

Time.	09–10.	10–11.	11–12.	12–13.	13–14.	14–15.	15–16.	Average density.
Stationary ...	15·1	4·9	4·2	3·1	5·8	7·1	12·0	7·0
Moving	11·3	11·2	6·0	7·1	15·4	16·3	30·9	11·5

Thus persons moving about these bamboos at a season of normally high *aquasalis* prevalence experience 65 per cent more attacks than persons remaining in one spot. In the heat of the day, 11–13 hours' activity is seen to be least. After 7 hours of continuous attraction by the stationary party of mosquitoes within 'range', it is seen that by mid-afternoon appreciable numbers are still proceeding to attack. As it is improbable that part of a completely static hungry population would remain unaffected by the accessible presence of blood over so long a period, it is concluded that the *aquasalis* population is not, by day, completely static, but that it must move at random until individuals suddenly come within attraction range of blood. There is seldom any appreciable air movement on the floor of the plantation, though the tops of the bamboos are usually heard steadily rustling in the wind.

How patchily distributed is the population is illustrated by two moving-party catches in March and April 1950. In the March catch, 6 man-hours between 08.55 and 10.35 attracted only one *aquasalis*. The weather was then fine. From 10.50 to 12.55, the weather was showery. Four man-hours in that period (4 stations worked) collected 40 specimens. But the rain may have affected activity. In the May catch, between 08.38 and 11.23, occupying 10 stations for 2 man-hours each, only the 08.38–09.08 and the 09.11–09.41 periods evoked no mosquitoes. There is no record of showers during the morning of the day concerned, and though there is

no significant difference in the total numbers captured on the two occasions, it would appear that on the day of the May catch the *aquasalis* population was more evenly distributed over the area searched than on the March occasion. On three occasions a sitting party had been posted in these bamboos between 20.00 and 21.00 hours, always at the close of days when the regular weekly afternoon catches (and also, as it proved, the subsequent next-morning catches) showed that there was a large daytime *aquasalis* population present. Not a single *aquasalis* attacked during these night catches. It is therefore concluded that when the light outside the plantation has fallen below the inhibitory-to-movement value of about 13 *l.c.* (Section VII-vii) there is a mass exodus of the bamboo population in search of blood. It is assumed that a reverse entrance of further unfeds takes place around dawn. The ovarian state of the evening and morning population is compared in Table XXXIV. A very interesting set of observations was obtained when a good blood supply was, for a short period, furnished within these bamboos. About July 1948 the Imperial College cleared a considerable area, and planted it with various crops, including an area under *Pennisetum purpureum*. To graze this off, a herd of fifteen dry cows was corralled nearby. Around the corral there was a high concentration of fed *aquasalis*, 2 ♂, 263 ♀ being captured, a density of 40·5 per man-hour. As their ovaries matured these females seem to have been drifting through the bamboos towards breeding water. During the month following the corralling of these cows searching* in the uncut bamboos between this clearing and the putative breeding grounds south and west of it produced 15 ♂, 307 ♀, a female density of 4·3 per man-hour with the following percentage stage distribution :

I	II	III	IV	V	VI	VII
48	3	0	4	17	17	11

It will be noticed that both in the original search and during this month, a few males were taken. Such are usually never seen here. Presumably they were following the females about.

During these searches for fed *aquasalis*, a second party was kept catching in the uncut bamboos around the cultivated clearing. The correlation between the two sets of catches was negligible, r = 0·06. After a month the cows were removed.

Resting density then fell to 1·5 ♀ per man-hour, and of the small total of 24 resters captured 22 were in Stage I, and the remaining 2 in Stages VI and VII. Though by that time the arrival of the first squatters to found Bamboo Village (*q.v.*) was diverting and reducing the attacker-catch in the uncut bamboos to the east of their area, the big drift through the uncut bamboos ceased with the removal of the cows from the I.C.T.A. clearing. This point should be borne in mind when the question of blood preferences in *aquasalis* (Section VIII) is considered.

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* These searches were made in the bamboo clumps and not by awaiting attack. We were led to this discovery of the resting places in pure stands of bamboo by the finding, about a fortnight before the cows arrived, of 2 abdomen I and 1 abdomen II in such spots. The single abdomen II was resting, during rain, between the stem and internodal fringe of adventitious roots, which carried off the rain running down the stem clear of the mosquito. It is obvious that the large unfed population that attacks must be normally sitting somewhere. The 48 per cent Stage I taken in these resting catches is of no interest. It is the 52 per cent of feds that is so important.

Searches of 1950 with bridge rebuilding.—From February to October 1950 the St. Joseph River Bridge situate at the eastern edge of these bamboos was under renewal, the road being carried on a temporary one-way diversion, necessitating night watchmen to regulate the traffic. These men must have furnished an immediate blood supply to the hungry bamboo *aquasalis* population. The area of the bamboos lying south of the road was therefore divided into three north-south sections, and searches for fed adults resting within bamboo clumps were conducted by men protected by '612' repellent. Four such yielded totals of :

	♂	♀	Fed per cent.
A : in vicinity of bridge ...	8	105	14·3
B : central area ...	2	79	5·1
C : western third ...	5	137	2·2

There was thus a distinct increase in the percentage of fed resting specimens in the vicinity of the human blood supply at the bridge. Of 6 specimens which gave an animal positive precipitin reaction, 4 were man +, the other 2 ox +. Having now described these Nelson Bamboos, references to them in the sections dealing with fertilization index (VII–x), and ovarian stages (VII–xi) will be comprehensible. So far the origin of the bamboo population has escaped discovery.

Over 8 weeks in January–March 1950, the correlation of adult catches at the regular catching spot in the bamboos with breeding in the irrigation drains and flooded road borrowpits half a mile to the westward of it was low and negative ($r = -0{\cdot}33$). Thus nearby breeding does not account for the adults in the bamboos.

About 2,000 feet beyond the eastern face of the bamboos are situated the Caroni Sugar Company's buffalo and mule stables on Curepe Savanah. The night population of engorged *aquasalis* in these stables is enormous. Dawn trap C2 (Section VII–xiii) is located north of these stables and quite close to the eastern face of the bamboos. Table XXXIII shows the usually high catches of *aquasalis* taken in it. Seven scrub-searches on Curepe Savanah, however, though showing fairly high per man-hour density, have a very low male percentage, indicating that the origin of the Savanah population is not close at hand, and in fact, save for a few springs in the south-west angle, there is no breeding on it. The figures are :—

Man-hours.			Abdominal stages.						
	♂	♀	I.	II.	III.	IV.	V.	VI.	VII.
162·66	99	2,009	233	455	606	377	146	151	41
Per man-hour ...	0·6	12·4	...	...	...	...	...	...	...
Percentage ...	4·7	...	11·6	22·6	30·2	18·8	7·3	7·5	2·0
Fed per cent ...	...	...	...	25·7	34·1	21·2	8·2	8·5	2·3

It was hoped that lines of search run west and south-west from around the stables would indicate the direction in which this Savanah population moves off for oviposition, and so show if the unfed bamboo population moves eastward to Trap C2 and the stables by night.

The results were not helpful, at 1,000 feet from the stables was found :—

Direction.	Man-hours.	♂	♀	I.	II.	III.	IV.	V.	VI.	VII.
West	9·0	19	58	1	28	18	8	1	1	1
South-west ...	3·5	9	35	4	6	9	16	0	0	0

At 2,000 feet westward only 1 ♀ was found (abdomen Stage VI). This point is within the bamboos. At 2,000 feet south-westward in scrub 3 ♂ only were found. The bank of the Caroni is reached at 4,900 feet, and between the 1,000 feet point and the bank no females were found at four evenly spaced stations.

If the Glaudon marshes are the source of both the Bamboo and Curepe Savanah *aquasalis* populations, it would appear that flight, without intermediate halts, takes place over more than a mile by mosquitoes not diverted by Glaudon Village in the north-east angle of the marshes. Whilst the enormous blood attraction of the Curepe and McLeod Stables (both up-wind from the marsh) might induce direct flight to blood, it appears improbable that a density in the bamboos of Stage I is almost as high as the total density around blood on the Savanah would arise from stable-ward flights halting for one daylight period within them. Only stained releases on a really large scale will serve to elucidate the problems of the origins of the two populations. (*To be continued.*)

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TABLE I.

Distribution of the tarsimaculatus-*group by degrees of latitude.*

Countries or states situate between latitudes.

Species.	♀ 4th palpal segment.	2nd hind tarsus, per cent, black.	Tucuman, Paraguay, South Brazil.	Rio de Janeiro, Sao Paulo, Parana.	Matto Grosso, M. Geraes, E. Santo.	Matto Grosso, Bahia.	R. G. do Norte, Paraiba, Pernambuco, Acre.	Amazonas, Para, Ceara, Maranhao, Piani.	Colombia, Fr. Guiana, Dutch Guiana.	Colombia, Venezuela, Br. Guiana, Panama.	Costa Rica to Guatemala, Trinidad to Martinique.	Br. Honduras, S. Mexico, Dominica to Haiti.	Mexico, South Texas, Cuba.
			30°–25°S.	25°–20°S.	20°–15°S.	15°–10°S.	10°–5°S.	5°–0°S.	0°–5°N.	5°–10°N.	10°–15°N.	15°–20°N.	20°–25°N.
albimanus Wd., 1821 ..	Black	50—75	..	..	..	..	..	..	+	+	+	+	+
oswaldoi Perry, 1922 ..	White	9—23	..	..	+	+	+	+	+	+	+	..	..
triannulatus Neiva & Pinto, 1922.	Black	23—50	..	..	+	+	+	+	+	+	+	..	..
strodei Root, 1926 ..	Var.	22—41	..	..	..	..	..	+	..	+	+	+	..
aquasalis Curry, 1932 ..	White	31—64	..	+	+	+	+	+	+	+	+	+	..
annomalophyllus Komp, 1936.	Black	25+	..	..	..	..	..	..	..	+	+	..	..
noroestensis Galvao & Lane, 1937.	White	16—33	+	..	+	+	+	+	..	..	..	..	..
rangeli Gab., C.-Gar. & Lane, 1940.	White	17—35	..	..	..	..	+	+	+	+	..	..	..
nunez-tovari Gabaldon, 1940.	White	17—45	..	..	+	+	...	+	+	+	..	..	..
benarrochi Gab., C.-Gar. & Lane, 1941.	White	37—55	..	..	+	+	+	+	..	+	..	..	..
konderi Galv. & Dam, 1942.	White	14—28	..	..	+	+	+	+	..	..	..	..	..
galvaoi Caus., Deane and Deane, 1943.	White	40—59	..	..	+	+	+	..	..	..	..	..	..

TABLE II.

A. aquasalis *recorded dissections.*

Locality.	Guts.	Number positive.	Oöcyst rate per cent.	Glands.	Number positive.	Sporozoite rate, per cent.	Author.
Brazil—							
Sao Paulo ...	43	1	2·3	23	0	0·0	Coutinho (1947—Table 41).
Rio de J. & Federal ...	1,403	27	1·9	1,260	4	0·3	Do. do.
Espirito Santo ...	41	0	0·0	41	0	0·0	Do. do.
Bahia	50	1	2·0	50	0	0·0	Do. do.
Sergipe	65	2	3·1	65	0	0·0	Do. do.
Alagoas	96	2	2·1	77	1	1·3	Do. do.
Paraiba	1	0	0·0	1	0	0·0	Deane, Causey & Deane (1948*—Table 14).
Rio Grande do Norte ...	215	5	2·3	211	0	0·0	Coutinho (1947—Table 41).
Ceara	921	7	0·8	925	2	0·2	Coutinho (1947—Table 41): Deane, Causey and Deane (1948—Table 14).
Maranhao ...	572	1	0·2	572	0	0·0	Coutinho (1947—Table 41).
Para	1,517	3	0·2	3,599	13	0·4	Deane, Causey & Deane (1948—Table 14). Galvao, Damasceno and Marques (1942).
Total ...	4,924	49	1·0	6,824	20	0·3	
French Guiana ...	117	0	0·0	117	0	0·0	Floch & Abbonenc (1943).
British Guiana ...	1,302	0	0·0	1,302	0	0·0	Giglioli (1945—p. 68) and *in litt.*

Trinidad—							
1928	...	...	...	200	3	1·5	de Verteuil (1931—p. 21).†
1931	80	4	5·0	...	...	...	Do. do.
1941-43	2,695	55	2·1	2,684	1	0·04	Ann. Reports, Malaria Division.
Total ...	2,775	59	2·1	2,884	4	0·1	
Grenada	346	4	1·2	794	4	0·5	Earle (1936—Table 3).
St. Lucia	219	8	3·7	297	3	1·0	Earle (1936—Table 4).
TOTALS	9,683	120	1·2	11,747	31	0·25	

* Deane, Causey and Deane do not include Coutinho's or Galvao *et al.*'s papers in their list of references.

† This record is a quotation, and no reference to the original work for details regarding exact locality and season over which the unspecified author worked, could be found. Downs, Gillette and Shannon make only passing reference to de Verteuil's work of 1931.

TABLE III.

A. aquasalis, *maxillary index by localities.*

Miss Harrison's analysis, rearranged geographically.

Country.	Locality.	Maxillary index, per cent.	Actual difference.	Difference / S. E. of difference.	Final analysis, race.	REMARKS.
Brazil	Rio de Janeiro	22·73			Group III	186 counts only.
			1·21	8·6		
Brazil	Para St. Maracanan	21·52			Group II	
			0·48	3·42		
French Guiana ...	?	22·00			Group III	
			0·32	2·28		
Dutch Guiana ...	Paramaribo	22·32			Group III	
			0·75	5·36		
British Guiana ...	Corentyne	23·07			Group IV	
			0·19	1·36		
Trinidad*	Mayaro	22·88			Group IV	
			0·02	0·142		
"	Pt. Fortin	22·90			Group IV	
			0·03	0·21		
"	Blanchisseuse	22·93			Group IV	Av. 2 counts.
			0·05	0·36		
"	Cedros	22·98			Group IV	

					0·10	1·21		
,,	...	...	Curepe	23·14			Group IV	Av. 7 counts.
					0·11	0·79		
,,	...	...	California	23·25			Group IV	
					0·01	0·071		
,,	...	...	Cumana	23·26			Group IV	
					0·06	0·43		
,,	...	...	Moruga	23·32			Group IV	Av. 13 post-D.D.T. counts.
					0·22	1·57		
Tobago	...	...	Plymouth	23·54			Group IV	
					0·02	0·142		
,,	...	...	Golden Grove	23·56			Group IV	
					0·64	4·57		
,,	...	...	Roxborough	24·20			Group V	
					3·45	24·64		
Grenada	...	...	Grenville	20·75			Group I	
					0·15	1·07		
St. Lucia	...	...	Dennery	20·60			Group I	
					0·30	2·14		
,,	...	...	Castries	20·90			Group I	
					0·36	2·57		
Antigua	...	...	?	21·26			Group II	
* All Trinidad average, 8 localities, 26 counts, excluding pre-D.D.T. Moruga,				23·06	0·01	0·071	Group IV	from British Guiana.

Omitting the Rio de Janeiro and the Leeward Island localities, not submitted for analysis, $\chi^2 = 2586{\cdot}5$ (n = 28), for which P is very small.

TABLE IV.

Meteorological figures: Imperial College of Tropical Agriculture, Trinidad.

1948.

Month.	Shade temperature.		Relative humidity, per cent.			Rainfall.		
	Maximum.	Minimum.	8 hours.	16 hours.	Minimum.	Inches.	Days.	Evaporation, c.c.
Jan. ...	85	68	83	67	58	2·16	19	26·4
Feb. ...	86	68	81	61	53	0·48	10	34·6
Mar. ...	86	69	77	66	56	1·00	16	36·6
Apr. ...	86	69	74	65	55	2·18	8	36·2
May ...	89	73	75	70	58	2·39	18	40·6
Jun. ...	86	73	86	75	67	12·61	26	24·7
Jul. ...	86	72	84	78	67	10·70	26	21·1
Aug. ...	88	72	84	78	65	7·84	24	21·0
Sep. ...	88	72	84	76	64	7·82	18	18·5
Oct. ...	87	72	81	79	66	8·79	23	19·0
Nov. ...	86	72	84	76	69	7·13	23	19·9
Dec. ...	85	69	83	70	65	1·22	18	24·6
TOTAL ...	...	...	...	...	...	64·32	229	...

TABLE IV—*contd.*

1949.

Month.	Shade temperature.		Relative humidity, per cent.			Rainfall.		
	Maximum.	Minimum.	8 hours.	16 hours.	Minimum.	Inches.	Days.	Evaporation, c.c.
Jan. ...	84	66	80	64	57	0·65	9	30·4
Feb. ...	85	67	79	62	57	1·98	15	33·9
Mar. ...	87	68	76	60	55	0·45	11	43·1
Apr. ...	89	71	69	57	53	0·34	8	52·4
May ...	89	72	72	62	57	2·85	14	49·5
Jun. ...	86	73	82	72	67	11·52	25	34·9
Jul. ...	86	71	81	72	66	8·94	17	30·1
Aug. ...	86	71	86	75	67	12·50	21	23·6
Sep. ...	88	71	86	74	68	8·12	19	25·0
Oct. ...	88	72	83	74	68	7·90	15	27·7
Nov. ...	87	70	85	77	66	7·45	21	24·8
Dec. ...	84	70	85	74	69	7·82	20	17·7
TOTAL ...	...	...	...	...	...	70·52	195	...

TABLE IV—*concld.*

1950.

Month.	Shade temperature.		Relative humidity, per cent.			Rainfall.		
	Maximum.	Minimum.	8 hours.	16 hours.	Minimum.	Inches.	Days.	Evaporation, c.c.
Jan. ...	83·0	70·0	79	69	66	6·14	21	23·1
Feb. ...	83·0	69·0	82	70	66	4·53	19	23·0
Mar. ...	84·0	68·0	78	65	50	1·60	13	30·6
Apr. ...	86·0	69·0	73	66	58	2·28	12	35·8
May ...	86·0	72·0	78	71	65	2·70	16	30·7
Jun. ...	85·0	73·0	84	76	70	11·05	26	23·3
Jul. ...	86·0	71·0	82	72	66	9·09	22	24·7
Aug. ...	87·0	72·0	87	78	69	13·32	25	24·7
Sep. ...	87·0	72·0	83	77	64	5·71	19	25·4
Oct. ...	88·0	71·0	83	75	63	9·20	16	29·9
Nov. ...	87·0	71·0	85	76	65	4·64	19	24·2
Dec. ...	85·4	70·0	88	75	64	6·71	19	24·6
TOTAL ...	...	...	...	...	...	76·97	227	...

TABLE V.

Caroni plain. Seasonal larval surveys.

Period.	Acreage surveyed.	Acreage wet.	Number of wet areas examined.	Number of positive breeding places found.	Wet areas found positive, per cent.	Total larvæ and pupæ found.	Average density per positive finding of 20 dips.*
Dry season: Mar.–Jun., 1948 ...	635	180	1,138	360	28·3	5,223	14·5
Rains: Jul.–Sep., 1948 ...	711	581	1,112	740	81·7	8,393	11·2
Post-rains: Oct.–Dec., 1947 ...	855	567	1,182	762	66·3	8,235	10·8

* Very approximately 1 sq. ft. surface water sampled per dip.

TABLE VI.

Relationship of breeding to salinity (pp. 100,000)—A. aquasalis.

Salinity to	Dry season.				Wet season.				Post-rains.			
	Percentage of total findings.	Percentage of total positive findings.	Percentage of total larvæ.	Average larval density per positive finding.	Percentage of total findings.	Percentage of total positive findings.	Percentage of total larvæ.	Average larval density per positive finding.	Percentage of total findings.	Percentage of total positive findings.	Percentage of total larvæ.	Average larval density per positive finding.
350	49	77	64	12·0	91	97	99	11·4	76	86	87	10·9
750	7	5	9	23·5	4	2·6	1	4·2	7	5	5	10·9
1150	5	9	19	30·1	1	0	0	0	4	3	3	10·6
1550	6	4	5	16·4	0·1	0	0	0	5	2	2	8·5
1950	5	1	1	24·7	0·5	0	0	0	5	2	2	11·7
2350	4	2	1	8·7	1·0	0	0	0	2	2	1	7·1
2750	0·3	0·3	0·1	4·0	2·5	0·4	0·1	0·1	1	+	+	2·0
3150	1	0	0	0	0	0	0	0	...	...	...	...
3550	1	0·5	0·1	2·0	0·1	0	0	0	...	...	...	...
3950	3	0	0	0	0·1	0	0	0	...	...	...	...
4350	3	0	0	0	0·1	0	0	0	...	...	...	...
4750	4	0	0	0	...	...	...	...	...	...	...	...
5150	4	0	0	0	...	...	...	...	...	...	...	...
5550	3	0	0	0	...	...	...	...	...	...	...	...
5950	2	0	0	0	...	...	...	...	...	...	...	...
6350	1	0	0	0	...	...	...	...	...	...	...	...
6750	1	0	0	0	...	...	...	...	...	...	...	...
7250	0·4	0	0	0	...	...	...	...	...	...	...	...
7550	0·3	0	0	0	...	...	...	...	...	...	...	...
7950	0	0	0	0	...	...	...	...	...	...	...	...
8000+	0·1	0	0	0	...	...	...	...	...	...	...	...

TABLE VII.

Pupal production in relation to salinity—A. aquasalis.

Salinity to	Dry season.			Wet season.			Post-rains.			Totals.		
	Positive findings.	Findings with pupæ.	Percentage of pupæ to total findings.	Positive findings.	Findings with pupæ.	Percentage of pupæ to total findings.	Positive findings.	Findings with pupæ.	Percentage of pupæ to total findings.	Positive findings.	Findings with pupæ.	Percentage of pupæ to total findings.
350	274	24	8·6	718	65	9·5	654	32	4·9	1,649	121	7·3
750	20	1	5·0	19	3	15·8	41	3	7·3	80	7	8·8
1150	35	4	14·7	0	0	0	23	6	26·1	57	11	19·3
1550	16	3	18·8	0	0	0	17	1	5·9	33	4	12·1
1950	3	1	33·3	0	0	0	15	4	26·7	18	5	27·8
2350	7	0	0	0	0	0	11	2	18·2	18	2	11·1
2750	1	0	0	3	0	0	1	0	0	...	...	...
3150	0	0	0	...	...	...	0	0	0	...	...	...
3550	2	1	50·0	...	...	...	...	...	...	...	...	...
Pupal percentage in total breeding (numbers).		16·3			11·2			14·0			13·5	
Pupal percentage in total breeding (times).		9·7			9·2			6·3			8·1	

TABLE VIII.

Relationship of breeding to light incidence—A. aquasalis.

Sunlight.					Light shade.					Heavy shade.				
Total breeding places examined.	Number with larvæ.	Positive, per cent.	Total larvæ.	Larval density.	Total breeding places examined.	Number with larvæ.	Positive, per cent.	Total larvæ.	Larval density.	Total breeding places examined.	Number with larvæ.	Positive, per cent.	Total larvæ.	Larval density.
2,257	1,568	69	19,380	12·4	800	270	34	2,286	8·5	102	20	20	66	3·3

TABLE IX.

Relationship of breeding to water clarity—A. aquasalis.

Clear.					Turbid.					Clear-brown.				
Total breeding places examined.	Number with larvæ.	Positive, per cent.	Total larvæ.	Larval density.	Total breeding places examined.	Number with larvæ.	Positive, per cent.	Total larvæ.	Larval density.	Total breeding places examined.	Number with larvæ.	Positive, per cent.	Total larvæ.	Larval density.
2,833	1,730	61	20,276	11·7	303	131	43	1,113	8·5	15	6	40	31	5·2

Table X.

Saline ammonia, pp. 10^5: Larvæ and pupæ per 100 dips—A. aquasalis.

Ammonia pp. 10^5 ..	0·001	0·002	0·003	0·004	0·005	0·006	0·007	0·008	0·009	0·01	0·02	0·03	0·04	0·05	0·06
Times found ..	15	25	4	34	24	12	16	4	10	20	22	5	6	..	4
Times with larvæ ..	9	18	3	26	16	8	13	4	8	10	13	2	5	..	2
Per cent times positive ..	60	72	75	79	67	67	81	100	80	50	59	40	83	..	50
Total larvæ found ..	1,215	727	40	3,443	837	2,417	647	708	501	436	1,054	45	435	..	85
Larvæ per positive finding, average.	135	40	13	132	52	268	50	177	63	44	81	22	87	..	42

Ammonia pp. 10^5 ..	0·07	0·08	0·09	0·10	0·15	0·20	0·25	0·30	0·35	0·40	0·45	0·50	0·55	0·60	0·65	1·0
Times found ..	3	1	..	8	1	4	8	2	3	3	1	2	..	1	..	1
Times with larvæ ..	2	0	..	6	1	3	6	2	3	2	1	1	..	1	..	1
Per cent times positive ..	67	0	..	75	100	75	88	100	100	67	100	50	..	100	..	100
Total larvæ found ..	412	0	..	406	35	746	1,312	980	370	554	583	594	..	222	..	20
Larvæ per positive finding, average.	106	0	..	68	35	249	187	490	123	277	583	594	..	222	..	20

TABLE XI.

Breeding in pool at mangrove edge, Laventille swamp, 1950: A. aquasalis.

(Main tree *Avicennia nitida*. When joined to nearby *Avicennia* cum *Laguncularia* quadrat not examined.)

Date, 1950.	Dips.	Salinity, per thousand.	Breeding by instars.						Remarks.
			I.	II.	III.	IV.	Pupæ.	Total.	
Jan. 31 ...	86	26·19	8	20	8	4	2	42	Water black.
Feb. 7 ...	...	...	...	...	...	...	...	...	No examination.
14 ...	75	15·06	1	1	1	14	0	17	Muddy brown.
22 ...	95	18·15	17	17	9	6	0	49	Muddy brown, very shallow.
28 ...	50	22·69	0	1	1	1	0	3	
Mar. 7 ...	90	18·97	1	0	0	0	0	1	Joined to quadrat.
14 ...	100	21·86	7	25	5	17	0	54	Very shallow, muddy brown.
21 ...	100	22·69	5	9	20	10	1	45	Very shallow, separated two portions, muddy brown.
28 ...	35	26·81	0	0	0	0	0	0	Puddles only.
Apr. 4 ...	...	...	...	...	...	...	...	...	Dry.
11 ...	...	...	...	...	...	...	...	...	Dry.
18 ...	...	...	...	...	...	...	...	...	Dry.
25 ...	...	...	...	...	...	...	...	...	Dry.
May 2 ...	...	...	...	...	...	...	...	...	Dry.
9 ...	...	...	...	...	...	...	...	...	Dry.
16 ...	...	...	...	...	...	...	...	...	Dry.
23 ...	...	...	...	...	...	...	...	...	Dry.
30 ...	...	...	...	...	...	...	...	...	Dry.
Jun. 6 ...	...	...	...	...	...	...	...	...	No examination, joined to quadrat.
13 ...	105	19·80	0	2	3	1	0	6	Muddy brown, up to 12 inches deep.
20 ...	105	19·80	0	0	0	0	0	0	
27 ...	105	22·27	0	0	0	0	0	0	Muddy brown, up to 12 inches deep.
Jul. 4 ...	105	25·57	0	0	0	0	0	0	Brown, ferrous iridescence, up to 12 inches deep.
11 ...	100	17·32	0	0	0	0	0	0	Clear, 8 inches, ferrous scum.

TABLE XI—*concld.*

Date, 1950.		Dips.	Salinity, per thousand.	Breeding by instars.						Remarks.
				I.	II.	III.	IV.	Pupæ.	Total.	
Jul.	18 ...	100	4·12	1	2	2	0	0	5	Clear, 4 inches.
	25 ...	100	12·79	0	2	5	0	0	7	Muddy, 4 inches.
Aug.	1 ...	...	...	...	...	...	...	...	...	No examination, joined to quadrat.
	8 ...	...	...	...	...	...	...	...	...	Do. do.
	15 ...	...	...	...	...	...	...	...	...	Do. do.
	22 ...	...	...	...	...	...	...	...	...	Do. do.
	29 ...	...	...	...	...	...	...	...	...	Do. do.
Sep.	5 ...	100	2·23	0	2	0	1	0	3	Clear, 6 inches.
	12 ...	...	...	...	...	...	...	...	...	No examination, joined to quadrat.
	19 ...	...	...	...	...	...	...	...	...	Do. do.
	26 ...	...	...	...	...	...	...	...	...	Do. do.
Oct.	2 ...	100	4·12	0	3	0	4	0	7	Nearly separated from quadrat, very shallow, black.
	10 ...	...	...	...	...	...	...	...	...	No examination, joined to quadrat.
	17 ...	...	...	...	...	...	...	...	...	Do. do.
	24 ...	...	...	...	...	...	...	...	...	Do. do.
	31 ...	...	...	...	...	...	...	...	...	Do. do.
Nov.	7 ...	...	...	...	...	...	...	...	...	Do. do.
	14 ...	...	...	...	...	...	...	...	...	Do. do.
	21 ...	...	...	...	...	...	...	...	...	Do. do.
	27 ...	...	...	...	...	...	...	...	...	Do. do.
Dec.	5 ...	100	7·42	7	3	0	5	1	16	
	12 ...	...	...	...	...	...	...	...	...	No examination, joined to quadrat.
	19 ...	100	4·54	0	0	0	3	0	3	
	28 ...	100	7·42	0	1	3	3	0	7	3 inches, muddy brown.
TOTAL 47 EXAM.		...	(+ 20)	47	88	57	69	4	265	
AVERAGE PERCENTAGES		...	15·99	17·9	33·2	21·4	26·0	1·5	...	

TABLE XII.

Breeding in Avicennia nitida *cum* Laguncularia racemosa *quadrat.*
Laventille swamp, 1950 : A. aquasalis.

Date, 1950.	Dips.	Salinity, per thousand.	Breeding by instars. I.	II.	III.	IV.	Pupæ.	Total.	Remarks.
n. 10 ...	...	...	...	...	...	...	...	...	Dry.
17 ...	80	2·68	0	0	1	1	0	2	
24 ...	...	...	...	...	...	...	...	...	Dry.
31 ...	...	...	...	...	...	...	...	...	Dry.
b. 7 ...	60	1·15	2	8	8	5	1	24	
14	85	21·45	1	2	1	3	1	8	Puddles, ferrous scum.
22 ...	...	...	...	...	...	...	...	...	Dry.
28 ...	...	...	...	...	...	...	...	...	Dry.
ar. 7 ...	100	19·39	2	0	0	0	0	2	Depth 1-2 inches, brown, turbid.
14 ...	...	...	...	...	...	...	...	...	Dry.
21 ...	...	...	...	...	...	...	...	...	Dry.
28 ...	...	...	...	...	...	...	...	...	Dry.
or. 4 ...	...	...	...	...	...	...	...	...	Dry.
11 ...	...	...	...	...	...	...	...	...	Dry.
18 ...	...	...	...	...	...	...	...	...	Dry.
25 ...	...	...	...	...	...	...	...	...	Dry.
ay 2 ...	...	...	...	...	...	...	...	...	Dry.
9 ...	...	...	...	...	...	...	...	...	Dry.
16 ...	...	...	...	...	...	...	...	...	Dry.
23 ...	...	...	...	...	...	...	...	...	Dry.
30 ...	...	...	...	...	...	...	...	...	Dry.
n. 6 ...	105	9·07	1	1	0	0	0	2	Depth 6 inches, muddy.
13 ...	65	51·15	0	1	1	0	0	2	Shallow puddles, brown.
20 ...	70	41·25	0	0	0	0	0	0	
27 ...	15	22·27	0	0	0	0	0	0	Muddy shallow pools.
l. 4 ...	70	36·30	0	0	0	0	0	0	Puddles only.
11 ...	100	17·32	1	0	0	1	0	2	Depth 2-3 inches, clear faint brown.

TABLE XII—*concld.*

Date, 1950.	Dips.	Salinity, per thousand.	Breeding by instars. I.	II.	III.	IV.	Pupæ.	Total.	Remarks.
Jul. 18 ...	100	2·06	2	1	0	0	0	3	Depth 2 inches, clear.
25 ...	...	...	...	...	...	...	...	...	Dry.
Aug. 1 ...	100	11·55	0	0	0	0	0	0	4 inches, clear brown.
8 ...	100	4·95	4	0	3	10	0	17	
15 ...	100	4·62	0	4	1	2	0	7	3 inches, clear brown.
22 ...	100	0·74	0	1	0	0	0	1	
29 ...	100	9·07	0	1	0	1	0	2	
Sep. 5 ...	25	3·34	1	3	3	3	1	11	Pools only.
12 ...	100	14·02	0	0	0	0	1	1	
19 ...	100	3·55	0	0	1	9	0	10	
26 ...	100	1·48	0	3	0	8	4	15	
Oct. 3 ...	100	8·25	0	9	2	7	2	20	Pools, clear brown, larval fish in shoals.
10 ...	100	0·74	0	1	0	0	0	1	
17 ...	100	0·82	0	3	0	2	0	5	
24 ...	100	1·40	1	2	1	4	0	8	
31 ...	100	1·48	0	3	0	2	1	6	
Nov. 7 ...	100	9·49	0	0	0	0	0	0	Depth 3 inches.
14 ...	100	18·97	0	0	2	0	0	2	
21 ...	100	12·37	0	0	0	1	0	1	
27 ...	100	6·19	0	2	0	0	0	2	
Dec. 5 ...	25	7·01	0	0	0	0	0	0	Depth to 2 inches, clear brown, much sun exposure.
12 ...	100	7·63	0	0	0	0	0	0	
19 ...	35	12·37	0	1	0	0	1	2	
28 ...	25	6·81	0	0	0	0	0	0	Depth 1½ inches, muddy.
TOTAL 51 EXAM.	...	(+33)	15	46	24	59	12	156	
AVERAGE PERCENTAGES	...	11·24	9·6	29·5	15·4	37·8	7·7	...	

TABLE XIII.

Breeding in Avicennia nitida ***quadrat, Laventille swamp, 1950*** **: A. aquasalis.**

Date, 1950.	Dips.	Salinity, per thousand.	Breeding by instars. I.	II.	III.	IV.	Pupæ.	Total.	Remarks.
Jan. 10 ...	...	...	...	...	...	...	...	...	Dry.
17 ...	40	3·09	0	0	0	0	0	0	
24 ...	...	...	...	...	...	...	...	...	Dry.
31 ...	...	...	...	...	...	...	...	...	Dry.
Feb. 7 ...	60	1·73	5	0	0	0	0	5	
14 ...	75	28·87	4	1	0	3	0	8	
22 ...	...	...	...	...	...	...	...	...	Dry.
28 ...	...	...	...	...	...	...	...	...	Dry.
Mar. 7 ...	100	27·84	1	0	0	0	0	1	A film of water only.
14 ...	...	...	...	...	...	...	...	...	Dry.
21 ...	...	...	...	...	...	...	...	...	Dry.
28 ...	...	...	...	...	...	...	...	...	Dry.
Apr. 4 ...	...	...	...	...	...	...	...	...	Dry.
11 ...	...	...	...	...	...	...	...	...	Dry.
18 ...	...	...	...	...	...	...	...	...	Dry.
25 ...	...	...	...	...	...	...	...	...	Dry.
May 2 ...	...	...	...	...	...	...	...	...	Dry.
9 ...	...	...	...	...	...	...	...	...	Dry.
16 ...	...	...	...	...	...	...	...	...	Dry.
23 ...	...	...	...	...	...	...	...	...	Dry.
30 ...	...	...	...	...	...	...	...	...	Dry.
Jun. 6 ...	105	9·90	2	1	0	0	0	3	Depth 6 inches muddy: canal re-excavation to here.
13 ...	75	42·08	1	0	0	0	0	1	Puddles 2–3 inches depth, brown.
20 ...	75	18·15	1	0	0	0	0	1	
27 ...	25	49·50	0	0	0	0	0	0	Puddles in footprints only.
Jul. 4 ...	70	37·83	1	1	0	0	0	2	Part pooled, par tunder 1 inch depth.

TABLE XIII—*concld.*

Date, 1950.	Dips.	Salinity, per thousand.	Breeding by instars. I.	II.	III.	IV.	Pupæ.	Total.	Remarks.
Jul. 11 ...	100	30·52	0	0	0	0	0	0	Depth 2 inches, clear brown. Some ferrous scum.
18 ...	100	4·12	0	0	0	0	0	0	Depth 3 inches, clear.
25 ...	...	...	...	...	...	...	...	...	Dry.
Aug. 1 ...	100	9·90	0	13	0	1	0	14	Depth 4 inches, turbid.
8 ...	100	3·30	5	7	13	4	0	29	
15 ...	100	12·99	0	12	2	2	0	16	Depth 6 inches, clear brown.
22 ...	100	4·45	2	46	12	16	4	80	
29 ...	100	10·72	11	47	67	104	4	233	
Sep. 5 ...	25	21·45	5	7	13	8	1	34	Pools only, brown.
12 ...	100	26·40	1	0	0	0	0	1	
19 ...	100	13·20	0	2	4	7	0	13	
26 ...	100	3·79	4	11	20	23	0	58	
Oct. 2 ...	100	14·02	0	0	2	0	1	3	Pools only, brown, larval fish.
10 ...	100	2·06	0	1	0	0	0	1	Depth 5 inches.
17 ...	100	4·95	0	1	2	8	0	11	
24 ...	100	10·72	0	0	0	5	0	5	
31 ...	100	12·79	0	1	0	0	0	1	
Nov. 7 ...	100	17·53	0	0	0	0	0	0	
14 ...	100	19·80	0	1	0	0	0	1	
21 ...	100	18·36	0	0	3	1	0	4	
27 ...	100	7·42	0	2	1	5	1	9	
Dec. 5 ...	100	19·90	2	1	0	1	1	5	Depth 1 inch, brown turbid.
12 ...	100	14·23	2	3	5	1	1	12	
19 ...	85	16·50	0	4	14	5	0	23	
28 ...	65	13·61	0	1	0	0	0	1	Depth ½ inch, turbid.
TOTAL 51 EXAM.	...	(+33)	47	163	158	194	13	575	
AVERAGE PERCENTAGES	...	15·81	8·2	28·3	27·5	33·7	2·3	...	

TABLE XIV.

Breeding in Rhizophora *mangle quadrat, Laventille swamp, 1950 :* A. aquasalis.

Date, 1950.	Dips.	Salinity, per thousand.	Breeding by instars. I.	II.	III.	IV.	Pupæ.	Total.	Remarks.
Jan. 10 ...	40	7·01	2	1	1	1	1	6	
17 ...	80	3·09	0	7	1	0	3	11	
24 ...	50	9·49	0	6	3	0	0	9	
31 ...	130	24·13	7	0	0	0	0	7	Relict pools.
Feb. 7 ...	60	1·32	10	8	3	8	4	33	
14 ...	80	4·95	8	2	0	0	1	11	Depth 3 inches.
22 ...	105	12·37	1	2	1	5	2	11	Relict pools.
28 ...	...	...	...	...	...	...	...	...	Area dry.
Mar. 7 ...	100	26·81	3	6	0	0	0	9	
14 ...	50	32·17	1	13	1	1	0	16	Relict puddles.
21 ...	?	23·10	2	4	0	6	0	12	Relict puddles.
28 ...	...	...	...	...	...	...	...	...	Area dry.
Apr. 4 ...	...	...	...	...	...	...	...	...	Area dry.
11 ...	100	47·85	2	1	1	0	0	4	Relict pools.
18 ...	...	...	...	...	...	...	...	...	Area dry.
25 ...	50	30·52	0	0	0	0	0	0	
May 2 ...	...	...	...	...	...	...	...	...	Area dry.
9 ...	70	41·25	0	0	0	0	0	0	
16 ...	65	42·90	0	0	0	0	0	0	Relict pools.
23 ...	...	...	...	...	...	...	...	...	Area dry.
30 ...	...	...	...	...	...	...	...	...	Area dry.
Jun. 6 ...	105	7·42	0	1	0	0	0	1	Depth 8–12 inches, 1 ♀ attacked, heavy rain earlier.
13 ...	105	39·60	0	0	0	0	0	0	Depth 4–6 inches, very turbid.
20 ...	105	26·40	0	3	0	0	0	3	
27 ...	105	34·65	0	2	1	0	0	3	Part dry—part 2–3 inches.
Jul. 4 ...	105	20·62	1	0	0	0	0	1	Depth 3 inches, tide rising, water only turbid.

TABLE XIV—*concld.*

Date, 1950.	Dips.	Salinity, per thousand.	Breeding by instars. I.	II.	III.	IV.	Pupæ.	Total.	Remarks.
Jul. 11 ...	100	29·70	2	0	4	1	0	7	Depth 4 inches, water nearly clear, tide falling, 1 ♀ attacked.
18 ...	100	2·89	4	11	2	4	0	21	Depth 4 inches, water clear.
25 ...	75	37·54	0	0	0	0	0	0	Relict pools, trees lopped down.
Aug. 1 ...	100	12·37	0	10	1	1	0	12	Depth 12 inches, clear brown larval fish, new quadrat
8 ...	100	4·12	13	15	14	22	0	64	
15 ...	100	11·12	3	13	1	0	1	18	Depth 6 inches, clear brown.
22 ...	100	13·61	2	3	1	1	1	8	
29 ...	100	10·72	11	6	14	9	0	40	
Sep. 5 ...	100	13·61	0	3	0	0	0	3	Depth 10 inches, clear.
12 ...	100	33·21	0	0	0	0	0	0	
19 ...	100	9·07	0	4	4	10	1	19	
26 ...	100	11·55	0	3	5	9	0	17	
Oct. 2 ...	100	13·20	0	4	2	3	0	9	Depth 6–8 inches, muddy.
10 ...	100	1·20	1	3	1	2	1	8	Depth 7 inches.
17 ...	100	7·01	1	1	2	0	0	4	
24 ...	100	12·17	1	2	4	1	0	8	
31 ...	100	9·49	0	1	1	1	0	3	
Nov. 7 ...	100	19·59	0	1	1	1	0	3	
14 ...	100	20·62	0	1	2	0	3	6	
21 ...	100	13·20	0	1	1	0	0	2	
27 ...	100	5·77	0	0	0	0	0	0	
Dec. 5 ...	100	5·77	4	3	1	1	1	10	
12 ...	100	12·17	8	3	1	0	0	12	
19 ...	100	16·50	0	2	5	1	0	8	
28 ...	100	2·47	0	1	0	3	0	4	Depth 8 inches, turbid.
TOTAL 51 EXAM.	...	(+44)	87	147	79	91	19	423	
AVERAGE PERCENTAGES	...	17·37	20·6	34·7	18·7	21·5	4·5	...	

TABLE XV.

Breeding in bare mud quadrat, Laventille swamp, 1950 : A. aquasalis.
Principal plant Ruppia maritima.

Date, 1950.	Dips.	Salinity, per thousand.	Breeding by instars. I.	II.	III.	IV.	Pupæ.	Total.	Remarks.
Jan. 10 ...	...	...	...	...	...	...	...	...	Not examined.
17 ...	...	...	...	...	...	...	...	...	Not examined.
24 ...	...	3·13	...	...	...	...	...	...	Depth 8 inches, *Spirogyra*.
31 ...	90	28·67	61	18	3	2	0	84	
Feb. 7 ...	60	2·31	1	7	0	2	0	10	*Spirogyra* nearly gone *Cladophora*.
14 ...	95	8·46	4	4	0	1	0	9	*Cladophora* only.
22 ...	93	21·45	42	57	24	4	0	127	1st signs *Ruppia*. *Prasiola* ? *crispa* on mud.
28 ...	50	29·91	2	2	1	0	0	5	
Mar. 7 ...	100	38·78	0	0	0	0	0	0	*Ruppia* submerged and *Lyngbya*, depth 6 inches, clear brown.
14 ...	100	43·73	0	0	0	0	0	0	*Ruppia* at surface, *Prasiola* and *Cladophora*.
21 ...	100	24·96	0	1	0	1	0	2	*Ruppia* at surface, *Prasiola*, depth < 1 inch.
28 ...	...	...	...	...	...	...	...	...	Dry since last week.
Apr. 4 ...	100	45·38	0	0	0	0	0	0	Depth 4–6 inches, *Ruppia* brown, procumbent.
11 ...	100	70·13	0	0	0	0	0	0	*Prasiola* on mud.
18 ...	...	...	...	...	...	...	...	...	Dry.
25 ...	55	23·92	0	0	0	0	0	0	No plant growth over mud.
May 2 ...	...	...	...	...	...	...	...	...	Dry.
9 ...	...	...	...	...	...	...	...	...	Dry.
16 ...	...	...	...	...	...	...	...	...	Dry.
23 ...	...	...	...	...	...	...	...	...	Dry.
30 ...	65	42·90	0	0	0	0	0	0	Depth 3 inches, no plant growth over mud.

TABLE XV—*contd.*

Date, 1950.	Dips.	Salinity, per thousand.	Breeding by instars. I.	II.	III.	IV.	Pupæ.	Total.	Remarks.
Jun. 6 ...	105	16·91	0	0	0	0	0	0	Depth 4–6 inches, no *Ruppia*, a little *Praxiola*, etc.
13 ...	105	29·76	0	0	0	0	0	0	Depth 4–6 inches, clear, no *Ruppia*. *Trichocorixa* +++ on mud.
20 ...	105	20·62	0	0	0	0	0	0	*Ruppia* regrowing.
27 ...	105	35·48	0	0	2	0	0	2	Depth 4 inches, clear, *Ruppia* 3 inches tall in patches.
Jul. 4 ...	105	45·38	0	0	0	0	0	0	Depth 4 inches, muddy brown. *Ruppia* submerged, no *Trichocorixa* but eggs on *Ruppia*.
11 ...	100	31·35	0	1	0	0	0	1	2–3 inches deep. *Ruppia* covered *Trichocorixa* eggs and hatched ++.
18 ...	100	10·72	0	1	0	0	0	1	3 inches, clear. *Ruppia* covered eggs.
25 ...	100	37·95	0	1	3	1	0	5	1–2 inches, clear. *Ruppia* all eggs, *Trichocorixa* nymphs +++.
Aug. 1 ...	100	28·05	3	5	0	1	0	9	Clear, 3 inches. *Ruppia* at surface covered eggs. *Trichocorixa* ++.
8 ...	100	8·25	2	2	3	6	1	14	*Ruppia* forming mats.
15 ...	100	13·20	3	4	0	1	0	8	4 inches, *Ruppia* brown, eggs darkened, no larvæ in mats. No *Trichocorixa* seen.
22 ...	100	14·44	12	32	8	7	0	59	*Ruppia* 5–9 inches tall, brown. Larvæ mostly in mats.
29 ...	100	32·17	0	0	8	12	0	20	*Ruppia* 6–10 inches, showing green again, no eggs. Insects sparse.
Sep. 5 ...	100	22·27	0	0	0	0	0	0	8 inches, clear.
12 ...	100	38·78	0	0	2	1	0	3	4 inches, *Ruppia* mostly regrown green, parts still brown.
19 ...	100	29·70	0	0	0	0	0	0	*Ruppia* recovered.
26 ...	100	61·88	2	0	0	0	0	2	*Prasiola* also seen.

TABLE XV—*contd.*

Date, 1950.	Dips.	Salinity, per thousand.	Breeding by instars. I.	II.	III.	IV.	Pupæ.	Total.	Remarks.
Oct. 2 ...	100	25·57	0	0	0	0	0	0	2–4 inches, *Ruppia* flourishing. *Prasiola* on mud.
10 ...	100	16·91	1	1	3	4	0	9	4 inches.
17 ...	100	20·62	0	0	0	1	0	1	
24 ...	100	28·87	0	1	2	9	0	3	4 inches, *Ruppia* brown, eggs again. *Trichocorixa* patchy +.
31 ...	100	19·80	0	0	0	0	0	0	2 inches, *Ruppia* brown.
Nov. 7 ...	100	29·49	0	2	2	2	0	6	2–3 inches, *Ruppia* brown.
14 ...	100	28·05	0	0	0	0	0	0	2–3 inches, *Ruppia* growth thick.
21 ...	100	21·86	0	0	1	0	0	1	4 inches, more eggs on smaller than large clumps. Cast skins ++.
27 ...	100	19·59	0	0	0	0	0	0	
Dec. 5 ...	60	17·74	1	3	0	0	1	5	3 inches, clear, low tide. Some patches brown. *Trichocorixa* not so numerous.
12 ...	100	21·04	0	0	0	0	0	0	6 inches.
19 ...	100	13·61	0	0	0	0	0	0	
28 ...	100	2·06	5	5	1	3	9	23	4 inches, red-brown water. Few *Trichocorixa* and eggs. *Prasiola* and *Spirulina*.
TOTAL 49 EXAM.	...	(+43)	139	147	63	49	11	409	
AVERAGE PERCENTAGES	...	26·18	34·0	35·9	15·4	12·0	2·7	...	

TABLE XVI.

Glaudon marshes. Cyperus articulatus. A. aquasalis. *Quadrat II to March 4, 1948 : Quadrat VI from June 11, 1948.*

Date.	Average depth in inches.	Temperature, °C.	Per 100,000.		Dips.	Per 100 dips.				Ratio : early : late larvæ.	Pupal percentage of total.
			Salinity.	NH_3.		Stages i-ii.	Stages iii-iv.	Pupæ.	Total.		
1948											
Feb. 5 ...	3	25·5	1,093	0·005	21	167	57	0	224	2·9 : 1	0·0
18 ...	<1	27·2	1,547	0·400	14	243	150	21	414	1·6 : 1	5·1
Mar. 4 ...	<1	28·3	1,691	0·250	19	74	37	0	111	2·0 : 1	0·0
18 ...	Dry	...	...	...	...	...	...	...	...	...	...
Apr. 1 ...	Dry	...	...	...	...	...	...	...	...	...	...
15 ...	Dry	...	...	...	...	...	...	...	...	...	...
29 ...	Dry	...	...	...	...	...	...	...	...	...	...
May 13 ...	Dry	...	...	...	...	...	...	...	...	...	...
27 ...	Dry	...	...	...	...	...	...	...	...	...	...
Jun. 11 ...	2	28·0	1,320	0·625	18	150	72	0	222	2·1 : 1	0·0
24 ...	8	28·0	198	0·312	10	280	600	10	890	1 : 2·1	1·1
Jul. 8 ...	4	26·7	202	0·001	10	330	110	10	450	3·0 : 1	2·2
22 ...	6	30·0	45	0·007	10	100	40	20	160	2·5 : 1	12·5
Aug. 5 ...	8	28·9	136	0·009	18	28	155	6	189	25·8 : 1	3·2
19 ...	10	28·9	132	0·006	18	200	83	0	283	2·4 : 1	0·0

Sep. 2 ...	8	28·9	33	0·005	18	44	72	0	116	1 : 1·6	0·0
16 ...	4	27·8	33	0·005	20	110	45	10	165	2·4 : 1	6·0
30 ...	4	26·5	330	0·004	20	30	5	0	35	6·0 : 1	0·0
Oct. 14 ...	14	28·0	45	0·002	20	20	0	0	20	∞	0·0
28 ...	5	26·5	289	0·005	20	20	0	0	20	∞	0·0
Nov. 11 ...	5	27·0	227	0·006	20	0	0	0	0	...	0·0
25 ...	5	27·0	169	0·005	20	5	40	0	45	1 : 8·0	0·0
Dec. 9 ...	3	24·5	433	0·425	10	0	0	0	0	...	0·0
22 ...	<1	24·0	949	0·011	10	0	0	0	0	...	0·0
1949											
Jan. 6 ...	Dry	...	...	...	...	...	...	...	...	...	...
20 ...	Dry	...	...	...	...	...	...	...	...	...	...
TOTAL 18 EXAM.	...	...	...	...	...	1,801	1,466	77	3,344	1·2 : 1	2·3
AVERAGE ...	5	27·3	493	0·082	...	...	...	...	...	...	...

TABLE XVII.

Glaudon marshes. Eleocharis mutata. A. aquasalis. *Quadrat VII to March 4, 1948 : Quadrat II from June 11, 1948.*

Date.	Average depth in inches.	Temperature, °C.	Per 100,000.		Dips.	Per 100 dips.				Ratio : early : late larvæ.	Pupal percentage of total.
			Salinity.	NH_3.		Stages i-ii.	Stages iii-vi.	Pupæ.	Total.		
1948											
Feb. 5 ...	2	27·5	1,608	0·045	23	17	22	0	39	1 : 1·3	0·0
18 ...	< ½	26·9	1,671	...	3	75	112	18	205	1 : 1·5	8·8
Mar. 4 ...	< ½	29·5	1,815	0·250	19	57	5	0	62	11·4 : 1	0·0
18 ...	Dry	...	...	...	...	...	...	...	...	...	...
Apr. 1 ...	Dry	...	...	...	...	...	...	...	...	...	...
15 ...	Dry	...	...	...	...	...	...	...	...	...	...
29 ...	Dry	...	...	...	...	...	...	...	...	...	...
May 13 ...	Dry	...	...	...	...	...	...	...	...	...	...
27 ...	Dry	...	...	...	...	...	...	...	...	...	...
Jun. 11 ...	3½	31·0	1,320	0·437	36	405	178	0	583	2·3 : 1	0·0
24 ...	11	29·0	233	0·021	20	20	140	0	160	1 : 7·0	0·0
Jul. 8 ...	8	26·6	194	0·001	20	320	60	0	380	5·4 : 1	0·0
22 ...	10	28·3	45	0·006	20	175	30	0	205	5·8 : 1	0·0
Aug. 5 ...	12	28·1	138	0·004	36	53	47	0	100	1·1 : 1	0·0
19 ...	13	17·8	70	0·007	36	131	55	0	186	2·4 : 1	0·0

Sep. 2 ...	11	28·0	26	0·008	36	30	36	0	66	1 : 1·2	0·0
16 ...	6½	27·0	22	0·009	40	58	42	0	100	1·4 : 1	0·0
30 ...	6	26·5	338	0·008	40	95	12	0	107	7·7 : 1	0·0
Oct. 14 ...	13	27·7	105	0·004	40	58	45	0	103	1·3 : 1	0·0
28 ...	6	27·2	237	0·006	40	50	17	5	72	2·9 : 1	6·9
Nov. 11 ...	5	27·0	226	0·004	40	35	15	0	50	2·3 : 1	0·0
25 ...	5	26·7	84	0·009	40	12	8	2	22	1·5 : 1	9·1
Dec. 9 ...	¾	25·0	513	0·101	40	0	0	0	0	...	0·0
22 ...	2	25·5	1,102	0·024	35	11	0	0	11	∞	0·0
1948											
Jan. 6 ...	3–Dry	25·0	1,815	0·350	20	25	15	0	40	1·7 : 1	0·0
20 ...	2	26·3	2,133	0·005	40	12	5	0	17	2·4 : 1	0·0
TOTAL 20 EXAM.	...	...	...	...	...	1,739	744	25	2,508	2·3 : 1	1·0
AVERAGE ...	6	27·4	684	0·065	...	...	...	...	...	...	...

TABLE XVIII.

Glaudon marshes. Paspalum vaginatum. A. aquasalis. *Average of quadrats IV and V throughout.*

Date.	Average depth in inches.	Temperature, °C.	Per 100,000.		Dips.	Per 100 dips.				Ratio: early : late larvæ.	Pupal percentage of total.
			Salinity.	NH_3.		Stages i-ii.	Stages iii-iv.	Pupæ.	Total.		
1948											
Feb. 5 ...	2½	28·5	1,289	0·009	45	18	15	0	33	1·2 : 1	0·0
18 ...	½	28·6	1,619	0·069	21	76	152	5	233	1 : 2·0	2·1
Mar. 4 ...	½	31·1	1,484	0·200	36	6	0	0	6	∞	0·0
18 ...	Dry	...	...	...	...	...	...	...	...	...	...
Apr. 1 ...	Dry	...	...	...	...	...	...	...	...	...	...
15 ...	n.r.	36·0	1,897	1·000	15	20	0	0	20	∞	0·0
29 ...	Dry	...	...	...	...	...	...	...	...	...	...
May 13 ...	Dry	...	...	...	...	...	...	...	...	...	...
27 ...	½	31·0	2,557	0·012	15	0	0	0	0	...	0·0
Jun. 11 ...	4	29·0	1,128	0·250	36	272	375	25	672	1 : 1·4	3·7
24	12	30·0	155	0·016	20	220	425	0	645	1 : 1·9	0·0
Jul. 8 ...	7	27·2	196	0·001	20	215	70	0	285	3·1 : 1	0·0
22 ...	10	29·5	27	0·008	20	210	110	10	330	1·9 : 1	3·0
Aug. 5 ...	9	28·6	99	0·005	36	19	33	3	55	1 : 1·7	5·5
19 ...	11	29·4	82	0·008	36	153	52	0	205	2·9 : 1	0·0
Sep. 2 ...	8	29·1	31	0·007	36	17	31	0	48	1 : 1·8	0.0

16 ...	10	27·8	35	0·007	40	24	0	1	25	∞	4·0
30 ...	6½	27·0	433	0·005	40	0	0	0	0	...	0·0
Oct. 14 ...	14	29·5	82	0·004	40	7	0	0	7	∞	0·0
28 ...	7	27·2	278	0·007	40	2	0	0	2	∞	0·0
Nov. 11 ...	5	27·2	268	0·004	40	12	7	0	19	1·7 : 1	0·0
25 ...	6	27·0	123	0·006	40	2	0	0	2	∞	0·0
29 ...	1½	25·2	451	0·275	30	0	0	0	0	...	0·0
Dec. 22 ...	1	26·3	1,237	0·015	30	10	0	0	10	∞	0·0
1949											
Jan. 6 ...	2	25·0	1,918	0·009	20	15	10	0	25	1·5 : 1	0·0
20 ...	2½	26·0	2,206	0·019	37	8	3	0	11	2·7 : 1	0·0
TOTAL 22 EXAM.	...	...	...	...	...	1,306	1,283	44	2,633	1 : 1	1·7
AVERAGE ...	5½	28·2	997	0·080	...	...	...	...	...	...	...

n.r. = no record.

TABLE XIX.

Glaudon marshes. Nelumbo amazonica. A. aquasalis. *Quadrat III throughout.*

Date.	Average depth in inches.	Temperature, °C.	Per 100,000.		Dips.	Per 100 dips.				Ratio: early : late larvæ.	Pupal percentage of total.
			Salinity.	NH_3.		Stages i-ii.	Stages iii-iv.	Pupæ.	Total.		
1948											
Feb. 5 ...	12	28·0	1,217	0·004	27	4	0	0	4	∞	0·0
18 ...	8	27·8	1,237	0·009	22	0	0	0	0	...	0·0
Mar. 4 ...	5	28·9	1,112	0·012	18	6	6	0	12	1 : 1	0·0
18 ...	2	30·0	1,382	0·011	23	0	4	0	4	∞	0·0
Apr. 1 ...	<½	30·0	2,454	0·062	18	0	0	0	0	...	0·0
15 ...	3	26·7	1,382	0·010	10	0	0	0	0	...	0·0
29 ...	½	32·8	2,269	0·060	10	0	0	0	0	...	0·0
May 13 ...	<½	30·5	2,764	0·075	...	0	0	0	0	...	0·0
27 ...	4	33·0	2,557	0·037	20	15	5	0	20	3·0 : 1	0·0
Jun. 11 ...	8	29·0	1,112	0·250	18	0	6	0	6	∞	0·0
24 ...	18	28·0	107	0·019	10	0	10	0	10	∞	0·0
Jul. 8 ...	12	27·2	198	0·002	10	0	0	0	0	...	0·0
22 ...	14	28·9	20	0·004	10	0	0	0	0	...	0·0
Aug. 5 ...	14	28·9	87	0·009	18	22	0	0	22	∞	0·0
19 ...	18	28·9	66	0·007	18	6	0	0	6	∞	0·0
Sep. 2 ...	18	26·7	29	0·004	18	27	11	0	38	2·5 : 1	0·0

1948	16 ...	16	26·7	29	0·006	20	0	0	0	0	...	0·0
	30 ...	12	27·5	577	0·006	20	0	0	0	0	...	0·0
	Oct. 14 ...	15	28·5	165	0·004	20	5	0	0	5	∞	0·0
	28 ...	18	27·0	309	0·004	20	0	0	0	0	...	0·0
	Nov. 11 ...	14	27·5	227	0·007	20	0	0	0	0	...	0·0
	25 ...	12	26·8	91	0·004	20	0	0	0	0	...	0·0
	Dec. 9 ...	5	24·5	390	0·006	20	0	0	0	0	...	0·0
	22 ...	6	26·0	1,320	0·007	20	35	0	0	35	∞	0·0
	1949											
	Jan. 6 ...	4	25·5	1,939	0·002	20	20	5	0	25	4·0 : 1	0·0
	20 ...	6	26·5	2,373	0·004	20	25	10	0	35	2·5 : 1	0·0
	TOTAL 26	9	...	...	...	...	165	57	0	222	2·9 : 1	0·0
	EXAM. AVERAGE ...	...	28·1	955	0·024	...	...	...	...	...	...	...

Table XX.

Glaudon marshes. Village well. A. aquasalis.

Date.	Average depth in inches below G.L.	Temperature, °C.	Per 100,000.		Predominant macroscopic growth.	Per 100 dips.					Ratio: early : late larvæ.	Pupal percentage of total.
			Salinity.	NH_3.		Dips.	Stages i–ii.	Stages iii–iv.	Pupæ.	Total.		
1948												
Feb. 5 ...	24	n.r.	...	...	...	...	...	...	...	...	...	...
18 ...	42	27·8	536	0·037	...	8	251	14	0	265	18·0 : 1	0·0
Mar. 4 ...	54	n.r.	577	0·125	*Hormidium*	19	16	0	0	16	∞	0·0
18 ...	60	27·8	619	0·250	,,	20	130	65	0	195	2 : 1	0·0
Apr. 1 ...	n.r.	26·7	660	0·375	,,	24	67	0	0	67	∞	0·0
15 ...	n.r.	26·7	454	0·250	,,	19	126	100	0	226	1 : 1·3	0·0
29 ...	n.r.	26·7	619	0·010	,,	20	10	0	0	10	∞	0·0
May 13 ...	Very low	28·1	619	0·500	Very little alga	12	518	76	0	594	6·8 : 1	0·0
27 ...	n.r.	29·0	742	0·375	*Nil*	16	169	94	0	263	1·8 : 1	0·0
Jun. 11 ...	n.r.	28·0	559	0·220	*Nil*	12	578	127	0	705	4·6 : 1	0·0
24 ...	G.L.	26·0	227	0·006	*Nil*	10	1,250	210	40	1,500	6·0 : 1	2·7
Jul. 8 ...	G.L.	26·7	95	0·004	*Nil*	10	150	80	20	2,501	1·9 : 1	8·0
22 ...	G.L.	n.r.	82	0·062	Scum	10	0	10	0	10	∞	0·0
Aug. 5 ...	<G.L.	27·8	70	0·075	*Nil*	12	76	95	8	179	1 : 1·2	4·5
19 ...	G.L.	n.r.	128	0·025	*Nil*	18	44	22	6	72	2 : 1	8·3
Sep. 2 ...	G.L.	27·2	59	0·037	*Nil*	18	50	56	0	106	1 : 1	0·0

16	...	G.L.	29·4	41	0·004	*Nil*	20	35	30	0	65	1·2 : 1	0·0
30	...	n.r.	26·5	45	0·004	*Nil*	20	30	85	0	115	1 : 2·8	0·0
Oct. 14	...	24	n.r.	54	0·004	*Nil*	20	0	0	0	0	...	0·0
28	...	24	n.r.	54	0·004	*Nil*	20	10	10	0	20	1 : 1	0·0
Nov. 11	...	14	n.r.	45	0·004	*Anabæna*	20	5	0	0	5	∞	0·0
25	...	12	27·0	41	0·002	,,	20	85	15	0	100	5·7 : 1	0·0
Dec. 9	...	24	25·5	41	0·004	*Nil*	20	40	30	0	70	1·3 : 1	0·0
22	...	36	25·5	45	0·007	*Nil*	21	95	0	5	100	∞	5·0
1949													
Jan. 6	...	48	25·5	59	0·005	*Anabæna spirogyra* +	20	5	5	0	10	1 : 1	0·0
20	...	54	25·0	49	0·007	Alga and scum	20	Record lost.					
TOTAL 26 EXAM.		...	27·0	...	...	...	...	3,740	1,124	79	4,943	3·3 : 1	1·6
AVERAGE	...	...	...	261	0·096	...	...	...	...	...	...	...	...

n.r. = no record. G.L. = ground level.

Table XXI.

Glaudon Village—outside captures, 1948 and 1949 : A. aquasalis.

Date.		1948.				1949.			
1948.	1949.	Male.	Female.	Male. p.m.-hr.	Female. p.m.-hr.	Male.	Female.	Male. p.m.-hr.	Female. p.m.-hr.
Jan. 29 ...	Feb. 3 ...	4	10	8·0	20·0	11	15	*22·0*	*30·0*
Feb. 18 ...	17 ...	2	7	4·0	14·0	9	9	9·0	9·0
26 ...	Mar. 3 ...	50	65	6·5	8·5	7	7	7·0	7·0
	17 ...	...	...	...	...	1	9	1·0	9·0
Apr. 1 ...	31 ...	2	2	4·0	4·0	0	0	0·0	0·0
	Apr. 13 ...	...	...	...	...	0	0	0·0	0·0
29 ...	28 ...	7	6	28·0	24·0	0	0	0·0	0·0
May 13 ...	May 12 ...	5	0	50·0	0·0	0	0	0·0	0·0
28 ...	26 ...	5	7	10·0	14·0	0	0	0·0	0·0
Jun. 11 ...	Jun. 10 ...	17	22	68·0	88·0	2	9	0·1	6·0
24 ...	23 ...	15	12	60·0	48·0	10	20	10·0	*20·0*
Jul. 8 ...	Jul. 7 ...	14	34	28·0	68·0	50	54	50·0	54·0
22 ...	21 ...	15	34	60·0	136·0	54	54	54·0	54·0
Aug. 5 ...	Aug. 4 ...	16	21	64·0	84·0	Area flooded 3 ft.			
19 ...	18 ...	4	13	16·0	52·0	19	21	19·0	*21·0*
Sep. 2 ...	Sep. 1 ...	2	19	8·0	76·0	47	91	47·0	91·0

16	...	15	...	4	25	16·0	100·0	31	33	31·0	33·0
30	...	29	...	4	4	16·0	16·0	21	32	*21·0*	*32·0*
Oct. 14	...	Oct. 13	...	4	12	16·0	48·0	25	52	25·0	52·0
28	...	27	...	14	31	18·7	41·7	10	12	10·0	12·0
Nov. 11	...	Nov. 10	...	4	37	8·0	74·0	16	32	*16·0*	*32·0*
25	...	24	...	2	13	4·0	26·0	24	52	24·0	52·0
Dec. 9	...	Dec. 8	...	0	12	0·0	36·0	56	113	56·0	113·0
22	...	22	...	1	24	3·0	72·0	2	3	2·0	3·0
Jan. 6	...	...	...	14	8	42·0	24·0	...	...	...	...
20	...	...	...	4	7	12·0	21·0	...	...	...	...
Total: Averages		...	...	209	425	12·3	25·0	395	618	18·3	28·9

1949. S.D. ♂ 18·2 ♀ 30·7.
S.E. ± 4·8 ± 5·3.

Differences not significant from the mean in italics.

TABLE XXII.

Streatham Lodge—north field—inundation irrigation.

Date, 1930.	Plant.	Height of rice plant in inches.	Water.	Still or flowing.	NH_3.	pH.	Calculated to 100 dips. *Nyssorhynchus* Sp. indet.	*aquasalis.*	*arcadiei.*	*albitarsis.*	*neomacultipalpus.*	Remarks.
Jun. 20 ..	Uncultivated cress and sedge.	..	Turbid puddles	S	0·100	6·7 (6·9)	0	0	0	0	0	
26 ..	,, ,,	..	Clearer puddles	S	0·030	6·6 (6·8)	0	0	0	0	0	
Jul. 3 ..	,, ,,	..	Cloudy puddles	S	0·175	6·4 (6·6)	0	0	0	0	0	
10 ..	Uncultivated	..	Flooded to 6 inches muddy.	S	0·005	6·6 (6·8)	5	0	0	0	0	
17 ..	Ploughed	..	4 inches turbid	S	0·0187	7·0 (7·0)	5	0	0	0	0	
24 ..	Transplanted	..	2 inches muddy	S	0·500	7·8 (7·9)	0	0	0	1	0	
31 ..	No growth	..	3 inches clear	S	0·0612	7·5 (7·7)	0	0	0	0	0	
Aug. 8 ..	Plants washed out	..	4 inches lt. turbid	F	0·0375	6·9 (7·0)	0	0	0	0	1	
15 ..	Replanted	9	Clear puddles	S	0·0875	7·8 (7·9)	0	0	0	0	0	
21 ..	Flood washed	18	A few puddles	S	?	?	0	0	0	0	0	
28 ..	.. ..	17	Clear puddles	S	0·4375	7·1 (7·4)	0	0	0	0	0	
Sep. 4 ..	.. ..	22	¼ inch slightly turbid	S	0·1350	7·2 (7·4)	0	0	0	0	0	
11 ..	.. ..	25	Puddles clear and turbid.	S	0·015	8·8 (8·8)	0	0	0	0	0	*Spirogyra.*
18 ..	.. ..	30	Puddles	S	?	8·4 (8·4)	0	0	0	0	0	Closterium ?
25 ..	.. ..	33	Odd puddles	S	?	?	..	..	..	6	2	Larvæ from water outside. *Spirogyra* and ? *Prasiola.*

Oct. 9	..	..	..	40	½ inch clear	S	0·015	6·7 (6·9)	0	0	0	0	0	Start flowering.
16	..	..	..	53	Dry	..	..	..	..	..	..	..	..	General flowering.
23	..	..	..	53	Dry	..	..	..	..	..	..	..	..	
30	..	..	..	53	1 inch pools	F	0·015	6·9 (7·1)	0	0	0	0	0	1·25 inch on Oct. 29.
Nov. 6	..	..	..	53	Dry	..	..	..	..	..	..	..	..	
13	..	..	..	53	Clear puddler	..	?	6·8 (7·0)	0	0	0	0	0	
20	..	..	..	53	Dry	..	..	..	..	..	..	..	..	
28	..	..	..	R	Dry	..	..	..	..	..	..	..	..	
Total: Averages	..	..	..	..	..	..	0·0790	..	10	0	0	7	3	

R = reaped. S = still. F = flowing.

Table XXIII.

Streatham Lodge—south field—inundation irrigation.

Date, 1950.	Plant.	Height of rice plant in inches.	Water.	Still or flowing.	NH_3.	pH.	Calculated to 100 dips. *Nyssorhynchus* Sp. indet.	*aquasalis.*	*oswaldoi.*	*albitarsis.*	*neomacultipalpus.*	Remarks.
Jan. 20 ..	Uncultivated grass	..	Dry except drain	..	0·015	6·4 (6·6)	11	0	0	0	0	
26 ..	,, ,,	..	Pools in grass	S	0·0062	6·4 (6·7)	0	0	0	0	0	
Jul. 3 ..	,, ,,	..	Puddles	..	0·250	6·2 (6·3)	0	0	0	0	0	
10 ..	,, ,,	..	Muddy flooded 12 inches.	S	0·0037	6·4 (6·6)	77	0	0	19	0	
17 ..	Ploughed	..	3 inches turbid	S	0·0125	6·5 (6·6)	0	0	0	0	0	
24 ..	,,	..	3 inches turbid	S	0·050	6·9 (7·2)	0	0	0	1	1	
31 ..	Transplanted	..	4 inches muddy	S	0·060	6·8 (7·0)	0	2	0	0	0	
Aug. 8 ..	No growth	12	4 inches turbid	F	0·0225	6·7 (6·8)	0	2	0	0	3	Much frog spawn.
15 ..	Little growth	10	1 inch clear	S	0·0062	7·1 (7·3)	? 3	0	0	0	0	Larvæ lost.
21 ..	.. ..	18	Pools turbid	S	0·3125	6·8 (6·8)	0	0	0	0	0	
28 ..	.. ..	21	Clear puddles	S	0·1875	7·2 (7·4)	0	0	0	0	0	
Sep. 4 ..	.. ..	24	Dry	..	..	..	..	..	..	..	..	
11 ..	.. ..	30	Puddles to ½ inch sheet	S	0·010	7·3 (7·6)	0	0	0	178	0	
18 ..	.. ..	33	2 inches turbid	S	0·005	6·7 (6·8)	3	0	0	0	0	
25 ..	.. ..	39	A few puddles	S	?	?	1	0	0	22	0	
Oct. 2 ..	.. ..	42	Scattered puddles	S	?	?	3	0	0	6	0	
9 ..	.. ..	51	2 inches cloudy	S	0·015	6·3 (6·5)	6	0	0	17	0	Flowering starting.

16	..	..	..	54	Clear puddles	S	0·0037	7·3 (7·3)	16	0	0	48	0	General flowering.
23	..	..	..	54	Dry	..	..	..	..	..	..	..	..	
30	..	..	..	54	Puddles	S	0·0075	6·7 (6·9)	1	0	0	0	0	
Nov. 6	..	..	..	54	Dry	..	..	..	..	..	..	..	..	
13	..	..	..	54	Clear puddles	..	0·0025	6·2 (6·4)	0	0	0	0	0	
20	..	..	..	54	Dry	..	..	..	..	..	..	..	..	
28	..	..	..	R	Dry	..	..	..	..	..	..	..	..	
Total: Averages		..	..	..	..	..	0·0571	..	121	4	0	291	13	

R = reaped. S = still. F = flowing.

TABLE XXIV.

Freeman Road—north field—canal irrigation.

Date, 1950.	Plant.	Height of rice plant in inches.	Water.	Still or flowing.	NH_3.	pH.	Calculated to 100 dips. *Nysorhynchus* Sp. indet.	*aquasalis.*	*sawadwi.*	*albimanus.*	*neomacculipalpus.*	Remarks.
Jun. 20 ..	Uncultivated grass	..	Clear rainpools	S	0·0112	6·6 (6·7)	0	1	0	0	3	
26 ..	Uncultivated	..	4 inches clear water	F	0·005	7·3 (7·4)	0	0	0	0	4	
Jul. 3 ..	Ploughed	..	6 inches clear water	F	0·100	7·0 (7·2)	0	0	0	0	0	
10 ..	,,	..	6 inches clear	F	0·0012	7·0 (7·1)	0	0	0	0	0	
17 ..	Transplanted	..	6 inches clear	F	0·0025	7·5 (7·7)	0	0	0	0	0	
24 ..	No growth	..	3 inches nearly clear	F	0·0062	7·6 (7·8)	0	2	0	0	0	
31 ..	Growing	12	Puddles	S	0·040	6·9 (7·0)	0	0	0	0	0	
Aug. 8 ..	.. ..	18	2 inches turbid puddles	F	0·025	7·0 (7·2)	0	0	0	0	0	
15 ..	.. ..	19½	Dry	..	..	..	..	..	..	..	..	
21 ..	.. ..	22	Clear puddles	S	0·0625	7·2 (7·4)	12	0	0	4	0	
28 ..	.. ..	30	3½ inches clear	F	0·0062	7·3 (7·5)	0	1	0	4	0	
Sep. 4 ..	.. ..	30	2½ inches clear	F	0·0012	7·7 (7·8)	0	0	0	0	0	
11 ..	.. ..	31	Turbid pools	S	0·005	7·5 (7·7)	0	0	0	0	0	Canal breached.
18 ..	.. ..	31	2 inches clear	F	0·005	7·8 (7·9)	0	0	0	0	0	
25 ..	.. ..	39	1½ inches clear	F	0·015	7·8 (7·9)	0	0	0	0	0	Flowering starting. Salinity 59 pp. 10⁶.
Oct. 2 ..	.. ..	49	2 inches clear	F	0·005	7·7 (7·8)	0	0	0	0	0	
9 ..	.. ..	42	2 inches clear	F	0·0025	7·7 (7·9)	0	0	0	0	0	Flowering general.

16	..	..	..	42	1¼ pool	S	0·200	7·4 (7·6)	0	0	0	0	0	
23	..	..	..	42	Clear, only inlet pool	S	0·0187	7·6 (7·8)	0	0	0	0	0	
30	..	..	..	42	½ inch clear	F	0·0075	7·8 (8·0)	0	0	0	0	0	
Nov. 6	..	..	..	42	Entrance pool only	S	0·030	7·9 (7·9)	0	0	0	0	0	
13	..	..	..	42	Muddy entr. pool	S	0·0025	7·1 (7·3)	0	0	0	0	0	
20	..	..	..	42	Clear puddles	S	0·040	7·4 (7·6)	0	0	0	0	0	? *Prasiola* on mud.
28	..	..	..	42	2½ inches clear	F	*Nil*	7·9 (8·0)	0	0	0	0	0	
Dec. 4	..	..	..	R	Puddles, clear	S	0·040	7·3 (7·4)	0	0	0	0	0	
TOTAL: AVERAGES		..	..	..	..	..	0·0255	..	12	4	0	8	7	

R = reaped. S = still. F = flowing.

TABLE XXV.

Freeman Road—south field—canal irrigation.

Date, 1950.	Plant.	Height of rice plant in inches.	Water.	Still or flowing.	NH_3.	pH.	Calculated to 100 dips. *Nymphorhynchus* Sp. indet.	*aquasalis.*	*oswaldoi.*	*albitarsis.*	*neomaculipalpus.*	Remarks.
Jun. 20 ..	Uncultivated grass	..	Pools, clear-brown	S	0·0075	6·9 (7·0)	0	4	0	0	0	
26 ..	Uncultivated	..	7 inches clear	F	0·0025	7·4 (7·5)	2	0	0	0	0	
Jul. 3 ..	,,	..	..	F	0·0087	7·4 (7·5)	0	0	0	0	0	
10 ..	,,	..	12 inches clear	F	0·0062	7·2 (7·3)	61	1	0	0	2	
17 ..	Grass cut for ploughing	..	4 inches clear	F	0·0125	7·4 (7·5)	0	2	0	1	0	
24 ..	Grass removed unploughed.	..	3 inches clear	S	0·0062	7·6 (7·8)	0	0	0	0	0	
31 ..	,, ,,	..	Turbid pools	S	0·0375	6·7 (6·8)	0	0	0	0	4	
Aug. 8 ..	Ploughed and planted	..	6 inches clear	S	0·0062	7·2 (7·4)	0	0	0	0	0	
15 ..	Transplants flattened	..	Turbid puddles	S	0·200	7·2 (7·3)	0	0	0	0	0	
21 ..	No growth	..	Turbid puddles	S	0·1875	7·7 (7·9)	0	0	0	0	0	Canal breached.
28 ..	.. ..	9	2 inches clear	F	0·0062	7·5 (7·6)	0	0	0	0	0	
Sep. 4 ..	.. ..	15	½ inch clear	F	0·0025	7·9 (8·0)	0	0	0	0	0	
11 ..	.. ..	18	Little turbid pools	S	0·020	7·4 (7·5)	0	0	0	0	0	
18 ..	.. ..	21	3 inches clear	F	0·005	7·8 (7·9)	0	0	0	0	0	Crabs +.
25 ..	.. ..	30	2½ inches clear	F	0·005	7·7 (7·8)	0	0	0	0	0	Salinity 41 pp. 10°.
Oct. 2 ..	.. ..	27	3 inches fairly clear	F	0·005	7·7 (7·8)	0	0	0	0	0	Crabs and fish.
9 ..	.. ..	27	2 inches clear	F	0·0025	7·7 (7·8)	0	0	0	1	0	Flowering starting.

16	..	.	..	30	½ inch pool	S	0·0437	7·3 (7·5)	0	0	0	0	0	
23	..	..	..	42	1½ inches clear	F	0·0037	7·8 (8·0)	0	0	0	0	0	Flowering genera..
30	..	..	..	42	¾ inch clear	F	0·0012	7·7 (7·9)	0	0	0	0	0	
Nov. 6	..	..	..	42	Pools	S	0·005	8·1 (8·1)	0	0	0	0	0	? *Prasiola*.
13	..	..	..	42	Turbid pools	S	0·0037	7·1 (7·3)	0	0	0	0	0	
20	..	..	..	42	Turbid pools	S	0·020	7·2 (7·4)	0	0	0	0	0	*Oscillatoria*
28	..	..	..	42	2 inches clear	F	0·005	7·8 (8·0)	0	0	0	0	0	
Dec. 4	..	..	..	R	Pools and puddles turbid.	S	0·0025	7·2 (7·4)	0	0	0	0	0	
TOTAL: AVERAGES	..	..	..	..	..	..	0·0198	..	63	16	0	2	6	

R = reaped. S = still. F = flowing.

TABLE XXVI.

Carapichaima ravine field—road run-off and seepage.

Date, 1950.	Plant.	Height of rice plant in inches.	Water.	Still or flowing.	NH_3.	pH.	Calculated to 100 dips. *Nyssorhynchus* Sp. indet.	*aquasalis.*	*oswaldoi.*	*albimanus.*	*neomacultipalpus.*	Remarks.
Jun. 14 ..	Ploughed	..	Muddy puddles	S	0·0225	6·7 (6·8)	0	0	0	0	0	
28 ..	,,	..	4 inches muddy	F	0·015	7·1 (7·2)	0	0	0	0	0	
Jul. 3 ..	Grass regrowing	..	Pools	S	0·025	8·1 (8·2)						
						9·4 (9·4)	0	0	0	0	0	*Spirogyra.*
10 ..	,,	..	6 inches muddy	F	0·015	6·0 (6·2)						
						6·5 (6·6)	46	0	0	17	0	L. 5 times + in alga. *Spirogyra.*
17 ..	Transplanted	..	Pools	S	0·125	8·4 (8·4)	0	2	2	8	1	*Spirogyra* ++.
24 ..	.. ..	11	Puddles	S	0·350	9·0 (8·8)	0	0	0	0	0	Alga-filled puddles.
31 ..	.. ..	21	6 inches muddy	F	0·0125	6·4 (6·5)	0	9	0	22	69	Alga nearly gone.
Aug. 8 ..	.. ..	30	3 inches little turbid	F	0·0062	6·6 (6·7)	0	0	0	130	3	*Spirogyra Ulothrix ? Oscillatoria.*
15 ..	.. ..	33	Puddles	S	0·0875	7·5 (7·5)						
						9·6+	9	0	0	15	0	*Spirogyra* and *Clostarium* ++.
21 ..	.. ..	42	Puddles	S	0·0375	7·9 (8·0)						
						9·2 (9·0)	0	0	0	0	0	*Spirogyra*
28 ..	.. ..	45	Puddles clear	S	0·5250	7·5 (7·6)	0	0	0	0	0	Dull day, *Spirogyra.*
Sep. 4 ..	.. ..	48	Puddles	S	?	?	0	0	0	0	0	
11 ..	.. ..	54	A few clear pools	S	0·030	7·9 (8·0)	0	0	0	0	0	
18 ..	.. ..	60	Dry	..	..	..	..	..	..	..	..	

25	..	..	..	>60	½ inch to puddles	..	0·015	6·8 (7·0)	12	0	0	0	0	Salinity 41 pp. 10⁶. Flowering starting.
Oct. 2	..	..	..	>60	Dry	..	..	..	..	..	..	..	..	
9	..	..	..	> 0	Puddles	..	?	?	0	0	0	0	76	
11	..	..	..	..	..	..	..	..	0	0	7	13	24	Over field generally.
16	..	..	..	>60	Very small puddles	..	?	?	0	0	0	44	0	Grain set.
23	..	..	..	>60	Dry	..	..	..	..	..	..	..	..	
30	..	..	..	>60	Dry	..	..	..	..	..	..	..	..	Lodging.
Nov. 6	..	..	..	R	Turbid puddles	..	0·0625	6·8 (6·9)	0	0	0	0	19	Cow dung in water.
13	..	..	..	R	Clear puddles	..	?	?	0	0	1	0	22	
20	..	..	..	..	Dry	..	..	..	..	..	..	..	..	
28	..	..	..	..	Dry	..	..	..	..	..	..	..	..	
Total : Averages	..	..	..	..	..	..	0·0592	..	67	11	10	249	214	

R = reaped. S = still. F = flowing.

TABLE XXVII.

Curapichaima flat field—rain-fed : water conserved.

Date, 1950.	Plant.	Height of rice plant in inches.	Water.	NH_3.	pH.	Calculated to 100 bpe.					Remarks.
						Nyssorhynchus Sp. indet.	*aquasalis.*	*oswaldoi.*	*albitarsis.*	*neomacculipalpus.*	
Jun. 14 ..	Grasses and sedge	..	Clear pools	0·037	6·1 (6·1)	0	0	0	0	0	
28 ..	Ploughed	..	3 inches muddy	0·025	6·0 (6·2)	0	5	0	0	0	
Jul. 3 ..	,,	..	3 inches very muddy	0·080	5·7 (5·9)	0	0	0	0	0	
10 ..	,,	..	5 inches muddy	0·025	5·3 (5·4)	4	0	0	0	0	
17 ..	,, grass growing	..	4 inches muddy	0·0125	6·1 (6·2)	0	0	0	0	0	
24 ..	Transplanted	..	2–3 inches muddy	0·100	6·1 (6·1)	0	0	0	1	0	Very warm water.
31 ..	.. ..	7	3 inches muddy	0·0112	5·5 (5·6)	0	0	0	27	3	
Aug. 8 ..	.. ..	12	3 inches turbid	0·0125	6·1 (6·2)	0	0	0	4	0	
15 ..	.. ..	13	2 inches little turbid	0·0062	7·0 (7·1)	1	0	0	11	0	Procumbent stems, all *Œdogonium.*
21 ..	.. ..	15	2 inches turbid	0·0125	7·1 (7·2)	0	0	0	6	0	,,
28 ..	.. ..	19	2 inches little turbid	0·0125	7·1 (7·2)	0	0	0	10	0	,, unhealthy. *Nitella oligospira.*
Sep. 4 ..	.. ..	25	2 inches little turbid	0·0125	8·2 (8·2)	0	0	0	38	0	
11 ..	.. ..	29	1 inch turbid	0·005	8·5 (8·4)	0	0	0	55	0	? *Oscillatoria.* ? *Lyngbya.*
18 ..	.. ..	33	1 inch little turbid	0·015	6·9 (7·1)	0	0	0	40	0	*Oscillatoria* 2 spp. *Anabæna.*
25 ..	.. ..	37	2½ inches clear brownish	0·0525	5·9 (5·0)	0	0	0	111	0	Salinity 33 pp. 10⁴ *Cyanoph plaques.*

Oct.	..	..	..	40	1½ inches clear	0·005	6·4 (6·5)	0	0	0	43	1	No alga.
9	..	..	..	46	1½ inches opalescent	0·010	6·3 (6·4)	0	0	0	94	0	Flowering, no alga.
16	..	..	..	46	1 inch ,,	0·0375	6·5 (6·6)	20	0	0	44	0	
23	..	..	..	46	1 inch ..	0·010	6·4 (6·5)	0	0	0	35	0	*Vaucheria*. *Desmidium*. A few *Xanthidium*.
30	..	..	..	46	Puddles 1½ inches clear	0·0125	6·5 (6·6)	0	0	0	65	0	
Nov. 6	..	..	..	46	Puddles opalescent	0·020	6·1 (6·2)	0	0	0	0	21	
13	..	..	..	46	Puddles opalescent	0·0675	4·9 (5·1)	0	0	1	1	30	
20	..	..	..	46	Puddles opalescent	0·010	6·2 (6·3)	0	0	0	1	0	
28	..	..	..	R	Dry	..	..	0	1	1	0	0	
Total: Averages	..	..	..	..	.. ..	0·0232	..	25	0	2	386	58	

R = reaped.

TABLE XXVIII.

Cress cultivation. Beds tabulated by age from planting.

	Number of observations.	Temperature, °C.			pH.			NH_3.		
		Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.
Spring ...	10	28·0	30·0	28·9	6·4 (7·0)	6·9 (7·2)	6·5 (7·0)	0·001	0·002	0·001
Channel ...	15	27·5	29·0	28·4	6·4 (6·8)	6·7 (7·0)	6·6 (7·0)	0·001	0·007	0·002
0–1 week ...	7	27·5	30·5	30·2	6·4 (7·0)	7·4 (7·4)	6·9 (7·2)	0·001	0·017	0·009
1–2 weeks ...	10	28·9	37·0	31·4	6·5 (7·0)	7·5 (7·2)	7·0 (7·1)	0·005	0·425	0·067
2–3 weeks ...	14	26·5	38·5	30·1	6·6 (7·0)	7·4 (7·3)	6·9 (7·2)	0·001	0·100	0·025
3–4 weeks ...	10	28·0	36·0	30·0	6·4 (7·0)	7·2 (7·4)	6·9 (7·2)	0·001	0·016	0·007
4–5 weeks ...	10	26·5	34·5	29·5	6·4 (7·0)	7·4 (7·4)	6·8 (7·2)	0·001	0·035	0·007
5–6 weeks ...	10	27·0	32·5	29·1	6·5 (7·0)	7·0 (7·0)	6·7 (7·1)	0·002	0·187	0·030
6–7 weeks ...	13	26·0	32·0	28·5	6·4 (7·0)	7·0 (7·2)	6·7 (7·1)	0·001	0·037	0·012
7–8 weeks ...	9	26·0	30·8	28·1	6·4 (7·0)	7·2 (7·3)	6·8 (7·1)	0·002	0·125	0·018
>8 weeks ...	15	24·5	31·4	28·6	6·5 (7·0)	7·4 (7·5)	6·8 (7·2)	0·001	0·062	0·015
Reaped ...	9	29·0	35·0	31·7	6·9 (7·0)	7·4 (7·5)	7·1 (7·3)	0·003	0·031	0·100
Abandoned ...	3	26·5	32·0	28·2	4·0 (4·6)	6·8 (7·0)	6·3 (6·8)	0·002	0·500	0·076

TABLE XXVIII—*concld.*

	Number of observations.	Salinity per thousand.			Breeding per 100 dips. *A. aquosalis.* Stages i-ii.			Stages iii-iv.			Pupæ.			Total.		
		Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.
Spring ...	10	20	20	20	2	65	29	0	25	13	0	2	0·5	7	90	41·5
Channel ...	15	16	54	25	0	70	17	0	25	7	0	8	1·0	0	85	23·0
0–1 week ...	7	20	62	31	0	25	7	0	0	0	0	0	0·0	0	25	7·0
1–2 weeks ...	10	16	33	24	0	85	27	0	35	5	0	25	3·0	0	140	30·0
2–3 weeks ...	14	16	59	27	0	40	8	0	45	6	0	10	1·0	0	55	16·0
3–4 weeks ...	10	16	59	24	0	65	12	0	20	3	0	0	0·0	0	85	15·0
4–5 weeks ...	10	16	29	21	0	50	9	0	10	1	0	5	0·5	0	55	10·5
5–6 weeks ...	10	20	33	25	0	150	19	0	30	4	0	10	1·5	0	160	24·5
6–7 weeks ...	13	16	45	23	0	50	14	0	20	4	0	5	1·0	0	75	20·0
7–8 weeks ...	9	20	41	25	0	65	14	0	25	6	0	5	0·5	0	65	20·5
>8 weeks ...	15	16	29	21	0	65	14	0	35	3	0	5	0·3	0	75	17·3
Reaped ...	9	13	66	28	0	170	42	0	15	4	0	5	0·5	0	185	47·0
Abandoned ...	3	20	25	23	0	130	35	0	15	7	0	5	1·0	0	140	43·0

TABLE XXIX.

Planorbis terversianus *D'Orb. Barataria cress beds. 1949.*

Period ...	...	< ¼ inch.	¼ inch.	½ inch.	¾ inch.	1 inch.	1¼ inches.	1½ inches.	1¾ inches.	2 inches.	Total.
Feb.–Apr.	...	129	91	157	691	931	1,114	1,534	350	9	5,006
Percentage	...	*2·6*	*1·8*	*3·1*	*13·8*	*18·6*	*22·3*	*30·7*	*7·0*	*0·2*	...
Aug.–Nov.	...	39	37	285	376	253	381	1,328	297	6	3,012
Percentage	...	*2·3*	*1·2*	*8·9*	*12·5*	*8·4*	*12·6*	*44·1*	*9·8*	*0·2*	...
Average percentage	...	*2·5*	*1·6*	*5·2*	*13·3*	*14·8*	*18·6*	*35·7*	*8·1*	*0·2*	...

TABLE XXX.

Maracas Valley—larval surveys, 1950-1951.

A. aquasalis with ? some *A. oswaldoi*: 20 dips each station.

Month.	Ortinola Estate. R.F.	River. Stations worked.	River. Stations positive.	River. L(e).	River. L(l).	River. Pupæ.	River. Total.	Side-streams. Stations worked.	Side-streams. Stations positive.	Side-streams. L(e).	Side-streams. L(l).	Side-streams. Pupæ.	Side-streams. Total.
Jan. ...	7·80	101	0	...	...	...	...	24	0	...	...	...	...
Feb. ...	7·50	113	0	...	...	...	...	24	0	...	...	...	...
Mar. ...	3·49	102	11	52	29	1	82	23	0	...	...	...	...
Apr. ...	1·78	93	14	74	39	0	113	28	3	49	19	0	68
May ...	2·95	92	10	17	25	0	42	15	2	29	23	1	53
Jun. ...	13·68	85	0	...	...	...	...	24	1	1	0	0	1
Jul. ...	8·80	92	0	...	...	...	...	29	1	4	11	0	15
Aug. ...	13·47	89	0	...	...	...	...	33	0	...	...	...	...
Sep. ...	6·09	87	0	...	...	...	...	28	0	...	...	...	...
Oct. ...	6·43	93	0	...	...	...	...	31	0	...	...	...	...
Nov. ...	10·27	89	0	...	...	...	...	30	0	...	...	...	...
Dec. ...	4·64	90	0	...	...	...	...	28	0	...	...	...	...
TOTALS ...	86·50	1,126	35	143	93	1	237	317	7	83	53	1	137

L(e) = young larvæ. L(l) = well-grown larvæ.

TABLE XXX—*concld.*

Month.	Ortinola Estate. R.F.	Pools. Stations worked.	Pools. Stations positive.	Pools. L(e).	Pools. L(l).	Pools. Pupæ.	Pools. Total.	Swamps. Stations worked.	Swamps. Stations positive.	Swamps. L(e).	Swamps. L(l).	Swamps. Pupæ.	Swamps. Total.	Remarks.
Jan. ...	7·80	6	1	5	0	0	5	2	0	...	...	...	...	Heavy rain daily.
Feb. ...	7·50	3	0	...	...	...	...	4	0	...	...	...	...	Jan. 6 to Feb.
Mar. ...	3·49	5	4	67	100	13	180	2	2	4	1	0	5	
Apr. ...	1·78	12	12	111	40	1	152	0	0	...	...	...	...	
May ...	2·95	3	1	5	3	0	8	4	1	0	1	0	1	Several side-streams flushed.
Jun. ...	13·68	11	0	...	...	...	...	0	...	...	...	...	...	
Jul. ...	8·80	7	0	...	...	...	...	2	0	...	...	...	...	
Aug. ...	13·47	1	0	...	...	...	...	4	0	...	...	...	...	Swamp rice, area C7.
Sep. ...	6·09	9	0	...	...	...	...	4	0	...	...	...	...	" " " "
Oct. ...	6·43	4	0	...	...	...	...	4	0	...	...	...	...	" " " "
Nov. ...	10·27	7	0	...	...	...	...	2	0	...	...	...	...	" " " "
Dec. ...	4·64	12	0	...	...	...	...	2	0	...	...	...	...	Rice reaped.
TOTALS ...	86·50	80	18	188	143	14	345	30	3	4	2	0	6	

L(e) = young larvæ. L(l) = well-grown larvæ.

Table XXXI.

Maracas Valley traps—monthly catches, 1948: A. aquasalis *only*.

1948.	C6.	C7.	C8.	C9.	C10.	C11.	Monthly total.	Percentage. C6.	C7.	C8.	C9.	C10.	C11.	Total of yearly percentage.	Remarks.
Jan. ...	44	268	401	63	145	294	1,215	*12·7*	*26·3*	*25·4*	10·5	*29·2*	*21·1*	22·4	
Feb. ...	17	300	498	76	107	237	1,235	4·9	*29·4*	*31·6*	*13·1*	*21·6*	*17·0*	22·8	3 weeks—C6.
Mar. ...	19	88	206	88	76	203	680	5·5	8·6	*13·0*	*15·2*	*15·3*	*14·5*	12·5	2 weeks—C6.
Apr. ...	16	19	9	8	10	40	102	4·6	1·9	0·6	1·4	2·0	2·9	1·9	
May ...	9	18	15	7	8	8	65	2·6	1·8	1·0	1·2	1·5	0·6	1·2	
Jun. ...	81	176	136	195	74	116	778	*23·3*	*17·3*	8·6	*33·7*	*14·9*	8·4	14·3	
Jul. ...	89	109	73	90	48	92	501	*25·6*	10·7	4·6	*15·5*	9·5	6·6	9·3	
Aug. ...	8	16	13	11	4	207	259	2·3	1·6	0·8	1·9	0·8	*14·9*	4·8	
Sep. ...	0	5	0	0	1	103	109	0·0	0·5	0·0	0·0	0·2	7·4	2·0	
Oct. ...	0	0	0	1	0	32	33	0·0	0·0	0·0	0·2	0·0	2·3	0·6	
Nov. ...	1	1	0	0	1	3	6	0·3	0·1	0·0	0·0	0·2	0·2	+	
Dec. ...	63	18	228	41	22	60	432	*18·1*	1·8	*14·4*	7·1	4·5	4·3	8·0	3 weeks—C9.
Total and averages	347	1,018	1,579	580	496	1,395	5,415	99·9	100·0	100·0	99·8	99·7	100·2	99·8	

Percentages exceeding normal monthly mean in italics.

TABLE XXXII.

Bamboo village—morning house captures, 1950 : A. aquasalis.

Last meal.	M.	O.	G.	H.	P.	D.	O/D	Σ	M.	O.	G.	H.	P.	D.	Neg.	Density per house.	A. I.
Jun. ...	22	0	0	0	0	0	0	22	22	0	0	0	0	0	3	1·4	100·0
Jul. ...	29	0	0	0	0	9	2	42	29	2	0	0	0	11	0	1·7	69·0
Aug. ...	17	0	0	0	0	2	0	19	17	0	0	0	0	0	2	0·8	77·8
Sep. ...	7	2	1	0	0	3	0	13	7	2	1	0	0	3	0	0·8	53·8
Oct. ...	0	0	0	3	0	0	0	3	0	0	0	3	0	0	0	0·6	0·0
Nov. ...	1	0	0	0	0	1	0	2	1	0	0	0	0	1	0	0·2	50·0
Dec. ...	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0·2	0·0
TOTALS AND AVERAGES ...	76	3	1	3	0	15	2	102	76	5	4	3	0	15	2	0·71	74·5

M = man. O = ox. G = goat. H = equine. P = pig. D = dog.

TABLE XXXIII.

Base line traps—Caroni Valley : A. aquasalis.

Percentages of total captures by 4-weekly periods—based on average daily catches.

Weeks.	A1.		B1.				
	Laventille.		New La Paille.				
	1946.*	1950.	1946.	1947.	1948.	1949.	1950.
1–4 ...	*13·8*	5·5	2·5	4·7	7·3	7·5	2·2
5–8 ...	*19·0*	3·9	5·3	3·7	4·5	8·0	1·3
9–12 ...	7·8	1·9	2·3	0·7	2·1	3·2	0·9
13–16 ...	2·0	0·6	1·8	tr.	1·6	1·7	1·3
17–20 ..	2·1	1·6	3·3	tr.	4·6	0·2	5·3
21–24 ...	*11·0*	9·7	*15·8*	0·1	*19·2*	1·5	6·9
25–28 ...	*16·1*	*20·9*	*18·2*	4·9	*35·4*	*20·5*	*14·0*
29–32 ...	*10·3*	*20·4*	*13·3*	*40·5*	9·9	*19·5*	*20·5*
33–36 ...	n.r.	*12·4*	*11·3*	*18·7*	5·5	7·3	*13·3*
37–40 ...	6·3	7·8	5·9	7·1	2·9	7·6	9·9
41–44 ...	4·6	7·4	6·1	7·1	2·4	9·9	7·9
45–48 ...	3·3	3·8	6·6	5·4	2·6	7·7	*10·7*
49–52 .	3·7	3·8	7·0	7·1	2·1	5·6	5·9
ACTUAL TOTAL ... (Capt. for year.)	115,448	18,405	320,692	411,933	559,102	137,802	373,459

* In 1946 Trap A1 was not at the same site as in 1950. The 1946 record is unreliable, as during weeks 33 to 36 the trap could not work, owing to it being surrounded by water. Significant differences from the mean italicized.

TABLE XXXIII—*concld.*

Weeks.	C2. Curepe.			D2. Streatham Lodge.			E1. Piarco.	
	1948.	1949.	1950.	1948.	1949.	1950.	1949.	1950.
1–4 ...	***17·4***	2·2	9·6	***13·4***	***13·4***	1·4	***14·1***	6·6
5–8 ...	***12·1***	1·7	5·6	9·2	9·0	2·5	***17·8***	7·9
9–12 ...	3·0	4·3	4·4	4·8	4·7	1·7	3·1	6·6
13–16 ...	2·5	0·1	7·0	2·7	0·9	3·7	2·1	6·6
17–20 ...	7·9	tr.	***10·4***	9·7	0·1	8·6	1·0	6·6
21–24 ...	***11·3***	0·4	***15·4***	***13·9***	0·7	8·0	1·6	7·9
25–28 ...	***19·3***	9·5	***23·5***	***19·6***	7·9	***34·6***	4·2	***15·3***
29–32 ...	8·1	***37·2***	7·1	9·2	***32·0***	***21·0***	***24·0***	***15·2***
33–36 ...	3·5	8·2	3·8	2·7	6·0	6·8	***10·4***	***10·5***
37–40 ...	3·5	***17·2***	4·8	1·9	***11·5***	6·8	***12·0***	2·9
41–44 ...	4·6	9·3	2·0	2·7	7·1	1·1	5·7	4·9
45–48 ...	3·2	5·0	3·2	2·1	3·5	0·9	2·1	3·7
49–52 ...	3·1	4·8	2·6	3·6	2·7	3·0	2·1	2·4
ACTUAL TOTAL ... (Capt. for year.)	323,851	427,346	293,586	49,451	32,408	17,737	3,015	2,363

TABLE XXXIV.

Ovarian classification of hungry females in Nelson bamboos : A. aquasalis.

1948 and 1949 monthly figures combined.

	Evening captures.								Morning captures.								Remarks.
	Ov. I.		Ov. II.		Ov. III.				Ov. I.		Ov. II.		Ov. III.				
	Number.	Per cent, monthly total.	Number.	Per cent, monthly total.	Number.	Per cent, monthly total.	Monthly total.	Per cent, annual total.	Number.	Per cent, monthly total.	Number.	Per cent, monthly total.	Number.	Per cent, monthly total.	Monthly total.	Per cent, annual total.	
Jan. ...	23	*13·9*	138	*83·6*	4	*2·4*	165	*15·5*	8	*14·3*	45	*80·4*	3	*5·4*	56	*8·2*	
Feb. ...	1	*2·4*	39	*95·2*	1	*2·4*	41	*3·8*	0	*0·0*	16	*100·0*	0	*0·0*	16	*2·3*	
Mar. ...	4	*12·5*	28	*87·5*	0	*0·0*	32	*3·0*	0	*0·0*	2	*100·0*	0	*0·0*	2	*0·3*	
Apr. ...	0	*0·0*	8	*100·0*	0	*0·0*	8	*0·8*	0	*0·0*	2	*100·0*	0	*0·0*	2	*0·3*	
May ...	1	*4·5*	20	*91·0*	1	*4·5*	22	*2·1*	0	*0·0*	1	*100·0*	0	*0·0*	1	*0·2*	
Jun. ...	9	*10·8*	72	*86·7*	2	*2·4*	83	*7·8*	2	*1·9*	95	*91·4*	7	*6·7*	104	*15·3*	
Jul. ...	34	*16·0*	162	*76·1*	17	*8·0*	213	*20·0*	12	*5·4*	200	*91·4*	7	*3·2*	219	*32·4*	1 ♀ in Ov. V., fertilized, attacked.
Aug. ...	16	*9·4*	136	*80·0*	18	*10·6*	170	*16·0*	3	*3·2*	88	*94·6*	2	*2·2*	93	*13·7*	
Sep. ...	4	*2·5*	151	*95·0*	4	*2·5*	159	*14·9*	1	*1·4*	70	*95·9*	2	*2·7*	73	*10·8*	
Oct. ...	4	*6·2*	57	*89·1*	3	*4·7*	64	*6·0*	6	*9·4*	57	*89·0*	1	*1·6*	64	*9·4*	
Nov. ...	1	*3·2*	28	*90·4*	2	*6·4*	31	*2·9*	0	*0·0*	24	*100·0*	0	*0·0*	24	*3·5*	
Dec. ...	5	*6·5*	70	*90·9*	2	*2·6*	77	*7·2*	0	*0·0*	24	*100·0*	0	*0·0*	24	*3·5*	
TOTAL ...	102	9·6	909	85·3	54	5·1	1,065	...	32	4·7	624	92·0	22	3·3	678		

TABLE XXXV.

Monthly totals of ovarian condition: A. aquasalis.

Christophers' classification, as modified by Roberts and O'Sullivan.

EVENING CATCHES.

Stage.	Jul.		Aug.		Sep.		Oct.		Nov.		Dec.		Jan.		Feb.		Mar.		Apr.		May		Jun.		Totals.	
	Number.	Per cent. total.	Number.	Per cent. total.	Number.	Per cent. total.	Number.	Per cent. total.	Number.	Per cent. total.	Number.	Per cent. total.	Number.	Per cent. total.	Number.	Per cent. total.	Number.	Per cent. total.	Number.	Per cent. total.	Number.	Per cent. total.	Number.	Per cent. total.	Number.	Per cent. total.
I ...	8	2	9	14	4	5	0	0	0	0	3	7	12	11	0	0	0	0	...	...	...	...	4	9	40	5
IIa ...	37	10	21	33	22	28	3	14	2	14	11	24	25	22	0	0	0	0	...	...	...	...	10	23	131	17
IIb ...	102	29	12	19	15	19	4	19	2	14	2	4	6	5	2	7	0	0	...	...	...	...	1	2	146	19
IIc ...	170	48	22	34	37	47	14	67	8	57	27	60	68	60	24	89	4	100	...	...	...	...	27	61	401	52
III ...	38	11	0	0	1	1	0	0	2	14	2	4	2	2	1	4	0	0	...	...	...	...	2	5	48	6
Total ...	355		64		79		21		14		45		113		27		4		0		0		44		766	

TABLE XXXV—*concld.*

MORNING CATCHES.

Stage.	Jul.		Aug.		Sep.		Oct.		Nov.		Dec.		Jan.		Feb.		Mar.		Apr.		May		Jun.		Totals.	
	Number.	Per cent, total.	Number.	Per cent, total.	Number.	Per cent, total.	Number.	Per cent, total.	Number.	Per cent, total.	Number.	Per cent, total.	Number.	Per cent, total.	Number.	Per cent, total.	Number.	Per cent, total.	Number.	Per cent, total.	Number	Per cent, total.	Number.	Per cent, total.	Number.	Per cent, total.
I ...	3	2	2	4	0	0	1	4	0	0	0	0	5	0	0	0	...	...	...	...	0	0	1	3	12	4
IIa ...	15	10	21	40	2	17	5	22	1	6	3	27	4	20	1	20	...	...	...	...	0	0	3	8	55	17
IIb ...	23	16	9	17	3	25	8	35	1	6	2	18	3	60	3	60	...	...	...	...	0	0	2	5	54	16
IIc ...	99	69	19	37	6	50	9	39	13	87	6	54	20	20	1	20	...	...	...	...	1	100	29	74	203	61
III ...	3	2	1	2	1	8	0	0	0	0	0	0	1	0	0	0	...	...	...	...	0	0	4	10	10	3
TOTAL ...	143		52		12		23		15		11		33		5		0		0		1		39		334	

TABLE XXXVI.

Dissections at 82 hours' p.c., percentages: dawn trap captures: A. aquasalis.

Ovarian stage.	Nov. 3, 1948.	Jan. 15, 1949.	Mar. 2, 1949.	Mar. 19, 1949.	Apr. 2, 1949.	Apr. 23–30, 1949.	May 4–7, 1949.	May 11, 1949.	May 28–31, 1949.	Jun. 11, 1949.	Jul. 1, 1949.	Jul. 20, 1949.	Aug. 6, 1949.	Aug. 19, 1949.	Sep. 19, 1949.	Oct. 1, 1949.	Oct. 15, 1949.	Nov. 13, 1949.	Dec. 6, 1949.	Average.
I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IIa	0	0	0	0	0	2	0	0	0	0	0	4	0	2	0	0	0	2	0	0
IIb	0	0	4	0	6	10	5	0	0	0	0	0	10	0	2	2	0	2	0	2
IIc	2	4	2	0	0	18	28	16	20	8	12	18	18	8	2	44	40	0	2	13
III	8	0	2	0	4	7	2	2	4	2	2	6	14	8	10	10	0	2	4	5
IV	8	6	0	4	6	2	10	2	6	0	8	16	6	0	0	8	0	0	0	4
V	82	*90*	*92*	*96*	84	*61*	*55*	80	70	*90*	78	*56*	*52*	82	86	*36*	*60*	*94*	*94*	76

S.D. 17·5. S.E. ± 4·2. Differences in stage V significantly different from mean italicized.

Dispersion of stage V percentages by months.

	30–40.	40–50.	50–60.	60–70.	70–80.	80–90.	90+
Jan. ...	...	...	...	...	...	+	...
Feb. ...	...	...	...	...	...	...	...
Mar. ...	...	...	...	...	...	...	+
Apr. ...	...	...	...	+	...	+	...
May ...	...	...	+	+	+	...	...
Jun. ...	...	...	...	...	...	+	...
Jul. ...	...	...	+	...	+	...	...
Aug. ...	...	...	+	...	...	+	...
Sep. ...	...	...	...	...	...	+	...
Oct. ...	+	...	+	...	...	...	...
Nov. ...	...	...	...	...	...	+	+
Dec. ...	...	...	...	...	...	...	+

TABLE XXXVII.

Intervals in days between density peaks, from daily caught traps and a bush quadrat. 1948: A. aquasalis.

Trap.	January.			February.			March.			April.			May.			June.		
	Intervals.	Number.	Average.	Intervals.	Number.	Average.	Intervals.	Number.	Average.	Intervals.	Number.	Average.	Intervals.	Number.	Average.	Intervals.	Number.	Average.
C1 ..	10.5.4.6	4	6·2	9.23	2	16·0	4.10.8	3	73·0	4.5.7.14	4	7·5	4.7.6.14	4	7·7	4.5.8.4	4	5·2
C2 ..	3.9.10.5	4	6·7	8.3.2.5.9	5	5·4	17.5.5.6	4	8·2	6.17.12	3	11·7	9.8.12	3	9·7	4.5.12	3	7·0
C4 ..	7.4.7.8	4	6·5	6.8.5.2.19	5	8·0	8.3.12	3	7·7	4.2.12.10	4	7·0	11.10.11	3	10·7	10.10.11	3	10·3
D1 ..	5.5.7	3	5·7	17.14.13	3	14·7	11.4.12	3	9·0	5.13.6	3	8·0	13.7.12	3	10·7	4.16.12	3	10·9
Q ..	..	..	..	..	..	..	9.5.7.6.7	5	6·8	9.5.12	3	8·7	4.10.7.6	4	6·7	7.4.7.7	4	6·2
Average trap interval.			6·3			9·5			8·1			8·4			9·5			8·1

Trap.	July.			August.			September.			October.			November.			December.		
	Intervals.	Number.	Average.	Intervals.	Number.	Average.	Intervals.	Number.	Average.	Intervals.	Number.	Average.	Intervals.	Number.	Average.	Intervals.	Number.	Average.
C1 ..	10.14.10	3	11·3	5.4.10.5.9	5	6·2	9.6.8.8	4	7·5	11.10.2.4	4	6·7	11.8.14	3	11·0	10.7	2	8·5
C2 ..	10.14.9	3	11·0	6.12.5.7.2	5	6·6	2.4.10.3.6	5	6·0	6.6.4.5.12	5	6·6	5.5.10.5.5.3	6	5·5	10.7	2	8·5
C4 ..	6.9.6	3	7·0	13.4.7.12	4	9·0	9.7.8.6	4	7·5	11.20	2	15·5	15.5.6.3.2	5	6·2	6.6.8	3	6·7
D1 ..	5.5.4.11	4	8·3	4.8.6.10.6	5	6·8	3.4.6.12.4	5	6·0	15.7.9	3	10·3	6.11.6.2	4	6·2	7.8.6	2	10·5
Q ..	3.20.4	3	9·0	9.14.5	3	9·3	10.11.6	3	10·0	10.4.11	3	8·3	6.9.2.6.6	5	5·8	8.4.4.9.4	5	5·8
Average trap interval.			9·3			7·0			6·7			8·9			9·4			6·7

TABLE XXXVIII

Precipitin reactions—outdoor bush resters, 1948: A. aquasalis.

Locality.	M.	O.	G.	H.	P.	D.	F.	M/O.	M/G.	M/H.	M/P.	O/G.	O/H.	O/P.	O/D.	O/F.	G/H.	G/D.	M/O/G.	M/O/H.	M/O/P.	M/G/D.	O/G/H.	O/G/D.	M/G/H/D.
Success	5	14	3	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	4	0	0	0	0	0	0
Barataria ...	3	13	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
El Socorro ...	1	43	8	15	0	1	1	0	0	2	0	10	5	0	0	0	0	0	1	0	0	1	1	0	0
N. la Paillo ...	2	73	13	6	2	0	0	1	1	0	0	15	8	1	1	0	1	1	0	1	0	0	2	0	1
Bordenal Savh. ...	0	84	0	5	0	0	1	0	0	0	0	1	2	0	0	1	0	0	0	0	0	1	0	0	0
Bamboo V. ...	2	11	1	6	0	0	0	1	2	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
N. Bamboos ...	2	2	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Curepe Savh. ...	1	70	5	8	0	0	0	0	0	0	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0
Do. (In Ramlila). ...	4	12	6	0	0	0	0	1	1	0	0	4	2	0	0	1	0	0	0	0	0	0	0	0	0
Curepe Quads. ...	0	95	1	11	0	0	0	2	0	1	0	1	6	0	0	0	0	0	0	0	0	0	0	0	0
Glaudon	1	91	0	0	0	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0
McLeod	2	56	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Govt. Farm ...	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
O. la Paillo ...	0	32	2	3	6	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Streatham L. ...	0	23	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL: AVERAGES ...	23	624	40	56	14	3	3	7	4	4	2	35	28	2	2	2	1	2	5	2	1	2	3	1	1

M = man. O = ox. G = goat. H = equine. P = pig. D = dog. F = fowl.

TABLE XXXVIII—*concld.*

Locality.	T +	T −	Percentage +	Σ bites.	M.	O.	G.	H.	P.	D.	F.	Percentage + bites. (A.I.) M.	O.	G.	H.	P.	D.	F.	Percentage multiple feeds.
Success	29	7	19	40	12	18	7	1	2	0	0	30	45	17	3	5	0	0	17·2
Barataria	22	8	27	23	3	13	1	1	4	1	0	13	57	4	4	17	4	0	4·5
El Socorro ...	89	2	2	112	5	60	21	23	0	2	1	4	54	19	20	0	2	1	16·9
N. la Paillo ...	129	8	6	167	6	102	34	19	3	3	0	4	61	20	11	2	2	0	25·6
Bordenal Savh. ...	95	3	3	101	1	88	4	5	0	1	2	1	88	4	5	0	1	2	3·2
Bamboo V. ...	25	1	4	31	6	14	4	7	0	0	0	19	45	13	23	0	0	0	12·0
N. Bamboos ...	7	0	0	9	3	4	1	1	0	0	0	33	44	11	11	0	0	0	28·6
Curepe Savh. ...	89	10	10	94	1	75	6	12	0	0	0	1	80	6	13	0	0	0	5·6
Do. (In Ramlila). ...	31	0	0	40	6	20	11	2	0	0	1	15	50	27	5	0	0	3	29·0
Curepe Quads. ...	117	30	20	125	2	103	3	17	0	0	0	2	82	2	14	0	0	0	8·5
Glaudon	97	4	4	101	1	94	2	0	0	3	1	1	94	2	0	0	3	1	3·1
McLeod	61	3	5	62	2	57	0	0	2	1	0	3	92	0	0	3	2	0	1·6
Govt. Farm ...	6	0	0	8	1	6	0	0	1	0	0	12	75	0	0	12	0	0	16·7
O. la Paillo ...	45	17	14	47	1	34	2	4	6	0	0	2	72	4	8	13	0	0	4·4
Streatham L. ...	25	0	0	25	0	23	1	0	1	0	0	0	92	4	0	4	0	0	0·0
TOTALS: AVERAGES ...	867	93	9·7	985	50	711	97	92	19	11	5	5·1	72·1	9·9	9·3	1·9	1·1	0·5	12·0

M = man. O = ox. G = goat. H = equine. P = pig. D = dog. F = fowl.

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CLINICAL TRIAL OF M-5943.

(A NEW CHLORO DERIVATIVE OF PROGUANIL.)

BY

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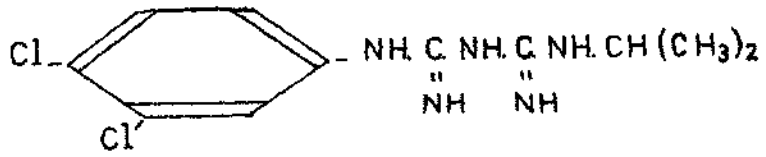
[September 21, 1951.]

A NEW diguanide known as 'M-5943' is of interest in representing an endeavour of the British workers in the field of antimalarial research to produce a further synthetic remedy for treatment of malaria in addition to proguanil. Chemically it is N_1-3 : 4-dichlorophenyl-N_5-isopropyl diguanide, differing from proguanil in having an extra chlorine group in position 3- of the benzene nucleus. It has the constitution as shown below :



The drug is reported to be much more active than proguanil against both the blood forms and exo-erythrocytic stages of *P. gallinaceum* and *P. relictum* but has the disadvantage of being more toxic in laboratory animals than proguanil (Curd *et al.*, 1950). With the hope that human malaria parasites may also be similarly susceptible to the drug, possibly with less toxic reactions, it was considered desirable to undertake clinical trials in patients especially with *P. vivax* infections. The results of the clinical trials of the drug on patients infected with Indian strains of malaria parasites in progress at the Calcutta School of Tropical Medicine are reported in this paper.

MATERIAL.

In this series, 32 patients mostly with *vivax* infection were treated with this drug in the Carmichael Hospital for Tropical Diseases. They included 28 *vivax*, 2 *falciparum* and 2 *malariæ* infections. They were studied particularly with the object of determining the time taken in controlling fever, the time taken for the asexual parasites to disappear from the peripheral blood, toxic effects if any and relapses. Four-hourly temperature charts were maintained and thick blood films stained by Field or J.S.B. technique were examined twice daily till the blood became negative for asexual parasites and thereafter once daily throughout the period during which the patients were kept under observation in the hospital.

DOSAGE.

The drug was available for experimental trial in the form of tablets, each tablet containing 10 mg. of the base.

The following regimes of treatment were tried in 22 and 10 cases respectively :—

Regime '*A*'—2 tablets (20 mg. base) t.d.s. for 10 days.

Regime '*B*'—2 tablets (20 mg. base) t.d.s. for 7 days, followed by 2 similar courses of 5 days at interval of 5 days.

Children were given proportionately smaller doses.

The distribution of the patients according to race, sex and age is shown in Table I.

TABLE I.

Distribution of patients according to race, sex and age.

Infection.	Total number.	Race.			Sex.		Age in years.		
		Bengalee.	Other Indians.	Non-Indians.	Males.	Females.	Up to 12.	12 to 20.	Above 20.
Vivax ...	28	17	10	1	26	2	6	6	16
Malariæ ...	2	0	2	0	2	0	0	0	2
Falciparum ...	2	2	0	0	2	0	0	1	1
TOTAL ...	32	19	12	1	30	2	6	7	19

RESULTS.

The immediate effects on the temperature and parasites are shown in Table II.

TABLE II.

Results of treatment.

Serial number.	Infection.	Duration of fever (days).	Duration of asexual parasites (days).	Gametocytes seen (days after treatment).	Stay in hospital (days).	Relapse after completion of treatment (days).
			REGIME 'A'.			
1	V	1	3	4	12	...
2	V	1	2	3	63	...
3	M	3	1	5	32	...
4	V	*	1	1	14	74 V
5	V	*	1	2	19	...
6	V	Fever (K.A).	1	0	63	...
7	V	3	3	5	15	...
8	V	1	2	3	29	...
9	V	1	2	2	27	...
10	V	3	2	3	30	...
11	V	1	1	1	42	50 (clinical).
12	V	3	3	3	13	70 V
13	V	2	4	4	22	41 V
14	V	3	4	5	21	61 V
15	V	3	5	5	13	...

V = *vivax*. M = *malariæ*.

* Refers to cases that were apyrexial when the treatment was started but had fever the day before.

TABLE II—*concld.*

Serial number.	Infection.	Duration of fever (days).	Duration of asexual parasites (days).	Gametocytes seen (days after treatment).	Stay in hospital (days).	Relapse after completion of treatment (days).
			REGIME 'A'—*concld.*			
16	V	2	2	4	13	...
17	V	4	2	5	15	...
18	V	2	1	1	26	...
19	M	*	1	8	53	...
20	V	1	2	3	13	...
21	F	1	2	3	25	...
22	F	3	3	0	17	...
			REGIME 'B'.			
1	V	3	3	4	33	..
2	V	*	2	2	29	...
3	V	1	3	3	50	...
4	V	3	2	4	40	...
5	V	2	2	6	10	70 V
6	V	*	2	3	30	..
7	V	2	2	3	30	...
8	V	2	3	3	11	...
9	V	1	5	4	37	...
10	V	2	3	5	30	...

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

* Refers to cases that were apyrexial when the treatment was started but had fever the day before.

The day-to-day effect of M-5943 on the temperature and asexual parasites are shown in Table III.

TABLE III.

Effect on asexual parasites and temperature.

Day of treatment.	Asexual parasites.					Temperature.				
	Positive.			Negative.		Raised.			Normal.	
	V	F	M	Number.	Percentage.	V	F	M	Number.	Percentage.
1st ...	28	2	2	0	0	23*	2	1	5	16
2nd ...	22	2	1	7	22	15	1	1	9	29
3rd ...	10	1	1	13	41	9	1	1	6	19
4th ...	4	0	1	7	22	1	0	1	9	29
5th ...	1	0	1	3	9	0	0	1	1	3
6th ...	0	0	0	2	6	0	0	0	1	3

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

* One *vivax* case with kala-azar was not included.

It is evident from the table that the asexual parasites disappeared from the peripheral blood by the third and fourth day of treatment in 63 and 85 per cent cases respectively. The corresponding figures as regards fever are 64 and 93 per cent. Asexual parasites were however found even up to the fifth day of treatment in two cases (6 per cent) and fever in one case (3 per cent).

RELAPSES.

All the patients were under observation in the hospital from 10 to 87 days (10 to 20 days—12 cases; 21 to 30 days—11 cases; over 30 days—9 cases) during which none of them had a relapse. After discharge from the hospital 16 patients (13 of 'A' and 3 of 'B' regime) could be followed up regularly for varying periods, 3 to 6 months. Five of the patients of regime 'A' (4 parasitic and 1 clinical) relapsed after 41, 70, 61, 74 and 50 days respectively, and one of regime 'B' after 50 days. The relapse rate appears to be over 30 per cent. Re-infection, of course, could not be definitely excluded.

GAMETOCYTES.

Gametocytes were present in peripheral blood in all the patients except two before the institution of treatment. They were apparently unaffected by the

treatment. In one quartan case, they were seen up to the 8th day. The disappearance of gametocytes is shown below :—

Day of treatment.	Gametocytes not found.	
	Number.	Percentage.
1st	0	0
2nd	3	10
3rd	3	10
4th	10	33
5th	6	20
6th and above ...	2	7

TOXIC ACTION.

Nine patients manifested some untoward effects. They complained of one or more of the following symptoms : nausea and vomiting (5 cases), pain in abdomen (2 cases), diarrhœa (1 case), giddiness (2 cases), dysuria (1 case), red patches on the body (1 case). They were usually noticed about 5 days after starting the treatment. The treatment was discontinued for a day or two and then reinstituted after the symptoms had disappeared.

ABSORPTION AND EXCRETION.

The excretion and absorption of M-5943 in human beings using regimes A and B were studied. The method of Spinks (1949) based on the modification of the original hydrolytic method for the determination of proguanil (Spinks and Tottey, 1946) was employed.

A study of whole blood concentration of M 5943 was possible in a very limited number of cases as about 40 to 50 c.c. of blood was needed for each estimation. In one case receiving regime B, the drug level in the blood was determined on the 4th, 6th and 7th day, four hours after the first morning dose and the values were 88, 175 and 200 μg. per litre respectively. The estimation was also done in the same patient on the following day just after the completion of the last 5-day course and the value was 71 microgrammes per litre. In another case with regime B, the concentration per litre on the 2nd, 4th and 7th day were 100, 182 and 200 microgrammes. With single dose of 60 mg., the drug concentration after 4 hours was found to be 80 μg./litre.

The excretion of M-5943 using regime A in four cases, and one case receiving a single dose of 60 mg., is shown in Table IV.

TABLE IV.

Excretion of M-5943 in urine in 4 patients receiving 20 mg. base t.d.s. for 10 days, and a patient receiving a single dose of 60 mg.

Time.	Daily M-5943 excretion (as hydrochloride) in urine (mg.).				
	20 mg. t.d.s. for 10 days.				60 mg. (single dose).
	1	2	3	4	5
1st 24 hours ...	8·2	10·2	7·5	8·6	12·5
2nd " ...	12·7	16·1	14·3	10·8	4·4
3rd " ...	14·8	18·4	17·6	10·6	1·6
4th " ...	16·9	20·8	21·5	12·5	0·2
5th " ...	15·6	19·6	25·6	14·9	...
6th " ...	21·2	25·5	24·8	14·8	...
7th " ...	16·8	22·7	20·6	16·7	...
8th " ...	19·6	24·2	22·8	18·5	...
9th " ...	18·0	20·5	19·7	16·4	...
10th " ...	13·7	21·5	22·5	14·5	...
11th " ...	4·0	12·4	10·3	10·2	...
12th " ...	3·9	10·6	8·4	8·1	...
13th " ...	2·8	6·4	6·3	3·6	...
14th " ...	2·1	4·5	2·9	1·2	...
15th " ...	1·9	3·2	1·8	0·5	...
16th " ...	1·2	2·1	0·7	...	...
17th " ...	0·7	0·9	...	...	...
18th " ...	0·4	...	...	...	...
19th " ...	...	...	...	...	...
20th " ...	...	...	...	...	...
TOTAL ...	174·5 or 25 per cent of intake.	239·6 or 35 per cent of intake.	227·3 or 33 per cent of intake.	161·9 or 23 per cent of intake.	18·7 or 27 per cent of intake.

It will be seen from the above table that the total urinary excretion of the drug varied from 23 to 35 per cent of the administered dose. The drug disappeared from the urine in 6 to 9 days with regime A and in 4 days with single dose. Presence of the drug was also tested in the urine of six other cases receiving regimes A and B after the courses of the drug were completed. With both regimes the drug was found to disappear in 6 to 10 days.

SUMMARY.

The results of treatment of 32 cases of malaria (28 *vivax*, 2 *falciparum* and 2 *malariæ*) with M-5943 are reported.

Two regimes of dosage were tried—regime 'A'—20 mg. t.d.s. for 10 days; regime 'B'—20 mg. t.d.s. for 7 days, followed by two similar courses of 5 days at intervals of 5 days.

The asexual parasites disappeared in 85 per cent and fever subsided in 94 per cent cases by the fourth day of treatment.

In treatment of malaria it appears to have no advantage over proguanil; its action is slow and *vivax* gametocytes are apparently unaffected.

The drug is more toxic than proguanil and even with daily 60 mg. doses toxic reactions were observed in 9 cases (i.e. 28 per cent) and therefore trial with higher dosage was not attempted.

Relapses are not uncommon and there appears to be no special advantage over quino-pamaquine or paludrine-pamaquine in treatment of relapsing *vivax* malaria.

Although the present series is small, it may be said that M-5943 has no advantage over other commonly used antimalarial drugs.

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ACTION OF PROGUANIL AND PAMAQUIN, SEPARATELY AND IN COMBINATION, AGAINST *P. KNOWLESI* IN *S. RHESUS* MONKEYS.

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THE present-day conception on the theory of relapses in malaria tends to incriminate the tissue forms which persist even after the establishment of the erythrocytic stages of the parasites liberated from the pre-erythrocytic schizonts. Existence of these forms in mammalian malaria has been demonstrated by Shortt and Garnham (1948) in sporozoite-induced *P. cynomolgi* infection in *S. rhesus* monkeys. In the case of avian malaria, e.g. *P. gallinaceum*, exo-erythrocytic stages can develop directly from the blood forms of parasites (Corradetti, 1941) but it is doubtful if the same happens in man and monkeys.

In simian malaria, unlike avian infection, it is believed that the relapse mechanism is similar to that in man. While radical cure of blood-induced infection of *P. knowlesi** in *rhesus* monkeys can be effected with active schizonticidal drugs, untreated cases of either sporozoite or blood-passaged infections have a tendency to relapse periodically for a period up to 2 to 3 years. This phenomenon may suggest the possibility of establishment of persisting tissue phase similar to that in *P. gallinaceum* infection as already discussed by Jaswant Singh *et al.* (1949).

* Untreated infection with the original strain of *P. knowlesi* in *rhesus* monkeys invariably resulted in the death of the animals. Due to the gradual attenuation of the virulence of the strain from 1947, as reported by Jaswant Singh *et al.* (1949), majority of the animals survive and there is a tendency towards chronicity after the crisis.

Although, pamaquin (also known as plasmoquine or plasmochin), a polyvalent gametocide, has some schizonticidal action against *P. vivax* and *P. malariæ* infections (Field, 1938), almost all workers are agreed that it is totally ineffective against the trophozoite stages of *P. falciparum* (Muhlens *et al.*, 1927; Sioli and Muhlens, 1927; Schulemann and Memmi, 1927; Green, 1929; James, 1931; Field, *loc. cit.*; Fairley *et al.*, 1947; Berliner *et al.*, 1948). With regard to the action against the trophozoites of *P. malariæ*, opinions differ and some consider that pamaquin is a powerful schizonticide against this parasite (Muhlens *et al.*, *loc. cit.*; Green, *loc. cit.*; League of Nations, 1937). Fulton and Yorke (1941) have reported that complete parasite clearance can be obtained with the minimum effective dose of pamaquin (3·3 mg./kg. of body weight) in *S. rhesus* monkeys infected with *P. knowlesi*. Its action in avian malaria is variable as shown by Manwell (1934) who observed that though pamaquin proved more effective than quinine against *P. elongatum*, its action is somewhat similar to quinine against the other species like *P. cathemerium*, *P. circumflexum*, *P. relictum* (*præcox*), etc.

Thus it would appear that, as a schizonticide, its action is variable according to the species of the parasites. In view of this, and the narrow margin of safety between therapeutic and toxic doses (Sinton and Bird, 1928), treatment of malaria with pamaquin merely for effecting a clinical cure is not recommended (Jaswant Singh, 1951). But the importance of pamaquin lies in its probable effect on the persistent tissue phase, believed to be responsible for the systematic relapses in *P. vivax* infection (Shannon, 1946; Berliner *et al.*, *loc. cit.*; Fairley, 1949). That the curative action of this antimalarial can be potentiated by the simultaneous administration of quinine has been shown by Sinton *et al.* (1930), Kligler and Mer (1931) and Sinton (1945). A combination of proguanil and pamaquin for the treatment of relapses has also been attempted by various workers with encouraging results (Fairley *et al.*, 1946; Hulshoff, 1950; Monk, 1948).

Though the minimum effective dose (M.E.D.) of proguanil against blood-induced infection of *P. knowlesi* in *S. rhesus* monkeys has been studied by the authors to be in the region of 0·02 gm. base per kilogramme of the body weight, they have observed that even after large doses of 50 mg. or higher, administered for 5 to 7 days, relapses were common (Jaswant Singh *et al.*, 1949). With the object of assessing, if radical cure of blood-induced infection of *P. knowlesi* can be attained or else if any significant changes in the relapse pattern are noticeable, therapeutic trials were undertaken with a combined dosage regime of proguanil and pamaquin in *S. rhesus* monkeys. The two antimalarials were also tried separately for comparative studies along with toxicity tests.

METHODS AND MATERIAL.

Altogether 42 monkeys were used for therapeutic tests (20 for proguanil, 10 for pamaquin, and 12 for proguanil-pamaquin compound*) and the technique adopted was similar to that reported earlier by Jaswant Singh, Nair and Basu (1950) and Jaswant Singh *et al.* (1950).

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* A product of Imperial Chemical Industries Ltd., from whom the antimalarials were obtained for this investigation.

Each proguanil-pamaquin compound tablet contains 0·1 gramme of proguanil (hydrochloride salt with 87·4 mg. base content) and 0·02 gramme of pamaquin (naphthoate salt with 9 mg. base content). In the series where proguanil or pamaquin was administered alone the same salts were used.

The dosage schedules employed in these trials varied. In case of proguanil, the daily dose ranged from 0·5 to 87·4 mg. of the base per kg. body weight of the animal. The dose of pamaquin when administered by itself corresponded to the amount of the base contained in proguanil-pamaquin tablets.

With regard to proguanil and pamaquin (combined), the dosages employed were again 0·5 to 40 mg. of proguanil base content with the appropriate amount of pamaquin contained in these tablets. In all cases the daily total amount of the drug was administered through a Ryle's tube in the form of a dilute suspension in water in two divided doses for 7 days.

Proguanil.—The results of daily observations in the series treated with proguanil under different daily dosage schedules are shown in Table I.

From Table I, it is evident that though there was prompt clearance of parasites in schedules 7 to 9, the dose of 40 mg. and above of proguanil per kilogramme of the body weight proved too toxic and was responsible for the death of some of the animals.

In non-toxic doses, 20 and 10 mg. dosage schedules appeared to be quite effective in the clearance of parasites from the peripheral circulation. The asexual parasites disappeared on an average in 84 and 96 hours respectively, while longer time (102 hours) was required for gametocyte clearance.

It was further observed that where prolonged observation was possible the dose of 10 mg. proguanil or above was effective in the sterilization of the blood-induced infection. In lower dosage schedules, relapses were frequent and the interval between the completion of the treatment and subsequent attack varied between 12 and 21 days.

Pamaquin.—The dosages of pamaquin used in the 10 monkeys in this series were 0·25, 0·5, 1·03, 4·12 and 8·24 mg. (per kg. of body weight) a day and they correspond to the proportionate amount of pamaquin that is combined with 2·5, 5·0, 20·0, 40·0 and 80·0 mg. respectively of proguanil in the proguanil-pamaquin tablets. Two monkeys were placed under each dosage regime.

Analysis of the results as shown in Table II indicates that pamaquin proved effective in the initial clearance of parasites from the peripheral blood in the dosage schedules 2 to 5. But schedule 5 (8·24 mg. pamaquin) showed adverse side-effects and was responsible for the death of one of the animals.

In non-toxic dosage, the regime with 4·12 mg. pamaquin effected gametocyte clearance in 84 hours and asexual parasites in 108 hours, almost reverse to what was observed in schedule 5 of the regime treated with proguanil.

The period between treatment and subsequent attacks in those in which complete sterilization was not obtained varied from 15 to 30 days.

From Table III, it will be noted that schedule 6 proved highly toxic to both the monkeys which succumbed during the course of treatment. Schedule 5, with 20 mg. proguanil and 2·06 mg. pamaquin, appeared to be very satisfactory in that

TABLE I.

Results of therapeutic trials with proguanil against P. knowlesi.

Schedule.	Total daily dosage (base) mg./kg. body weight.	Serial number of the monkeys employed in each dosage regime.	Time taken (in hours) for clearance of parasites.			Average interval between cessation of treatment and relapse (in days).	Remarks.
			Total.	Asexual.	Sexual.		
1	0·5	1	264	264	192	21	Severe relapse.
		2	288	288	192	20	,, ,,
		3	...	...	...	...	Inactive.
2	1·0	1	162	162	162	12	.....
		2	186	186	186	12	.....
3	2·5	1	144	144	96	...	Died after 15 days' intercurrent disease.
		2	180	180	144	...	Cured.
4	5·0	1	132	132	132	12	.....
		2	132	132	84	...	Cured.
5	10·0	1	96	96	96	...	,,
		2	108	96	108	...	,,
6	20·0	1	84	84	184	...	,,
		2	120	84	120	...	Died after 13 days, spleen smear showed parasitic infection.
7	40·0	1	108	96	108	...	Died after receiving 200 mg. (dose).
		2	96	96	96	...	Cured.
		3	132	96	132	...	Died after 38 days.
8	43·7	1	114	114	96	...	Died after receiving 279 mg. (base).
		2	...	...	...	...	Died after the second dose.
9	87·4	1	84	84	84	...	Died after receiving 480·7 mg. drug (base).
		2	72	72	68	...	Died 3 days after treatment.

parasite clearance could be attained in 72 hours (average). In lower dosage schedules 3 and 4, asexual parasite clearance was observed in 102 and 114 hours

TABLE II.

Results of therapeutic trials with pamaquin against P. knowlesi.

Schedule.	Total daily dosage (base) mg./kg. body weight.	Serial number of the monkeys employed in each dosage regime.	Time taken (in hours) for clearance of parasites.			Interval between cessation of treatment and relapse (in days).	Remarks.
			Total.	Asexual.	Sexual.		
1	0·25	1	...	...	...	...	Not active.
		2	...	...	...	...	" "
2	0·51	1	132	132	96	20	Active but no effect on relapse rate.
		2	120	120	96	30	.....
3	1·03	1	84	84	60	...	Cured.
		2	132	132	60	16	No effect on relapse rate.
4	4·12	1	108	108	84	...	Died 1 month after treatment.
		2	108	108	84	18	Not effective against relapse.
5	8·24	1	48	48	24	...	Died 3 days after treatment.
		2	60	60	36	...	Cured.

respectively. Gametocytes disappeared much quickly in 54 and 66 hours (average) respectively.

The total number of animals in which observations for at least 3 weeks could be undertaken was 8; two each under schedules 2 to 5. While parasitic relapses occurred in 25 and 33 days in animals under schedule 2, no parasites could be detected in respect of both the animals under schedule 3, and one animal each under schedules 4 and 5 during a short observation period varying from 20 days to one month.

Prolonged observation, however, for over 2 months was possible in case of one animal each under the last two schedules mentioned above. When the blood smears did not show any parasites for 2 months, spleen was removed from both the animals. In one case (schedule 4) patent parasitæmia was established 21 days after, while in the other case (schedule 5), it was 34 days after splenectomy.

RESULTS OF TOXICITY TESTS.

PAMAQUIN.

Acute toxicity.—Thirty-two animals were used for toxicity tests with pamaquin (Table IV). Although adverse side-effects like excessive salivation, retching,

TABLE III.

Results of therapeutic trials with proguanil-pamaquin compound against P. knowlesi.

Schedule.	Total daily dosage (base) mg./kg. body weight.	Serial number of the monkeys employed in each dosage regime.	Time taken (in hours) for clearance of parasites.			Interval between cessation of treatment and relapse (in days).	Remarks.
			Total.	Asexual.	Sexual.		
1	0·5 mg. proguanil and 0·05 mg. pamaquin.	1	...	...	...	...	Inactive.
		2	264	264	172	...	.....
2	1·0 mg. proguanil and 0·10 mg. pamaquin.	1	168	168	72	25	.....
		2	108	108	96	33	Severe relapse.
3	2·5 mg. proguanil and 0·25 mg. pamaquin.	1	96	96	24	...	Escaped from cage 20 days after treatment.
		2	108	108	84	...	Died 1 month after treatment. (Intercurrent disease.)
4	5·0 mg. proguanil and 0·51 mg. pamaquin.	1	108	108	108	...	Died 21 days after treatment. (Intercurrent disease.)
		2	120	120	24	81	Relapse occurred 21 days after splenectomy.
5	20·0 mg. proguanil and 2·06 mg. pamaquin.	1	60	60	...	...	Died 1 month after treatment. (Intercurrent disease.)
		2	84	84	...	93	Relapsed 33 days after splenectomy.
6	40·0 mg. proguanil and 4·12 mg. pamaquin.	1	72	72	48	...	Died after receiving 280 mg. proguanil base and 99·88 mg. pamaquin base.
		2	...	...	...	...	Died with parasitæmia after receiving 160 mg. proguanil base plus 32·96 mg. pamaquin base.

vomiting, anorexia, tachycardia, rapid respiration and restlessness were detectable in monkeys receiving single doses of pamaquin, no death occurred up to a maximum dose of 50 mg. per kilogramme of the body weight. When the dose was increased to 75 mg., 3 out of 6 animals died between 1 and 5 days. This schedule may be considered as L.D. 50.

The effect of large doses of proguanil-pamaquin compound when given as a single dose to 60 monkeys is shown in Table V.

TABLE IV.

Toxicity tests with pamaquin in monkeys.

Schedule.	Single dose (base) mg./kg. body weight.	Number of monkeys tried.	Number died.	Percentage mortality.	Interval in hours between drug administration and death.
1	75	6	3	50	Two in 24 and 48 hours; one in 5 days.
2	50	5	0	...	...
3	40	5	0	...	...
4	30	6	0	...	...
5	20	4	0	...	...
6	10	6	0	...	...

TABLE V.

Results of acute toxicity tests with proguanil-pamaquin compound.

Schedule.	Single dose (base) mg./kg. body weight.	Number of monkeys tried.	Number died.	Percentage mortality.	Interval in hours between drug administration and death.
1	300 (with 30 mg. pamaquin).	5	4	80	In 2 cases within 3 minutes and in the remaining 2, 4 minutes and 1 hour.
2	250 (with 25 mg. pamaquin).	6	5	83	In 3 cases within 3 minutes and in the remaining 2 within an hour.
3	200 (with 20 mg. pamaquin).	8	3	37·5	In one 3 minutes, one within an hour and one within 4 hours.
4	100 (with 10 mg. pamaquin).	10	3	30	All three within 4 minutes to 1 hour.
5	75 (with 7·5 mg. pamaquin).	10	1	10	Within an hour.
6	60 (with 6 mg. pamaquin).	4	1	25	" "
7	55 (with 5·5 mg. pamaquin).	10	0	0	...

It will be noted that altogether 55 animals were placed under the different schedules. On account of the variable number of animals utilized under different dosage regimes, correct appraisal of the mortality rate is difficult. However, it is clear that all the 10 animals survived the dose of 55 mg. proguanil and 5·5 mg. pamaquin as per schedule 7. As higher doses proved too toxic to some of the monkeys, this schedule is regarded as L.D. 0, whereas L.D. 50 appears to be in the region between 200 and 300 mg. of proguanil with the corresponding doses of pamaquin.

DISCUSSION.

Effective clearance of parasites with proguanil from the peripheral blood in blood-induced *P. knowlesi* infections in *rhesus* monkeys has been previously reported by Jaswant Singh *et al.* (1949). In the present series of investigations, the authors observed that parasite clearance was attained more or less in all the dosage schedules. The time for asexual parasite clearance with the lowest dosage schedule was 276 (average) hours as compared to 78 hours (average) with 87·4 mg. proguanil. But it is evident that proguanil in doses of 40 mg. or above is too toxic. In non-toxic therapeutic doses, clearance of asexual parasites was attained quickest in 84 hours (average) with a dose of 20 mg. (per kg. body weight), though action against the gametocytes was not so prompt (102 hours).

Fulton and Yorke (*loc. cit.*) observed in *P. knowlesi* infection that a dose of 3·3 mg. of pamaquin caused complete disappearance of parasites within 120 to 144 hours. In the present series, asexual parasite clearance was attained on an average in 54 hours with a dose of 8·24 mg. pamaquin which, however, proved very toxic. In non-toxic doses comparatively rapid clearance was noticed in doses of 1·03 mg. (average 108 hours), while more prompt response was attained against the gametocytes. Thus, while asexual parasite clearance was quicker with proguanil, gametocytes disappeared more promptly with pamaquin. This more or less confirms the previous observations that pamaquin acts slowly on the dividing forms of *P. knowlesi* (Nauck, 1934) and that it is a powerful gametocidal drug (Field, *loc. cit.*; Coatney, 1935; Ciuca *et al.*, 1942; Dick and Bowles, 1947; and Fischer, 1949).

The combined dosage regime of proguanil and pamaquin proved too toxic in the schedule with 40 mg. proguanil and 4·12 mg. pamaquin, but the next lower dosage (20 mg. proguanil with 2·06 mg. pamaquin) showed the highest therapeutic efficiency in non-toxic doses, of all the series. Asexual parasite clearance was observed in 72 hours as against 84 hours with 20 mg. of proguanil and 108 hours with 4·12 mg. pamaquin when either of the drugs was administered alone. Thus, it would appear that the addition of a small dose of pamaquin (2·06 mg.) was responsible for the enhanced schizonticidal activity of (20 mg.) proguanil. Similar effect against gametocyte clearance is also noticeable. It will be observed that sexual parasite clearance was attained on an average of 84 hours with the dosage schedule of 1·0 mg. proguanil with 0·1 mg. pamaquin, the same as was observed with 4·12 mg. pamaquin alone. In higher dosage schedule with proguanil-pamaquin, more prompt response against gametocytes was apparent which cannot be accounted for with the dosage of pamaquin alone which is combined with proguanil.

Effects of proguanil on relapse rates of *P. vivax* infection have been discussed by Johnstone (1946) and Andrews *et al.* (1947). Similar studies were made with pamaquin (Sinton and Bird, *loc. cit.*), quinine and pamaquin (Sinton *et al.*, *loc. cit.*; Fieldman *et al.*, 1947; Alving, 1948) and with proguanil and pamaquin (Monk, *loc. cit.*). During their preliminary studies against blood-induced *P. knowlesi* infection, Jaswant Singh *et al.* (1949) reported that though sterilization of the infection occurred in many cases when treated with proguanil, it was not always the case. During the present investigations they have obtained similar results. In non-toxic but effective therapeutic dosage schedules it was observed that under proguanil regime (schedules 4 to 6) there had been no relapses in 4 out of 6 animals, while under pamaquin regime (schedules 2 to 4) sterilization of infection occurred in only one out of 5 animals (1 animal under schedule 3 died during the course of observation). But those animals in which relapses occurred, the period between treatment and relapses was longer under pamaquin (16 to 30 days) regime than with proguanil (12 to 20 days). This period seems to be very much prolonged (25 to 93 days) when the animals are treated with a combined dosage regime of proguanil and pamaquin. In view of the fact that relapses occurred in all the animals under schedules 2 to 5 where prolonged observation was possible, this lengthening of the interval between treatment and relapses seems to be the main advantage.

As compared to the regime under proguanil, the relatively higher relapse rates with pamaquin (either alone or in combination with proguanil), which is believed to have action against the exo-erythrocytic forms, would indicate that such developmental phase of blood-induced infection does not exist and therefore sterilization depended mainly on the schizonticidal action of the drugs administered. Hence better results were obtained with proguanil.

From the results on the toxicity trials with pamaquin it is observed that L.D. 0 and L.D. 50 is 50 mg. and 75 mg. (per kilogramme body weight). In dogs and cats the maximum tolerated doses have been determined to be 20 and 7·5 mg. respectively (Fulton, 1942). Thus, it would appear that unlike dogs and cats, monkeys are less susceptible to this drug.

In some cases death of the monkeys was delayed after oral administration of large doses of pamaquin. Such delayed death in fowls has also been recorded by Kohlschutter *et al.* (1943). Many of the toxic side-effects of pamaquin in monkeys observed during the studies are in conformity with those reported by earlier workers (Wiselogle, 1946).

In their previous studies, Nair *et al.* (1951) reported that the maximum tolerated dose of proguanil is 55 mg. (kg. body weight) and that 40 to 60 per cent mortality was caused by doses ranging from 150 to 500 mg. With a dose of 550 mg., 70 per cent of the animals died, while 600 mg. resulted in 100 per cent (L.D. 100) death.

In the present series when proguanil was administered concurrently with pamaquin in approximately one-tenth the dose of the former, a combined single dose up to 200 mg. proguanil and 20·6 mg. pamaquin, it was observed that the mortality rate was less than what was observed with the same dose of proguanil alone (Nair *et al.*, 1951). But when the dose of proguanil-pamaquin was raised to 250 to 300 mg./kg. of proguanil and proportionate dose of pamaquin,

the death rate in the monkeys increased up to 80 to 83 per cent as against 40 to 60 per cent produced by proguanil. Monk (*loc. cit.*) made similar reports in human cases that when pamaquin is combined with a high dose of proguanil, toxic symptoms were noticeable in a larger number. This is believed to be due to the fact that proguanil displaces pamaquin from tissues and toxic reactions are due to high pamaquin level in plasma.

SUMMARY.

1. In non-toxic therapeutic doses quicker schizonticidal action was attained with proguanil than with pamaquin.

2. The effects against gametocytes were quite different with pamaquin which was found to be more potent than proguanil.

3. A dose of 20 mg. proguanil with 2·06 mg. pamaquin showed the highest therapeutic efficiency than either of the drugs administered alone. Thus, it would appear that addition of a small dose of pamaquin had potentiated the action of proguanil.

4. In non-toxic doses gametocyte clearance was obtained much quicker with the combined dosage regime than either of the drugs administered alone.

5. Relapses occurred in all the regimes though less with proguanil. However, the interval between treatment and relapses appeared to be significantly prolonged in the combined regime of proguanil and pamaquin.

6. L.D. 0 in respect of pamaquin and proguanil-pamaquin was determined to be 50 and 55 mg. respectively per kg. of body weight of monkeys. L.D. 50 was found to be 75 mg. in case of pamaquin and between 200 to 250 mg. with paludrine-pamaquin compound.

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STUDIES ON TOXICITY OF PROGUANIL IN *S. RHESUS* MONKEYS.

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REPORTS on toxicity and pharmacology of proguanil in laboratory animals by several workers are available (Davey, 1946; Butler *et al.*, 1947; Schmidt, Hughes and Smith, 1947; Schmidt *et al.*, 1947; Vane, 1949; Findlay, 1951). In mice the minimal lethal dose of proguanil hydrochloride varies slightly with the body weight (Davey, *loc. cit.*). According to Butler *et al.* (*loc. cit.*), acute toxicity of this drug varies with the species of the animal and the method of administration. On their observations that mice and rats are highly susceptible to proguanil, unlike chicks, they concluded that man probably behaves more like chicks than mice and rats. Schmidt, Hughes and Smith (1947) recorded that in *rhesus* monkeys, even after a single oral dose of 320 mg. of the drug per kg. body weight, no case of death occurred. But when it was given intramuscularly, a dose of 160 mg./kg. proved fatal. Similarly, Findlay (1951) records observation after 500 mg./kg. of the drug administered orally to a baboon *Cercopithecus aethiops centralis* and observed no toxic reaction. On the basis of this he reported that monkeys are less susceptible to proguanil than rats and mice. On the other hand, Jaswant Singh *et al.* (1949) observed some deaths in *rhesus* monkeys during the course of proguanil hydrochloride treatment with much lesser doses than used both by Schmidt, Hughes and Smith (1947) and Findlay (1951). This necessitated a detailed investigation on the various lethal doses of proguanil (intravenous lactate and orally hydrochloride) in *rhesus* monkeys. The present paper deals with the acute and chronic toxic manifestation after oral administration. The results of toxic manifestations after intravenous injection of proguanil lactate have already been reported by Jaswant Singh, Nair, Basu and Ray (1950).

METHODS AND MATERIAL.

The method of estimating the lethal doses (L.D. 0, L.D. 50, L.D. 100) was followed in this work as described by Jaswant Singh, Nair and Basu (1950) and Jaswant Singh, Nair, Basu and Ray (1950). The weight of the experimental monkeys ranged from 5 to 15 lbs. and the dosages employed were in terms of the base of the drug per kg. body weight. In all cases the drug was given in the form of a dilute suspension as a single dose on empty stomach in the morning. Drug was administered with the help of Ryle's stomach tube

TABLE I.

Acute toxicity tests with proguanil in monkeys.

Single dose mg./kg. (base).	Number of monkeys tried.	Number died.	Mortality percentage.	Interval between drug administration and death.	Lethal dose.
600	2	2	100	2 immediately	100
550	10	7	70	7 immediately	...
500	10	4	40	1 immediately, 2 within 5 to 12 hours, 1 within 13 to 24 hours.	
450	10	4	40	1 within 1 hour, 1 within 2 to 4 hours, 2 within 5 to 12 hours.	
400	10	4	40	4 immediately	
350	10	4	40	1 immediately, 2 within 1 hour, 1 within 1 to 2 hours.	
300	10	6	60	2 immediately, 4 within 1 hour.	
250	10	4	40	3 immediately, 1 within 2 to 4 hours.	50
200	10	5	50	1 immediately, 1 within 1 hour, 3 within 13 to 24 hours.	
150	10	4	40	1 within 1 hour, 3 within 2 to 4 hours.	
100	10	2	20	2 immediately	
75	10	2	20	2 within 2 to 4 hours	
70	10	2	20	2 within 2 to 4 hours	
65	10	1	10	1 within 2 to 4 hours	
55	10	0	0	.....	0

(attached to a syringe). The monkeys were then watched carefully for any symptoms like copious salivation, vomiting, restlessness, rapid breathing, etc. The interval between the cessation of drug administration and death was also recorded. Those that did not show any toxic manifestation were kept under observation for a minimum period of 2 weeks.

The daily dose of the drug was given in two divided doses, morning and evening, and was continued for 7 to 14 days in monkeys undergoing chronic toxicity tests. Each monkey after cessation of treatment was kept under observation for

TABLE II.

Results of chronic toxicity tests with proguanil in monkeys.

*Daily dosage in mg. of the base per kg. body weight.	Number treated.	Number died.	REMARKS.
5 mg. for 7 days	12	0	.....
5 mg. for 14 days	9	0	.....
10 mg. for 7 days ...	13	0	.....
10 mg. for 14 days	2	0	.....
15 mg. for 7 days	5	0	.....
20 mg. for 7 days	8	1	Death on the 4th day of drug administration. Slight congestion of lungs on post mortem.
30 mg. for 7 days	3	0	.....
40 mg. for 7 days ...	2	0	.....
45, 55, 60 and 80 mg. for 7 days ...	4 (one in each dose).	0	.....
Five courses of 5, 7·5, 10, 12·5 and 25 mg. for 7 days each with an interval of 20 to 30 days between each regime.	1	0	.....
Three courses of 5, 10 and 20 mg. for 7 days.	2	0	.....
Three courses of 10, 12·5 and 25 mg. for 7 days.	3	1	Death on 3rd day during the third course of treatment. Post mortem typical of proguanil toxicity.
Two courses of 20 and 35 mg. for 7 days	2	0	.....
Four courses of 10, 20, 35 and 70 mg. for 7 days.	1	0	.....

* Given in two equal parts at 10 a.m. and 5 p.m.

1 to 6 months. In a few cases two to five courses of the drug were given to the same animals, and in some the subsequent dosage was increased progressively, doubling the previous dosage.

RESULTS.

Sixteen dosage series were followed ranging from 55 mg. base of the drug per kg. body weight to 600 mg., using in each series 10 monkeys, except in the 600 mg. series in which only 2 monkeys were included. The results are recorded in Table I. The highest dose per kg. body weight of the animal which gave rise to no mortality (L.D. 0) was 55 mg. The doses which caused 40 to 50 per cent mortality (L.D. 50) were between 200 and 500 mg., and for 100 per cent mortality it was 600 mg. The total number of deaths recorded due to acute toxic effect of proguanil was 53 and the interval between administration of the drug and death was either immediate or within 3 minutes in 43·4 per cent, between 4 minutes to 1 hour in 20·8 per cent, 2 to 4 hours in 18·9 per cent, 5 to 12 hours in 7·5 per cent, 13 to 24 hours in 7·5 per cent and between 25 to 72 hours in 1·9 per cent of the monkeys. The toxic reactions produced by the drug before death of the monkeys and post-mortem findings were almost the same as those observed in monkeys receiving bromoguanide (Jaswant Singh, Nair and Basu, 1950), except that the salivation noticed after proguanil administration was not so marked as with bromoguanide. The details of chronic toxicity tests are given in Table II.

In some cases only one course of proguanil was given in 5 to 40 mg. daily doses. In others the course was repeated two to five times. The drug was given in 5 to 80 mg. per kg. a day, gradually increasing the dosage each time the course was repeated.

It will be noticed in Table II that, out of 67 monkeys receiving treatment regimes, death occurred in only 2 monkeys, one under 20 mg. single dosage regime and the other receiving 25 mg. during the third course.

Post-mortem examination of the animal that received 20 mg. dosage revealed no gross changes attributable to the toxic reaction of the drug. The post-mortem appearance of the lung in the other case revealed congestion and œdema as often seen in monkeys which died due to toxic doses of proguanil.

DISCUSSION.

The acute lethal doses worked out by Davey (*loc. cit.*), Schmidt, Hughes and Smith (1947), Schmidt *et al.* (1947) and Butler *et al.* (*loc. cit.*) in different laboratory animals when given orally may be summarized as follows—in mice L.D. 0, 15 to 75 mg./kg.; L.D. 50, 23 to 80 mg./kg. and L.D. 100, 30 to 100 mg. In rats, the corresponding figures are 80 to 100, 100 to 200 and 800 respectively. In chicks, the maximum non-lethal dose was 200 mg./kg., and the dose required to produce 50 per cent mortality, 400 to 600 mg./kg. Schmidt *et al.* (1947) and Findlay (1945), finding that in the experiments conducted by them 400 to 500 mg./kg. of the drug was not lethal to monkeys, came to the conclusion that though mice were found to be most

susceptible to the toxic reactions with proguanil when compared to rats, monkeys were less so, compared to both mice and rats. The present findings show that in North Indian *S. rhesus* monkeys, while the maximum oral non-lethal single dose and L.D. 100 are 55 and 600 mg./kg. base, respectively, 150 to 500 mg. dosage caused 40 to 60 per cent mortality. The same drug when given intravenously in the form of lactate, the L.D. 0, L.D. 50 and L.D. 100 was worked out as 33, 48 and 56 mg./kg. base respectively (Jaswant Singh, Nair, Basu and Ray, 1950). It becomes apparent that there is hardly much difference in the results of toxicity studies after oral drug administration in rats as observed by other workers and those recorded in *rhesus* monkeys in the present series.

Butler *et al.* (*loc. cit.*) have shown that delayed deaths due to acute toxicity of proguanil occur in mice and rats from 1 hour to even 24 hours following intravenous and intraperitoneal injection, but in chicks all deaths occur within 15 minutes. Hughes *et al.* (1947) have demonstrated that even in mice immediate death can be effected by simultaneous injection of prostigmine and proguanil. It may be presumed that delayed deaths may be due to interference with enzyme system. In chicks, administration of the drug in toxic doses maintained a fairly high level of proguanil in the plasma but in the same doses only a low concentration was noted in rats. It is likely that the degradation products formed in mice and rats are toxic, whereas in chicks and human beings the metabolic processes of proguanil may either be different or, even if similar, degradation products are formed that are rapidly destroyed and excreted. Whatever be the mode of the breakdown processes it is interesting to note that in the present series 64 per cent of the animals died within 1 hour of the oral administration of the drug in toxic doses, and in approximately 2 per cent death occurred within 24 to 72 hours. With intravenous administration (Jaswant Singh, Nair, Basu and Ray, 1950), however, the interval between the injections and death of the animals was 1 to 37 minutes except in one case which died after 20 hours.

The main toxic features observed in the acute toxicity tests in monkeys include disinclination of food, salivation, rapid breathing and coma. Post-mortem findings in such cases consisted of bloating of stomach and intestine, frothy exudate from trachea, bronchi, bronchioles and alveoli. Findings somewhat similar to these have been reported by other workers during their pharmacological and toxicity investigations in the various laboratory animals. In dogs, the drug induces copious salivation, loss of appetite, extreme cachexia and cardiac arrhythmia (Spinks, 1946; Hughes and Schmidt, 1947). When given in sub-lethal doses to rats, mice, dogs and monkeys, the intake of food is invariably reduced (Findlay, 1951; Hughes and Schmidt, *loc. cit.*). Further, there is a temporary fall in blood pressure, increased rate of respiration in anæsthetized or pithed cat. In these animals, as well as in man, gastric secretion is inhibited (Vane, 1949; Burn and Vane, 1949; Doll and Schneider, 1948).

The results obtained in the chronic toxicity tests are not always consistent. Schmidt, Hughes and Smith (1947) recorded that monkeys receiving 40, 80 and 160 mg. proguanil per kg. body weight succumbed after 22, 18 and 7 days' treatment, whereas during the present observation it is noticed that an oral dose of 20 to 40 mg. of the base a day for 7 days was responsible for the death in some animals. As compared to these, Davey (as quoted by Findlay, 1951) observed that 60 mg./kg. of

proguanil (salt) given intravenously twice daily caused a few deaths in chicks after 2 or 3 days' medication and that the minimal lethal doses in rats and mice are 50 and 20 mg./kg. respectively. From Table II it may be seen that during the present investigation, dosages ranging from 5 to 80 mg./kg. a day, in two divided doses, administered for 7 to 14 days to 67 apparently healthy monkeys except in one case (with a dose of 25 mg.), caused no death attributable to the toxic effects of proguanil. The effect on the other hand in the infected ones is somewhat different as observed by Ray *et al.* (1951) in which it was found that 5 out of 7 monkeys infected with *P. knowlesi* and treated with 40 to 87·4 mg./kg. of proguanil died due to toxic effects. From these results it may be presumed that healthy monkeys can tolerate a much higher dose than those with acute malarial infection. This is in conformity with the observation made on chicks that the maximum tolerated dose in respect of healthy birds is higher than the parasitized ones (Wiselogle, 1946).

SUMMARY.

Acute and chronic toxic effects after oral administration of proguanil hydrochloride were assessed in *S. rhesus* monkeys using 219 animals and L.D. 0, L.D. 50 and L.D. 100 were determined to be 55, 200 and 600 mg./kg. base respectively. The chronic toxic dose varied between 20 to 40 mg./kg. There is also evidence to show that in doses much higher than the therapeutic level, *S. rhesus* monkeys infected with *P. knowlesi* are more prone to the toxic effects than healthy ones.

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ISOLATION OF A STRAIN OF *P. INUI* FROM MIXED INFECTION IN MALAYAN MONKEY.

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INFECTIONS with one or more plasmodia in oriental monkeys like the *M. irus** (*M. cynomolgus*, *S. irus*), which are commonly found in Malaya, were reported by Napier and Campbell (1932), Knowles and Das Gupta (1932) and Sinton and Mulligan (1932). After rapid serial passages through *rhesus* monkeys (*M. mullata mullata*, *S. rhesus*, *M. rhesus*), the last two workers were able to isolate pure strains of *P. knowlesi* and *P. cynomolgi* from *M. irus* monkeys with mixed infections (Sinton and Mulligan, 1932, 1933*a*, 1933*b*, 1933*c*; Mulligan, 1935). These monkeys had been imported from Singapore and purchased in a Calcutta market. After isolation of *P. cynomolgi* from the more virulent parasite, *P. knowlesi*, these workers encountered in some of the experimental monkeys which had showed apparently a pure strain of *P. cynomolgi*, another (third) plasmodium of somewhat low virulence. This became evident only after about 6 months when the infection with the predominant species, *P. cynomolgi*, had attained a low-grade chronicity. Subsequent passage to a *rhesus* monkey showed again an apparent infection with *P. cynomolgi* in the early stages though 5 months later the new species became readily detectable. After a sub-passage to a fresh animal, a pure strain of a plasmodium, different from *P. cynomolgi*, was isolated. Morphologically, this parasite was found to be similar to *P. inui* Halberstadter and Prowazek, 1907. This parasite in the

* The classification of primates is after Pocock (1939).

original (*M. irus*) monkey and in some *rhesus* monkeys after sub-passages had evidently remained in a latent form along with the two other plasmodia. The original host (*M. irus* No. 56) had therefore been harbouring a triple infection of the three species of simian parasites (Sinton, 1934*b*).

Sinton (1934*a*) concluded that the periodicity of the schizogony cycle of this parasite was 72 hours, and not 48 hours as reported earlier (Sinton and Mulligan, 1933*a*).

Donovan (1920) observed a species of plasmodium in smears collected from one of the many *M. sinicus* (*S. sinicus*, *M. radiata*) monkeys from Kallar, Nilgiris, South India. He considered the species to be *P. cynomolgi*. This observation has not been confirmed and according to Mulligan and Swaminath (1940), 'It is possible that the infection was *P. inui* with which *P. cynomolgi* was at that time confused'. The same authors report from findings of a strain of *P. inui* in a young specimen of *M. sinicus* obtained from the foothill area in the vicinity of Kallar. These appear to be the only two authentic instances of plasmodial infection in Indian species of monkeys belonging to the sub-family Cercopithecinæ. No evidence of any infection in North Indian *rhesus* monkeys has so far been recorded.

The present report records the findings of (1) an extremely low parasitic infection in *rhesus* monkeys after they had received inoculation of samples of infected blood from Malayan monkeys—*M. irus*, received through the courtesy of Dr. Edeson, Institute for Medical Research, Federated Malaya State; (2) Progressive increase in the course of parasitæmia after splenectomy in experimental *rhesus* monkeys; (3) Existence of two morphologically distinct species of parasites, one of which was readily detectable to be *P. cynomolgi*; (4) Subsequent study showed the second species to be morphologically identical with *P. inui* having a 72-hour periodicity; (5) Comparative study of the pathogenicity of the new strain with the *P. inui* strain maintained in these laboratories since it had been isolated by Sinton (1934*b*); and (6) Certain morphological variations in *P. inui* in the earlier stages particularly after splenectomy (hitherto unreported).

MATERIALS AND TECHNIQUES.

Monkeys.—Altogether 36 North Indian *rhesus* monkeys have been utilized for sub-passages, splenectomy, and for studies on morphology and pathogenicity. The monkeys were procured from the neighbouring districts of Delhi.

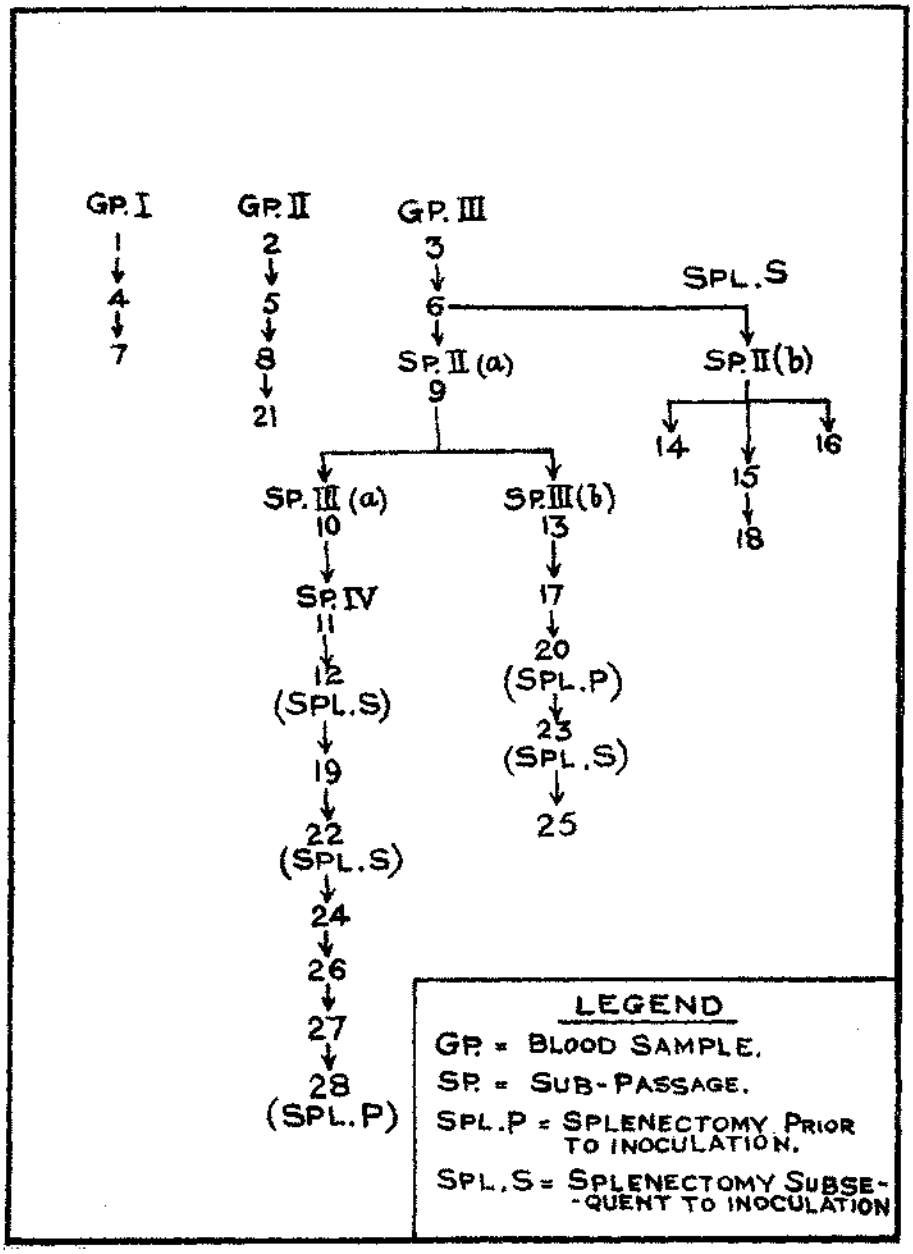
Original infective inoculum.—Defibrinated and oxalated blood was received from Malaya in capsules containing three samples (I, II, III)* each showing extremely scanty infection in thick films. The capsules were packed in chipped ice in a thermos flask and was sent over by air from Tampin *via* Singapore.

Although samples of blood I and III were from the same monkey, to avoid loss of the strain in case of accidental death, they were inoculated in two separate *rhesus* Monkeys 1 and 3.

Monkey 1 received 2 c.c. of blood (Sample I) intravenously, while Monkey 3 was inoculated with 2·5 c.c. blood from Sample III intravenously and 1 c.c. intraperitoneally. Out of 4·5 c.c. of blood of Sample II, 3 c.c. was inoculated

* Samples I and III were stated by Dr. Edeson to be from the same *M. irus* monkey.

CHART 1.



intravenously and 1·5 c.c. intraperitoneally in Monkey 2. The dose of the inoculum depended on the amount of the blood received per sample.

Infective inoculum during sub-passages.—During the earlier part of the investigation, the number of parasites inoculated was not calculated due mainly to the extremely low infection and 10 to 20 c.c. of blood from donor monkeys was injected intravenously to the recipients. Subsequently, however, calculated dose of parasites was injected and it varied between 5 and 50 million parasites per kilogram of the body weight of the animal.

Route.—Unless specifically mentioned as in the case of the first passage, the inoculation was given by the intravenous route in all cases.

Blood examination.—As a routine, all smears were stained with J.S.B. stain (Jaswant Singh and Bhattacharji, 1944) and the modified technique was followed (Jaswant Singh, 1950). Side by side, panoptic method of staining, as recommended by Green (1932), was also adopted for comparative study of stippling. For detailed study of the pigment, J.S.B. stain filtrate* (unpublished) was extensively used and it was found to be more useful for observation of the early appearance of granules.

Splenectomy.—In view of the scanty infection even after second passage in *rhesus* monkeys, the question of removal of spleen in some monkeys was considered. While prompt results were achieved in case of Monkey 6, there was no such response in respect of Monkey 2. Neither subsequent serial passages from Monkey 2 nor splenectomy in Animals 5, 8 and 21 resulted in any increase in parasitæmia. This part of the study had to be abandoned on account of extremely low infection.

As a rule left paramedian incision was preferred during laparotomy for removal of the spleen and chloroform or in some cases 'kemithal' (Jaswant Singh *et al.*, 1952) was used as an anæsthetic.

Sub-passages and isolation.—Inoculation of blood in *rhesus* Monkeys 2 and 3 showed patent parasitæmia on the 11th day of inoculation, detectable in thick films only, but the infection remained extremely low throughout in both cases and it was not possible at this stage to hazard any opinion on the identity of the species. Sub-passages from these animals were carried out as shown in Chart 1.

Monkey 1, the recipient of Sample I, showed no parasitic infection for 14 days when it developed acute diarrhœa and died suddenly. Soon after its death, 40 c.c. of heart blood was inoculated in Monkey 4. Sixteen days later, a few parasites were detected in the thick smear. After subsequent passage in Monkey 7, parasitæmia was detected on the 11th day and the intensity of infection appeared to be similar to that seen in Monkey 9 (second sub-passage from Monkey 3, recipient of Sample III). Both these animals were inoculated on the same day from their respective donors. From daily blood smear examination, it became clear that parasitæmia was gradually on the increase in Groups I and III. As blood Samples I

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* *J.S.B. stain filtrate.*—Subsequent to the publication of J.S.B. stain (Jaswant Singh and Bhattacharji, 1944), the technique of the preparation was modified in which Solution I (methylene azure) is subjected to filtration after boiling. The precipitate recovered from the filter paper is dried, scraped and then grounded up with buffer salt (1·75 gm. disodium hydrogen phosphate) into a fine powder. This is then dissolved in 50 c.c. water and used after allowing it to mature for 3 to 7 days. The filtrate contains a small amount of the azure in solution and stains the parasite lightly thereby making the pigment more prominent. The granules retain the same hue as seen in dehæmoglobinized (in water) blood smears but with the advantage of bringing out clearly the parasite outline. The smear is stained first for a second only in J.S.B. II and then for a minute in the filtrate. Longer treatment in the filtrate does not affect staining.

and III were from the same monkey. Series I was discontinued and serial passages were continued in Group III as shown in Chart 1.

After the second sub-passage to Monkey 9 in Group III, splenectomy was carried out in Monkey 6 on the 15th day after it had received the infective inoculation from Monkey 3. Subsequent examination of blood smears from Monkey 6 showed gradual increase in parasitæmia and during the following 12 days 4 to 6 parasites could be detected in every field of the thin film. This facilitated study of the morphology of the parasites and at this stage two morphologically different species of parasites were detectable, one of which was identical to *P. cynomolgi*. It appeared to be the predominant species. On account of the relatively low density and certain peculiar morphological appearances, the identity of the second species was difficult and this necessitated further serial passages for isolation of the species.

From Chart 1, it will be observed that only one animal (Monkey 9) received inoculation from No. 6 prior to the removal of its spleen, while three others received inoculation after splenectomy. For convenience, these have been shown as sub-passage II(*a*) in Monkey 9 and II(*b*) in Monkeys 14, 15 and 16. Monkey 14 received inoculation when mostly rings and early trophozoites were detected in the blood of the donor Monkey 6. Twenty-four hours later when the blood of donor showed well-developed trophozoites and a few schizonts, inoculation was made in Monkey 15. After another 20 hours, when schizonts and rings predominated, blood from the same donor was injected intravenously to No. 16. In all the three animals *P. cynomolgi* predominated during the early stages of the course of infection, but in developed infection during the sub-acute stage, the second species became noticeable in larger numbers. Subsequent passage at this stage to Monkey 18 from No. 15 showed similar course of events and the second species was not detected until the acute phase of *P. cynomolgi* infection was over.

With regards to sub-passage II(*a*), III(*a*) and III(*b*), the animals received infective inoculation irrespective of the stage of development of the parasite in the donors. After the third passage from No. 9 (5th from 3rd), comparatively higher parasitæmia was evident. Subsequent inoculations were carried out only when mature schizonts of the second species were detectable in the blood of the donors. The time of inoculation, therefore, depended mainly on the existence of mature schizonts of the second species irrespective of the number or the course of infection.

After several such serial passages it was noticed that only one species of parasite (the second species) and not *P. cynomolgi* could be detected in Monkeys 12, 19 and 22. Removal of spleen in Monkey 12, after the infection appeared to be extremely low, resulted in a progressive rise of infection in which both species could be detected. But after splenectomy in Monkey 22, only the second species was detected in larger numbers and no infection with *P. cynomolgi* could be seen in this animal or in any of the subsequent sub-passages in Monkeys 26, 27 and 28. Parasitic infection in Monkey 26 and of the later series appeared to be comparatively heavy and attained a peak showing 5 to 10 parasites per field of the thin film. After detailed morphological study of this parasite, and in the absence of *P. cynomolgi*, it was considered that a pure strain of a species identical to *P. inui* was isolated. This was confirmed by the determination of the duration of the schizogony cycle, which showed a 72-hour periodicity.

With regard to the sub-passage line III(*b*), both the species were detectable in Monkey 20 from which spleen had been removed 2 days prior to the inoculation. Subsequent passages to Monkeys 23 and 25 showed presence of the same two species of parasites at certain stages of the infection and thus no further sub-inoculation was carried out.

MORPHOLOGICAL STUDY.

As a rule it is not always easy to establish definite identity of a species of plasmodium by examination of thick films only and more so, if the infection is scanty. Naturally, therefore, the problem becomes more acute when one has to deal with more than one species.

In the present studies it was possible to determine the species clearly when progressive rise in parasitæmia was noticed after splenectomy in Monkey 6 and, as mentioned previously, it became clear at that stage that more than one species of plasmodia were detectable.

One of the species showed the following characteristic features of *P. cynomolgi* :—

1. Pronounced increase in size of the host cells with marked stippling which appeared fairly early.
2. Ring forms showing accessory chromatin dots.
3. Trophozoites markedly amœboid.
4. Number of merozoites in mature schizonts varying between 12 and 20.
5. Pigment appearing in well-developed trophozoites and of a coarse nature. The colour of the pigment in the trophozoites golden brown and brown or dark brown in schizonts and gametocytes.
6. Gametocytes considerably larger than the normal size of red blood cells.

The morphology in respect of the second species, however, was different, firstly, parasitic density was lower than in *P. cynomolgi*, and secondly, certain morphological variations were encountered at certain stages of the infection and the parasitic forms appeared to be at variance with the usual description of the three species. Such peculiarities were as follows :—

1. Early nuclear divisions almost simulating accessory chromatin granules.

2. Highly amœboid trophozoites with filiform tendril-like cytoplasm, apparently unattached to chromatin dots. The same was noted in respect of linear or arched chromatin in some cases.

3. six to 16 merozoites in mature schizonts.

4. The host cells, slightly enlarged ; or some even smaller than the normal red blood cells. In some cases the host cells of the schizonts, mature or otherwise, were found to be either deformed, oval or oblong, a feature commonly encountered in *P. knowlesi* infection.

This added to the earlier difficulties when the question of a third species had not been overruled altogether. These variations were invariably encountered in splenectomized animals, as well as during the initial stages of infection in some of the monkeys inoculated earlier.

Morphological study was rendered much easier from fifth sub-passage when there was increased parasitæmia in Monkey 12 and apparently the second species only could be detected.

It was at this stage that it was realized that the appearance of peculiar forms mentioned above was more or less a transient feature noticeable during the initial rise of the infection. This phenomenon was not noticeable after the seventh sub-passage, but it was more or less a prolonged feature in splenectomized animals. However, the general morphological character of the second species showed the following constant features during the later sub-passages.

Asexual forms : 1. *Large vesicular nucleus.*—Occasional suggestion of a nuclear vacuole. In rings and trophozoites the chromatin was often found divided in two and in some three granules of unequal size.

2. Persistent and marked vacuole.

3. Slight amœboid character of the parasite as compared to *P. cynomolgi.*

4. Many of the parasites showed the characteristic stellar and ' pupal ' forms so commonly seen in *P. inui.*

5. Ten to 18 merozoites in mature schizonts.

6. *Pigment* appeared early, in the form of fine yellowish granules, and scattered over a wide area, abundant in trophozoites and of light brown or golden brown colour. During the subsequent developmental stages, pigment is found in clumps of dark brown colour near the periphery than in the centre. In mature schizonts, pigment is near the centre and in clumps.

7. *Host cell :* (*a*) *Enlargement.*—Slight enlargement of the R.B.C. infested with well-developed parasites is frequently encountered. But during the chronic stage this phenomenon is not so clear on account of variation in the size of the normal red cells—anisocytosis.

Soon after the peak some of the infested cells appear to be smaller than normal red cells. The parasites themselves, particularly schizonts, appeared very small and seem to occupy only a part of the cells. These are apparently the crisis forms (Afridi, 1938).

(*b*) *Stippling.*—Although with Leishman or Giemsa's stain it is possible to see stipplings in *P. inui*, they are well shown in preparations stained with J.S.B. or by Green's panoptic method. The stippling, however, is neither such a dominant feature nor so numerous as seen in *P. cynomolgi.*

Sexual forms.—The gametocytes are definitely smaller than those of *P. cynomolgi.* There is usually slight enlargement of the infested red cells, particularly in respect of the macrogametocytes. The cell outline is often found to be indefinite and occasionally ' ghost-like '. In some cases they appear to be extracellular.

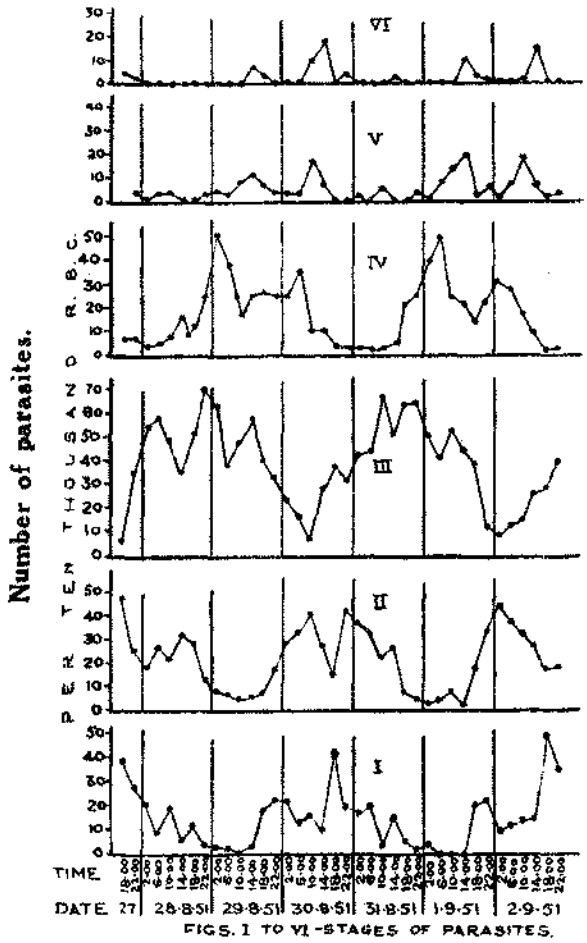
The female and male forms are easily distinguishable from the character of the nuclei, which are compact in case of the former and diffused in microgametocytes. These observations point out that the species is identical to *P. inui* Halberstadter and Prowazek, 1907.

DURATION OF SCHIZOGONY CYCLE.

In view of the earlier difficulties about the identification of the second species of the parasite, particularly due to certain morphological variations, the periodicity of the asexual cycle after isolation of the pure strain was determined and this was 72 hours (Chart 2).

CHART 2.

Duration of schizogony cycle of P. inui.



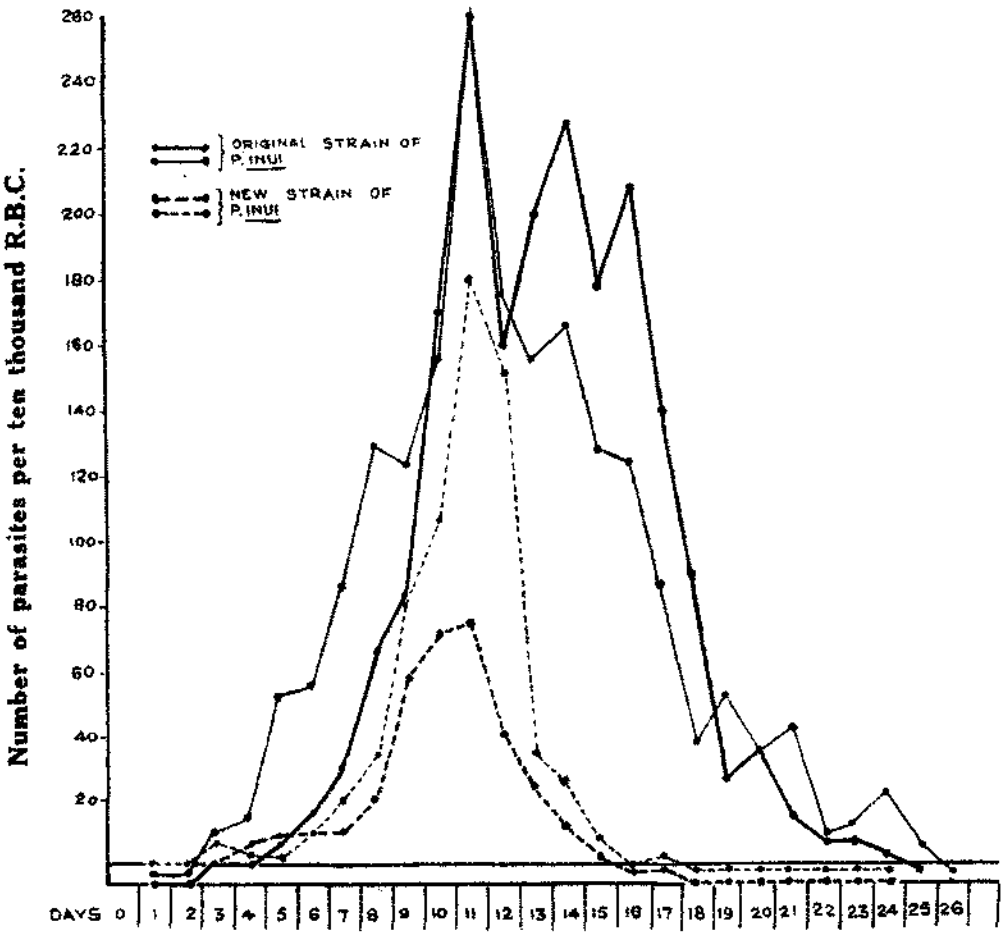
I. Rings.
II. Early trophozoites.
III. Developing trophozoites.
IV. Fully developed trophozoites.
V. Segmenters.
VI. Schizonts (mature).

The technique followed was after Mulligan (1935) and was found to be extremely useful. Further it was observed that a clearer picture could be obtained if the differential parasite count was done during the earlier stage of the infection when the number of parasites are fewer, as existence of more than one brood usually complicates the issue particularly during the acute phase of the infection.

The maximum number of mature schizonts and early ring stages of the main brood were observed to be at 14.00 and 18.00 hours respectively and were detectable at interval of 72 hours. The presence of a minor brood preceding the major one by 24 hours as shown in Stages I and VI of the Chart is apparent.

CHART 3.

Course of infection.



Dose of inoculum—5 million parasites per kilogram body weight.

PATHOGENICITY.

Comparative study has been made of the dose of inoculum, pre-patent period, and course of infection in respect of the newly isolated strain of *P. inui* and the original strain maintained in these laboratories. From Chart 3 it will be observed that :—

(*i*) The pre-patent period was same in all the animals.

(*ii*) Peak of infection reached on the 11th day after patent parasitæmia was established, be it with the new or the old strain.

(*iii*) The intensity of infection in the two animals inoculated with the old strain was much higher than that in the animals infected with the new strain.

(*iv*) The crisis is acute in infection with the new as compared to the original strain, in which case a high level parasitæmia was detectable for a much longer period.

(*v*) Decline in parasitæmia was attained 5 days after peak in respect of the new strain while this occurred 11 days after in case of the original strain.

(*vi*) Infection was apparently more severe with the original than with the new strain.

DISCUSSION.

In human malaria, with mixed infection the phenomenon of predominance of one species of parasite over the other at certain stages is well known. Sinton and Mulligan (1933*a*, 1933*b*) have amply demonstrated this inherent character of the simian plasmodia during their studies on mixed infections in *M. irus* monkeys. In their series it was observed that there was complete absence of *P. inui* during certain stages of mixed infection, on account of the predominance of *P. cynomolgi* over the other. This was responsible for the delay in establishing the existence of *P. inui* infection in the monkeys (Sinton, 1934*b*).

During the present studies the effect of splenectomy on the course of infection was apparent, as within a short period it had been possible to observe the existence of two species of plasmodia and the authors are inclined to believe that under similar situations this step is the obvious choice. While identity of *P. cynomolgi* could be easily established, certain morphological variations in the second species described earlier made immediate diagnosis somewhat difficult. Further, progressive rise in infection in splenectomized animals caused considerable anæmia with concomitant anisocytosis. On account of this, some of the erythrocytes infested with *P. inui* appeared to be enlarged. This has been already stressed by Sinton (1934*b*), who mentioned about the same difficulties experienced by some workers.

The reasons for morphological variations like the occurrence of filamentous processes of the cytoplasm, distortion of the infested cells, etc., as reported earlier, are not quite clear. But as such changes were noticed to be persistent feature in Monkey 6 for several months and that such peculiar forms were encountered in other infected animals which were subjected to splenectomy, it was considered that

the morphological aberrations were possibly related in some way to the removal of the spleen. But such filamentous character of the cytoplasm and some of the peculiarities encountered in respect of chromatin were seen in some of the earlier sub-passages as well.

Sinton and Mulligan (1933*c*) have mentioned of reports of various workers like Berenberg Gossler, Gonder and Rodenwaldt, Blanchard and Langeron, who had observed definite morphological changes in different species of plasmodium after passage to new hosts or after splenectomy. But they themselves failed to observe any such variation in respect of *P. knowlesi* and *P. cynomolgi*, whether after passage from the natural hosts (*M. irus*) to *rhesus* monkeys or after splenectomy in animals infected with one or the other species. No such morphological variations have been reported by Sinton (1934*b*) in respect of *P. inui* during serial passages. As he had not made any observation on the morphological variations after splenectomy, it is difficult to conjecture whether any such variations could have occurred in respect of the strain of *P. inui*. Talliaferro (1932), however, reports of morphological difference in the same species of plasmodium in different Panamanian monkeys which are not the natural hosts of *P. brasilianum*. Wolfson (1939) mentions of morphological variations in *P. lophura* when inoculated from canaries (natural host) to ducks which are considered to be the unusual hosts. Commenting on such morphological and certain physiological variations in avian plasmodia, Hewitt (1940) suggests that physico-chemical differences may exist between the red cells of canaries, ducks and chickens. He further observes that morphology and physiology of a species may be considerably changed by the introduction into an abnormal host.

On the basis of such analogies the authors are inclined to believe that the morphological variations of *P. inui* encountered are primarily due to the lack of balance between the plasmodium and the host which may be considered as an abnormal host for this parasite, the natural host being *M. irus* of Malaya. That the *rhesus* monkeys are abnormal hosts for *P. knowlesi* is borne out by the fact that infection is very severe in these animals when inoculated from *M. irus*, which do not seem to suffer from any ill effects.

As these variations were found to persist in the splenectomized animals and were of a transient nature during the primary rise of the infection in some of the earlier passages, it may be argued that the host-parasite relationship was not fully established during the early series, and was more or less completely absent in splenectomized animals. When, however, the balance was fully established as in the later passage series, the typical morphological characteristics became evident. But when any of the animals was subjected to splenectomy, highly amœboid forms or filamentous type of the parasite became not an uncommon feature.

Morphological characters of plasmodia are specific for each parasite and this helps in the identification of the species of parasite. Similarly each species follows its own periodic cycle. The duration of the schizogony cycle in some parasites is 24 hours as in the case of *P. knowlesi*. In others like *P. cynomolgi*, *P. vivax* and *P. falciparum*, the duration is of 48 hours, while it is 72 hours in respect of *P. inui*, *P. malariæ* and *P. brasilianum* (Talliaferro, *loc. cit.*). Thus, it is more or less a group character of the parasite. Hence, morphological study and the duration of the schizogony cycle should normally confirm the identification of a parasite, and on

the basis of these characteristics it is established that the second species of plasmodium isolated from the blood of the Malayan monkey showing a mixed infection is a pure strain of *P. inui* Halberstadter and Prowazek, 1907.

Comparative study on the pathogenicity of the newly isolated strain against the original strain show that although the general course of infection in the early stages of the infection and the peak are similar, the intensity of the infection with the same dosage of inoculum is somewhat different. With the original strain the peak parasitæmia is considerably higher than with the new strain. Further, while in the new strain the crisis is abrupt, this is not so in the case of the original strain in which the parasitæmia retains a more or less sustained level for a longer period. Whether this difference is associated with number of sub-passages or to the difference in the strain can only be analysed after further studies.

SUMMARY.

Inoculation of infected blood from *M. irus* to *rhesus* monkeys showed extremely scanty infection, and identity of the parasite in the earlier stages was not possible. Subsequent passages did not improve the situation but splenectomy resulted in increased parasitæmia in some of the animals. One of the species was identified as *P. cynomolgi*. After repeated sub-passage it was possible to isolate a pure strain of *P. inui* which had a 72-hour periodicity.

Certain morphological variations of this species observed in the earlier stages and in some animals which were subjected to splenectomy were considered to be due to unstable host-parasite balance.

The intensity of infection with this strain of parasite seemed to be slightly lower than that with the original strain maintained in these laboratories during the last 17 years.

ACKNOWLEDGMENT.

The authors wish to acknowledge their grateful appreciation for the helpful co-operation of the Director, Institute for Medical Research, F.M.S., and of Dr. Edeson of the same Institute for his kindness in sending the blood samples from Malaya.

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Indian Journal of Malariology, **5**, 3, *September 1951.*

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

III. Latency, relapse and immunity in albino rats with blood-induced infections.

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[September 24, 1951.]

In a previous paper, Ramakrishnan and Satya Prakash (1950) reported that a certain number of rats with blood-induced infections of *P. berghei* developed chronicity, exhibited latency* and relapsed†. The present report deals with systematic observations during periods ranging from 6 to 42 weeks of 75 albino rats with latent infections.

MATERIAL AND METHODS.

The rats were from the same source as reported before by Ramakrishnan and Satya Prakash (*loc. cit.*). The majority of animals were full-grown adults about a year old but the correct age was not known except in 30 which belonged to litters born in our own colony. All the rats were on a standard diet and housed under identical conditions.

The route of inoculation was intraperitoneal and the dose of infection in every case was 8×10^5 parasites approximately. Thick and thin blood smears of the experimental rats were stained by the J.S.B. method (Jaswant Singh and Bhattacharji, 1944) and examined daily throughout the period of observation. Thick smears were examined for about 3 minutes and confirmed by examination of 100 fields of thin smear before declaring a slide negative.

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* Latency = A complete disappearance of parasites from the peripheral blood continuously for at least two weeks following overt parasitæmia as judged by microscopic examination of blood smears.

† Relapse = Repatency of parasitæmia in the peripheral circulation preceded by a spell of latency.

Splenectomies were performed under Nembutal anæsthesia (15 mg. per 20 gm. weight) administered intraperitoneally. Superinfections were carried out with the homologous strain of parasites, the route and dose were the same as for the primary inoculation. Sub-inoculations during latency were carried out with 0·2 c.c. of blood diluted in citrate saline (2 per cent) injected into the test animal which in every case was a *Rattus rattus*. For every sub-inoculation, two test animals were used and were observed for a period of 10 to 15 days before declaring the sub-inoculation negative.

RESULTS.

One hundred and eighty-three rats were inoculated in batches, about once a week, and 170 of them took the infection. Ninety-five of the infected animals died during the acute stage within about 15 days of the inoculation and the remaining 75 were able to overcome the primary parasitæmia which became latent. The majority of the animals of this group were under observation till they died, while a few were utilized for other experiments after a minimum period of 8 weeks' observation. Table I gives the details regarding sex distribution and the results of inoculations :—

TABLE I.

Results of inoculation 8×10^5 parasites in 183 rats.

Animal group.	Number inoculated.	Number positive to inoculation.	Number refractory to first inoculation.	Number developed chronic infections.	Number died during the acute phase.
Males ...	103	95	8	49	46
Females ...	80	75	5	26	49
TOTALS ...	183	170	13	75	95

The average pre-patent period was 2·1 days with a minimum of one to a maximum of 12 days. The primary periods of patent parasitæmia in the 75 animals that developed latency were as shown in Table II :—

TABLE II.

Mean duration of primary parasitæmia in the different groups of animals.

Animal group.	Number of animals.	Mean duration of primary patent parasitæmia in days.
Males ...	49	19·8 (S.D. = 3·8)
Females ...	26	14·1 (S.D. = 5·2)

S.D. = standard deviation.

The difference between the patent periods in males and females is statistically significant. Thus, the females that were able to overcome the primary parasitæmia did so earlier than the males.

PARASITIC RELAPSE.

Out of the 75 animals with latent infections, 37 (50 per cent) had one parasitic relapse and 8 (10·7 per cent) had two such episodes. The periods at which the relapses occurred are shown in Table III :—

TABLE III.

Parasitic relapses in the animals under observation by months.

Months.	Number under observation.		Number that had one relapse.		Number that had second relapse.	
	M	F	M	F	M	F
1	49	26	2	2	...	...
2	49	26	21	9	1	...
3	42	21	2	1	4	...
4	34	15	...	...	1	...
5	20	13	...	...	...	...
6	15	6	...	...	...	...
7	9	3	...	...	1	...
8	5	3	...	...	1	...
9	3	5	...	...	...	...
10	2	5	...	...	...	...
11	1	4	...	...	...	...

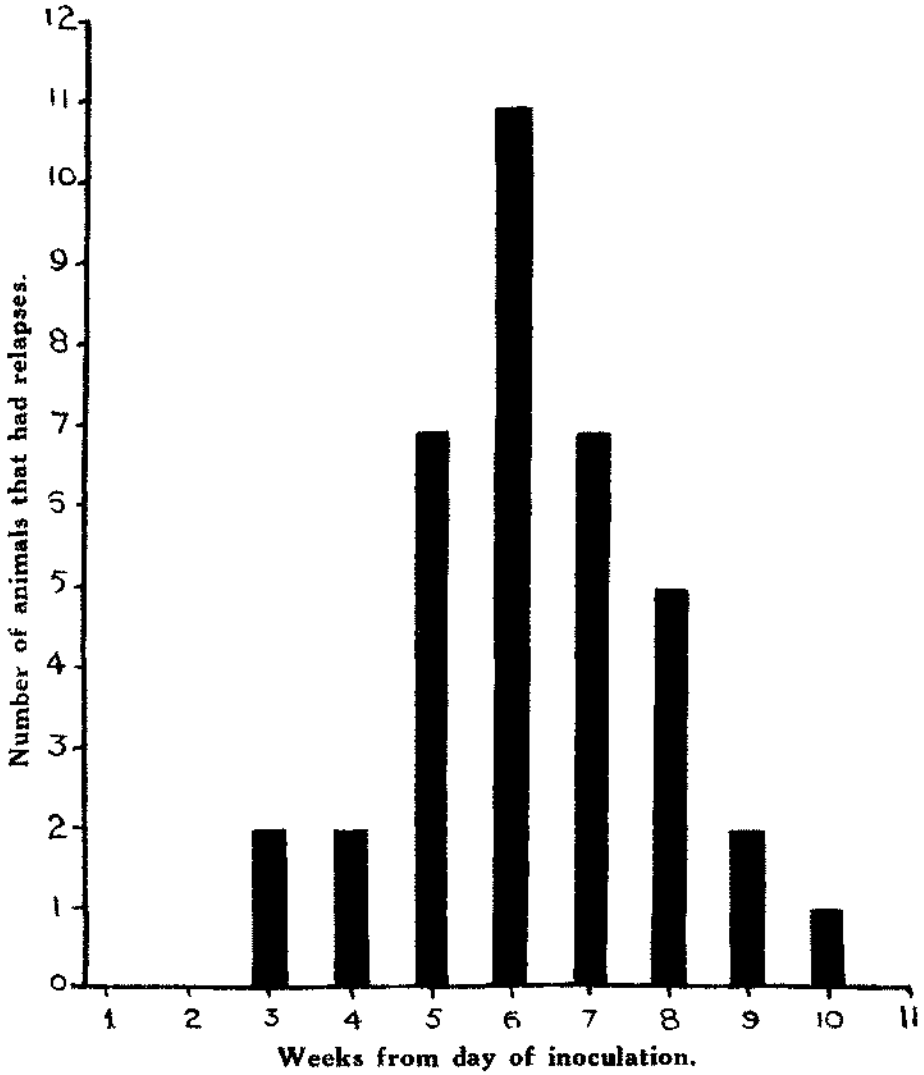
M = males. F = females.

Sixty per cent of the males and 46 per cent of the females had one parasitic relapse during latency. Grouping both the sexes together, 30 out of 37 (81 per cent) relapsed in the second month from the day of inoculation, while 4 and 3 relapsed in the first and third months respectively. The distribution of the relapses was about equal in fifth, sixth, seventh and eighth weeks. Graph 1 shows number of males and females that relapsed for the first time in weeks. A second relapse occurred in 8 males; four in the third month from the day of inoculation and the remaining four, one each in the second, fourth, seventh and eighth months.

respectively. In all these observations, the parasitæmia during relapses was low and transient, rarely lasting for more than 3 days (in 2 animals it lasted for 9 days).

GRAPH 1.

Number of animals that relapsed for the first time in weeks.



DURATION OF INFECTION.

Among the 75 animals in which the infection became latent, one male (R134) had undergone a spontaneous cure during latency following the primary parasitæmia. In the rest of the animals the parasitæmia was sub-patent for varying

periods. Periodically blood from some of the latent animals was sub-inoculated into *R. rattus* to determine the infective status of the donor. The details and results are given in Table IV :—

TABLE IV.

Results of sub-inoculation experiments.

Animal groups; sub-inoculation results.		Total.	Period in weeks from day of inoculation when the sub-inoculations were made and their results.																		
			8	12	13	14	16	17	18	19	20	21	22	23	24	25	26	29	31	33	36
Males	Number sub-inoculated.	15	..	..	1	1	1	3	1	..	2	1	2	1	1	..	..	1	..	..	..
	Number positive	9	..	..	1	0	1	2	1	..	1	1	2	0	0	..	..	0	..	..	..
Females	Number sub-inoculated.	15	3	1	1	..	1	..	..	1	1	2	..	..	..	1	1	..	1	1	1
	Number positive	6	1	1	0	..	1	..	..	1	1	1	..	..	..	0	0	..	0	0	0

Sub-inoculation experiments showed that untreated latent infections persisted for a minimum period of 6 months.

IMMUNITY TO HOMOLOGOUS SUPERINFECTION.

Of the 75 latent animals, 9 were subjected to homologous superinfection during different periods of latency. One of the 9 was subjected to a similar treatment for a second time. The details of the experiments are depicted in Table V :—

TABLE V.

Details of homologous superinfections during latency by weeks.

Animal group and the number of superinfection.		Weeks.						Totals.
		5	21	22	26	27	42	
MALES	First superinfection	5	1	...	1	1	...	8
FEMALES	First superinfection	...	...	1	...	...	...	1
	Second superinfection	...	...	...	...	...	1	1

There was not a single case in which the homologous superinfection gave rise to patent parasitæmia in the blood indicating that immunity was of a sufficient

degree to take care of the inoculum. Judged by the results of superinfection it can be concluded that the animals were immune up to a minimum period of 27 weeks or seven months, while one rat was so up to the forty-second week or eleventh month from the day of primary inoculation. These results confirm those obtained by sub-inoculation of blood during latency.

RESULTS OF SPLENECTOMY.

Eight animals were splenectomized at different intervals during latency after the primary parasitæmia. Four died within 5 days and one on the tenth day of the operation without the parasitæmia in the peripheral blood becoming patent. Of the remaining 3, one (No. 176) showed parasitæmia on the eighth day of splenectomy and died 12 days later. Another (No. 134) had a spontaneous cure as determined by negative sub-inoculations before and after splenectomy. The third (No. 179) had a parasitic relapse on the seventh day of splenectomy and the post-splenectomy parasitæmia was patent without any remission for 73 days before it assumed a second latency.

Certain changes were noted in some of the parasites after splenectomy. A greater proportion of the parasites was found to be in mature erythrocytes and the trophozoites markedly amœboid. Pigment was visible in the parasites during the growing stages. These characters of the parasite were in marked contrast to descriptions that have been previously reported by Ramakrishnan and Satya Prakash *(loc. cit.)* and detailed studies of the change in characteristics are in progress.

AGE OF RATS IN RELATION TO LATENCY.

It has already been stated that 30 of the animals used in the experiments were born in the local colony and of known ages. The behaviour of infection in them has been analysed separately in Table VI :—

TABLE VI.

Number of animals of known age inoculated and number that showed latency.

Age in weeks.	Number of animals used.		Number that showed latency.	
	Males.	Females.	Males.	Females.
13	4	1	1	1
16	8	3	5	...
17	2	2	1	...
18	1	2	...	1
20	2	...	2	...
21	2	3	1	1
TOTALS	19	11	10	3

Two of the 13 weeks old animals as well as others older were able to overcome the primary parasitæmia and developed latent infections.

DISCUSSION AND CONCLUSIONS.

Parasitic relapses are well known in blood-induced infections of malaria. Recently, Corradetti and Verolini (1951) reported the rate and intervals of relapses in blood-induced infections of *P. malariæ* in man and *P. cynomolgi* in *Macacus rhesus* monkeys. In their series, the relapse rate both in man and monkeys was 100 per cent and the infections lasted throughout the 35 weeks of observation in the case of man and 39 weeks in the case of monkeys. In no case was there any mortality during the acute stage of the disease.

In these series, however, as stated above, of the 170 animals that took the infection after the first inoculation, 95 animals died during the acute stage and only 75 became latent of which only 37 (50 per cent) relapsed once and 8 (10 per cent) relapsed for the second time during the observations. From Table III, it appears that *P. berghei* has a definite rhythm for relapsing, altered to a little extent by the individual reactions of the host. Thus, it is seen in the Graph that 27 out of 37 or 73 per cent of the first relapses occurred in fifth to the seventh weeks of infection.

The length of primary patent parasitæmia in the males and females was 19·8 and 14·1 days respectively and the difference is statistically significant. It appeared that the females were able to overcome the infection in a shorter time than the males. This difference in immunity, however, did not bear any relation to the relapsing periods as the majority of the first relapses occurred in both the sexes in the second month of infection.

Our observations on sub-inoculation of blood from latent animals indicated that the infection lasted at least 22 weeks. Negative results within this period in some animals may have meant one of three things, namely, (*a*) the animal under test underwent a spontaneous cure, (*b*) the parasites in the inoculum were either absent or too few in numbers, or (*c*) the recipient animal was refractory to the inoculation. As already stated in most of the experiments, two *R. rattus* were used for every sub-inoculation. In some cases an animal was negative on sub-inoculation but was positive on a subsequent occasion and it was probable that the parasites were too few or absent in the first inoculum. It is concluded that the parasites were present in the peripheral blood but at a sub-patent level throughout the latent period.

The immunity seemed to last throughout latency as evidenced by the ineffectivity of homologous superinfection. On the other hand, rat No. 134, as stated previously, had undergone a spontaneous cure during latency and, when this animal was given a second homologous infection, parasitæmia developed after the usual pre-patent period of 2 days and behaved as in a non-immune in duration and intensity.

In blood-induced infections as pointed out by Corradetti and Verolini (*loc. cit.*), Bignami's interpretation of the mechanism of relapses that they were dependent on the continued existence of erythrocytic parasitics, probably explains the phenomena of relapse. It would appear that the immunity was a delicate host-parasite relationship and at times the parasite got the upper hand of the defence

mechanism of the host. But, as the majority of the relapse episodes took place within a specific period in the present observations, relapses seemed more a characteristic of the parasite than any undue fluctuations in the host immunity.

The possibility of the existence of a secondary tissue phase has to be borne in mind. Van den Bergh *et al.* (1950) described the presence of a secondary tissue phase of development in histiocytes and capillary endothelium similar to that seen in avian malaria. This has not been confirmed by other workers and has been questioned by Galliard and Lapierre (1950). In the present series, no such forms were encountered in post-mortem organ smears examined as a routine, although nucleated precursors of erythrocytes were found parasitized, especially in heavy infections as recorded previously by Ramakrishnan and Satya Prakash (*loc. cit.*). Parasites in such cells were unpigmented but appeared in all other respects identical to the ones seen in mature erythrocytes. Whether these can be considered as exo-erythrocytic forms or not requires further study. Organs of certain animals have been preserved for detailed examination.

Age could be a factor responsible for some of the animals developing latent infections. As there were no animals younger than 13 weeks in these series, the earliest age at which untreated infections were capable of becoming chronic cannot be stated. Raffaele and Baldi (1950) found that in 6 very young rats (a few days old) the parasitæmia was intense as in the case of mice, and they all died during the acute phase of the infection. They also found in 4 rats, 4 to 9 weeks old, that 3 contracted the infection which became latent in two.

SUMMARY.

Seventy-five out of 170 albino rats with blood-induced infection of *P. berghei* developed latency and were observed during a period ranging from 6 to 42 weeks. The remaining 95 animals died during the acute phase, within about 15 days.

Fifty per cent of the animals with latent infection showed first parasitic relapse, majority of which occurred in the second month after infection. Ten per cent showed second parasitic relapse, most of which occurred in the third month.

In 30 animals of known ages (13 to 21 weeks old), latency occurred in all age groups; the factors which enable animals to overcome the primary infection do not seem to be due to age only but certain individual variations.

Sub-inoculations of blood from latent animals showed that infections can last for a minimum period of 6 months. Homologous superinfections during latency did not give rise to any patent parasitæmia and confirmed the above findings.

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

IV. The reaction of blood-induced *P. berghei* in albino mice to quinine and sulphadiazine.

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INTRODUCTION.

SYSTEMATIC screening of several known antimalarial drugs against *P. berghei* has been reported by Goodwin (1949), Schneider, Decourt and Montezin (1949) and Thurston (1950). *P. berghei*, being different in its reactions to drugs from some of the other parasites, both avian and mammalian, on which similar trials have been carried out, leads one to believe that the physiology of this parasite must differ in some fundamental way from that of others. The reaction of a parasite to drugs depends on three cardinal factors, namely its physiology, immunity status and the metabolism of the drug in the host. If the latter two factors are the same, some ideas on the physiological needs of the parasite may be gathered. It is with this view that the present study was undertaken and the results are reported.

MATERIALS AND METHODS.

The source of supply of albino mice and the strain of parasites were the same as described previously by Ramakrishnan and Satya Prakash (1950).

The route of inoculation was intraperitoneal and the dose was always 5 million parasites per mouse except in 2 animals, each of which received 2 million

parasites. The requisite amount of blood to be inoculated was estimated after calculating the number of parasites per 10,000 erythrocytes in the donor. The total erythrocyte count of the donor was not always made, but presumed to be 7 millions as judged by a few sample counts.

No selection was made as to age of the 87 mice used, as the majority of them were from outside sources and were utilized depending on their availability. Both sexes were represented in all the experiments, but were not distributed in equal numbers.

The quinine preparations used were injectible sterile solution of quinine di-hydrochloride (6 grains in 2 c.c.) manufactured by Lister Antiseptics and Dressings Co., Ltd., Calcutta, and quinine bi-hydrochloride salt manufactured by N. V. Bandoengsche, Kinine Fabriek, Bandoeng, Java. Unfortunately, the base content was not known for either of the preparations. The sulphadiazine used was the injectible sterile solution of sulphadiazine sodium (each ampoule containing the equivalent of 1 gramme of sulphadiazine in 4 c.c.) prepared by May & Baker, Ltd., Dagenham, England.

Drugs were not administered on the day of inoculation except occasionally, but only when the parasitæmia became patent. Both quinine and sulphadiazine were given orally with a pipette in aqueous solutions in the morning and evening on 3 consecutive days. Ordinarily no difficulty was experienced in drug administration and if an animal was not co-operating, a few minutes' rest had to be given between mouthfuls; removal of the water bottle for about an hour prior to drug administration was found useful.

Blood smears (thick and thin), stained by the method of Jaswant Singh and Bhattacharji (1944), were examined daily from the day of inoculation up to 10 days from the 1st day of giving drug. Smears were declared negative after examination of the thick smears for approximately 3 minutes each and 100 fields of the thin smears. In positive smears, parasites were counted against 10,000 erythrocytes with an Ehrlich's eyepiece.

Undrugged animals for comparison were observed separately from each batch of mice inoculated. Some of the mice given quinine earlier and their controls were used in the sulphadiazine series. The day of inoculation and the days on which the parasitæmia were compared in the drugged and comparison animals were identical in each experiment.

RESULTS.

The degree of parasitæmia was not closely related to the dose inoculated. Thurston (*loc. cit.*) used different doses ranging from 50,000 to 150 million parasites per mouse and reported similar results. Table I gives the course of infection in 13 of the mice used for comparison.

Even with identical doses and route of inoculation the pre-patent period varied greatly in different mice. In these series, parasitæmia did not progressively increase as in Thurston's (*loc. cit.*). There were two periods of 'crisis', one around the 5th day and the second about the 10th. The untreated mice died on various days ranging from 7 to 22 days after inoculation.

TABLE I.

Course of infection in undrugged mice after giving 5 million parasites intraperitoneally.

Serial number of mouse.	Total number of parasites per 10,000 R.B.Cs. on days following inoculation.											
	1	2	3	4	5	6	7	8	9	10	11	12
152	2	32	173	224	271	1,236	1,256	298	535	209	282	448
163	0	0	5	21	29	89	78	129	164	118	124	403
164	0	0	3	5	16	134	157	343	D	...	...	...
165	0	2	7	92	118	389	D	...	...	...	...	...
185	4	175	228	325	430	392	598	380	356	247	629	555
186	0	161	433	372	645	465	319	1,049	678	357	429	833
187	0	0	0	0	0	0	37	367	312	D	...	...
190	0	0	0	13	209	260	201	427	446	599	234	609
195	0	0	0	0	0	1	126	638	1,155	D	...	...
201	0	28	142	202	286	267	299	473	460	699	226	513
202	12	138	213	264	245	317	275	246	384	687	308	284
220	0	0	0	0	0	5	101	418	364	D	...	...
224	2	3	9	246	456	282	311	D	...	...	...	...

D = died.

In the quinine series different dosage schedules were tried ranging from 12 mg. to 120 mg. per 20 gm. weight of the mouse spread over 3 days, the drug being given in two equal doses daily. The course of infection in the drugged and comparison animals is shown in Table II.

In doses up to 60 mg. per 20 gm. weight, during the first 7 days, the parasitæmia was less in the quininized animals, while after the 7th day (or 4th day from the last day of drugging) in most of the drugged animals it was in excess of that of

TABLE

Daily average parasitæmia per 10,000 erythrocytes during 10 days the corresponding

Total dose in mg. per 20 gm. body weight.	1		2		3		4		5	
	E.	C.	E.	C.	E.	C.	E.	C.	E.	C.
12	0*	0	0	0·5	0	146	100	128	397	185
18	0·2	1	2·2	3	8·6	20	39	81	109	111
24	50	0·79	110	190	154	239	43	393	66	1,243
30	5·5	32	526	173	567	224	157	271	241	1,236
36	0*	0	0	1	2	6	7	57	82	74
42	0*	0	4·5	1	15	6	10	57	73	74
60	49	229	241	285	238	332	165	222	122	325
90	399	...	846	...	481	...	693	...	210	...
96	6·3	1·5	92	4·5	11	123	0·2	228	0	141
102	4	1·5	5·5	4·5	6·5	123	0	228	0	141
114	3	1·5	4	4·5	2	123	2	228	0	141
120	28	1·5	55·5	4·5	22	173	0·5	228	0	141

E = experimental animals.

* Drug administered from the day following inoculation.

† No control.

untreated ones. From this it was presumed that quinine was excreted within 4 days after which period the plasma concentration of the drug was below the effective level. It was probable that in the untreated animals, from the 7th day onwards, the defence mechanism of the host was able to gain partial control of the infection, while in the untreated animals, due to decrease in numbers of parasites before the 7th day, the host was not able to develop the same tolerance.

II.

from the 1st day of drugging in animals treated with quinine and untreated controls.

6		7		8		9		10		Number of animals.
E.	C.	E.	C.	E.	C.	E.	C.	E.	C.	
...	...	175	345	...	...	120	920	210	1,220	2
360	213	679	188	227	158	220	173	230	95	5
142	937	466	377	249	426	211	232	145	304	3
161	1,256	528	298	188	535	316	209	142	282	2
208	D	73	...	129	...	...	...	...	...	2
194	239	175	73	190	129	226	64	405	118	2
347	494	867	421	673	482	774	463	448	817	6
1,062	...	1,337	...	2	...	11	...	83	...	2†
0	158	0·25	101	2·8	209	77	182	105	D	4‡
0	158	0	101	0	209	0	182	0	...	...
0	158	0	101	0	209	0	182	0	...	...
0	158	0	101	0	209	0·5	182	2·0	...	...

C = comparison animals. D = died.

‡ Five animals were used—4 animals became negative for 3 or more days following treatment. In the fifth, there was 99 per cent reduction in parasitæmia for 2 days. This animal has not been taken up for the analysis of data.

Similar trials as above were conducted with sulphadiazine and the dosage schedules ranged from 0·03 to 3·0 mg. per 20 gm. mouse weight spread over 3 days, drugging. The course of infection in the drugged and comparison animals is depicted in Table III.

P. berghei was sensitive even to as small a dose of sulphadiazine as 0·03 mg. per 20 gm. weight of the mouse, as evidenced by lower parasitæmia in the initial

TABLE

Daily average parasitæmia per 10,000 R.B.Cs. during 10 days from untreated

Total dose in mg. per 20 gm. body weight.	1		2		3		4		5	
	E.	C.	E.	C.	E.	C.	E.	C.	E.	C.
0·03	1·5	58	5	76	32	110	50	119	24	224
0·06	8	58	15	76	0·7	110	0	119	0	224
0·12	75	168	132	331	7	350	0	538	0	426
0·3	638	115	150	84	1·5	273	0	296	0	359
0·6	568	146	184	101	0	193	0	213	0	253
1·2	135	115	113	84	1	273	0	296	0	359
1·8	135	115	129	84	0	273	0	296	0	359
2·4	686	115	148	84	0	273	0	296	0	359
3·0	781	1,946	82	382	1	611	0	1,372	0	807

E = experimental animals.

* When experimental animals were negative for 10 continuous days blood was sub-inoculated and

stages following treatment. The parasitæmia was higher than in the untreated mice on the 7th, 8th and 9th days, a phenomenon which also occurred in the quinine series and the explanation is probably the same as stated above. With 0·06 mg. per 20 gm. weight of the mouse and doses higher than this, there was a total clearance of parasites from the peripheral blood for varying periods. The parasites appeared to be eliminated completely with doses of 1·2 mg. and above, while with the different doses between 0·06 and 0·6 mg. per 20 gm. mouse weight the parasites were not patent in the peripheral blood for different periods and reappeared.

An attempt was made in 4 animals to determine the minimum single dose which would effectively clear the parasites and the details of the experiment are given in Table IV.

Single doses ranging from 1·0 to 2·5 mg. per 20 gm. weight cleared the peripheral blood for a period of 11 to 16 days following drugging, after which the

III.

the 1st day of drugging in animals treated with sulphadiazine and controls.

6		7		8		9		10		Number of animals.
E.	C.	E.	C.	E.	C.	E.	C.	E.	C.	
69	373	342	261	636	403	456	370	462	609	3
2	373	2	261	55	403	267	370	262	609	3
0	459	1	715	57	467	315	302	234	529	3
0	1,045	0	318	0	445	0	196	0	117	2
0	923	0	910	0	923	0	196	2·5	117	2
0	1,045	0	319	0	445	0	196	0	117	1 cured*.
0	1,045	0	319	0	445	0	196	0	117	1 cured*.
0	1,045	0	319	0	445	0	196	0	117	3 cured*.
0	2,268	0	656	0	2,386	0	D	0	...	2 cured*.

C = comparison animals. D = died.

the recipient animals were observed for 10 to 15 days.

parasites became patent again. A dose of 1·2 mg. per 20 gm. weight given divided over 3 days seemed to sterilize the blood of parasites, while 2·5 mg. given as a single dose cleared the parasites only temporarily. This was probably due to the plasma concentration of the drug not remaining at an effective level for a sufficiently long period as it did when a smaller dose was spread over 3 days.

DISCUSSION.

The aim of the experiments, as already stated, was to compare the reaction of *P. berghei* from that of others to two of the known antimalarials. From the results shown in Table I, it was seen that the parasitæmia varied from day to day even in untreated animals, despite all the known conditions remaining the same. To compare the reactions of the parasite to different drugs it was considered necessary to depart from the usual definitions of minimum effective dose generally adopted in screening antimalarials based on Class I effect of quinine (Shannon, 1946). In

TABLE IV.

The effect of single doses of sulphadiazine orally administered to mice infected with P. berghei.

Number of animals.	Dose in mg. per 20 gm. body weight.	Parasitæmia per 10,000 R.B.C's. during 10 days from the 1st day of drugging.										Repatency of parasites on the day.
		1	2	3	4	5	6	7	8	9	10	
1	1	650	43	2	0	0	0	0	0	0	0	12th.
1	1·5	596	17	4	0	0	0	0	0	0	0	11th.
1	2	709	37	1	0	0	0	0	0	0	0	15th.
1	2·5	701	35	0	0	0	0	0	0	0	0	16th.

these experiments the minimum effective dose of a drug was considered to be that dose which cleared the peripheral blood for a minimum of 3 consecutive days when administered orally on 3 consecutive days commencing from a day on which the parasites were patent and was in accordance with Class II effect of Shannon (*loc. cit.*). Results were not comparable to those of others quoted above, as in most cases in this series drug administration was commenced during the patent parasitæmia and the methods of evaluation and analysis were different.

According to the definition given above, the minimum effective dose of quinine and sulphadiazine were found to be 96 mg. (16 mg. twice daily for 3 days) and 0·12 mg. (0·02 mg. twice daily for 3 days) per 20 gm. weight respectively. The quinine equivalent of sulphadiazine for blood-induced infections of *P. berghei* calculated in accordance with the formula of Marshall (1946) is $\frac{96}{0{\cdot}12} = 800$, which is slightly higher than the figure 727 quoted by Thurston (*loc. cit.*).

The tardy reaction of *P. berghei* to quinine in the present series appeared to be an important difference between this parasite and the others. In Tables II and III, the daily parasitæmia recorded were the average of the group of drugged and undrugged animals respectively for each dose tried. Any individual variations in the absorption and plasma concentration of drug were allowed for by the method of averages.

P. berghei seemed to be highly sensitive to sulphadiazine and 1·2 mg./20 gm. weight sterilized the blood of parasites (Table III). Coggeshall (1940) suggested that species with a rapid rate of metabolism like *P. knowlesi* are most sensitive to sulphonamides. The high sensitivity of *P. berghei* to the drug lends support to this view, as in mice it has a rapid rate of metabolism and has an asexual periodicity of 24 hours (Ramakrishnan and Satya Prakash, *loc. cit.*) like *P. knowlesi* in monkeys.

Marshall *et al.* (1942) and Richardson *et al.* (1946) working on *P. lophura* and *P. knowlesi* respectively found that the mode of action of sulphadiazine on the parasites was similar to that on bacteria, due to the drug's affinity to para-aminobenzoic acid which is essential for the parasite metabolism. Thurston (*loc. cit.*) confirmed this in relation to *P. berghei* and concluded that some fundamental difference in metabolism of the different species of parasites affected their susceptibility to sulphonamides. Below is a list of some plasmodia and their reaction to sulphadiazine :

Reaction of some plasmodia to sulphadiazine.

Species of plasmodium.	Response to sulphadiazine.	Investigator.
P. vivax ...	Clinical cure ...	Coggeshall *et al.*, 1941.
P. falciparum ...	Do.	Do.
P. knowlesi ...	Highly sensitive* (Q.E. 175). ...	Richardson *et al.*, 1946.
P. cynomolgi ...	Sensitive ...	Coggeshall *et al.*, 1941.
P. inui	Do.	Do.
P. gallinaceum ...	Sensitive* (Q.E. 0·8) ...	Thurston, 1950.
P. lophura ..	Slightly sensitive ...	*A survey of anti-malarial drugs, 1941–1945.* Wiselogle, 1946.
P. cathemerium ...	Do. ...	Do.
P. relictum var. *malalinum*	No sensitivity ...	Manwell, Counts and Coulston, 1941.
P. circumflexum ...	Do. ...	Do.
P. nucleophilum ...	Do. ...	Do.
P. berghei	Highly sensitive ...	Thurston, 1950.

* Quinine equivalent.

P. berghei would appear to resemble the plasmodia of monkey and man more than others.

Certain mice being refractory to the first inoculation was already referred to and a detailed account has been published (Ramakrishnan and Satya Prakash, *loc. cit.*). In the present series of 87 mice inoculated, 10 proved refractory during a minimum observation period of 10 days. To confirm the refractoriness, blood of 4 such mice were sub-inoculated into 8 *Rattus rattus* with negative results.

For some reasons, not known, the refractoriness in the present series was considerable as compared to other published results (Vincke and van den Bulcke, 1949) and the most probable cause was some factor inherent in the strains of mice available to the authors. As it is important to be certain that the animal is

infected before drugging, it is considered that drugging should commence only after parasitæmia is patent in blood-induced infections.

SUMMARY AND CONCLUSIONS.

1. The minimum effective dose has been defined to be the smallest dose of the drug which cleared the peripheral blood of parasites for at least 3 consecutive days when administered orally in divided doses on 3 consecutive days of patent parasitæmia.

2. *P. berghei* was highly sensitive to sulphadiazine, the minimum effective dose of which was 0·12 mg., while that of quinine was 96 mg. per 20 gm. mouse weight.

3. In the present series, 11·5 per cent of the animals was refractory to the first inoculation confirming previous results in spite of the fact that the dose of inoculation was 5 million parasites. These results made it necessary to commence drugging only after patent parasitæmia was established.

4. *P. berghei* in its sensitivity to sulphadiazine resembled the malaria parasites of monkey and man.

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STUDIES ON THE BIONOMICS OF *ANOPHELES AQUASALIS* CURRY, 1932 (contd.).*

Part II.

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[April 12, 1951.]

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* The earlier sections of this paper have appeared in the *Indian Journal of Malariology*, Vol. 5, No. 3 (September 1951), p. 293.

ADULT BIONOMICS.

(I) ACTIVITY PERIODS.

SHANNON (diary, Nov. 1–7, 1942), from personal experience with all-night catching, was of the opinion that from midnight to 04.00 hrs. *aquasalis* activity is practically nil. This opinion was put to numerical proof in June–August 1950, the season covering maximal density. The five dawn traps used in the host preference experiments described in Section VIII were modified by closing up the louvres under the roof, and cutting sliding shutters in the sides that could be opened and closed at any desired hour*. It was found that the bait animals were liable to take a few mosquitoes with them into the traps unless they were put in before 16.00 hrs. Traps were brushed out and pyrethrum sprayed each morning. Each Sunday night of these experiments the shutters were kept closed with the animals 'in', as a test for unenterability. Only six night experiments a week were therefore possible. The opening and closing of the individual traps was done by two field men provided with an alarm clock modified into a watchman's recording clock, as described by McArthur (1949). As only one species of bait, the ox, was used, it was possible to avoid any preference error arising from individual trap locations (though they were all in a line at 100 ft. intervals), or individual animal attraction differences, by using in a weekly rotation different traps for the five periods into which the night was divided, whilst not alternating the bait animals. These periods were 18.00–20.30, 20.30–23.00, 23.00–01.30, 01.30–04.00 and 04.00–06.00 hrs. During the course of these experiments, sunset occurred from 18.19 to 18.25, and sunrise from 05.38 to 05.50. Fortunately during these two months there occurred no dawn-squalls to destroy the captures resting on the screens. The totals, by periods, of 54 night captures, which with the unobserved test Sundays, covered just over two complete lunar cycles, were (*aquasalis* only) :—

	I	II	III	IV	V	Tota
	(18.00–20.30)	(20.30–23.00)	(23.00–01.30)	(01.30–04.00)	(04.00–06.00)	
Number caught	40,295	11,179	19,630	9,755	12,084	92,943
Percentage ...	43·5	12·2	20·8	10·5	13·0	

Activity, therefore, continues throughout the night, but after 20.30, at a reduced intensity.

The figures also furnished data in respect of the effect of moonlight on flighting time, as first shown by Ribbands (1946) for *A. funestus* in West Africa. Correlation coefficients, as used by Ribbands, were not calculated, as it is obvious

* In a series of preliminary experiments much trouble was experienced in making these traps unenterable when baited, mosquitoes gaining access through cracks at the corners of the louvres, and round the door. Not only had the louvres to be completely closed up, but screens of tar-soaked sacking, tightly tied to nails, had to be placed over the two doors after putting in the bait animal. After this 'unauthorized' entrance was reduced to nil, as proved by the Sunday 'blank' test runs.

that *A. aquasalis* is in the main a crepuscular mosquito, and its main activity period is in the first two hours of darkness. The catches up to 20.30 hrs. were therefore particularly studied. To render the results more clear cut, only nights were totalled during which, up to 20.30, there either was a moon throughout or no moon at all (neglecting very short periods when the moon either set a few minutes after the sun, i.e. before full darkness, or rose a few minutes before 20.30 hrs.). This subdivision furnished data for 24 nights with moonlight immediately succeeding sunset and continuing up to 20.30 hrs. and for 25 nights with no moon between sunset and 20.30 hrs.

Moon to 20.30 hours.				No moon to 20.30 hours.			
Period.	Total night's catch.	Catch to 20.30 hours.	Percentage.	Period.	Total night's catch.	Catch to 20.30 hours.	Percentage.
Jun. 16–30	28,067	11,899	42·4	Jun. 6–14 ...	6,133	1,581	25·8
Jul. 17–28 ...	27,391	15,609	57·0	Jul. 2–13 ...	21,821	6,442	29·5
				Jul. 31–Aug. 5 ...	5,370	2,447	45·6
Totals ...	55,458	27,508	49·6	...	33,324	10,470	31·4

The very large first period catch during the six moonless nights at the beginning of August render the percentage differences less striking than the June and July figures alone indicate, but there seems little doubt that *aquasalis* activity is greater in the first two hours after sunset on moonlit than on moonless nights. This fact cannot be without some bearing on intensity of transmission. Though the Trinidad villager, like all inhabitants of the tropics, 'turns in' early, they do not go to bed with the sun, and, as the precipitin results in D.D.T. sprayed areas in Table XXXVIII show, there is much out-of-doors (probably including verandah) biting before the house proper is finally retired to, at least by adults. Everywhere in the world, human outdoor activity is generally greater on moonlit than on moonless nights, and the high-crepuscular activity of *aquasalis* thus renders it less susceptible to residual spraying control than species which bite later in the night.

As stated earlier, after collecting from the traps was completed after 6 a.m., these were brushed out and lightly pyrethrum-sprayed to ensure that they should be empty of mosquitoes for the following night's operation. This procedure was taken advantage of to study why it is possible to collect stragglers in houses after daylight. The first, main, collection having been made and the bait animal removed, the brushing out and spraying was done with the trap doors closed, and stragglers remaining in the dark of the animal compartment were thereby driven through the V-screen to the trap compartment, from which a second collection was then made. The results were interesting, as indicating that it is the late entrants (and *ipso facto* the late feeders) that tend to straggle in houses after daylight. The figures below show the percentage of each period's catch which were obtained after driving such stragglers to the screens.

Residual percentage of 9 weeks' catches not moving to screen at dawn.

Period.	I	II	III	IV	V
	0·9	1·6	1·4	1·8	2·0
	Average percentage of stragglers			1·3	

The increase is not regular, and the percentage is only markedly different from the average for the first and the last two periods. Some 24 per cent of a night's catch are not full-fed by dawn (Section V). Also it is likely that feeders of the early night possibly indulge in a second 'nip' later (Section VIII). Thus the period differences are unlikely to be very well defined; but the figures definitely bring out what a minute proportion of the total *aquasalis* which have been in a house by night remain for morning collections for dissection, and therefore how unrepresentative must be dissection results based on house captures of this species.

It is very difficult to assess the effect of rain on activity. During a light drizzle biting certainly continues, but really heavy rain appears to stop movement. In Trinidad, rain around sunset is less usual than at other periods of at least the day. Figures for periodical distribution of the total day's precipitation are not available.

(II) DAY-TIME RESTING PLACES.

Though the introductory section records that the amount of day-time resting in houses in Brazil is to some extent controlled by the nature of the surrounding terrain, it has long been known that, in Trinidad at least, there is an almost *en masse* departure of adults from houses by first sign of dawn, only stragglers remaining, as shown in the preceding sub-section. Such stragglers serve for the only probably human-fed material available for dissection. Shannon took advantage of this mass departure-tropism to devise his 'dawn-trap' for this species. The trap consists of two compartments, one containing a bait animal to which mosquitoes gain access through louvres along the sides under the overhanging roof (the interior of this compartment is quite dark), and the other, oriented east, with metal gauze end and sides, separated from the animal compartment by a V-screen. The engorged mosquitoes, perceiving the first light*, desert the vicinity of the host and pass through the V-screen into the trap compartment, which has

* Shannon also suggested that it might be the pre-dawn wind that caused the exodus. Observations within a trap at 20.00 and 04.00 hours, conducted on 2 full moon and 2 new moon nights, throw doubt on both Shannon's heliotropic and anemotropic hypothesis of this migration. On a full moon night large numbers were found to have passed eastward through the V-screen even as early as 20.00 hours, and even within the animal compartment, quite dark except for the screened eastern end, there were more resting on the screen than on the sides or roof, as if immediately on feeding there is a tropism towards even the faint light of the night sky, much more marked on full moon nights. During the observations of September 26, there was a total lunar eclipse around midnight. On that night at 20.00 hours, 210 specimens were counted on the trap compartment front screen; at midnight this had fallen to 30, and at 04.00 hours, had again risen to 166. These observations suggest that specimens feeding within a house vacate it very soon *p.c.*, which has an important bearing on the length of contact with surfaces treated with a chlorinated hydrocarbon that this species can be forced to undergo.

a separate side door for entrance for collection from the gauze end and sides. So strong is this positive photo-tropism that, when an early morning rain squall strikes the screens, the mosquitoes remain thereon and are beaten to the floor in a soggy mass rather than move back into the comparative darkness and shelter of the back of the trap compartment, even though they would be unlikely to find their way through the reverse direction of the V-screen into the complete shelter of the animal compartment. Collection has to be made early in the morning, or the trapped insects are preyed upon by ants and hunting spiders.

But all that could be discovered about the natural day resting places of those insects which had left the vicinity of the host at dawn was a paragraph in Shannon's first report to The Rockefeller Foundation (covering the period September 28 to November 8, 1941), that he failed to attract resting adults with the keg-trap used in the U.S.A. for *A. quadrimaculatus*, even when placed in a stable where *aquasalis* was feeding every night. But among bamboos growing around a stable, he collected 20 ♂, 2 ♀ on the outside of fallen internodal sheaths. He seems to have paid no further attention to this problem. Realizing that until the natural day resting places were discovered little progress could be made on adult bionomics, at the time of starting work in the autumn of 1947 the author concentrated on this. Artificial shelters, a thatched dome over vegetation and a pit roofed with leaves, not only failed to attract the insects, but freshly fed specimens introduced into these had vacated them by the following morning.

A first glimmer of success was achieved when field assistant de Verteuil found a few adults resting among dried banana sheaths affording very little shade. Searches were then initiated in low scrub and the first day's work produced 3 ♂, 101 ♀, the latter in all stages of digestion from abdomen I to abdomen VII, together with single specimens of *A. oswaldoi* and *A. neomaculipalpus*. As Professor Kirkpatrick of the Imperial College remarked 'had any general insect collecting by sweeping ever been done locally, the natural resting places of *aquasalis* would have long since been known'.

During 1948, random searches all over the area produced 2,239 ♂, 4,942 ♀ *aquasalis* and 173 ♂, 256 ♀ *neomaculipalpus*, but only 1 ♂, 2 ♀ *oswaldoi* and 4 ♂, 12 ♀ *albitarsis*. The maximum per man-hour catching rate, using 1 cm. diameter glass tubes with an aspirating mouthpiece, then found was 95·8 ♂, 77·8 ♀ per man-hour*, with an average for that year, all localities, of 3·7 ♂, 8·3 ♀ per man-hour. In subsequent years routine collections in selected clumps of scrub vegetation around representative villages and other localities have been the source of data for local seasonal measurements of fluctuations in density. The quite high correlation that exists between larval and adult density measurements has been referred to in the account of breeding in the saline marshes described in Section VI (ii).

With this discovery *aquasalis* became as amenable to investigations into its adult bionomics as any of the house-resting† species of the Old World, in fact rather

* A few higher values have since been found, up to 130 ♀ per man-hour, but the author thinks this is near the physical limit of the method.

† Recent literature has coined the more elegant term 'exophilic' for species which rest outside and not within habitations, human or animal. But outside resting species may feed either within the habitation, or out of doors. The additional terms 'exo-' and 'endo-phagic' seem therefore required to describe both the resting and feeding tropisms of any species.

more so, as house resters are a special part of the general population of any species. Certainly dissection work is hampered in that a general outside resting population has fed, according to its degree of anthropo- or zoo-phily, on many other animals than man, but outside catches afford a true measurement of the anthropophily of a species (this is discussed in detail in a later section), by furnishing material for precipitin reactions.

(iii) MORTALITY DURING THE GONOTROPHIC CYCLE.

Another most useful measurement such catches furnish is that of the rate of mortality during the gonotrophic cycle, by classifying the catches into the seven abdominal stages of Boyd's (1930) modification of Sella's original method. The Nyssorbynchus spp. known to the author are less easy to classify abdominally than those of other sub-genera, owing to the fact that the pleuræ have grey markings, and hence the degree of digestion is less easy to note, but using a set of fieldmen the senior of which have now long become familiar with the method, the percentage of error is probably fairly constant, at least from 1949 onwards. The data almost invariably show considerably larger numbers in Stage VI than in Stage V, and many more in Stage VI than in Stage VII. Decision between Stages VI and VII rests upon the presence or absence of a trace of black blood along the ventral side of the base of the abdomen, rather difficult to see in *aquasalis*. Possibly the increased numbers of Stage VII recorded in 1948 over 1949 are caused by men then learning this method neglecting or not observing this trace of residual blood. *Per contra*, it may be that some abdomens are classified as Stage VI which are really VII. But attainment of Stage VII in *C. fatigans* is not always equivalent to readiness for oviposition (Senior White, 1934), and admitting that there is an error in the method, the author considers that Stage VII percentages (on which all the calculations of survival rates as later discussed are based) can be taken as substantially representing numbers ready for movement for oviposition. There is nothing like the 'pile up' of Stage VII so noticeable in *C. fatigans* completing digestion in houses (Senior White, *loc. cit.*).

Experience has shown that *aquasalis* seldom rests by day out of doors more than a foot above ground, though the experiments described in sub-section (viii) reveal it temporarily resting at all elevations up to at least five feet at dusk. The micro-climates affecting outside resting are discussed later.

Taking first the densities, male percentages, variation in stage percentages and mortality from fresh fed, as indicated by taking the freshly fed percentage as 100, and summing backwards from the ultimate, mature ovary (Stage VII), survival rate, the major collection results are shown in Table XXXIX. Every collection made is not tabulated, but, from 1949 onwards, only those continued over the major part of a year. It is obvious that a totalization of the table would be of no value, owing to the varying breeding conditions (as indicated by male percentage and per man-hour densities) in the various localities searched. Survival rate to Stage VII is also influenced by the yet unmeasured fluctuations in the density of predators, which probably vary with both locality and season. But it is obvious that it is a reasonable estimate to reckon that no more than 5 per

cent of fresh-feds survive to mature ovary. In many long period collections the percentage is much lower. The import of this terrific mortality on the vectorial capacity of *aquasalis* is considered in Section IX.

Individual collections fall into two categories, those in which mosquitoes in Stage VII are found and those in which they are not.

Densities at which Stage VII found.

[170 collections (1948-49) yielding 14,354 Stage II and 849 Stage VII. Survival rate 5·9 per cent.]

Per man-hour ...	1–10	11–20	21–30	31–40	41–50	51–60	61–70
Average density ...	5	15	25	35	45	55	65
Number of collections ...	69	41	24	8	7	7	4
Percentage of total collections.	40·6	24·1	14·1	4·7	4·1	4·1	2·4
Percentage of Stage VII numbers.	11·8	5·5	6·4	4·7	5·5	4·2	7·7

Per man-hour ...	71–80	81–90	91–100	101–110	111–120	121–130	131–140
Average density ...	75	85	95	105	115	125	135
Number of collections ...	5	1	2	0	0	1	1
Percentage of total collections.	2·9	0·6	1·2	0	0	0·6	0·6
Percentage of Stage VII numbers.	8·7	4·8	5·3	0	0	11·9	30·8

The survival rate is shown in Chart 2.

Mr. Cope of the Imperial College was good enough to submit these data to statistical analysis, calculating the regression of survival (y) on density (x). He finds :—

Linear regression : $t10 = \sqrt{\frac{182{\cdot}2}{41{\cdot}52}} = 2{\cdot}095$. Not significant.

PARABOLA : ANALYSIS OF VARIANCE FOR QUADRATIC REGRESSION :

	SS	DF	MS	F	P
SS regression ...	442·1	2	221·1	12·81	1 per cent.
Remainder (error) ...	155·3	9	17·26		
Total	597·4	11			

∴ Parabola gives good fit to data.

CUBIC: ANALYSIS OF VARIANCE FOR CUBIC REGRESSION:

	SS	DF	MS	F	P
SS to cubic regression.	472·2	3	157·4	10·6	1 per cent.
Remainder (error) ...	125·2	8	15·65		
Total	597·4	11			

∴ 3rd degree curve gives significantly good fit to curve (but apparently no better than parabola).

EQUATION TO PARABOLA:

$Y = 8{\cdot}942 - 1{\cdot}60906\,(x - 12{\cdot}667) + 0{\cdot}07508\,(x^2 - 223{\cdot}67)$

[x is here 1/5 of actual density classes.]

$= 11{\cdot}631 - 1{\cdot}60906x - 0{\cdot}07508x^2$

$\frac{dy}{dx} = -1{\cdot}60906 + 0{\cdot}15016x = 0$

$\therefore\ x\ (\text{min}) = \frac{1{\cdot}60906}{0{\cdot}15016} = 10{\cdot}715\ (\text{x5 factor}) = 54$

INDICATING A MINIMUM SURVIVAL AT DENSITY OF ABOUT 54 PER MAN-HOUR.

EQUATION TO CUBIC:

$Y = 8{\cdot}942 + 0{\cdot}186836\,(x - 12{\cdot}667) - 0{\cdot}088248\,(x^2 - 233{\cdot}667) + 0{\cdot}003899\,(x^3 - 4600{\cdot}6) = 9{\cdot}258 + 0{\cdot}186836x - 0{\cdot}88248x^2 - 0{\cdot}003899x^3.$

$\frac{dy}{dx} = 0{\cdot}186836 - 0{\cdot}176496x + 0{\cdot}011697x^2 = 0.$

Root for minimum gives $x = 11{\cdot}45$ (x5 factor) $= 57{\cdot}25$.

Hence, combining both equations, minimum survival is at a catching density of about 56 per man-hour.

The parabola and cubic curves are shown together with the crude curve in Chart 2. From the analysis it appears that the higher survival rate at low and even more so at very high densities is a genuine phenomenon. It is obviously '*concurrently density dependent*' (Solomons, 1949) and it remains to speculate how a density-dependent control phenomenon can be less effective at low and at very high densities than at intermediate values. It is suggested that at very low densities the insects are so well protected by their chosen ecological niches as to largely escape their predators, while at very high densities the predators cannot keep pace with them. The observed predators [sub-section (iv)] are animals of slower reproduction than mosquitoes. The factor that Solomons calls 'suppression', i.e. a time-lag in density dependence, probably supervenes. The 'limiting factor' here is the slower rate of reproduction of the predators.

Densities at which Stage VII is not found.

378 collections (1948-49). The distribution of these are:

Average density per man-hour.	5	15	25	35	45	55	65	75
Numbers	282	56	21	9	4	1	1	1
Percentage	74·6	14·8	5·5	2·4	1·1	0·3	0·3	0·3
Average density per man-hour.	85	95	105	115	125	135	...	...
Numbers	1	0	1	0	0	0	...	...
Percentage	0·3	0	0·3	0	0	0	...	...

89·4 per cent of the findings with no Stage VII are found at densities 5 and 15. Above densities of 45, there are few such findings. The figures above appear to represent a hyperbola with an asymptote near 55, suggesting that at densities exceeding 45, there is always a large enough population to ensure some survival to Stage VII, whereas at low densities Stage VII is less frequently found, e.g. the density 5 per man-hour represents only 40·6 per cent of the number of collections yielding Stage VII, whereas the same density yielding no Stage VII represents 74·6 per cent of the total number of collections in which this stage is absent.

In connection with mortality figures the very high oöcyst: sporozoite ratio (49 : 1) recorded in Table XLVI requires consideration. This ratio indicates an exceptional degree of mortality. Either *aquasalis* as a species is constitutionally very short-lived, or the ratio is the result of the high degree of predator mortality it suffers. In either case it is this mortality which, in Trinidad, renders *aquasalis* so comparatively innocuous as a malaria-vector.

(IV) PREDATORS.

These have been very insufficiently studied, owing to the fact that none of the field staff have had any general entomological training, and the author has had so little opportunity himself for this very time-consuming observational work.

Now neither *A. aquasalis* nor *A. neomaculipalpus* rests in bushes in the usual anopheline attitude, head and body at about 45° to the vertical, and the hind legs separately raised. *A. aquasalis* generally rests on a grass stem or leaf stalk situate at any inclination to the ground, with its body more or less parallel to or even across the stem, the hind legs frequently being passed around the stalk and always apposed below the body. The white patch thus made by the conjoined hind tarsi is then very conspicuous. From time to time one or other hind leg (very seldom both together)* is waved in a circle and brought back to the original apposition.

* Nielsen and Greve (1950), p. 234, record the same habit of moving *one* of the hind legs and of making very short flights, in day-time resting *Ædes cantans* in Denmark.

A. neomaculipalpus is more anopheline-like in its resting attitude, but the hind legs, again apposed and conjoined along the tarsi, are directed in a line with the body-axis, not raised. In this species there is no white tarsal area to direct attack to a non-vital spot.

Even without attack *aquasalis* is not by day completely static. Periodically the palps are vibrated, but less frequently than the hind legs. It is not disturbed by the twigs used for resting moving as the bushes are swayed by the wind, nor does a sudden increase in light incidence, owing to wind-movement of the bush canopy, disturb it. It seems to realize that in a few seconds light will return to a suitable value. Having on one occasion two specimens (*a* and *b*) in view sitting within 3 inches of one another, in half an hour one remained absolutely still (it had only one hind tarsus) but in the same time the other hopped thrice within an area about 2 inches in diameter. No reason for the change of position could be observed. Records on three other specimens are:

(*c*) 10.25 hrs. first seen.
10.30 hrs. circled hind legs and vibrated palps.
10.45 hrs. circled hind legs, palps still.
10.48 hrs. hopped 3 inches, circled legs, then flew out of vision.

No predators approached during this period, and the insect seemed quite indifferent to drifts of cigarette smoke. The reason for flight was not apparent.

(*d*) 10.40 hrs. first seen.
10.49 hrs. circled legs.
10.57 hrs. circled legs.
11.02 hrs. hopped 2 inches; after resettling circled legs, and did not move again whilst under observation.

(*e*) 10.51 hrs. first seen, on twig.
11.02 hrs. flew nearer ground (here covered with fallen leaves) and rested on a twig at right angles to the axis.
11.25 hrs. had not again moved.

During these observations specimens have been seen attacked by Myrmecine ants and by hunting spiders, neither yet identified. A specimen was seen resting undisturbed within 4 inches of a runway along which ants were transporting pupæ. Another was thrice unsuccessfully attacked by an ant, once from its front, twice from behind. After each abortive attack it moved a few inches and resettled.

A few successful attacks have been seen, and both the mosquito and its predator secured. This is more difficult with hunting spiders than with ants as the former often drop into the ground debris with their prey. The ant (which is smaller than *aquasalis*), if successful, seems to secure a grip on the abdomen. It was not until two years later that opportunity was found for further observations. By that time there were sufficient technicians (though all are quite untrained in natural history) to do this observational work in the author's absence. These men have reported a large lizard and a small frog (neither yet identified) as hunting in scrub in which adults were resting. But a few dissections of both animals showed that though they are omnivorously insectivorous, no mosquito remains were

found. They may be useful predators nonetheless. The men reported no attacks by ants or hunting spiders, and such further observation as the author himself found time for, confirmed this. Again no visible reason was seen for the 'leg-circling' behaviour, which goes on even when no other insect is anywhere near. Nonetheless, this 95 per cent reduction during one gonotrophic cycle continues and remains unexplained. If the reduction is due to predators operating during the night, the author does not know any method of observing it, for though *aquasalis* is not greatly disturbed by electric torchlight, other animals well may be. Solomons (*loc. cit.*) has emphasized that a density-dependent phenomenon, such as this reduction has been proved to be [Section VII (ii)], is not due to climatic causes. Thus the cause of the reduction from fresh-fed to mature-ovary abdomen remains entirely unexplained.

When flighting to attack at dusk, *aquasalis* frequently runs the gauntlet of a large population of a crepuscular dragon-fly, identified by Lieut.-Colonel F. Fraser as *Gynacantha bifida* Rambur. Colonel Fraser informed the author that in this species the remains of up to 30 specimens of mosquitoes have been found in a single stomach, but the dissections of a few guts have shown nothing but the remains of small Coleoptera. Colonel Fraser adds that this dragon-fly is also partial to small Lepidoptera.

This dragon-fly, of course, plays no part in the mortality of day-time resting *aquasalis*. It is present almost throughout the year, though it is very much more common in autumn than at other seasons. During 52 weekly observations in 1949 it was noticed on 34 occasions. On 16 of these, its appearance preceded sunset by an average of 10 minutes (nearly all these occasions were during its maximal autumnal density), and on 18 occasions it appeared after a lag averaging nearly 10 minutes from sunset. It commences its flights well up in the air, and it is only towards dusk that it hunts low over the ground. At any observed elevation it may be catching mosquitoes, but its massed descents around dusk suggest that it was then largely interested in the mosquitoes attacking the bait animals used in these observations. Before light incidence has fallen to 0 foot-candles it has disappeared, and as regards *aquasalis* it can only at most reduce the initial attack flight. It is inactive by the time *neomaculipalpus* attacks.

(V) PLANTS PREFERRED FOR DAY RESTING.

Naturally these must differ according to the flora of the locality, but pure stand quadrats, each 25 ft. × 25 ft., of ten species noticed to be common or favoured by *aquasalis*, together with an equal-sized quadrat of mixed bush as found in that particular locality, and a quadrat packed with coconut husks, which was found on an estate near Cedros to be much favoured for day-time resting, were established. The results are set out in Table XL.

The experiment had to be conducted in the only, and made available, portion of a neglected coconut garden from which the necessary area was cleared of undergrowth. Palm shade is not considered unfavourable to resting, but the water table was too high, and certain plants, particularly *Flemingia strobilifera*, known from general searches to be a favoured resting place, grew poorly, even though such drainage as the lie of the land permits of, was put in. The nearly equal sex proportion indicates the proximity of nearby breeding.

In considering the results of this experiment it must be remembered that these were literally 'pure stand' quadrats, and that the grass understorey was removed and kept out, even from the comparison quadrat of natural mixed bush. Nonetheless, practically one-third of the total catch came from the mixed bush. Owing to the breakdown of the photometer only one set of light measurements in these quadrats was possible. These were made on a heavily overcast to drizzly morning, and it seems best to consider the results as the percentage reduction at ground level of the light incidence above the plant concerned. Except for the Hamilea, the Cassia, and the low-growing Borrerria, the percentage of reduction was not strikingly different between the various quadrats. Saturation deficiency was measured at ground level. The largest catch in a pure stand quadrat was at the highest saturation deficiency (S.D.) in the fern *Dryopteris impressa*, but this is followed by the Borrerria catch at a S.D. of nil, and differences due to light reduction and S.D. will require much further investigation under clear skies during the dry season. They will also be investigated when the grass understorey has been permitted to re-establish itself.

A second experiment was conducted in this plantation, on the effect of height of grass on density of resting population. During 1949, a series of 23 fortnightly catches was made in this highly prolific area, not only for measurements of seasonal fluctuations in density (which averaged 13·9 ♂, 15·0 ♀ per man-hour), but also for measurements of light incidence, as considered in the following sub-section (vi). On completion of that series, the scrub beneath the coconuts in the area searched was eradicated, leaving only the grass understorey, which was periodically cut back. This was then searched on 24 fortnights. On the same occasions, some nearby scrub with grass beneath coconuts was searched for comparison. In 1950, the density in the comparison scrub was very much lower than in 1949, though the nearby B1 trap catches (Table XXXIII) were nearly treble the 1949 total, and this can only be considered to be a reflection of the fact that the comparison area of 1950 did not afford such attractive resting facilities as the scrub cut out after 1949, though the two sites were quite close to one another. It will be seen that in the comparison scrub the sex ratio was the same as in 1949.

A. aquasalis.

(Barataria coconut quadrat after scrub clearing under grass, compared with adjacent scrub area as searched in 1949.)

	Searches.	Man-hours.	Per man-hour density.		Remarks.
			♂	♀	
1949	23	191	13·9	15·0	Scrub and grass.
1950	24	1,360	1·0	1·2	Scrub and grass.
1950	24	1,360	0·04	0·13	Grass only.

When the pure grass plot is considered, it is observed (firstly) that the sex ratio has greatly changed, being 1 : 3 instead of approximately equal. Secondly, that as regards the females, the per man-hour density is very much less than that in even the poor-yielding scrub. But in these totals the height of the grass was not considered. This was average-measured at each search, with the following interesting findings. Quite fortuitously, 12 searches were made in each of the two height categories.

	♂		♀	
Height of grass in inches ...	2–8	9–16	2–8	9–16
Average catch per man-hour ...	3·6	8·3	8·8	15·4

The height of the grass does not affect the resting sex ratio, but it most definitely does the actual numbers resting, which are doubled when the grass exceeds 8 inches in height*. From this grass experiment one fact of great public health importance emerges, though naturally it will require a pilot experiment on a much larger scale than 1/10th of an acre before it can be used for proposals to Government for the biological control of *A. aquasalis*.

If grass alone is present and is kept short, resting of adults, both newly emerged and feds, is greatly discouraged. The only practical way by which to keep grass at a low height is by grazing, for the cutlass-cutting of grass that one now sees everywhere where Indians keep cattle in Trinidad is extremely wasteful of man-power. An increased cattle population will serve to divert even further *aquasalis* from man. But one must proceed with caution, involving much experimentation. Causey and Deane's data quoted in Section I show that if the outside terrain is inimical to resting, *aquasalis* goes into houses both to feed and to rest. To cause this locally might well be disastrous. That cattlefeds, however, should come to a bad end for lack of resting places during digestion could only be advantageous. Now, man, furnished though he be with the whole current armamentarium of chemical warfare, appears nowhere to have succeeded in eradicating any anopheline *so long as a particular species is fought on its own ground.* Nothing could be more eloquent of defeat than Dowling's (1950) paper on attempts to eradicate *A. gambiæ* from Mauritius by D.D.T. Here is an oceanic island where everything seemed favourable for the D.D.T. eradication of the house-haunting *gambiæ*. Instead, *gambiæ* has abandoned the D.D.T.-contaminated house as a resting place, and like *aquasalis* in Trinidad, has continued to pursue its nefarious activities from out of doors.

Among his papers, when the author succeeded as Second Imperial Entomologist that strange genius the late F. M. Howlett, notes for a paper on biological control were found. He was referring to the chemical warfare of pre-War I against insects, and comparing it with his discovery of how to take advantage of the chemo-tropisms of insects. He concluded, 'Instead of beating blindly against the web of life, we may one day learn how to take the threads apart, and weave anew a pattern to our liking'. The public health worker, urged by preventable mortality, has beaten

* Many other comparisons of heights were tried before the critical value of 8 inches was arrived at. None of them gave such clear-cut differences, and they further had the disadvantage of the numbers of catches at different heights being unequal.

blindly, with an ever-more-efficient armamentarium, against the web of life, until his efforts have resulted in the twin disasters of over-population and soil erosion. So long as national disputes are settlable in terms of adult human densities, over-population may have its points. Would that a supra-national and dictatorial power would enforce the taking apart of the human web of life, and 'weave anew a pattern to our liking!'

Meanwhile, if eradication be not possible—and it is certainly not so in continental areas and continental islands like Trinidad—efforts to control to a reduction to a density at which transmission is no longer possible must be continued. This density will vary with the vectorial efficiency of the particular vector species of a locality. 'Blind-swiping' with chlorinated hydrocarbons will undoubtedly achieve such reduction far more swiftly than biological control, but the ever-recurring cost of chemical warfare against insects is high, often beyond the financial capacity of a local administration. Where as in the case of *aquasalis*, biological control by increasing the bovine population will simultaneously conduce to greater economic well-being, it is indubitably the method to be preferred in all cases where it is feasible and where a policy of *festina lente* can be safely pursued.

(VI) THE MICRO-CLIMATES OF DAY-TIME RESTING PLACES.

In the absence of apparatus capable of the measurement of temperature and humidity in exact spots, it has only been possible to investigate the light incidence at which resting is found. The instrument used was a 'Weston' photometer reading from 1 to 10,000 foot-candles, without a 'Viscor' filter in the target, and thus recording both visible and ultra-violet light together. The author considers the instrument used in Portugal by Cambournac and Hill (1940) to have had a 'Viscor' filter, as at that time the Weston Company only supplied instruments graduated to 0·1 foot-candle (which Cambournac and Hill's paper shows they used), with 'Viscor' filters. The exclusion of ultra-violet light may have been without much significance under the circumstances in which these authors' measurements of evening light within stables were made, but its exclusion in measurements made in day time in the open would certainly be undesirable, as certain insects at least are known to appreciate light in the ultra-violet. The instrument was very sluggish at the lowest values (below 2 foot-candles). In 1950, the Weston Company modified the instrument. The scales now read 0–1,000 : 0–100 : 0–2. For work in very bright light, a filter attachment is slipped over the target. With this in position the scales read × 10. The delicacy of measurement is thus greatly enhanced. Two sets of observations were made with the instrument in its original condition. The procedure in both was to place the photometer target as closely as possible below a resting insect (if this was too near the ground the target was apposed as close to it as possible). The first series was taken at random all over the area. In the second series the target was then withdrawn and the insect captured and examined for digestion stage. This was slower work than mere light measurement near an insect not requiring capture for identification. The first series, in January-February 1949, yielded 265 ♂, 1,020 ♀. It was hoped, at this dry season, to obtain maximal tolerance values, but in Trinidad cloudless skies are the exception, the great cumulus masses of the north-east trades nearly always proceeding across the sky. In any case, observations could not be confined to near

noon, but were made between 09.00 hrs. and midday. The second series, comprising 425 ♂, 341 ♀, were taken once weekly between March and December in scrub beneath the shade of tall coconut palms in the very neglected plantation referred to in sub-section (v). Observations were not made during actual rain, though from May onwards the sky was generally much more overcast than during the taking of the first series.

When tabulated separately, no striking differences were seen in the average values, save that in the first series the male resting value averaged 27·9 foot-candles, and in the second 67·2 foot-candles, indicating that at least the shade of the tall coconut palms did not affect the second set of results. The two series have therefore been tabulated together in Table XLI. It is seen that 97·6 per cent of all males and 96·3 per cent of all females were found resting at below 301 foot-candles. No males were found at values higher than 600 foot-candles, and with a single exception the remaining females were not found at over 2,500 foot-candles. Therefore, 300 foot-candles can be taken as the limiting value above which resting is negligible, though 1 per cent of the females are found resting at values which the male will not tolerate. Not only does the diurnal motion of the sun and passing clouds affect light incidence. When wind movement of the scrub caused often large variations in incidence, readings were taken between guests. As stated previously, resting *aquasalis* is not disturbed by momentary high fluctuations in intensity.

The abdominal classification of the specimens observed in the second series was undertaken to see whether there was any variation in the light incidence preference during the gonotrophic cycle. The results are given below:

Average light incidence for resting in abdominal Stages I to VII.

Stage ...	I	II	III	IV	V	VI	VII	Total
Number ...	86	62	44	39	35	36	25	328
Average foot-candles	83·2	93·3	71·5	56·4	66·7	76·7	58·2	Mean: 79·0

The S.D. of these values is 13·9, the S.E. = ± 0·8.

The differences from the mean are therefore all statistically significant. Their biological significance is, however, not easy to understand. Tentatively it is suggested that there is some relationship between light incidence and saturation deficiency. A freshly engorged insect may be able to tolerate a greater deficiency than one unfed, or with mature eggs, but the rise in tolerance in Stages V and VI is on this hypothesis inexplicable.

The insects concerned in this series were all definitely *resting*, not even the Stage I showing any inclination to attack the observers.

(VII) THE MICRO-CLIMATE AT WHICH RESTING *aquasalis* IS ACTIVATED.

This series of observations was made once weekly around sunset, using a bait animal, to observe the initiation of *aquasalis* and *neomaculipalpus* evening activity, fluctuations in density, and in the relative proportions of the anopheline species attacking the baits; which in 1948 were a group of men *versus* a horse, and in 1949 a horse *versus* an ox. In the presence of the animals, the human catchers were not attacked. Throughout the two years, reading of an Assmann psychrometer were made at ten-minute intervals for the best part of an hour before the start of attack, and continued until observations were terminated at the moment when a representative catch of about 100 specimens had been secured. Simultaneous light measurements were made in the second year only*. Only one first attack observation at each set up of the experiment was, of course, possible.

It soon became apparent that saturation deficiency, up to the sunset maximum of 9 mm. encountered locally, was not related to activation, and the values of light incidence is alone considered in Table XLII. Activation is apparently entirely controlled by light incidence.

It is seen that for the 64 first attacks recorded the mean light incidence is 4·0 foot-candles, and that nearly 83 per cent of all the attacks occurred from 0 to 13 foot-candles. Above 13 foot-candles, the distribution of the remainder is completely random, and 13 foot-candles is considered to be the highest value at which *aquasalis* is normally activated. Values above this appear to be result of disturbance of individual insects. Disturbance seems able to activate this species, when avid, at full daylight values, which was also found for *Ædes cantans* by Nielsen and Greve (*loc. cit.*). For instance, 5 men eating their lunch around the experimental quadrat at Bordenal [Section VIII (ii)] sitting on short grass near the bush, caught 26 *aquasalis* (25 in Stage I and 1 in Stage II) attacking them. The author was not at the time present, but on a subsequent occasion, at this spot, at 09.45 hrs., 5,100 foot-candles 12 inches above, and 900 foot-candles within the grass, were recorded. To reach the men the hungry females in the scrub must have passed through the higher value. On another occasion, during the surveys of the Glaudon marshes, the author was attacked by a solitary female about 10.30 hrs., obviously disturbed shortly after oviposition by the men dipping out a quadrat. The sky was cloudless, and the light anything up to or above 10,000 foot-candles. Light values at the moment of sunset have not been recorded, as clouds on the western horizon over the Gulf of Paria make it so very variable, but the 13 foot-candle value is passed at any time up to at least 15 minutes before sunset, and the records show that the earliest record of *aquasalis* attack occurred 12 minutes before sunset: this figure may however be one affected by the disturbance of one specially hungry insect. There was a lag averaging 10 minutes in 1948 and 17 minutes in 1949 between sunset and time of first attack.

An exception to these conclusions is furnished by light incidence values within the deep shade of the Nelson Bamboo Plantation, where attack proceeds all day. [The special conditions of this plantation are considered in Section VI (vi)].

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* This long series of meteorological observations is on file in the Malaria Division for the use of any worker who may be concerned with evening conditions in the tropics. Their existence has been notified to the Bureau of Animal Populations, Oxford.

A set of observations at 11.00 hrs. under the clear skies of February 1949 in and just outside this plantation gives the following light values: they can be accepted as maximal:

Grass verge in full sun	8,600	foot-candles.
Grass under shade of the bamboos ...	1,700	,,
Grass under Flemingia shade on verge ...	125	,,
Beneath Flemingia in sun on verge ...	58	,,
Beneath 3 ft. high Flemingia ...	6·5	,,
On grass at entrance to bamboos ...	191	,,
On sunpatch on path in bamboos ...	5,050	,,
In shadow on path	77	,,
At 'regular catching spot' (*vide infra*) ...	215	,,
Drain bottom below psychrometer station	74	,,
In centre of a bamboo clump ...	36	,,
In hole in stump trash	23	,,
Under broad-leaf plant in shade ...	29	,,
Under ferns in sunpatch on path ...	170	,,

For two years, weekly morning and evening catches and observations were made at what is called 'the regular catching spot' in the above table. This was on the edge of a deep drain beside a path. The actual times were variable, but all were between 16.00 and 17.00 hrs. in the afternoon, and around 09.30 hrs. in the morning. The results of 66 evening and 52 morning observations during which attacks occurred and light was measured are given in Table XLIII. There is nothing remarkable, when noon-time light intensity at this spot is as high as 215 foot-candles and attack occurs at this hour, that both the average afternoon and morning attack incidence in these bamboos should exceed 13 foot-candles: what has to be and is still not explained is why, in this plantation, the insect is active at much higher values than in the open.

Saturation deficiency in this plantation is low, the usual relative humidity, both evening and morning, exceeding 80 per cent and frequently passing 90, though the minimum recorded in the drought of April 1949 were 62 in the afternoon and 65 in the morning. During this period there were no *aquasalis* captures, but this was as probably due to the lack of breeding places as to saturation deficiency being too high for activity.

(VIII) EVENING MOVEMENT.

Whilst making the observations on biting preferences with different species of animals set forth in Section VIII (ii), it was noticed that, as the evening grew later, specimens (the freshly engorged ones were particularly noticeable in the torch beams) appeared to rest at greater heights on the leaves of the experimental bush quadrat than at the start of the evening attack. To test this observation numerically, the bush-face adjacent to two bait animals tethered about 10 ft. outside it was divided into levels from 1 to 5 ft. by strings stretched across poles. Catches were made by 5 men, each allotted one level of mosquitoes resting on the outer leaves within easy reach of their tubes *. The tubes were changed every

* In this experiment tubes 13 cm. long by 3 cm. diameter, with an inverted V-opening, were used by the catchers, as it was necessary to work fast.

10 minutes, the run commencing from the moment when observation of the bait animals indicated that attack had fully developed. This occurred only after the then unmodified photometer had fallen to 0 foot-candles. Five 10-minute periods each night were worked, and three nights catches were made. At the same time the Assmann psychrometer (only one instrument was then available) was alternated between ground level and 5 ft., and read every 5 minutes, experience having shown that it would come into equilibrium within that time.

The results are set out in Table XLIV. The level of the highest percentages of the period catches (with one exception, 18.10–18.20 hrs.) gradually moved upwards during the first 40 minutes of the evening attack. Estimated by total numbers caught, intensity of movement was greatest between 18.20 and 18·40 hrs. But the most striking fact brought out by these catches is the very small percentages found engorged, in spite of the two bait animals, which were seen to be heavily attacked. Apparently what was caught on the leaves of the bush-face were not in the main fed specimens moving back into bush shelter, but hungry insects drawn to the face by blood attraction (including that of the catchers), and pausing there before 'taking off' across some 10 ft. of low grass to attack the bait animals. But as so small a percentage were engorged, it cannot be substantiated that as time goes on engorged specimens rest at higher levels, but only that larger numbers appear higher on the bush-face. As it is known that, before activation, practically all day-time resting occurs in the lowest 12 inches of vegetation, the activation process therefore appears to involve two movements, first an upward one within shelter, and then an outward one towards blood. The return (engorged) flight is to all levels, but wherever they go to, freshly fed specimens off the bait animals frequently do not immediately return to the bush, unless they straightway fly deep into it. Figures obtained during the same observations for *neomaculipalpus* show maximum engorged resting between 12 and 48 inches. As this species does not bite above the level of the hocks this means an upward engorged flight.

During two runs another bait animal was tethered south of the hut used in the experiments described in Section VIII (ii), so that the wall of this formed the most convenient landing ground other than short grass. Of 57 *aquasalis* captured on the hut wall at all levels, 80 per cent were freshly engorged : of 80 *neomaculipalpus*, 91 per cent. These are very different values to those obtained on the bush-face, but do not aid in ascertaining whether the majority of those engorging on the animals near the bush-face depart.

The meteorological observations indicated that the air was usually slightly warmer at the 4 ft. than at ground level, but during one run (Oct. 26) there was a temperature inversion lasting 15 minutes between 18.01 and 18·11 hrs. and on December 19 there was a temperature inversion throughout the run, the ground being warmer than the 4 ft. level by 0·5° to 2·0°F. Saturation deficiency was always higher at 4 ft. than at ground level, except in 5 of the 21 double readings, when the two levels were equal. The differences never exceeded 4 mm., which at temperatures between 75° and 80°F. is perhaps without effect on the mosquito. Psychrometers are notoriously untrustworthy at very high humidities, and considering that the two levels were read only at 5 minutes' differences, the meteorological figures afford no clue to difference in densities at the various levels, and are not reproduced.

(IX) SWARMING AND MATING.

Nielsen and Greve (*loc. cit.*) consider that there is practically no relationship between male swarming and the act of mating, it being fortuitous that a very few females get mixed up with the male swarms and are then fertilized. With a resting areal density of at least 10 per sq. ft. (much higher than anything recorded for *aquasalis*) they observed fewer matings within their enormous swarms of *Ædes cantans* and *Ædes communis* than is here recorded, and they are driven to generalizations regarding where the mass-mating occurs, which they state to be in the air in the afternoon and among the vegetation in the morning. They apparently carried out no spermatheca dissections in support of their supposition (p. 243) that females found near the ground during the night had already been fertilized. These authors consider (p. 255) that the rationale of swarming is to mix males from different breeding places and so prevent in-breeding, the females appearing to be very static throughout their lives and to lay their eggs in the breeding places from which they themselves emerged. This hypothesis cannot apply to all species of mosquitoes. *A. albimanus* and *Ædes sollicitans*, for example, indulge in mass female flights.

As regards the phenomenon in *aquasalis*, the few data so far accumulated show that, though they are, relatively to the males, in a large minority, females are far from rare in the swarms. In a swiftly fading light it is very difficult to capture an individual pair without getting other specimens into the net. Of the few females certainly taken *in cop.*, in every case the gut has been empty, but 5 out of 7 had the spermatheca fully or partially filled. A few more females taken in the swarms but not actually seen *in cop.* have also been dissected. In every case, again, the gut has been empty, but in only 2 out of 12 has the spermatheca been filled. Copulation and ejaculation of spermatozoa are successive events, and pairs may have been captured before ejaculation had occurred.

From January 1951, for a year, the minute of swarm formation, i.e. from when this is sufficiently dense to be noticeable (though frequently the swarm is later seen to thicken), is being plotted against the time of sunset, as done by Nielsen and Greve (*loc. cit.*). So far, unlike in Denmark, swarm formation has always followed and not preceded sunset, which is quite understandable on the very great differences in evening light intensity between 10° and 56°N. latitude.

To date, 17 observations of the moment of swarm formation show that this has followed sunset by 15 to 26 minutes, average 20 minutes. Disappearance of the swarm,—it is impossible to be sure whether this is dispersal, or due to lack of further visibility,—has, for 10 observations, followed its formation in from 9 to 22 minutes, average 18 minutes. The close average concordance is suggestive. Only 4 observations, all in August 1950, of the actual light incidence at moment of swarming have been made, and this has varied from 0·5 to 5·0 foot-candles. So soon as the photometer has been returned, a sufficiently long series properly to evaluate this will be collected. During these observations other species have been seen. On August 1, 1950, among a very dense swarm of *aquasalis*, in which 15 sweeps of a large net caught 648 ♂ and 5 ♀, 19 ♂ *oswaldoi* were taken, but no females of that species. It is therefore impossible yet to decide whether this was a joint mating swarm, as described for *A. subpictus* and *A. sundaicus* by Rao, Roy and

Rao (1942), or whether the males of the two species were both waiting to mate with female *aquasalis*. If cross-mating occurs, this is a matter of great importance. Should any hybrids eventuate from such cross-matings, they would presumably be sterile, and thus cross-mating would tend to the reduction of breeding of both species, as tried by van der Plank (1947) with Glossina.

On January 19, 1951, a sweep in a nearby, secondary, swarm produced one ♂ *albitarsis*, but whether this was a pure *albitarsis* swarm was not certain. A quite separate, but again adjacent, swarm of *Mansonia* (?) *titillans* was seen on November 1, 1950. Swarms of Chironomids, Tipulids, and ants have frequently been noticed, and the observer must exercise care that the swarm under observation is definitely one of *aquasalis*.

With few exceptions, swarming has always been observed over grass adjacent to, but not actually above, the south-west angle of the experimental bush quadrat on Bordenal Savanah. It has seldom been directly above, but nearly always a few feet to leeward of the bait animal, over a corner of grassland adjacent to a rice plot which is wet from June to November. It has never been observed actually over the tall scrub of the quadrat.

Sets-up of meteorological instruments all round the usual location of the swarm alone would indicate whether its location is influenced by delicate differences in light, humidity, wind or radiant heat. But it seems quite impossible that fertilization of the vast female *aquasalis* population of the Caroni Plain is the result of a few widely dispersed swarms. Such swarms must exist everywhere, but the number of observers necessary to determine how many swarms simultaneously exist in even a limited area would be quite unobtainable, considering that visibility at swarming time is reduced to a few feet of distance and that only against the sunset sky. In any case, if the so far recorded sex proportion in these swarms is general, the author must agree with Nielsen and Greve (*loc. cit.*) that the swarms are not the main locus of fertilization, which remains unknown.

The swarms are not constant in height. They are usually first seen at about 10 ft. above ground. They may later descend to 2 to 3 ft. But they were not influenced by blowing out the musical notes B and G found by Nielsen and Greve (*loc. cit.*) to succeed in ' calling the swarm down '. Further experiments on these lines would best be made with a variable audio oscillater, not easy to arrange in an isolated locality.

(X) FERTILIZATION.

This was studied during the weekly catches in the Nelson Bamboo Plantation. Specimens attacking the human bait men were captured before they could actually bite (the men were fully clothed and wearing gum boots), and placed in a Barraud cage. Evening captures were given sugar water or raisins overnight so that the ovary, on dissection the following morning, should be in the same condition as at the time of attack.

For 19 months, from January 1948 through to July 1949, the spermatheca of each specimen was dissected out and crushed to show presence or absence of spermatozoa. This procedure is not infallibly accurate, the dissection, and the

coverslip pressure, may force out too early the contained spermatozoa, and, if these are scanty, the specimen may be recorded as unfertilized, whereas actually it had been so. Christophers *et al.* (1936) observation that an empty spermatheca is more difficult to rupture by light pressure than a full one is undoubtedly correct, but this difference cannot safely be used to record an insect with an apparently empty spermatheca as 'fertilized'. Only the visible presence of spermatozoa was recorded. The ovarian stage of each specimen was also recorded. The results are tabulated as under:

Time of capture.	Ovary Stage I.			Ovary Stage II.			Ovary Stage III.		
	Number caught.	Number fertilized.	F.I., per cent.	Number caught.	Number fertilized.	F.I., per cent.	Number caught.	Number fertilized.	F.I., per cent.
Afternoon ...	88	47	53·4	666	457	68·6	25	22	88·0
Morning ...	22	5	22·7	465	352	75·7	19	15	78·9
Total: Average ...	110	52	47·3	1,131	809	71·5	44	37	84·1

F.I. = Fertilization index of Christophers.

These figures emphasize the observational error. An ovary in Stage III must be that of an insect requiring a second feed during one gonotrophic cycle. This is quite common in *aquasalis* (*vide infra*), but it is impossible that a female with a Stage III ovary should have proceeded thus far in the cycle without fertilization, and the fertilization index of these Ovary III specimens must really have been 100. Either the spermatozoa of a copulation had all been used in the previous ovulation, or the spermatheca had been artificially emptied by the dissection procedure.

But the data clearly demonstrate that, in the *aquasalis* population of these bamboo plantations, the origin and nature of which is discussed in Section VI (vi), not only are many of the Ovary I specimens unmated but a considerable number of Ovary II's also. The very different fertilization indices (Christophers *et al.*, *loc. cit.*), i.e. the fertilized percentage in Ovary I, between evening and morning captures suggest that the morning population largely comprised those which had emerged too late for a mating flight before entering the bamboos for day-time resting shelter; whilst their much smaller numbers in the morning than in the afternoon suggest that among the Ovary II are a proportion which mated, but did not feed the night previous. Other data, presented in Section VI (vi), suggest that these bamboos are a 'staging post' between emergence and/or oviposition and feeding, though they normally contain no source of blood, and so serve to select a certain section of the *aquasalis* population which elsewhere is indistinguishably mingled in the total population.

(XI) OVARIAN STAGES.

The captures in the Nelson Bamboo Plantation used in studying fertilization index was continued until December 1949, the findings for 24 months being set forth in Table XXXIV. It is seen that, in agreement with the fertilization index, there are only one-third as many Ovary Stage I females in these bamboos in the morning as in the afternoon, and Ovary II dominates the picture both evening and morning. There is no other locality here where persistent attack by *aquasalis* occurs all day with presence of no other blood supply than that of the capturers themselves. That many of the attacking specimens are in at least their second ovulation is indicated by the numbers the dissection of which reveals relict eggs in the ovaries. All specimens in this condition were in Ovary II and none in III. The distribution of the findings of specimens with relict eggs appears to be seasonal. Weekly dissections were carried on for 26 months. During this period :—

Relict eggs in ovary.

Period.	Time of catch.	Times found.	Number dissected.	Relicts.	
				Number.	Per cent.
Dec.–Feb. ...	Evening	8	73	10	14
Dec.–Feb. ...	Morning	4	20	4	20
Jul.–Sep. ...	Evening	3	74	4	5
Jul.–Aug. ...	Morning	5	152	7	5

The solitary exception to these two seasons of relict eggs was a single specimen caught in the morning during the very severe drought of May 1949. Why relict eggs, which, though believed to be in the nature of an accident, must indicate some interference with normal oviposition, should virtually be confined to the beginning and close of the period of maximum production is quite obscure, but their occurrence may indicate increased longevity at the seasons of their prevalence. If this is so, it is a fact of importance with regard to malaria transmission.

Subdivision of ovarian Stage II.—This stage in Christophers *et al.* (*loc. cit.*) classification covers a range of individual ovarian conditions which are not comprised in any other of his five stages. In Stage II, one may find (*a*) an ovary, containing very few yolk granules, which it is believed may be developed without fertilization, (*b*) an ovary with a thick cluster of yolk granules but not yet obscuring the nucleus, (*c*) an ovary with its oviduct closed, as during development, or open, as immediately *post partum*, when a batch of eggs has just been laid, and the next ovarioles, developed to Stage II, occupy each follicle. Roberts and O'Sullivan (1948) therefore

proposed a division of Stage II into 3 substages, which can be keyed out as under* :—

1. Oviducal canal obviously patent ... ... IIB
   Oviducal canal not apparent, closed 2

2. Yolk granules very few, scattered, follicles lightly packed, and tracheoles usually knotted apically ... ... IIA

   Yolk granules dense, though nucleus not entirely obscured. Tracheoles usually straightened out apically ... IIC

Substage IIA is assumed to be the ovary of a nullipara undergoing its first development. IIB, an ovary immediately *post partum*. IIC, an ovary which is probably undergoing its second ovulation, as the tracheoles are straightened as they stretch over the large eggs at the first maturing of the ovary, and do not recurl at their tips after oviposition. A IIC ovary with knotted tracheoles should therefore be a *nullipara*, but the proposers did not attempt to further divide Stage IIC, nor did the author consider it safe to do so. Dissection traction may straighten tracheole endings. Another difficulty is that the oviducal canal may be obviously patent in one ovary but not in the other. This may be an artefact due to traction during dissection. If one canal was patent, the ovary was classified as IIB.

During the Nelson Bamboo investigations this subdivision of Stage II was made through a year† with results as in Table XXXV, in which, as the numbers are small, the four or five weekly dissections of each month are added together. To complete the general picture, Stage I and III ovaries are also included. It is only in August and in January, and then in the evening catches only, that a noteworthy increase in the percentage of newly emerged specimens is found.

Though it has been shown that there is a complete 'turnover' of the *aquasalis* population in these bamboos during the late evening, the various months are, below, compared for the catches of both periods together, as this special area is merely attracting each month a selected portion of the general population of the Caroni Plain, which elsewhere cannot be separated into its evening and morning conditions. With the start of heavy breeding, after the onset of the rains, in *July*, it is seen that the expected increase of Stages I and IIA (*nulliparæ*) does not eventuate, the proportion is, in fact, below the average of the year. But the numbers which, having just oviposited, by evening enter the bamboos (presumably after the morning catches were made before 10 a.m.) is greatly increased, though such insects have largely departed by the morning following. *August* shows the picture one would expect in July, with a high percentage, both evening and morning, of Stages I and IIA. In *September* the evening percentage of IIA remains high, but in the morning it is those which have just oviposited that show an increase,

* Macan (1950) has recently proposed a method of subdivision of ovary Stage II and Stage III, each into three substages. He makes no use of the patency or otherwise of the oviducal canal, however.

† The observations ran from August to July. In tabulating, July has been transposed to the head of the Table, to bring out changes from the onset of rains more clearly.

which is not apparent in the evening population of the month. As shown in Table XXXV, output during the rest of the wet season then becomes stabilized, save that in *October* Stages IIA and IIB remain above the average. *November* totals are too small to make speculations based on percentages profitable. In *December* and *January* the evening numbers of *nulliparæ* are high, but those which have just oviposited are very few. After the onset of the dry season numbers are again too small to make percentage-based speculations profitable. Taking the year's totals, the morning and evening percentages of the various stages are seen to be very similar, save that Stage III requiring a second meal for ovulation are twice as numerous in the evening as in the morning.

(XII) FLIGHT RANGE.

The only experiments so far possible, owing to inability to mass-breed this species, have been carried out by staining series of 50 engorged specimens taken from Dawn Traps. Assuming midnight as the average time of feeding, the specimens were 9 hours *p.c.* when stained. Two per cent watery solutions of eosin and methylene blue were used. After staining, the insects were released in two quadrats, one of pure *Flemingia strobilifera* and the other of mixed scrub, both with a grass understorey. The two quadrats were recaught 24 and 48 hours after the stained insects were released. The bush surrounding the quadrats was also searched on both days over about a 30 ft. circle. The results of 4 such experiments combined are:

	Total catch at 24 hours.	Stained catch.	Stained, per cent.	Percentage of stained release recaught.	Stained recaptures. Abdominal stages.					
					II	III	IV	V	VI	VII
Flemingia ...	51	15	29·4	3·7	0	2	2	6	4	1
Mixed scrub ...	37	7	18·9	1·7	2	1	1	1	2	0
Total : Average	88	22	25·0	2·75	2	3	3	7	6	1

Catches after 48 hours were all negative for stain. So also were catches at 24 and 48 hours in the surrounding scrub. It therefore appears that engorged *aquasalis* is not very static after the first period of dusk following a feed, and that when released for movement by that dusk, this movement is to some considerable distance. The large proportion of the newly-engorged stained releases which have attained approaching ovary maturation in 33 hours, requires further investigation.

(XIII) DENSITY FLUCTUATIONS*.

When the daily records of trap captures are examined, it is immediately apparent that there are very great fluctuations in numbers captured. During 1948 four traps and during 1949 seven traps were operated every day, and their records moving-averaged. Following Ribbands (1944), a rise is accepted as a ' peak ' when it is 120 per cent or more over the previous ' low '. To be accepted as significant a ' low ' must be below 80 per cent of the previous peak. In Table XXXVII, the intervals between density peaks in four traps and a bush quadrat†, all caught out daily, are set out for the year 1948. In that year, for 165 peaks in the traps and in the quadrat-catches, the interval-distribution between peaks in days is as under, expressed as percentages of the total peaks.

Days ...	2	3	4	5	6	7	8	9	10	11	12
Percentage ...	4·8	4·2	12·1	13·3	12·7	6·1	7·3	6·1	9·1	5·4	7·3
Days ...	13	14	15	16	17	18	19	20	21	22	23
Percentage ...	2·4	3·6	1·2	0·6	1·8	0	0·6	0·6	0	0	0·6

The average interval value, 7·8 days, is much below the length of a generation as deduced by daily larval dipping as described in Section V (ii), whilst 47·1 per cent of the total peaks occur at the very short interval of 2 to 6 days. Mr. Ribbands, discussing these results, suggested that they be re-examined considering only outstanding peaks with over 250 per cent rise. Using this criterion there were 59 peaks, distributed as under:

Days ...	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Numbers ...	5	2	1	0	1	4	3	4	1	1	1	2	2	3	1
Days ...	20	21	22	23	24	25	26	27	28	29	30	31	32	33	
Numbers ...	5	1	0	1	3	2	1	2	0	1	0	3	0	0	

* Measurements of density based on bait captures, whether by means of traps or on baits exposed in the open, are, as pointed out by Phillips (1930), not actual measurements of density, but of ' density-activity ', and it is possible to conceive of activity being greatly reduced, either by meteorological conditions or by a passing satiety induced by a transient but very copious blood supply, such as would occur where herds of cattle are driven through an area by night, so that the activity factor in the densities recorded by night traps or by morning resting captures would be so changed that apparent density would be very greatly altered. (Cattle-driving by night has not been seen in Trinidad, but it may well occur in other parts of the mainland *aquasalis*-distribution area, and it certainly occurs in India, and probably in other parts of the tropics where it is deleterious to the health of the animals to drive them through the heat of the day.) Again, the measurement of resting density by day-time outdoor catches will be affected by the density-activity of the previous night. Thus, both the trap catches and those in the bush quadrats are really measurements of density-activity only. The measurement of true fluctuations in actual density would appear only to be possible by measurement of density of ' newly-emerged ', i.e. Ovary Stage I, specimens. This would involve the dissection of every Abdominal Stage I specimen, and is obviously impracticable on a large scale. Nonetheless, it seems well again to point out that all work on densities are really no more than measurements of ' density-activity '!

† Owing to unavoidable trampling whilst catching, 2 quadrats, near one another, were ' used ' and ' rested ' alternately, to allow the lowest vegetation storey to recover.

Days ...	34	35	36	37	38	39	40	41	43	46	66	72	114
Numbers ...	0	1	0	1	1	1	0	1	1	1	1	1	1

Of these, peaks at intervals of 35 days and over may be neglected, being beyond the extreme limit found for emergence-to-emergence by even laboratory breeding, which is always less favourable than natural conditions for development. Fifty of these major peaks are then left. Nearly one-third of these are below the developmental cycle minimum, and after 31 days there are frequent negative periods. Whilst these fluctuations in peak-to-peak interval (by either criterion) throw no light on the exact length of the cycle, they clearly indicate that the length of the developmental cycle is very flexible, but probably never exceeds one month, the limit tentatively placed upon it by Shannon (diary).

The distribution in time of the 120 per cent criterion peaks in the three traps C1, C2 and C4, situated at ½, ½ and 1 mile intervals from just south of the Caroni River northward, was not simultaneous.

Grouping the peaks at each of these 3 traps it is found :

Peaks on the same night in all 3 traps ...	... 3
Peaks on consecutive nights in all 3 traps	... 1
Peaks on the same night in traps C1 and C2	... 5
Peaks on consecutive nights in traps C1 and C2	... 12

These figures might suggest a northward influx travelling towards the hill-foot, or a simultaneous rise in density over a distance of 2 miles. Actually, neither phenomenon seems to occur. The remaining peaks (much the most numerous) showed no sign of a directional succession. In 1949, when 5 adjacent traps used for comparing the relative attraction of various baits [Section VIII (ii)] were operated simultaneously with trap C2 of the 1948 series situate at ¼ mile distance, again operated daily, for comparison, it was found that peaks in the 5 adjacent traps seldom occurred simultaneously, and in fact both the number of peaks and the intervals between them differed for each trap*.

The 120 per cent criterion for a peak was again applied :

Trap.	Bait.	Number of peaks.	Average interval, days.	Percentage of peaks at under 12 days, interval.
C2 ...	Ox	43	8·3	88
Ca ...	Ox	39	8·8	77
Cb ...	Man	38	7·3	96
Cc ...	Horse	40	8·6	75
Cd ...	Pig	35	7·8	94
Ce ...	Goat†	23	8·6	70

* This *phenomenon furnishes additional evidence in favour of the calculation put forth in* Section IX, that the blood attraction range of a trap is in the vicinity of 40 yards only. It is also confirmatory of the conclusions of Zulueta (1950, 1950*a*) that the blood attraction range is very short.

† The goat-bait trap was not started until May.

The average interval between peaks for the whole series is 8·1 days, somewhat longer than the 7·8 days found from the 1948 figures. It is, however, certainly safe to assume: (*i*) that the average interval between density peaks is about 8 days, (*ii*) that many peaks occur at intervals much less than the minimum generation cycle of *aquasalis*, which as deduced from larval instar peaks (Section V) is 12 days.

The only possible explanation for density peaks occurring at intervals of less than the developmental cycle is gonotrophic discordance (Rao, 1947), i.e. the need for more than one blood meal during a single ovulation period. Excluding for the moment consideration of the phenomenon of feeding on more than one animal during a single engorgement, which is considered under precipitin reactions in Section VIII (i), it was found, by tubing engorged specimens taken from dawn traps, that of 31 insects, 2 (6 per cent) required a second feed in the laboratory before they could be induced to oviposit, though 96 per cent of those tested showed the spermatheca filled. In another experiment, using unfed attackers from the Nelson Bamboos, which were then human-fed in the laboratory, of 52 tubed for oviposition, only 19 survived to do so, but of these:

Laid after 1 feed ... 4 = 21 per cent.
Laid after 2 feeds ... 12 = 63 per cent.
Laid after 3 feeds ... 3 = 16 per cent.

The phenomenon of requiring more than one feed to mature a batch of eggs therefore appears to be common in *aquasalis*. Rozeboom (1936), breeding *A. albimanus*, records that this species will occasionally take two or three meals between layings. A similar phenomenon is recorded by Hocking and McInnes (1948) for *gambiæ* in East Africa. Strict 'textbook' gonotrophic concordance therefore does not seem to be the rule in at least certain species, including *aquasalis*. The phenomenon is obviously of considerable epidemiological importance, since an anopheline feeding on man more than once between layings runs a correspondingly increased chance of ingesting gametocytes and of distributing sporozoites, the extreme case being *A. atroparvus* under gonotrophic dissociation in Northern Europe.

The density fluctuations considered above are short period, and must in some manner be related to the gonotrophic cycle. But there remains for consideration *long period density fluctuations*, the cause of which is climatic. For this study, the year has been divided into thirteen 4-weekly periods, and the trap catches over these are shown as percentages of the annual totals. The Imperial College meteorological records (Table IV) must be used for comparison all over the area though, as stated earlier, local rainfall figures show considerable differences over a few miles. The results for the base traps of each line run northward from the Caroni are set forth in Table XXXIII. The total catches for the year set out at the foot of each year's percentage column show the almost incredible numbers in which *aquasalis* is produced. For each trap it must be remembered that the figures are the outcome of attack on a single ox exposed as a bait per night, and that the attraction range of a trap is believed to be in the region of 40 yards radius.

Turning now to the percentage distribution of the total annual catches in each trap over the thirteen 4-weekly periods into which the year is divided, it will be seen from Table XXXIII that there are very great annual fluctuations in the

4-weekly percentages of the annual total catches. In the table, catches of over 10 per cent of the 13 months' totals in a month are italicized to bring out the months of maximum production each year. The figures will be far more valuable when many more years' records are available, but very broadly, nearly half the year's total captures are taken between weeks 21 and 36 (end of May to early September) and as transmission by *aquasalis* is certainly a function of density, control measures should be concentrated upon this period, unless it be (Section IX) that longevity, resulting in the highest oöcyst rate, is increased after the season of maximum production, which would be quite in conformity with what is known regarding several other vectors. Fluctuations in rainfall (Table IV) as measured at the Imperial College do not serve to explain the annual differences, as precipitation on the Caroni Plain is so local, and in addition precipitation in the valleys of the Northern Range affects the extent of breeding area. Unfortunately, there is not enough malaria on this plain to make fluctuation in dispensary attendances comparable with fluctuations in *aquasalis* density.

(XIV) DENSITY FLUCTUATIONS IN OUTSIDE RESTING.

This was studied in 1949 in 6 localities, Barataria, El Socorro, New la Paille, Bordenal Savanah (generally), McLeod and Old la Paille (Table XLV). Collections were commenced during the breeding minimum of April. The monthly percentages cannot therefore be compared with those found in the traps tabulated in Table XXXIII, but the combined figures are useful for comparisons, and give a fair cross-section of density across the Caroni Plain as a whole. No material from these catches was sent for precipitin tests, as it was felt that the 1948 tests (Table XXXVIII) did not require broad scale amplifications, but rather detailed study of food changes in individual localities in comparison with density, which are subsequently considered. The totals of these catches are shown in Table XLV.

It is seen that following the initial outburst of breeding after the rains were established, density did not fluctuate significantly from the mean for the rest of the year. Though there is a steady output of *aquasalis*, there is some evidence that there is a reduction in numbers surviving to mature ovary as the year goes on. It is seen that around these 6 villages, reduction during the gonotrophic cycle was twice as severe in 1949 as was found during the random catches of 1948, when 10·5 per cent of the feds survived to Stage VII as against only 5·4 per cent in 1949. As stated earlier, there is some doubt if this difference is not an artefact caused by greater experience in abdominal classification in the second year's work, but if the phenomenon is genuine then adult predators suffered much less during the drought of 1949 than did larval predators.

The whole subject of predators cries out for study. With no trained entomologist to assist, the subject has perforce been neglected.

(XIVa) FLUCTUATIONS IN HOUSE RESTING DENSITY.

The fluctuations are very marked, and even in non-D.D.T'd villages day-time resters are so rare that searches before the onset of the rains are hardly worth the making. Table XLVIII shows the fall-off in numbers which occurs from November, though it is not on a per house or on a time basis. Table XXXII shows how low

are the per house numbers through the subsequent season in 14 houses of the same village, the same houses being searched on each visit. A house in New la Paille village was therefore hired, made as entrance-proof as is normally possible, and two rooms fitted with an entrance and an exit trap respectively. Between week 24 and 39, daily searches of these traps yielded 106 in-coming and only 25 out-going *aquasalis*, together with 8 specimens which had entered the room with the inwards trap and one specimen which had not entered the exit trap, but remained in that room. These figures do not indicate any higher degree of entrance than one per room per night, even where a widow with young children formed the bait. There is of course great animal deviation in New la Paille, and results might have been very different if a house in a suburban or negro village area had been obtainable, but this could not be arranged.

(XV) POPULATION 'BUILD-UP' AFTER THE DRY SEASON MINIMUM.

The figures in Table XXXIII show that there is in normal years a population minimum between weeks 16 and 20, i.e. in April-May, and that thereafter high proportions of the total annual output occur for the following 3 or 4 months. But in the spring of 1949, there occurred exceptionally dry weather, which afforded the opportunity of studying the 'build-up' phenomenon under clearer conditions than usual.

The dry season of 1949 'anticipated', the December 1948 rainfall at the Imperial College being 1·22 inches only, the lowest figure since the records there commenced. The drought continued to May 27 (in week 21), when 1 inch fell across the Caroni Plain generally. But for the 5 months December to April only 4·64 inches was recorded at the College Station. Professor Hardy of the Imperial College states that if the local fall in any one month is less than 2·4 inches it is all lost by evaporation, and none of the rain enters the soil for crop growth. It can therefore be accepted that a precipitation in excess of that figure is required to produce or maintain surface water for anopheline breeding. Thus, during those first 5 months of 1949 it is obvious that the amount of breeding water was steadily diminishing. The evaporation figures (Livingstone wet-bulb atmometer) show increases of evaporation in 1949 over 1948 as under (c.c.):

Jan.	Feb.	Mar.	Apr.	May.	Jun.
4·0	(—0·7)	4·5	16·2	8·9	10·2
Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
9·0	2·6	6·5	8·7	4·9	(—6·9)

From January to May, therefore, the excess evaporation of 1949 over 1948 amounted to 33·2 c.c., April being a serenely dry month in which only 0·34 inch fell with only 16 showers in the lowest category, 'under 0·40 inch', distributed over 8 days, an average of 0·02 inch per shower.

Breeding as indicated by the weekly average figures used in compiling Table XXXIII fell to an exceptional minimum. The base-line traps B1, C2 and D2 were all reduced to nil catches on occasional days. (Trap B1 has been known to capture 14,257 *aquasalis* in a single night.) The weekly evening catches on Curepe Savanah were all nil from April 13 to June 10, and those in the Nelson Bamboos from April 7 to June 2. Likewise (Table XXI) the Glaudon adult catch was nil from March 31 to May 26. So far as could be ascertained, the only *aquasalis* breeding on the Caroni Plain in April and May 1949 was in the hill-foot springs feeding the cress bed area, and the area irrigated from the San Juan River. During this period, therefore, *aquasalis* must have been reduced to near the 'minimum effective breeding population' that serves to maintain a species from local extinction.

The build-up of the B1 trap captures after the onset of the rains is shown in the table below from the nadir in week 14 of 1948 and in week 16 of 1949. The actual percentage increases from these minima are best expressed as their logarithms rather than as their actual values:

Week	14	15	16	17	18	19	20	21
1948								
Average daily catch ...	148	502	475	422	1,095	1,622	996	1,578
Logarithmic increase, per cent	...	2·53	2·52	2·45	2·87	3·04	2·83	3·02
1949								
Average daily catch ...	...	...	3	6	11	17	13	15
Logarithmic increase, per cent	...	...	...	2·00	2·56	2·75	2·63	2·70

Week	22	23	24	25	26	27	28
1948							
Average daily catch ...	1,868	4,601	11,520	10,907	10,478	6,136	4,778
Logarithmic increase, per cent	3·10	3·49	3·89	3·87	3·85	3·62	3·51
1949							
Average daily catch ...	21	62	247	247	1,071	983	1,424
Logarithmic increase, per cent	2·84	3·31	3·91	3·91	4·55	4·15	4·91

In actual figures, the increase from the nadir to week 26 was 4,139 per cent in 1948 and 35,700 per cent in 1949, which indicates the enormously more rapid build-up after a severe drought than in a normal year, though up to the end of June 1949

(week 26) a total fall of 12·24 inches in 10 weeks had not served to build up the catch to anything approaching the numbers of 1948. But conditions around trap B1 in 1949 were being interfered with by some drainage and embanking being carried out by the Public Works Department. The very much more rapid *rate* of build-up in 1949 than in 1948 is considered to have been due to the fact that during the drought the larval predators suffered as severely as did *aquasalis*, but being fish and insects of slower reproduction, took longer than the Anopheles to re-build their populations. Trap C2, the putative breeding grounds for which were not affected by the P.W.D. drainage, was, by July 1949, producing around 7,000 per night, against a maximum of 4,000 in 1948, again suggesting that the first effect of the cessation of the drought was an escape from density-dependent control of breeding.

The logarithm increases as in the table above are plotted in Chart 3. In 1949, the start, almost from zero, of the build-up is slower than the start from appreciable numbers at the nadir of 1948, but rapidly catches up with it, and then far surpasses it, by week 28 percentage increase being logarithm 4·91 compared with 3·51.

(XVI) INFILTRATION FROM THE CARONI PLAIN INTO THE NORTHERN RANGE VALLEYS.

There is a certain amount of malaria, as indicated by school spleen rates, in villages in the Santa Cruz and Maracas Valleys down which the San Juan and St. Joseph Rivers pass to the Caroni Plain. At the tops of these valleys the problem of its causation is complicated by the presence of *A. bellator*, but in their lower stretches this vector is absent.

De Verteuil (1931) produced evidence for mass migration of *aquasalis* adults at the commencement of the rains, whereby, he claimed, freshly formed breeding places were populated.* Shannon (Annual Reports, 1941–1945) also considered that *aquasalis* flew further in wet than in dry weather, and believed that the malaria of these side valleys was due to mass infiltration from the plain. If this were so, it is obvious that specimens captured in the upper traps in these valleys should show a larger percentage of 'late wings' of Perry's classification than captures in the traps situated among the breeding grounds. Accordingly, from September to December 1947, a weekly classification was carried out, but no evidence was found for this. The small captures in the upper valley traps were uniformly found to be in Wing I, pointing to local breeding. Such 'old wings' as were found in this investigation (all in Wing III, none in Wing IV) were all from the lower traps, and may have been no more than the result of the overcrowding which occurs in these prolific traps. So far as could be arranged, traps were located up these valleys from Morvant above the Laventille Mangrove Swamp (A-line); to Mount St. Benedict above the Streatham Lodge irrigation area (D-line), at half-mile intervals; for 1946 on the 'A-line' and the B (Santa Cruz) line, and for 1948 on the C (St.

* Stone and Reynolds (1939) showed that *aquasalis* eggs will survive at least one month's stranding on mud from seepage areas which had been without standing water during that period. No month locally is entirely rainless, and therefore the supposed mass migrations may be due to reactivation of eggs stranded during the dry season.

Joseph) and D lines. The percentage reduction in the annual catches from the base trap (= 100) upwards were :

A2	A3	A4	A5						
30	12	8	2						
B2	B3	B4	B5	B6	B7	B8			
61	13	0·1	0·1	0·2	0·2	0·8			
*...	...	C4	C5	C6	C7	C8	C9	C10	C11
		70	0·3	0·1	0·4	0·6	0·3	0·3	0·3
...	D3	D4	...	..	D7				
	55	1·6			1·6				

But these density reductions are not really explained by distance from breeding grounds, except in the case of the A-line. As the lower (swampy and most prolific) areas of the plain are left, human population density steadily increases to the hill-foot, along the whole of which there is ribbon development along the Eastern Main Road. Up the A-line there is as heavy human population round trap A3 site, in Morvant settlement, as around trap A4. On this line, trap A5 only was in lightly populated hill-side cultivation. Also only on this line was there no breeding other than in the plain, and on this line alone there is seen a steady rate of trap population decrease with distance from breeding. On the B-line the ascent of the valley was not commenced until trap B5. Traps B2 to B4 were in the thickly populated El Socorro plains area. There is certainly little breeding around these traps, but the reduction up to at least B4 is the result as much of diversion by other sources of blood as by increasing distance from breeding. The C2-C11 line is more instructive. Traps C5 and C6 were situated immediately south and north of the old Spanish capital town of St. Joseph, and the Maracas Valley is not really entered until trap C7. From that trap to C11 breeding shows no further reduction. The D-line had only one uphill trap, D7. Up to trap D3 there is breeding in the rice lands. Between D3 and D4 the thickly populated St. Augustine-Tunapuna area was passed through. Traps D5 and D6 failed to operate owing to bait animal difficulties. D7, on a spur below the Benedictine Monastery, showed no more reduction over D2 than did D4.

The Maracas Valley.—Only the C-line, the Maracas Valley, furnished adult catches which appeared worth comparing with detailed larval surveys, and these have already been described in Section VI. The only evidence for influxes from the plain was furnished by the trap catch figures for one week in December 1948. During this week trap C8 showed two large catches (for the area) of 101 on 16th and 51 on 18th, catches on the remaining days running nil to 4. Small rises, 16 and 12, on the same days at C9 were well above the average daily catch of the week, and trap C11 doubled its catch on December 21. On the strength of these findings, trap C8 was kept working for a further year, but the phenomenon was not repeated. The author is convinced, in the light of the 1940 monthly larval surveys set forth

* C2 and D2 used as base traps. C1 and D1 were situated south of the river.

in Table XXX, that sudden rises in these high valley traps are the result of purely local breeding and that there is no real evidence for mass infiltrations from the plain. Such infiltration would have to be made against a katabatic wind, and meteorological investigations, suggested by Wing-Commander Frost, R.A.F., Air Ministry Meteorologist, Caribbean Area, involving three simultaneous theodolite observations of 'no lift' illuminated pilot balloons have not been feasible.

As just stated, in 1948, a line of 6 dawn traps at ½-mile intervals was located up this valley, between the 100 ft. and 500 ft. contours. The results, by the usual 4-weekly periods, are set forth in Table XXXI. No detailed larval survey was possible until 1950, by which time the trap line had been removed, hence it is not possible directly to compare the adult catches of 1948 with the larval catches of 1950-51. The latter are set out in Table XXX, in which the ½-mile circles around the former trap sites are combined for each month as (*a*) the effective dispersal flight range of *aquasalis* is still unknown, (*b*) how much wind-induced movement of imagines exists is also unknown. The larval survey table shows that there was light breeding from March to May, followed by an almost complete cessation of breeding (save in one neglected drain, classed as a side stream, near trap C11 in July) right up to February, when just as breeding was recommencing after a premature dry period at the end of December, heavy rains all through January again washed out all the breeding places.

Comparison of Tables XXX and XXXI suggests that it is only in years of unusual rainfall distribution that there is any *aquasalis* production in this valley after the onset of the rains, and that the considerable (though not high) catches of December 1948 were an exceptional phenomenon, related to the commencement of the long drought of early 1949. The valley is quite closely inhabited by a population mainly engaged in cacao cultivation. A rough census within a few furlongs of the road shows a man : ox + equine ratio of 14 : 1, and thus there does appear a distinct risk of insufficient animal deviation, but adult resting densities have always been so low that precipitin material has not been obtainable. Further, production is so light that there cannot well be sufficient density for transmission even if man alone was fed on. In the absence of *A. bellator*, as far up the valley as trap C11 (it exists, by human bait catches, at the head above the 500 ft. contour), it is concluded that autochthonous *aquasalis* malaria in the Maracas and similar valleys does not exist, for the author contends that there is no evidence of mass infiltrations from the plain below as an alternative explanation. It was well said by a Professor of Medicine in Calcutta that 'malaria saves the doctor from thinking out a diagnosis' and the clinical malaria that is reported from these valleys requires fuller substantiation by encouraging general practitioners to make more effective use of the free blood-film examination service provided by the Malaria Division. The population is not stable, it goes down to the Caroni Valley Plain to cinemas, shops and 'tamashas' of all kinds. Locally contracted malaria below the *bellator* zone in the side valleys of the Northern Range appears to be a clinical myth unsubstantiated by evidence supportable by either spot-mapped spleen or blood examinations. Detailed investigations (which are exceedingly difficult in an undisciplined population) into the daily movement of both clinical and properly diagnosed malaria patients would alone reveal where any true infections have been contracted. (*To be continued.*)

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Table XXXIX.*

A. aquasalis, outside resting captures, Caroni Valley, Trinidad.

Year.	Locality.	Total captures.		Density per man-hour.		Percentage by abdominal stage.							Survival rate from fresh-fed.						Male percentage.
		♂	♀	♂	♀	I.	II.	III.	IV.	V.	VI.	VII.	II.	III.	IV.	V.	VI.	VII.	
1948 ..	93 general searches	2,239	4,942	3·7	8·3	17·5	40·0	8·4	7·4	8·1	9·9	8·7	100	51·5	41·4	32·3	22·5	10·5	31·2
1948-49	Curepe Sav. daily catches in scrub quadrats (12½ months).	285	2,922	0·8	8·2	6·7	61·2	6·8	8·0	6·4	7·5	3·4	100	52·0	40·9	28·5	18·0	5·6	8·3
1949 ..	6 villages monthly searches, April to December.	3,826	7,237	8·1	15·4	17·4	20·7	14·2	12·6	12·3	18·4	4·4	100	75·0	57·8	42·6	27·7	5·4	34·8
1949 ..	Bordenal Sav. general (8 months).	1,242	2,594	9·7	20·2	15·1	21·2	13·5	12·2	14·5	19·1	4·4	100	74·6	58·7	44·4	27·3	5·2	32·4
1949 ..	Bordenal quadrat pre-experiment (7 months).	1,845	2,037	5·8	6·4	47·0	18·3	9·5	7·7	6·1	9·4	1·9	100	65·6	47·5	32·8	21·4	3·6	47·5
1949 ..	New la Paille coconuts (10 months).	2,653	2,863	13·9	15·0	40·3	19·6	13·4	10·9	8·5	5·9	1·4	100	67·2	44·7	26·5	12·2	2·3	48·1
1950 ..	Success village ..	7,753	2,425	5·8	1·8	43·7	21·7	5·3	7·7	8·5	10·8	2·3	100	61·5	52·0	38·3	23·1	4·0	76·2
1950 ..	New la Paille ..	2,753	4,067	12·4	18·3	28·6	31·0	5·6	8·4	9·2	14·7	2·5	100	56·6	48·7	37·0	24·1	3·5	40·4
1950 ..	Bamboo village ..	769	2,300	1·5	4·5	15·2	45·9	6·3	7·0	8·2	15·7	1·5	100	48·9	41·2	32·9	20·3	1·8	25·1

* Table I to Table XXXVIII have appeared with Part I of this paper in the *Indian Journal of Malariology*, Vol. 5, No. 3, September, 1951, pp. 293–404.

TABLE XL.

A. aquasalis—*total of 15 fortnightly outside resting searches of 1–5 man-hours per quadrat—July to December 1950.*

	Natural mixed bush.		*Cordia macrostachya.*		*Flemingia strobilifera.*		*Hamilea* sp.		*Urena lobata.*		*Cassia tora.*		*Stachytarpheta cayenensis.*		*Borreria verticillata.*		Sp. indet. (wild canna).		*Dryopteris impressa.*		*Acroceras ? zizanoides.*		Filed coconut husks.		TOTALS.	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
By sexes ...	115	112	23	13	6	7	8	12	30	37	18	18	10	9	36	37	3	3	46	72	26	35	4	12	325	367
Totals ...	227		36		13		20		67		36		19		73		6		118		51		16		692	
Percentage of totals.	32·8		5·2		1·9		2·9		9·7		5·2		2·7		10·5		0·9		17·1		8·8		2·3		...	
Light reduction: Percentage to S.D. mm.	13		14		13		38		12		29		16		22		*		8		*		†		...	
	3·5		0·5		1·0		2·0		1·5		2·0		0·0		0·0		...		3·0		*		†		...	

* Growth too poor for measurement.

† No measurement possible.

TABLE XLI.

A. aquasalis, *light incidence in resting spots of male 691, female 1,361.*

Foot-candles ...	0/5	6/10	11/20	21/30	31/40	41/50	51/60	61/70	71/80	81/90	91/100
Male ...	23	63	127	88	65	51	46	33	38	23	20
Female ...	19	82	184	188	149	130	96	71	52	44	40
Male percentage ...	...	...	...	...	...	...	...	...	...	...	83·5
Female percentage	...	...	...	...	...	...	...	...	...	...	77·5

Foot-candles ...	101/110	111/120	121/130	131/140	141/150	151/160	161/170	171/180	181/190	191/200
Male ...	14	15	7	11	9	6	7	1	2	3
Female ...	39	34	31	17	21	12	14	12	12	8
Male percentage ...	...	...	...	...	...	...	...	...	...	10·9
Female percentage	...	...	...	...	...	...	...	...	...	14·7

Foot-candles ...	201/210	211/220	221/230	231/240	241/250	251/260	261/270	271/280	281/290	291/300
Male ...	1	4	5	1	5	0	1	3	1	1
Female ...	7	12	6	8	12	1	3	2	3	3
Male percentage ...	...	...	...	...	...	...	...	...	...	3·2
Female percentage	...	...	...	...	...	...	...	...	...	4·1

TABLE XLI—*contd.*

Foot-candles ...	301/310	311/320	321/330	331/340	341/350	351/360	361/370	371/380	381/390	391/400
Male ...	2	1	1	0	1	1	0	2	2	0
Female ...	1	2	3	0	1	2	0	2	1	0
Male percentage ...	...	...	...	...	...	...	...	...	...	1·3
Female percentage	...	...	...	...	...	...	...	...	...	0·8

Foot-candles ...	401/410	411/420	421/430	431/440	441/450	451/460	461/470	471/480	481/490	491/500
Male ...	0	0	0	0	2	0	0	0	0	0
Female ...	0	0	2	2	1	1	0	1	1	2
Male percentage	...	...	...	...	...	...	...	...	...	0·3
Female percentage	...	...	...	...	...	...	...	...	...	0·7

Foot-candles ...	501/510	511/520	521/530	531/540	541/550	551/560	561/570	571/580	581/590	591/600
Male ...	0	0	0	0	1	0	1	1	1	0
Female ...	1	0	1	0	0	1	2	1	0	1
Male percentage ...	...	...	...	...	...	...	...	...	...	0·4
Female percentage	...	...	...	...	...	...	...	...	...	0·6

TABLE XLI—*concld.*

Foot-candles ...	601/620	621/640	641/660	661/680	681/700	701/720	721/740	741/760	761/780	781/800
Male ...	0	0	0	0	0	0	0	0	0	0
Female ...	0	1	1	0	1	1	0	0	0	1
Male percentage	...	...	...	...	...	...	...	...	...	0
Female percentage	...	...	...	...	...	...	...	...	...	0·4

Foot-candles ...	801/820	821/840	841/860	861/880	881/900	901/920	921/940	941/960	961/980	981/1,000
Male ...	0	0	0	0	0	0	0	0	0	0
Female ...	1	0	0	1	0	0	0	0	0	0
Male percentage ...	...	...	...	...	...	...	...	...	...	0
Female percentage	...	...	...	...	...	...	...	...	...	0·2

Foot-candles ...	1,001/1,500	1,501/2,000	2,001/2,500	2,501/3,000	3,001/3,500	3,501/4,000	4,001/4,500	4,501/5,000	5,001/5,500	5,501/6,000	6,000 +
Male ...	0	0	0	0	0	0	0	0	0	0	0
Female ...	1	1	1	0	0	0	0	0	0	0	1
Male percentage	...	...	...	...	...	...	...	...	...	...	0
Female percentage	...	...	...	...	...	...	...	...	...	...	0·4

TABLE XLII.

A. aquasalis, *light incidence at first evening attack.*

Foot-candles	0	0·5	1·0	1·5	2·0	2·5	3·0	3·5	4·0	4·5	5·0	5·5	6·0	6·5
Number ...	2	4	5	13	3	5	5	...	...	...	3	...	1	...
Foot-candles	7·0	7·5	8·0	8·5	9·0	9·5	10·0	10·5	11·0	11·5	12·0	12·5	13·0	
Number ...	1	...	2	...	2	1	1	...	1	...	2	...	2	
											No. 53.	Percentage total		82·8
Foot-candles	13·5	14·0	14·5	15·0	15·5	16·0	16·5	17·0	17·5	18·0	18·5			
Number ...	...	...	...	...	...	...	...	...	...	...	...			
Foot-candles	19·0	19·5	20·0	40	46	50	61*	68	98*	142	220			
Number ...	2	...	1	1	1	1	1	1	1	1	1			
									No. 11.	Percentage total				17·2

S.D. = 3·2. S.E. = ± 0·4. Mean = 4·0 foot-candles.

TABLE XLIII.

A. aquasalis, *light incidence during attacks in Nelson bamboos.*

	0/5	6/10	11/20	21/30	31/40	41/50	51/60	61/70	71/80	81/90	91/100
Evening values	6	13	23	8	8	2	1	4	...	...	...
Percentage ...	...	...	...	...	...	...	...	...	...	...	...
Morning values	1	1	10	7	8	7	7	3	3	...	1
Percentage ...	...	...	...	...	...	...	...	...	...	...	...

	101/110	111/120	121/130	131/140	141/150	151/160	161/170	430	Total.	Average.
Evening values	...	1	...	...	...	...	...	...	66	22·2
Percentage ...	...	98	...	...	...	...	...	...	...	...
Morning values	1	...	...	1	1	1	1	1	53	53·2
Percentage ...	...	92	...	...	...	...	...	...	...	...

TABLE XLIV.

A. aquasalis, ♀ *catches on Bordenal quadrat face.*

(Summation of 3 series of observations.)

Level period.	0–12 INCHES.			12–24 INCHES.			24–36 INCHES.			36–48 INCHES.			48–60 INCHES.			Totals.	Percentage of total.	Total fed.	Percentage fed
	Caught.	Percentage of total.	Fed.	Caught.	Percentage of total.	Fed.	Caught.	Percentage of total.	Fed.	Caught.	Percentage of total.	Fed.	Caught.	Percentage of total.	Fed.				
18.00–18.10 ..	12	14·8	0	18	22·2	0	20	**24·7**	4	18	22·2	1	13	16·1	2	81	7·6	7	8·6
18.10–18.20 ..	23	9·8	0	59	**25·2**	3	57	24·4	3	43	18·4	1	52	22·2	7	234	22·0	14	6·0
18.20–18.30 ..	15	5·3	2	74	26·1	7	50	17·6	3	81	**28·5**	3	64	22·5	1	284	26·7	16	5·6
18.30–18.40 ..	20	7·4	0	52	19·3	10	62	23·0	4	60	22·3	2	75	**27·9**	4	269	25·3	20	7·4
18.40–18.50 ..	12	6·1	1	33	16·8	4	46	23·5	6	52	26·5	3	53	**27·0**	2	196	18·4	16	8·2
Total by levels ..	82		3	236		24	235		20	254		10	257		16	1,064		73	6·9
Percentage of grand total		7·7			22·2			22·1			23·9			24·2					

Mean catch = 42·4. S.D. = 21·6. S.E. = ± 1·4.

Table XLV.

A. aquasalis. *Six localities. Monthly catches. April to December 1949.*

Month.	Man-hours.	Male.	Female.	Density per man-hour.		Male percentage.	Percentage of female catch.						
				Male.	Female.		I.	II.	III.	IV.	V.	VI.	VII.
Apr.	39·50	6	3	**0·2**	**0·1**	67	0	67	0	0	0	0	33
May	41·00	8	2	**0·2**	**0·05**	80	0	100	0	0	0	0	0
Jun.	49·00	213	297	4·3	6·9	42	1	13	10	11	17	30	18
Jul.	44·50	1,326	2,179	**29·8**	**49·0**	38	13	25	16	10	12	19	5
Aug.	54·25	859	1,085	**15·8**	20·0	44	18	22	9	15	11	20	5
Sep.	46·50	381	943	8·2	20·3	29	18	27	13	11	10	19	3
Oct.	60·75	379	829	6·2	13·7	33	23	18	18	14	12	13	2
Nov.	61·00	286	723	4·7	11·9	28	25	10	18	15	12	19	2
Dec.	74·00	368	1,176	5·0	15·9	24	21	17	13	13	15	16	4
Totals : Average ...	470·50	3,826	7,237	8·1	15·4	34·6	17·4	20·7	14·2	12·6	12·3	18·4	4·4

S.D. = Male 9·3. Female = 14·1.

S.E. = Male ± 3·1. Female = ± 4·7.

Densities significantly different from the means in bold figures.

TABLE XLVI.

Trinidad. Dissections of various species as recorded in annual reports.

Species.	1941.				1942.				1943.			
	G.	+	Gl.	+	G.	+	Gl.	+	G.	+	Gl.	+
aquasalis	488	5	500	0	2,177	50	2,163	1	30	0	21	0
oswaldoi	10	0	10	0	21	0	21	0	...	...	...	...
albitarsis	7	0	9	0	16	0	18	0	...	...	...	...
neomaculipalpus ...	31	0	30	0	49	0	48	0	17	0	...	...
apicimacula	3	0	3	0	3	0	3	0	...	...	...	...
bellator	1,304	6	1,382	1	1,263	10	1,348	0	6	0	...	...
homunculus	5	0	5	0	5	0	5	0	...	...	...	...

Species.	1945.				1946.				TOTALS.			
	G.	+	Gl.	+	G.	+	Gl.	+	G.	+	Gl.	+
aquasalis	504	0	306	0	593	1	330	0	3,792	56	3,320	1
oswaldoi	...	...	...	...	...	...	...	...	31	0	31	0
albitarsis	6	0	...	...	...	...	...	...	29	0	27	0
neomaculipalpus ...	26	0	...	...	...	...	...	...	123	0	78	0
apicimacula	...	...	...	...	...	...	...	...	6	0	6	0
bellator	...	...	...	...	...	...	...	...	2,573	16	2,730	1
homunculus	...	...	...	...	...	...	...	...	10	0	10	0

Infection Rates: *A. aquasalis* Gut 1·47 Glands 0·03 Ratio 49 : 1
A. bellator Gut 0·62 Glands 0·04 Ratio 15 : 1

G. + = gut infected. Gl. + = salivary glands infected.

Table XLVII.

A. aquasalis. *Bordenal quadrat.*

Man-hours:	♂	♀	Per man-hour. ♂	Per man-hour. ♀	Abdominal stages. I.	II.	III.	IV.	V.	VI.	VII.	♂ Per cent.
(i) Results of catches under normal conditions.												
315·9	1,845	2,037	5·8	6·4	958	372	195	158	123	192	30	47·5
Per cent	...	...	...	...	47·0	18·3	9·5	7·7	6·1	9·4	1·9	...
Fed per cent ...	...	...	...	...	...	34·5	18·1	14·7	11·4	17·8	3·6	...
(ii) Results of post-experiment catches.												
47·05	265	919	5·6	19·5	243	644	1	19	2	9	1	22·4
Per cent ...	...	...	...	...	26·4	70·1	0·1	2·1	0·2	1·0	0·1	...
Fed per cent ...	...	...	...	...	...	95·3	0·2	2·8	0·3	1·3	0·2	...

TABLE XLVII—*concld.*

(iii) Precipitin results: normal condition catches.

M.	O.	G.	H.	M/O.	M/G.	M/H.	O/G.	O/H.	M/G/H.	Σ+		M.	O.	G.	H.	Negatives.
12	84	0	16	2	1	3	1	9	1	147		19	96	3	29	6
											Percentage	12·9	65·3	2·1	19·8	4·5

(iv) Precipitin results: post-experimental catches.

M.	O.	G.	H.	M/O.	M/H.	O/G.	O/H.	G/H.	M/O/H.	M/G/H.	O/G/H.	Σ+		M.	O.	G.	H.	Negatives.
15	206	8	331	2	3	1	54	3	2	1	1	698		23	266	14	395	16
													Percentage	3·3	38·1	2·0	56·6	2·6

Normal conditions: percentage multiple bites 13·2.
Experimental conditions: percentage multiple bites 11·0.

TABLE XLVIII.

A. aquasalis *house catches, bamboo village, 1949: Dissections.*

Month.	Number dissected.	Gut +.	Gland +.	REMARKS.
Aug. ...	377	0	0	
Sep. ...	655	1	0	2 half-grown oöcysts.
Oct. ...	590	0	0	
Nov. ...	547	1	0	1 large oöcyst.
Dec. ...	167	0	0	
TOTAL ...	2,336	2	0	
Infection rate ...	...	0·1	0	

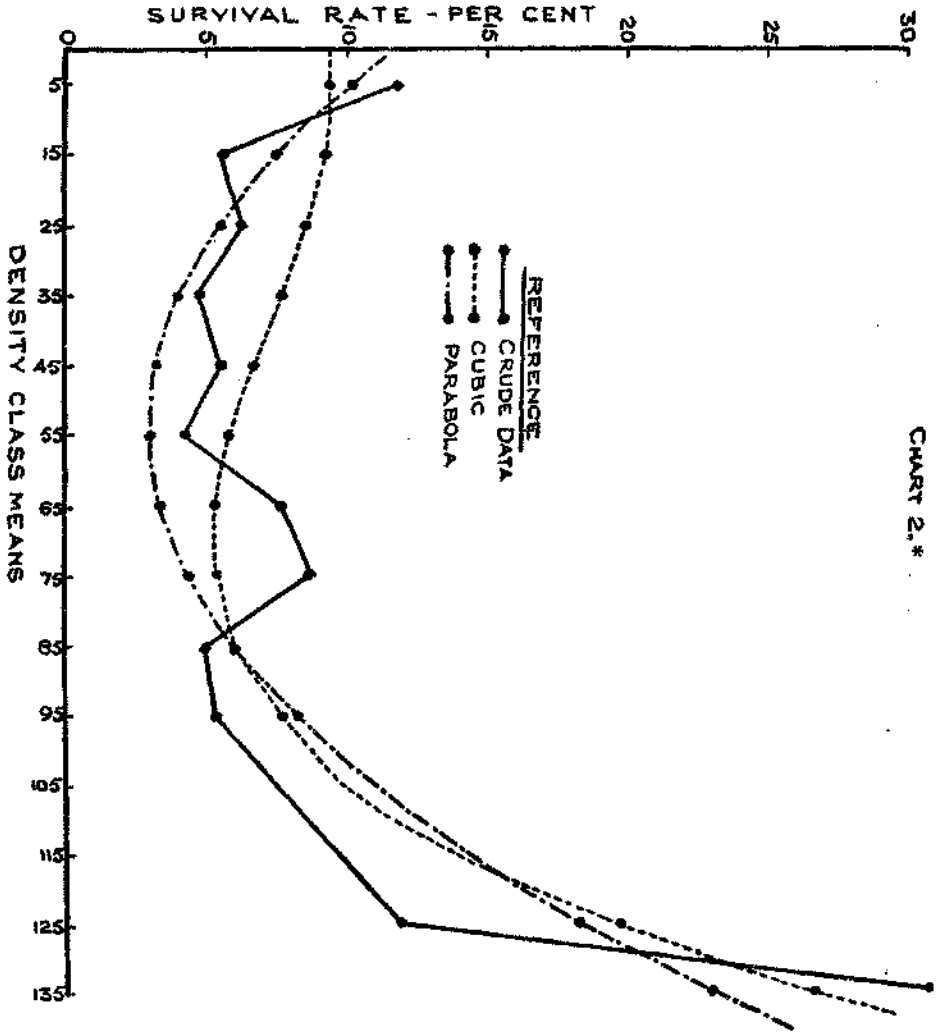

*Chart 1 has appeared with Part I of this paper in the *Indian Journal of Malariology*, Vol. 5, No. 3, September 1951, pp. 293–404.

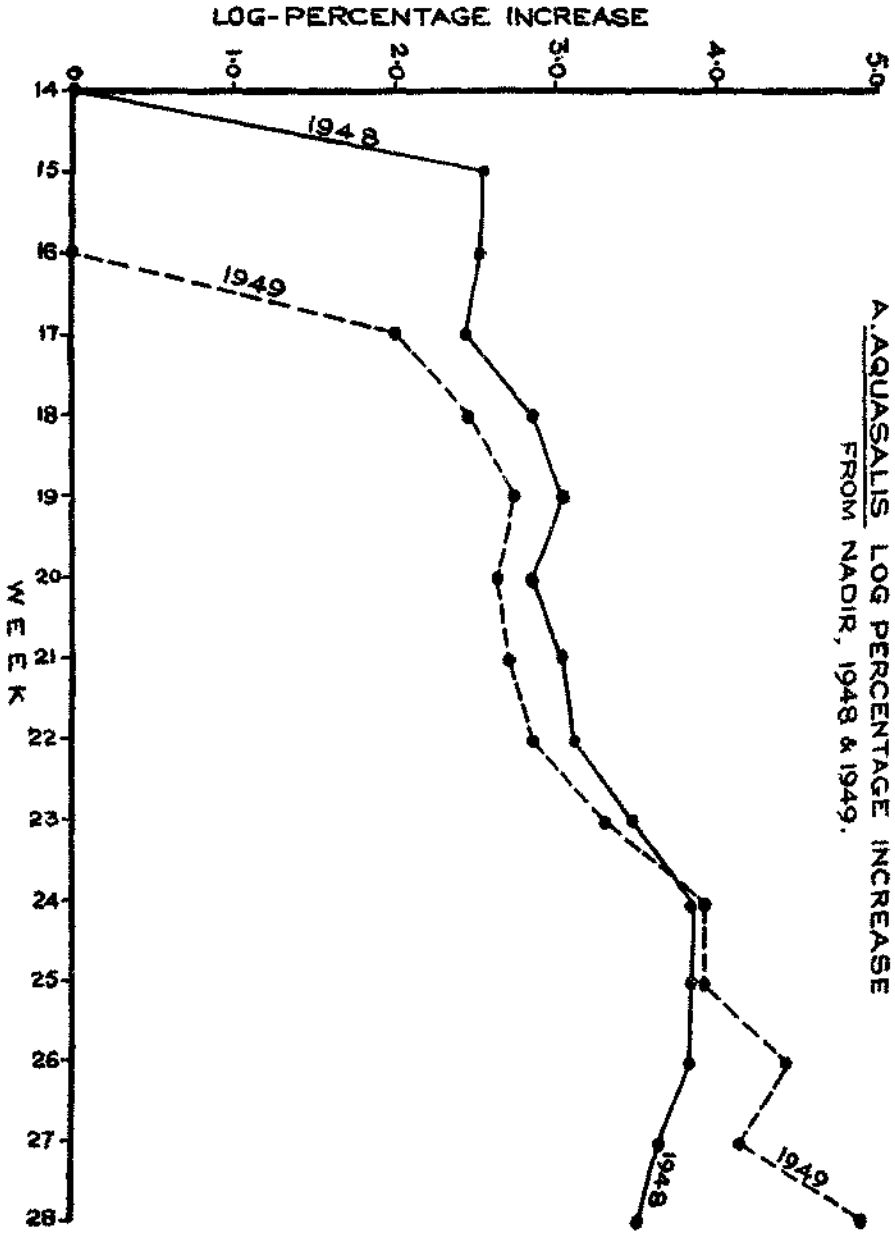

CHART 3.
A. AQUASALIS LOG PERCENTAGE INCREASE
FROM NADIR, 1948 & 1949.

Indian Journal of Malariology, **5**, *4*, *December 1951*.

STUDIES ON NOCTURNAL ACTIVITIES OF *A. FLUVIATILIS* JAMES, 1902.*

BY

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INTRODUCTION.

THIS paper records certain observations made on the nocturnal movements of *A. fluviatilis* James, 1902, more especially with regard to its time of entry into houses, time of feeding and the relation between blood meal, blood digestion,

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fertilization, ovarian development and oviposition. These observations were made in a village called Kallar with a population of about 600. It is situated at the foot of the Nilgiris (North Latitude 11° 19′ and East Longitude 76° 55′) at a distance of six miles from Mettupalaiyam on the Southern Railway. The village is sparsely populated by aborigines who work as labourers on the arecanut plantations in the foothills. Except for one hamlet of the village in which there is a compact group of nine huts, all the other huts in the village are discrete and scattered. Each hut is a single-room tenement with a verandah in front. It is usually dark and ill ventilated and made of cut bamboos plastered with mud on both sides and roofed with thatch. The inmates generally sleep in the verandahs sheltered by the eaves of the roof.

Russell and Jacob (1942) have described in detail the epidemiology of malaria in Kallar. They have shown that the village is hyperendemic for malaria and that high infection rates are encountered in *A. fluviatilis* at all times of the year. As the village is situated at the foot of the hills and surrounded by plantations, wind movements have little effect on the nocturnal movements of the mosquitoes. Temperature and humidity are both compatible with all phases of mosquito activity throughout the year.

GENERAL METHOD OF STUDY.

Mosquito collections were made by well-trained experienced men. As far as possible, collections were made in each hut to the last mosquito available both from inside the hut and in the verandah including the outside of the walls of the hut, employing adequate men and time for the purpose. Mosquitoes were captured with suction tubes from inhabited huts at quarterly intervals during the night, the first quarter ending at 9 p.m. (Indian standard time), the second at 12 midnight, the third at 3 a.m. and the fourth at 6 a.m. As there was but slight variation in the actual time of sunset in different seasons of the year, no special allowance was made on this behalf.

Mosquitoes collected on each occasion were identified immediately under a bright light and placed in separate containers according to the classes to which they belonged. The classification adopted is as follows and self-explanatory :—

Unfed	abdomen thin, without trace of blood.
Freshly fed	abdomen distended with fresh blood; ovaries undeveloped.
Partially gravid	abdomen distended with partially digested blood; ovaries partially developed.
Gravid or nearly fully gravid ..	abdomen distended with mature ovaries with or without a small ventral streak of blood.

TIME OF ENTRY OF *A. FLUVIATILIS* INTO HOUSES.

Table I shows the number of *A. fluviatilis* caught in each quarter of the night in each of the four quarters of the year. Altogether 22 observations were made in different periods of the year covering all seasons, out of which 13 were made in the

same hut which was isolated from all other huts, 3 were made in a second similarly isolated hut, 2 others in a third and equally isolated hut and 4 others were made in the compact hamlet referred to above. As there were no material variations in the observations made on these different occasions, they have been pooled together and shown in Table I for the different seasons of the year and in different quarters of the night.

TABLE I.

A. fluviatilis (females) caught in huts in each quarter of the night in each quarter of the year.

Quarter of the year.	QUARTER OF THE NIGHT.				
	I.	II.	III.	IV.	TOTAL.
Jan.-Mar. ...	165	258	202	109	734
Apr.-Jun. ...	104	247	200	79	630
Jul.-Sep. ...	194	271	191	85	741
Oct.-Dec. ...	79	108	99	47	333
TOTAL ...	542	884	692	320	2,438

I = ending 9 p.m. II = ending 12 midnight.
III = ending 3 a.m. IV = ending 6 a.m.

The data furnished in Table I show that about 58 per cent of *A. fluviatilis* entered the huts before midnight and 42 per cent after midnight. The period of maximum entry, however, would appear to be in the second and third quarters of the night during which period as much as 64 per cent entered the huts. The proportion that entered in the last quarter of the night was the least.

These findings are in agreement with those of Nursing, Rao and Sweet (1934) who recovered 53 per cent in two collections made before midnight in an endemic area in Mysore State in tents in which a human bait was placed. Senior White (1941) made catches from a human-baited trap-net at 2 a.m. and 6 a.m. in the Satpura Ranges and noted that *A. fluviatilis* were attempting to feed at 2 a.m. In a subsequent study Senior White, Ghosh and Rao (1945) found that numbers of *A. fluviatilis* entering between midnight and 5 a.m. were much greater than those entering before midnight or after 5 a.m. till just before dawn. On the other hand, Viswanathan and Ramachandra Rao (1943) and Viswanathan *et al.* (1944) concluded that in North Kanara District, about 71 per cent of *A. fluviatilis* entered houses during the first quarter of the night. These workers made periodical collections in a tent in which 2 or 3 members of the staff spent the night under the protection of mosquito nets. The above difference in the period of maximum entry into houses may well be due to different regional behaviour of the mosquito as suggested by Viswanathan and his co-workers (*loc. cit.*).

TIME OF FEEDING OF *A. FLUVIATILIS.*

TABLE II.

Feeding activities of A. fluviatilis *at different quarters of the night.*

Observations.	I quarter ending 9 P.M.			II quarter ending 12 midnight.			III quarter ending 3 A.M.			IV quarter ending 6 A.M.		
	Unfed.	Freshly fed.	Total.	Unfed.	Freshly fed.	Total.	Unfed.	Freshly fed.	Total.	Unfed.	Freshly fed.	Total.
1	10	5	16	14	12	26	9	18	27	2	5	7
2	3	0	3	27	12	39	14	11	25	1	11	12
3	5	0	5	4	4	8	1	12	13	0	5	5
4	5	1	6	7	10	17	5	14	19	0	12	12
5	1	2	3	20	5	25	5	19	24	0	12	12
6	38	2	40	62	20	82	30	47	77	2	34	36
7	0	0	0	2	13	15	1	5	6	0	3	3
8	28	2	30	103	22	125	46	27	73	2	29	31
9	13	1	14	10	11	21	3	24	27	0	11	11
10	14	2	16	6	10	16	1	10	11	1	5	6
11	4	0	4	5	4	9	0	2	2	0	4	4
12	6	0	6	8	14	22	1	14	15	0	11	11
13	5	4	9	1	10	11	3	7	10	0	2	2
14	3	0	3	0	6	6	1	5	6	0	3	3
15	8	0	8	9	2	11	4	3	7	0	4	4
16	2	7	9	3	13	16	0	22	22	0	13	13
17	10	1	11	28	3	31	29	6	35	13	10	23
18	20	5	25	30	37	67	5	55	60	1	32	33
19	11	1	12	12	11	23	1	8	9	1	3	4
20	5	5	10	24	95	119	3	84	87	0	42	42
21	7	3	10	11	44	55	2	58	60	0	13	13
22	8	0	8	14	42	56	0	26	26	0	11	11
Total ...	206	42	248	400	400	800	164	487	651	23	275	298
Per cent at each quarter.	83	17	...	50	50	...	25	75	...	8	92	...

Out of a total of 2,438 specimens of female *fluviatilis* that gained entry into human dwellings, as many as 629 were unfed. Of these, 442 or 70 per cent entered before midnight. The bulk of these, however, entered in the second quarter of the night. Again, of a total of 1,997 unfed and freshly fed specimens, the relative proportions of the two groups in each quarter of the night were 83 : 17 in the first, 50 : 50 in the second, 25 : 75 in the third and 8 : 92 in the fourth quarters. These data show that while feeding may and does take place in all quarters of the night, it is relatively of the lowest proportions in the first quarter and highest in the last.

While in the last section it was stated that the bulk of the entry of female *fluviatilis* took place in the second and the third quarters of the night, the bulk of the entry of the female *fluviatilis* ready for feeding took place in the second quarter of the night. While the very high proportions of the fed mosquitoes in the last quarter of the night is understandable, the fact that appreciable feeding does occur even in the earliest quarter makes it hazardous from the point of malaria transmission as pointed out by Viswanathan and Ramachandra Rao (*loc. cit.*). As against 317 mosquitoes which were found to feed in the first and last quarters, 887 fed in the middle two quarters, showing that the maximum feeding and probably, therefore, the maximum transmission of malaria occur during this part of the night.

BEHAVIOUR OF UNFED *A. FLUVIATILIS*.

Unfed specimens were collected early in the night from the compact group of huts in the hamlet referred to above and dusted with printers' dust of distinctive colours after first lightly etherizing them. When the mosquitoes revive and begin to fly about, loosely attached dust particles are shaken off the body. However, the phenomenon of auto-dusting referred to by Senior White *et al.* (1945), which was said to have vitiated the results of some of his experiments, was not discernible in any of these investigations. The mosquitoes which became moribund after dusting were discarded, and those that recovered were allowed to fly into clean glass chimneys. They were released from these chimneys at about the beginning of the second quarter of the night inside an isolated hut. Recaptures were made from the same hut during the subsequent quarters of the same night. Catches were made for 30 minutes before midnight, before 3 a.m. and before 6 a.m. In some cases collections were also made after 6 a.m. and they are shown as under the fifth quarter. Out of 279 unfed mosquitoes thus released, 61 or nearly 22 per cent were recaptured later in the same night. Table III furnishes the data of such recaptures :—

TABLE III.

Number of marked unfed female fluviatilis *subsequently recaptured.*

Class of mosquitoes.	Number recaptured.				Total.
	II quarter ending 12 midnight.	III quarter ending 3 a.m.	IV quarter ending 6 a.m.	V quarter after 6 a.m.	
Unfed ...	11	10	1	...	22
Freshly fed ...	3	25	9	2	39
Total ...	14	35	10	2	61

Out of the 61 specimens recaptured, 22 remained unfed all through the night, while the remaining 39 were found to have taken a blood meal. Of the former, 11 specimens were observed to remain in an unfed condition up to about two hours after release, 10 up to five hours and one up to eight hours. As regards the fed mosquitoes, 3 specimens had commenced feeding within about two hours of the release, 25 within two to five hours and 9 after five hours. In the last collections, 2 fed females were recaptured; these might have been overlooked in the earlier collection or stayed outdoors after feeding and returned to the hut subsequently.

Out of 61 mosquitoes recaptured during the same night, only 14 were captured at the end of the second quarter. The remaining 47 mosquitoes had obviously left the hut. They were in a state fit for taking a meal of blood. The source of blood meal was available in the hut and yet such a large proportion appear to have left the hut without taking a blood meal. How much of it is due to artificial interference associated with the experiment itself and how much of it truly reflects what happens in nature every night, it is difficult precisely to determine. However, considering the large numbers that left the hut it would seem reasonable to assume that such outward movements do, in fact, occur with respect to *fluviatilis* mosquitoes which enter houses in a condition ready to feed. While their continued presence in the hut without feeding may represent an absence of conditions ideal for feeding due either to the movement of the source of blood meal or other causes, the egress of the mosquitoes from the hut is not so readily understandable except as a reflection of the natural nocturnal activity of the mosquito itself. Further support is lent to this view inasmuch as 10 mosquitoes returned to the hut even so late as the last quarter, one of which was still unfed at the end of the last quarter. Yet another point of interest which arises from these data is the apparent restriction in the range of movement of the mosquito from the breeding grounds to the place of feeding and outdoor places or shelter. If the stained mosquitoes which moved out of the hut in this experiment at the beginning of the second quarter had migrated into other huts in search of a blood meal, one would not expect such a considerable return in the later quarters of the night into the same hut, because it is not permissible to assume that there is any homing instinct. If a movement does take place from hut to hut, it must be assumed to be indiscriminate and the re-entry into any particular hut can only occur on the basis of randomization. The fact, however, that a sufficiently large number of stained mosquitoes were recaptured in the same hut at different quarters of the same night would seem markedly to restrict the range of movement of the mosquito. In fact, inasmuch as the breeding grounds were close by, our experience would seem to point to the hypothesis of a more or less 'closed circuit' for *fluviatilis*.

BEHAVIOUR OF SPECIMENS WHICH HAD FED ONE NIGHT EARLIER.

Freshly fed mosquitoes were separated from the morning catches, dusted as described above and released into a hut in the forenoon. Recaptures were made from the same hut during the different quarters of the same night of release. Out of 542 mosquitoes thus released on 11 different occasions, 42 or nearly 8 per cent were recaptured (Table IV).

TABLE IV.

Abdominal condition of 42 dusted A. fluviatilis *females which had fed one night earlier.*

Class of mosquitoes.	I quarter ending 9 p.m.	II quarter ending 12 midnight.	III quarter ending 3 p.m.	IV quarter ending 6 a.m.	V quarter from 6 a.m.	TOTAL CAUGHT.
Partially or nearly fully gravid.	14	3	2	2	1	22
Collapsed abdomen containing old digested blood.	3	..	.	...	..	3
Freshly fed	1	3	4	1	8	17
TOTAL	18	6	6	3	9	42

Compared to the proportion recaptured in the previous experiment, the number recaptured in the present experiment was very much smaller. Out of 42 specimens recaptured, 17 were freshly fed and 3 had their abdomens in a collapsed condition with old digested blood. At first sight it would appear that the 17 specimens which apparently fed within 24 hours of the previous feed show a very rapid gonotrophic cycle of development, almost a 24-hour cycle. As will be seen later, however, we feel that the specimens which fed within 24 hours did so not after oviposition after the previous meal of blood but merely to assist the full development of the ovaries in the same gonotrophic cycle. Only fully fed mosquitoes were released in this experiment. Apparently a single blood meal, even when full, was not adequate in a certain proportion of the cases for complete ovarian development. The 3 specimens in which the abdomen was found to be in a collapsed condition and there was evidence of old digested blood, represent the class of mosquitoes in which blood digestion takes place more rapidly than ovarian development. In such cases, for full ovarian development, a second blood meal in the same gonotrophic cycle would appear to be necessary.

Furthermore, if this phenomenon is confirmed, there is a definite stimulus for the 20 mosquitoes to return to the hut during the same night, viz. for the purpose of feeding. If we exclude these 20 mosquitoes, out of 522 mosquitoes released, only 22 have actually re-entered. This would appear to show that a very considerable degree of outdoor resting is resorted to by *A. fluviatilis* during part of the gonotrophic cycle and that their entry into houses is very largely motivated by the functional stimulus of an urge for blood meal. Relatively few numbers of the partially digested or partially gravid mosquitoes would appear to come back

to huts during the night, although in daytime catches in human huts an appreciable proportion of mosquitoes in this gonotrophic condition was found.

BEHAVIOUR OF SPECIMENS WHICH HAD FED TWO NIGHTS EARLIER.

Freshly fed *fluviatilis* females were obtained as before, stained and released also as stated earlier, but recaptures were made from the same hut on the second night after the day of release. Out of 350 specimens thus released, 17 per cent or 58 were recaptured as shown in Table V :—

TABLE V.

Abdominal condition of 58 dusted A. fluviatilis *females which had fed two nights earlier.*

Class of mosquitoes.	I quarter ending 9 p.m.	II quarter ending 12 midnight.	III quarter ending 3 a.m.	IV quarter ending 6 a.m.	V quarter from 6 a.m.	TOTAL.
Unfed	7	11	4	...	2	24
Partly fed ...	...	2	...	1	1	4
Fully fed ...	...	5	8	8	7	28
Gravid	1	...	...	...	1	2
TOTAL ...	8	18	12	9	11	58

The 17 per cent of recapture in this experiment is more in accord with the first experiment in which 22 per cent of unfed specimens were recaptured. This experience, coupled with the fact that, out of the 56 recaptured specimens which had oviposited after the previous blood meal, as many as 32 obtained a fresh blood meal within 48 hours of the previous feed, shows that the gonotrophic cycle is presumably of 48 hours' duration for the bulk of the specimens.

BEHAVIOUR OF GRAVID SPECIMENS.

Four hundred and eighty-two gravid mosquitoes were released at about noon and recaptures were made during the different quarters of the same night in the same hut. Fifty-seven mosquitoes or about 12 per cent were thus recaptured. Among these, 24 were unfed and 33 had taken a blood meal. Out of these 24 unfed specimens, 15 were recaptured before midnight and it is, therefore, permissible to assume that if they were not captured, they might have fed during the same night itself. This observation shows that oviposition and feeding may take place the same night in a vast majority of cases.

BEHAVIOUR OF SPECIMENS WHICH HAD LAID EGGS IN THE LABORATORY AND LATER FED ON GLUCOSE.

Gravid females were isolated in individual vials and allowed to oviposit. The specimens which were undamaged after eggs were laid were placed in a cage and fed on 10 per cent glucose solution. In two separate experiments, 59 females marked with printers' dust were released at noon in a deep drain close to an experimental hut in which *fluviatilis* larvæ were normally breeding. On release, the female mosquitoes were seen to go into the crevices of the drain but not into the open. Of the 59 marked specimens thus released, as many as 22 or nearly 40 per cent were recaptured during the same night, 12 in the first quarter, 4 in the second and 6 in the third. Fourteen of these were recaptured before they had had time to have a blood meal. The very high proportion of recapture in the nearest hut lends further support to the hypothesis of 'closed circuit' referred to earlier. When oviposition had already been completed before dusk, a very large number appear to enter houses even in the first quarter of the night ready for feeding.

INCIDENCE OF DOUBLE BLOOD MEALS IN *A. FLUVIATILIS* AND ITS RELATION TO OVARIAN DEVELOPMENT.

A female mosquito may take double blood meal within the same night when the earlier meal is for some reason not full. This phenomenon is fairly well known. Some females which had taken only a partial blood meal do not seem to have the opportunity to have a subsequent blood meal during the same night. In the authors' experience in Kallar, as many as 10 per cent among the freshly fed mosquitoes appear to be partially fed. This roughly indicates the proportion of the number of females which have only a partial blood meal at the end of the night. The number that fail to get the full blood meal on the first occasion of feeding would naturally be higher than 10 per cent. In order to have some idea of the actual proportion that take only a partial blood meal on the first occasion of feeding, the relative proportions of partially and fully fed females were recorded in different quarters of the night. Out of 1,204 fed females, as many as 202 or 17 per cent were found to have had only a partial blood meal when captures were made in different quarters of the night, without giving the partially fed females any further opportunity to get a second blood meal within the same night. Thus, out of every 100 mosquitoes as many as 17 take only a partial blood meal at the first attempt. Of these 17, however, 7 mosquitoes apparently manage to get subsequent blood feeds within the same night so that early the following morning they are reckoned as fully fed. In other words, about 7 per cent of the total fully fed mosquitoes collected on any one morning would appear to have had at least 2 blood meals the previous night. The phenomenon of the blood meals may be due to disturbances of some kind preventing the completion of feeding either on account of movement by the host of predilection or a disinclination on the part of the mosquito itself to complete the process. Apart from the double feeding that occurs in the same night, there is also indication in the present data as stated earlier to show that even in the same gonotrophic cycle double feeding occurs on different nights. Out of 127 specimens dissected, 12 or 9·4 per cent were found to contain both fresh blood and residue of an earlier blood meal. This may be due to one of two causes. Firstly, oviposition may take place even in the presence of a residue of the blood meal so

long as the ovarian development is completed. Secondly, ovarian development may not take place at the same rate as blood digestion betokening a condition of gonotrophic discordance mentioned by Venkat Rao (1947) and, in such cases, in order to facilitate further development of the ovaries the mosquito may be obliged to take a second blood meal within the same gonotrophic cycle and on this account one may encounter both fresh blood meal as well as residue of the old blood meal in the gut of the same specimen. Venkat Rao (*loc. cit.*) has recorded that this phenomenon of gonotrophic discordance occurs in *A. annularis* and *A. culicifacies*. While the positive experiments referred to by him are no doubt conclusive, the presumptive evidence furnished by him in his Table I as well as in the tabular data of Senior White quoted by him does not necessarily betoken gonotrophic discordance. In the case of some anophelines especially *A. vagus* it has been observed that the fully gravid female may drop eggs in the cages even though no special facilities for egg laying such as a petri dish with water or moist filter paper are provided. Thus, in dissections in the laboratory of females kept for a few days for allowing the blood to get digested, one often finds ovaries in Stages II and V. The specimens whose ovaries are found to be in Stage II may well have dropped their eggs when they are kept in captivity.

The importance of a double blood meal in the same night or in different nights within the same gonotrophic cycle arises out of its effect on the quantum of malaria transmission. In the fascinating formula evolved by MacDonald (1950), the biting index is reckoned as 0·5, presumably because the gonotrophic cycle is of 48 hours' duration and anophelines feed every other night. This factor would, however, need to be slightly increased in order to allow for the double feeding in the same night or double feeding in the same gonotrophic cycle. Under the conditions of the experiments herein recorded the biting index would appear to be 0·6 instead of 0·5, if allowances are made for double blood meals either on the same night in particular or within one gonotrophic cycle in general.

FERTILIZATION, OVARIAN DEVELOPMENT AND BLOOD DIGESTION.

The number of freshly fed females examined during the different quarters of the night for the presence or absence of sperms was 298. All these specimens with the exception of 4 or 1·4 per cent were found to be fertilized. Among the fertilized females the proportion of the primiparous females was not determined. Some indication of the proportion of primiparous to multiparous females among the unfed individuals is available from the fact that, out of 56 unfed females dissected, 46 had their ovaries in Stage II and all of them were fertilized and therefore multiparous. Ten unfed females had their ovaries in Stage I and were therefore primiparous. Of these, 7 were fertilized and 3 were not. It would thus appear that roughly one-fifth of the female *fluviatilis* feeding every night consists of primiparæ and among the latter, though mating generally occurs prior to feeding, it is by no means absolutely necessary. The most impressive feature noted in the course of dissections carried out *at night* was the brisk circular movement of the sperms in spermathecæ. When similar specimens were examined during the day, the spermathecæ were found filled with sperms which showed varying degrees of activity but without any circular movement.

In the cases of mosquitoes which were partially or fully fed and dissected after the complete digestion of the blood meal, record was also made of the condition of ovaries and of the presence or absence of sperms. The results are set out in Table VI :—

TABLE VI.

State of ovarian development and presence or absence of sperms.

	Number examined.	Number with mature ovaries.	Number with sperms.	Number without mature ovaries.	Number with sperms.
Partly fed ...	27	0	0	27	24
Fully fed ..	182	167	165	15	13
TOTAL ...	209	167	165	42	37

As was expected in Anopheles mosquitoes, none of the 27 partly fed females had mature ovaries when the digestion of blood was completed. While in 3 specimens among them the non-maturation of the ovaries may be ascribed to lack of fertilization, in the remaining 24 there was definite evidence of fertilization and yet the ovarian development was incomplete perhaps showing that the size of the blood meal plays an important part in ovarian development. Out of the 182 females which had fully fed, 92 per cent were found to contain mature ova, while in 8 per cent ovaries had failed to reach maturity, showing perhaps that even one full blood meal is not adequate in all cases for full ovarian development.

SUMMARY AND CONCLUSIONS.

1. This paper records the nocturnal movements of *A. fluviatilis* in a hyper-endemic village in the foothills of the Nilgiris in South India.

2. Fifty-eight per cent of female *fluviatilis* in different stages of gonotrophic cycle are found to enter human residences before midnight but the period of maximum entry is in the second and third quarters of the night (64 per cent).

3. Seventy per cent of female *fluviatilis* which are ready for feeding enter before midnight but, while feeding occurs all through the night, the bulk of it and presumably, therefore, the bulk of malaria transmission occur during the second and third quarters of the night.

4. Twenty-two per cent of 279 unfed female *fluviatilis* released into a hut on different occasions were recaptured in the same hut in different quarters of the same night. Relatively small numbers commenced feeding within two hours of release but from 2 to 5 hours after release a good number had fed. This experiment demonstrates a considerable degree of outward movement during the night from the place of feeding even before feeding is commenced. But such

movement appears to be restricted in its range since a good number re-enter the same hut (in much larger proportions than on the basis of randomization) indicating perhaps a phenomenon of 'closed circuit' between a breeding place, a feeding place and outdoor shelters very close to them.

5. In the case of female *fluviatilis* in a partial stage of development of gonotrophic cycle, a very considerable number appear to stay out after moving out of the hut in which they were released. Those specimens which on account of the inadequacy of the previous blood meal have not developed their ovaries proportionate to blood digestion, re-enter the hut during the night of release and feed within 24 hours of the previous meal.

6. A good proportion of female *fluviatilis* which had fed the previous night and were released into a hut in the forenoon are found to re-enter the hut in different quarters of the second night after the release and take their feed indicating a gonotrophic cycle of 48 hours' duration.

7. Gravid specimens released at noon into a hut return to the hut during the same night after oviposition and take a blood meal showing that feeding may occur on the same night on which oviposition takes place.

8. This phenomenon is naturally even more perceptibly observed in the case of mosquitoes which were allowed to oviposit in the laboratory and released at noon in a drain. A very great majority return to the hut the same night for a blood meal.

9. A certain proportion of female *fluviatilis* take a double blood meal in the same night thus increasing the hazard of malaria transmission. A further proportion take a second blood meal on successive nights within the same gonotrophic cycle presumably because their previous blood meal, even when full, is not adequate for complete ovarian development. This further increases the biting index of this species. Apart from partial blood meal being inadequate for full ovarian development, there is evidence of 'gonotrophic discordance' with respect to *A. fluviatilis*.

10. While mating generally takes place before a blood meal, it is by no means the rule. About one-fifth of the females taking a blood meal every night would appear to consist of primiparæ. During the night the sperms in the spermathecæ have a brisk circular movement, but during the day while the sperms were active, there was no such circular movement.

ACKNOWLEDGMENT.

The authors are very grateful to Drs. D. K. Viswanathan and T. Ramachandra Rao for their valuable suggestions and helpful criticisms.

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A FUNGUS (CHYTRIDINÆ SP.?) RESEMBLING PLASMODIAL SPOROZOITES IN THE SALIVARY GLANDS OF *A. ANNULARIS* VAN DER WULP, 1884.

BY

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[November 1, 1951.]

INTRODUCTION.

THE report deals with micro-organisms believed to be a fungus (Chytridinæ sp.?) in the salivary glands of *A. annularis* encountered in the course of experiments to establish mosquito transmission of *Plasmodium berghei* (Vincke and Lips, 1948). In fresh preparation, the micro-organisms bore a very close resemblance to plasmodial sporozoites and were easily mistaken for the latter. The finding is considered to be important as a possible source of error in epidemiological studies in the field and full details are therefore reported here.

EXPERIMENTAL PROCEDURE.

Mice, rats (white as well as brown) and garden squirrels were infected with blood of rats infected with *Plasmodium berghei*. Mosquitoes were fed upon these animals when gametocytes were present in the blood. The number of gametocytes

ranged from 4 to 59 per 10,000 erythrocytes and in many instances several batches of mosquitoes were fed on the same animal on successive days as long as gametocytes were present in the circulating blood.

All mosquitoes used were hatched in the laboratory. As a rule, the mosquitoes were starved for 24 hours before feeding. After feeding, the mosquitoes were stored and maintained on glucose water in the insectary where the average maximum and minimum temperatures were 82·3 and 80·4°F. respectively, with a mean humidity of 74·5 per cent.

Surviving specimens were dissected from the 5th day onwards up to the 20th day in normal saline and on a few occasions in a mixture of equal parts of rat serum and saline. Many fresh preparations of the salivary glands were air-dried and fixed in methyl alcohol and stained either with Giemsa, Leishman or J.S.B. (Jaswant Singh and Bhattacharji, 1944).

RESULTS.

A total of 3,652 female specimens of *A. annularis* were fed on gametocyte carriers, of which 1,832 were dissected for examination of midgut and salivary glands. Two hundred and sixty-six or 14·5 per cent of the specimens showed sporozoite-like bodies in the middle lobes of the glands, situated both in the acini as well as the ducts of the lobe, but oöcysts were not seen in any gut preparation.

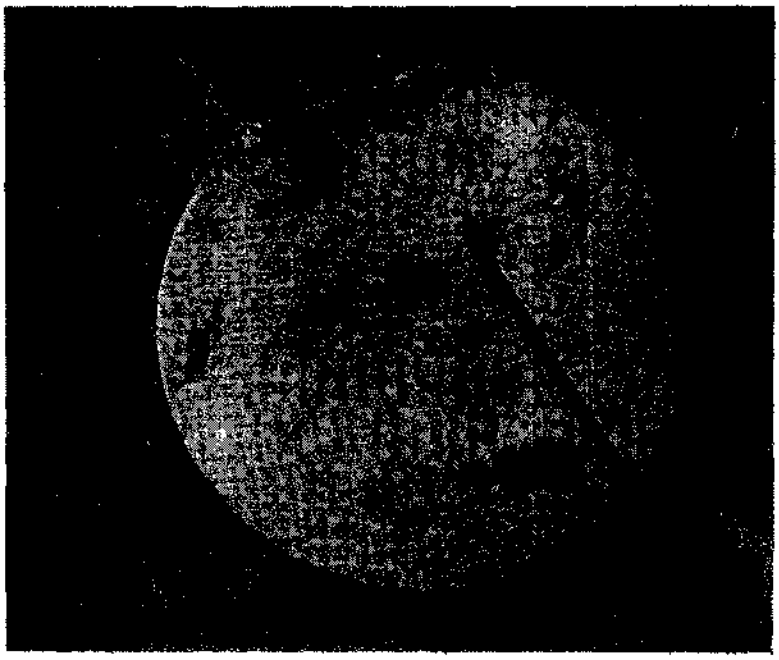
In some instances the lobe was packed with the bodies which, on crushing the gland, were seen to come out singly as well as in groups just like sporozoites. They were non-motile and measured 6 to 10 microns in length and about 0·5 micron in thickness. The majority were rod-shaped and rarely a few had the typical sickle shape of sporozoites. Examined under the high-power objective (1/6) they looked typically like plasmodial sporozoites (Plate II—microphotograph).

On staining, the great majority of them took the eosin stain and appeared uniformly red and rarely a few stained blue and revealed a red chromatin-like nucleus. Camera lucida drawings of these bodies in the fresh as well as stained preparations are seen in Plate III. Over 75 per cent of the 'positive' preparations were inoculated by various routes into 10 white mice, 13 white rats, one brown rat and 2 squirrels, which were all under observation for a minimum of 8 weeks and some for over 14 weeks. Daily examination of blood was negative.

The micro-organisms in the salivary glands of *A. annularis* were intriguing, as no reference to a similar phenomenon could be found in literature available to the authors. In the fresh preparations, they looked so much like sporozoites but on staining they did not reveal the typical characteristics. Further, a careful search for oöcysts in the midgut preparations, as well as animal inoculations of the sporozoite-like bodies, proved negative.

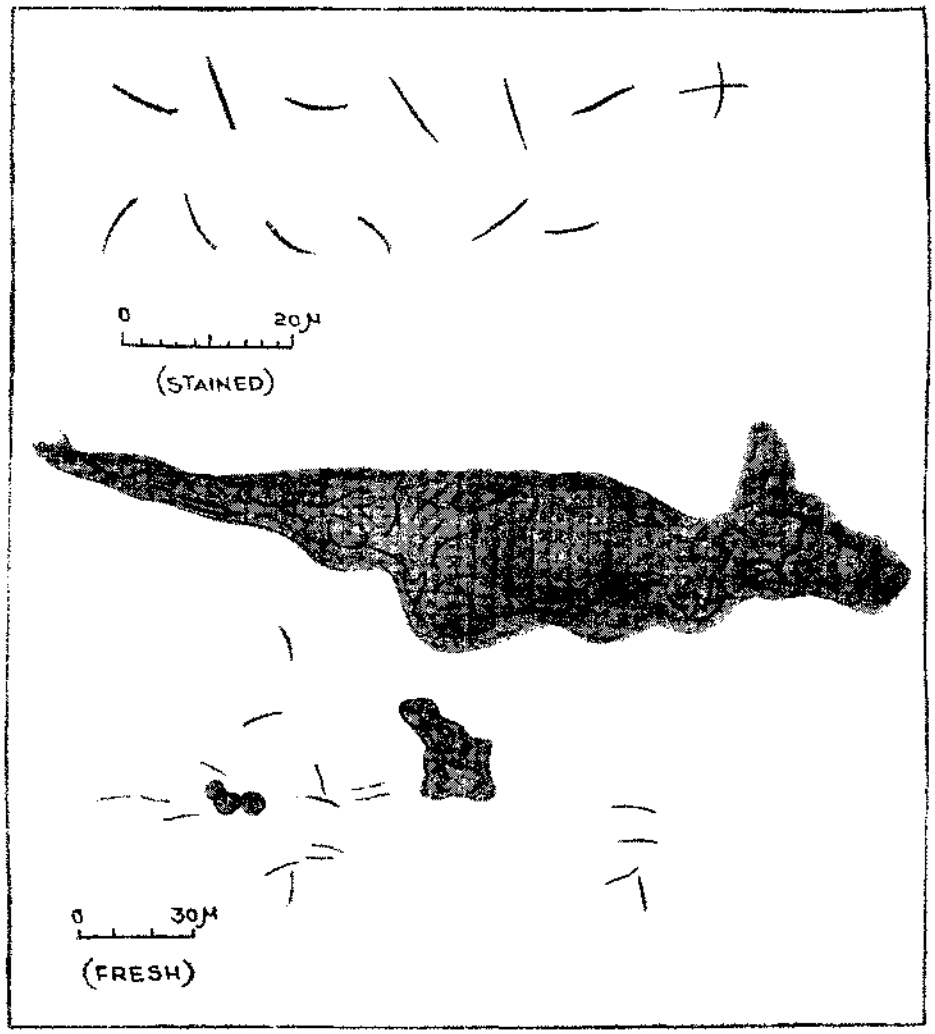
Adult specimens of *A. annularis* were captured in a village, Salimpur, a few miles away from Delhi. Out of 23 dissected, 3 showed in the salivary glands, bodies similar to the ones encountered in the laboratory before. A second collection of adult specimens of *A. annularis* was made from the same village and 18 out of 46 dissected showed the bodies in the salivary glands. They were injected

PLATE II.



Microphotograph showing fresh preparation of the fungus resembling sporozoites.

PLATE III.



Camera lucida drawings of stained and fresh preparations of the fungus resembling sporozoites.

intraperitoneally and subcutaneously into a clean *rhesus* monkey* whose blood, examined every day for 8 weeks after the inoculation, was negative. In none of the mosquitoes dissected, however, was there any evidence of oöcysts in the midgut preparations. Those that had fresh blood in them showed mammalian erythrocytes.

One hundred and four laboratory-bred female specimens of *A. annularis* were fed on clean white rats and the survivals were dissected after an interval of 7 days. 13·4 per cent of the specimens showed bodies similar to those encountered before. This proved positively that these bodies could not be sporozoites.

Fifty-six specimens were fed only on glucose water for a few days and on dissection the sporozoite-like bodies were seen in 29 per cent of the salivary gland preparations. In order to see if these bodies were present in the glands of freshly hatched mosquitoes, 90 such specimens of *A. annularis* were dissected with negative results.

All other species of mosquitoes† fed on infected rodents were negative to oöcysts and sporozoites with the exception of a single specimen of *A. stephensi* (type) which showed typical sporozoites and has been previously reported (Ramakrishnan and Satya Prakash, 1950).

DISCUSSION.

The identification of sporozoites in fresh preparations of infected salivary glands is comparatively easy due to special characteristics of the organisms. This appears to be the first instance where micro-organisms which closely resembled plasmodial sporozoites were encountered in the salivary glands of *A. annularis*. They have nevertheless proved to be not sporozoites by their peculiar staining reactions, the absence of oöcysts in the whole series, negative animal inoculations, and the fact that these bodies were found in mosquitoes fed on clean animals as well as glucose exclusively. Three facts which stand out prominently are that the sporozoite-like bodies were found in the glands of specimens of *A. annularis* only; the second is that they were not found in freshly hatched mosquitoes which shows that whatever the nature of the bodies, it was something that had to grow and required some time for its development; and thirdly, other species of mosquitoes treated in identical manner did not, in a single instance, show such or similar bodies.

Investigations on *A. annularis* were coming to an end in May, when larvæ became scarce. Just about the end, a few specimens fed on infected rats showed fungal masses in the gut which were seen as large clumps of mycelial threads with a dense opaque centre and short filaments radiating in all directions. It is possible that a fungus grows in the gut of the mosquito and its mycelial bits find their way to the salivary glands.

Dr. H. N. Ray of the Veterinary Research Institute, to whom a description of the bodies, drawings, photographs and stained preparations were sent for opinion,

* In the past a few experimental monkeys had been released in the vicinity of Salimpur. It was considered that a natural transmission of simian malaria was a possibility.

† *A. stephensi*, *A. subpictus*, *A. jamesi*, *A. ramsayi*, *Ædes* and *C. fatigans*.

considered that the bodies were a fungus probably referable to Chytridinæ sp. ?*.

Fungus mycelial bodies in the salivary gland preparation of mosquitoes caught in the field can be mistaken for sporozoites in the fresh preparation even by the trained eye and lead to wrong conclusions in epidemiological studies. Christophers, Sinton and Covell (1939) mention various other objects which may be mistaken for sporozoites such as (1) the wrinkles seen on the chitin of the œsophageal diverticula; (2) certain acicular crystals sometimes seen in the region of the glands. To these may be usefully added the present finding of the fungus in the salivary glands of *A. annularis*. Thus, it is important not to identify sporozoites in the absence of oöcysts and characteristic staining reactions.

SUMMARY.

Some *A. annularis* fed on rats with gametocytes of *P. berghei* and dissected at different intervals showed bodies closely resembling sporozoites in the salivary glands, and identical bodies were seen in specimens which were fed on clean rats or on glucose exclusively. Similar bodies were also seen in 30·5 per cent of wild-caught *A. annularis* from villages around Delhi.

The absence of oöcysts in the entire series, the bodies not showing the characteristic staining reactions of sporozoites, as well as negative animal inoculations proved that they were not sporozoites, and are believed to be mycelial bits of a fungus Chytridinæ sp.?.

The importance of characteristic staining reactions and the presence of oöcysts in the gut are stressed in the identification of sporozoites in field epidemiological studies.

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* Dr. Ray in a personal communication states that, in 1943, he had once seen some similar bodies in the smear preparation of tests of a tick, *Ornithodorus savigiya*, but since it was not followed up in a systematic manner, no further remarks could be offered on the subject.

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DARAPRIM (50-63)* IN SIMIAN MALARIA.

BY

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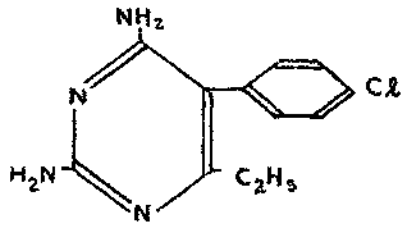
FOLLOWING the discovery of Diaz de Leon in 1937, that sulphonamides, though chemically unrelated to the well-known antimalarials like quinine, mepacrine and pamaquin, have some antimalarial activity, Curd *et al.* (1945) were prompted to investigate the pyrimidine compounds. On the presumption that their activity might be due to competition with the parasites for certain essential requirements of the organisms as suggested in the case of mepacrine, these workers began with the pyrimidine derivatives of aniline, and were able to synthesize active compounds like M-2665, M-2666 and M-3349 (Curd and Rose, 1946*a*, 1946*b*). These compounds may be considered as definite milestones in the progress of the development of proguanil (M-4888). The turning point from the pyrimidine derivatives to proguanil was M-3349. Though pyrimidine provided some degree of activity, it was later realized that the ring system was not altogether essential. The same group of workers made certain variations by replacing the ring with

* Received through the courtesy of the Wellcome Laboratories of Tropical Medicine, London.

According to the World Health Organization terminology, daraprim is now known as pyrimethamine.

diguanide and they were finally able to demonstrate high activity of proguanil (Curd *et al.*, *loc. cit.*; Curd and Rose, 1946*c*; Findlay, 1951).

On the other hand, Hitchings *et al.* (1948) observed that some of the pyrimidine compounds were antagonistic to pteroglutamic acid in the same way as proguanil. This led to further studies and several substituted 2 : 4-diaminopyrimidines were developed (Russell and Hitchings 1951; Falco, Russell and Hitchings, 1951; Falco, DuBreuil and Hitchings, 1951). Falco *et al.* (1951) reported high grade activity of some of these compounds against *P. gallinaceum* and *P. berghei*, and particularly daraprim (50–63), an aryl and alkyl substituted 2 : 4-diaminopyrimidine (*see* structure below) which has been considered to be many times more active than proguanil.



DARAPRIM.

(2 : 4-diamino-5-*p*-chlorophenyl-6-ethyl pyrimidine.)

The present paper reports observations on the activity of daraprim against blood-induced *P. knowlesi* infection in North Indian *rhesus* monkeys. Studies on the acute toxicity tests have also been recorded. High degree of refractoriness of a proguanil-resistant sub-strain of the same species to this compound has recently been reported by Jaswant Singh *et al.* (in press).

MATERIALS AND METHODS.

Host.—Sixty-six *rhesus* monkeys were utilized for therapeutic trials and assessment of the M.E.D. They did not have malarial infection before. Prior to these investigations, they were subjected to the routine procedures of blood smear examination and tuberculin test (Jaswant Singh *et al.*, 1951). Monkeys used for toxicity studies had been previously treated for acute primary experimental infection and some of them were either completely cured or had chronic infection but showed no patent parasitæmia at the time of investigation. In each case at least a month had elapsed after completion of treatment before these animals were used for toxicity tests.

All animals were weighed prior to the commencement of drug administration but those included in the higher therapeutic dosage schedules were weighed again on the day following a 7-day course and the difference in weight, if any, was recorded.

Parasite.—A strain of *P. knowlesi* maintained in these laboratories by serial blood passages to monkeys for well-nigh 20 years was used for the studies. In

untreated infection, parasitæmia attains a level of 20 to 30 per cent of the erythrocytic cell infection though the mortality rates, during recent years, have been noted to be quite low on account of the attenuation of the strain as reported earlier by Jaswant Singh, Ray and Nair (1949, 1950).

The dose of inoculum in all cases was 5 million erythrocytic parasites per kg. of body weight. Ehrlich's eye-piece, adjusted to count 100 erythrocytes per microscopic field of the oil-immersion objective, was used for enumeration of parasites. One hundred such fields were examined for determination of parasite rate per 10,000 R.B.C. Negative results were recorded when no parasites were detected in 200 fields of the thick film.

Drug administration.—The dosage schedules were calculated in terms of mg. of the base content per kg. of body weight. Suspension of the drug was administered for therapeutic trials daily for 7 days with the help of a Ryle's tube on empty stomach. Medication was commenced when intensity of the infection in monkeys attained a level of 0·1 to 0·2 per cent of the erythrocytic cell infection.

Index of antimalarial activity.—The minimum effective dose (M.E.D.) was determined by recording the minimum dose of antimalarial which caused class II effect as described by Shannon (Wiselogle, 1946), wherein complete clearance of asexual parasites is attained by the day following the termination of the course of treatment. In higher therapeutic doses, sterilization of the blood-induced infection (class III effect) was determined by splenectomy and a subsequent follow-up for a fortnight. The operation was performed when no parasites were detectable for at least a period of 30 days after completion of the treatment.

Toxicity tests.—Thirty-three monkeys were placed under different schedules for assessing acute and chronic toxic effects. The drug was administered on empty stomach as a single dose. Toxic manifestations were recorded from the beginning.

Animals which died during or soon after termination of treatment were subjected to post-mortem examination. Histopathological studies were made on tissues collected during post-mortem examination and compared to biopsy materials collected from normal monkeys.

Tissues were fixed in formal saline, and after necessary processing, the sections were stained with hæmatoxylin and eosin.

RESULTS OF THERAPEUTIC TRIALS.

Preliminary trials for determination of plasmodicidal activity and the range of dosage schedules required to be explored were conducted on 18 monkeys. On account of the somewhat structural similarity of daraprim to proguanil, the initial range selected was from 0·01 to 2·0 mg. base per kg. of body weight. Drug was administered daily for 7 days.

As shown in Table I, complete asexual parasite clearance was obtained under all schedules from 110 to 144 hours.

Determination of the M.E.D.—For assessing the minimum dose that would effect complete clearance of the asexual parasites by the day following the termination of the 7-day course, another lot of 14 monkeys were treated with dosage schedules lower than those shown in Table I.

TABLE I.

Determination of range of dosage schedules.

Schedule number.	Dose in mg./kg.	Number of animals used.	Activity.	Parasite clearance in hours (average) if class II.	Number relapsed.	Day of relapse.	Number cured.	Remarks.
I	0·01	2	Class II effect	144	2	20 for both	*Nil*	
II	0·02	2	,,	144	2	7 and 30	,,	
III	0·05	2	,,	120	2	7 and 20	,,	
IV	0·125	2	,,	120	2	7 and 21	,,	
V	0·25	2	,,	120	2	12 and 15	,,	
VI	0·5	2	,,	120	2	30 for both	,,	
VII	1·0	3	,,	112	1	30	2	
VIII	2·0	3	,,	110	*1	35	2	*Relapsed after splenectomy.

TABLE II.

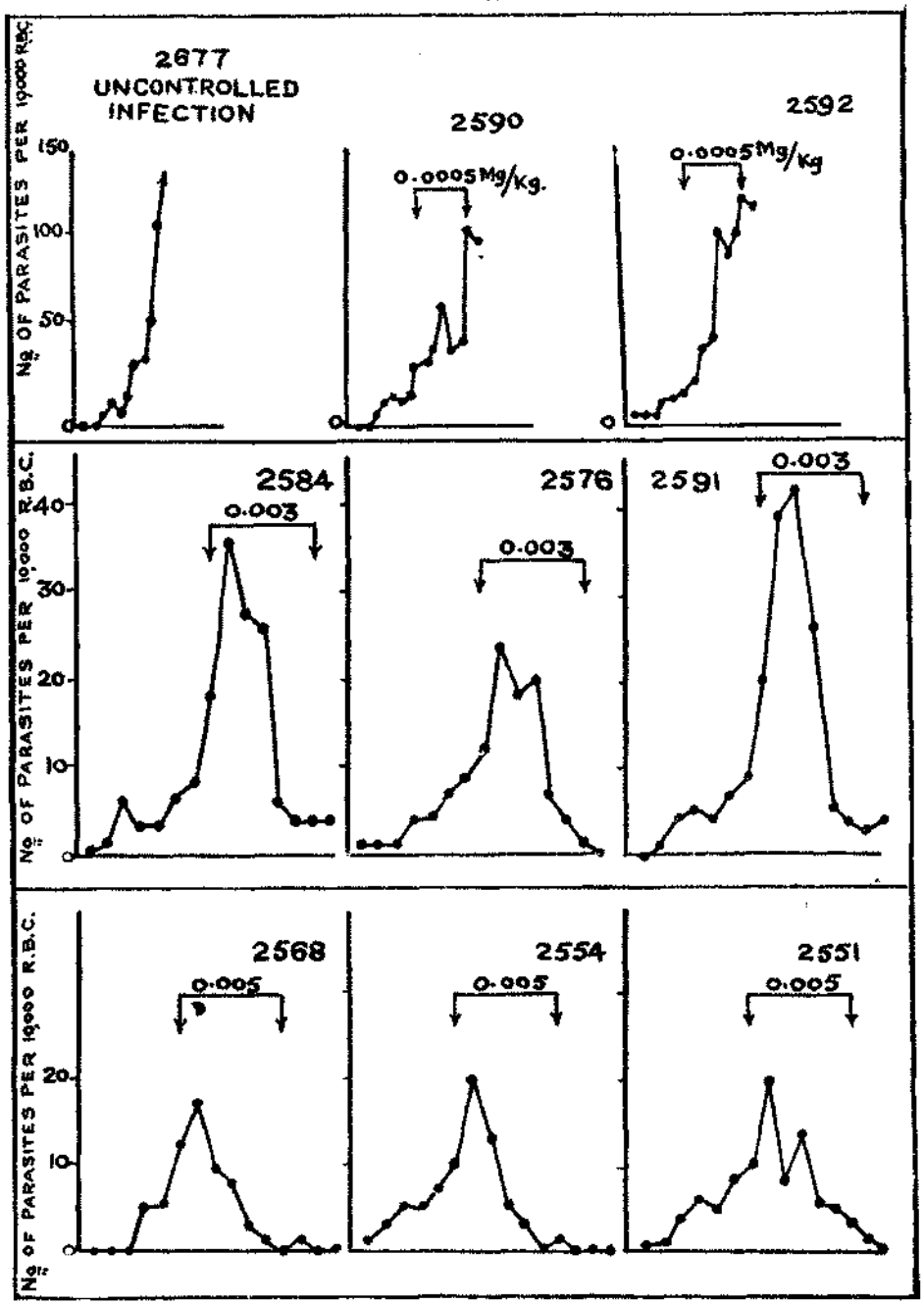
Determination of the minimum effective dose.

Schedule number.	Dose in mg./kg.	Number of animals used.	Number under class I effect.	Class II effect.		Remarks.
				Number of animals.	Average time for parasite clearance (hours).	
I	0·0003	2	Ineffective	...	...	
II	0·0005	3	,,	...	...	
III	0·001	3	3	...	...	
IV	0·003	3	2	1	168	
V	0·005	3	...	3	144	M.E.D.

Schedules I and II as per Table II were ineffective. In the next higher dosage there was deceleration in the course of parasitæmia (class I effect) but elimination of parasites from the peripheral circulation was not obtained. However, class II effect was attained in all the three animals under Schedule V and parasite clearance attained in an average of 144 hours. Parasitic relapses occurred in each case.

The course of infection in untreated and treated monkeys under the different schedules shown in Table II appear in Chart 1.

CHART I.



Class I and II types of plasmodicidal effect.

Sterilization of blood-induced infection.—Higher dosage schedules, as shown in Table III, were employed for determination of the minimum therapeutic dosage that would effect complete sterilization of the blood-induced infection (class III effect). Side by side, the maximum dose that could be tolerated by the animals without any appreciable loss of weight was also determined. Altogether 33 monkeys were utilized for these investigations.

TABLE III.

Higher dosage schedules.

Schedule number.	Dose in mg./kg.	Number of animals used.	Weight in lb. (average).			Activity—class II.			Cure.	Remarks.
			Initial.	Final.	Difference.	Asexual clearance in hours (average).	Gametocytes in hours (average).	Recrudescence in days.		
I	4·0	5	10·8	10·6	−0·2	110·4	158·4	15 and 26	3	Class III effect.
II	5·0	5	11·5	11·45	−0·05	100·8	163·2	...	5	,,
III	7·5	5	9·27	9·0	0·27	96·0	139·2	...	5	,,
IV	10·0	5	10·4	9·7	0·7	96·0	150·0	20 and 32	3	,,
V	12·5	5	11·5	10·4	1·1	91·5	120·0	...	5	,,
VI	15·0	5	9·5	7·8	1·7	81·6	120·0	...	4	One died due to toxic effect.
VII	20·0	3	Died during or soon after completion of treatment.							

Asexual parasite clearance was attained between 110·4 and 81·6 hours in Schedules I to VI. A dose of 20·0 mg./kg. proved too toxic and all the three animals died either during or soon after termination of the course. Though sterilization occurred in all cases under Schedules III, V and VI, there was appreciable loss of body weight under Schedules V and VI.

Gametocyte clearance under all schedules ranged from 120 to 163·2 hours though individual variations extended from 96 hours in a few cases under Schedules IV to VII to a maximum of 240 hours in one case treated with 4 mg./kg. dose.

Acute toxic effects.—The results of the single heavy oral dose of daraprim have been shown in Table IV. In all cases where the animals succumbed, toxic symptoms appeared within 1 to 36 hours following drug administration.

TABLE IV.

Acute toxic dosage schedules.

Schedule number.	Dosage, mg./kg.	Number of animals.	Mortality.		Mortality per cent.
			Number. died.	Hours after drug administration.	
I	150	3	3	1–4	100
II	100	5	3	1–3	60
III	75	5	3	2–4	60
IV	60	5	3	3–5	60
V	50	5	2	3–36	40
VI	40	5	...	...	0

From Table IV it would appear that the L.D. 0, L.D. 50 and L.D. 100 are 40, 55 and 150 mg./kg. body weight respectively.

The various acute toxic manifestations appeared in the following sequence. The first signs were excessive salivation, retching and vomiting, followed by convulsions lasting for several hours in some cases, paralysis and finally death.

Post-mortem examination.—Post-mortem examination revealed congestion of some of the organs including brain and liver but the brunt appeared to be on the kidneys which were heavily congested and in some cases appeared much darker.

There was no appreciable changes noticeable in the lungs, heart and intestines.

Histopathological findings.—Examination of the section of tissues collected during post-mortem examination showed the following changes as compared to the biopsy materials from normal monkeys. Major changes appeared to be in the kidneys and in the liver.

Kidneys.—Degenerative changes in the convoluted tubules were conspicuous. The lining cells appeared to be swollen with narrowing of the lumen. Nuclei of many cells showed either pyknotic changes or were absent.

Liver.—Acute toxic effects of the drug in the liver were manifested in the apparent swelling (cloudy) of the parenchyma cells which appeared mostly either round or oval rather than the polygonal type as seen in the normal liver. There were considerable changes in the nuclei.

Detailed studies on the acute and chronic toxic effects of the drug on different organs will appear in a subsequent report.

DISCUSSION.

During the preliminary studies with daraprim against *P. knowlesi* it was observed that doses ranging from 0·01 to 2·0 mg./kg. body weight caused class II effect similar to that observed with a dose of 0·02 (M.E.D.) or more of proguanil against the same species of plasmodium (Ray *et al.*, 1951). Subsequent studies showed that activity of daraprim with a minimum dose as low as 0·005 mg./kg. was also similar to that attained with the M.E.D. of proguanil. On that basis, the minimum effective dose (M.E.D.) of daraprim was considered to be 0·005 mg./kg. Asexual parasite clearance under both the drugs, proguanil and daraprim, was attained in about 6 to 8 days in every case. Thus it is evident that daraprim is effective in very small doses and is four times more active than proguanil. Falco *et al.* (1951) had previously reported that some of the 2 : 4-diaminopyrimidine compounds are highly active against *P. gallinaceum* and *P. berghei* and that the drugs were also active against the blood forms of *P. cynomolgi*. The same authors had further observed that against *P. gallinaceum* and *P. berghei*, daraprim (50-63) is 60 and 200 times respectively more active than proguanil. Working with *P. cynomolgi*, Schmidt (1951) has observed that it is 30 times as effective as proguanil.

In the higher dosage schedule (Table III), asexual parasite clearance occurred between 110 and 96 hours in doses ranging from 4 to 10 mg., although slightly better results were noticeable with 12·5 and 15 mg. dosage. But the last two doses appeared to be ill tolerated by the animals as manifested by appreciable loss of weight. The clearance in the lower dosage schedule with 1 or 2 mg. (Table I) does not appear to be significantly different from that observed with higher dosage schedules. Thus it would appear that the rate of parasite clearance is not directly proportional to the dosage, and the speed is more or less comparable to that observed under proguanil treatment (Ray *et al.*, 1951). Compared to the effect of chloroquine under similar dosage schedules (Jaswant Singh, Ray, Basu and Nair, 1951), action of daraprim is significantly slower. These observations are in accord with the findings of Schmidt (1951), who has observed that action of daraprim is 'closely similar to that of chloroguanide and distinctly different from that of chloroquine'.

More or less the identical biological activities of daraprim and proguanil may be attributed to considerable structural similarity between these two drugs. Furthermore, Carrington *et al.* (1951) have recently succeeded in isolating a tenfold active *in vivo* metabolite of proguanil which also displays a marked structural resemblance to daraprim and, in fact, it can be considered as a hybrid of the chemical structures of these two main drugs. These recent chemical and biochemical investigations suggest that perhaps proguanil and daraprim have a similar mechanism of action with quantitative variation in therapeutic intensity.

Action of daraprim against gametocytes is slower still and in some cases the sexual forms persisted for several days after completion of the course. Except in ranges well-nigh toxic level, the time for gametocyte clearance was more or less the same in the lower dose and there was no correlation between the dose of anti-malarial and the rate of clearance of parasites.

In majority of the cases treated with doses lower than 5 mg./kg., sterilization of blood-induced infection was not attained. But in 5 mg./kg. or higher doses, complete elimination of the infection was attained in most cases. Thus it would appear that in higher therapeutic doses, which were not toxic, class III effect was evident in the majority but not so in every case.

From acute toxicity studies it is observed that a single oral dose of about 150 mg. may be recorded as L.D. 100, whereas 40 to 60 per cent mortality occurred between 50 and 100 mg. dosage. Nair *et al.* (1951) had reported that 40 to 60 per cent of the monkeys died with doses of proguanil ranging from 150 to 500 mg., whereas L.D. 100 is 600 mg./kg. of proguanil. Daraprim therefore appears to be four times as toxic as proguanil.

It would appear that M.T.D. in monkeys with acute primary infection is lower than those not showing any detectable parasitic infection. Similar observations were reported by Nair *et al.* (1951) during their studies on the toxic manifestations of proguanil. These findings are similar to those observed in chicks (Wiselogle, 1946).

The lesion in the kidneys definitely points out that the drug sets up an acute tubular nephritis similar to that observed after administration of heavy metal salts like mercuric chloride (Boyd, 1947). Cloudy swelling of the cells of the liver is attributable to the same exogenous toxic stimuli and is considered to be the beginning of a degenerative change.

Jaswant Singh *et al.* (in press) have shown during their studies on the effect of daraprim against proguanil-resistant sub-strain of *P. knowlesi* that a dose of 8 mg./kg. of the drug was required to produce the same effect as with 0·005 mg. to the proguanil-resistant strain, though Rollo (1951) was able to show only a two and a half times cross-resistance against a proguanil-resistant strain of *P. gallinaceum*. Schmidt (1951) reports that 'development of resistance of daraprim appears to occur with relatively great frequency and rapidity' in *P. cynomolgi* and that the character is retained after mosquito passage. The same worker has further observed that daraprim-resistant strain is equally refractory to proguanil. On the basis of existing evidence the authors are not in a position to hold the same optimistic view that daraprim 'will prove to be of value in the suppression treatment of human malaria, and specially in the treatment of proguanil-resistant strains' as expressed by Falco *et al.* (1951).

SUMMARY.

The minimum effective dose of daraprim was established to be 0·005 mg./kg. body weight and thus compared to the M.E.D. of proguanil (0·02 mg./kg.), it is four times more active.

Action against the asexual forms of the erythrocytic parasites in blood-induced infection is slow and is more or less comparable to that of proguanil. The rate of clearance is not proportional to the dose.

Gametocyte clearance is slower still and in some cases they persisted long after completion of the treatment. In non-toxic range there was no relation between the dosage schedule and the speed of gametocyte clearance.

Blood-induced infection was sterilized in most of the cases with dosage of 5 mg. and above though parasitic relapses did occur in as high a dose as 10 mg./kg.

Compared to the acute toxic effects of daraprim and proguanil, the former was four times as toxic as proguanil. Post-mortem examination and histopathological studies were carried out and there is evidence to show that degenerative changes were mostly noticeable in the kidneys and to a somewhat lesser degree in the liver.

Concurrent studies against a proguanil-resistant strain have shown that the sub-strain is highly refractory to daraprim.

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EFFECT OF PROGUANIL ON SPOROGONY CYCLE OF *P. KNOWLESI*.

Part I.

BY

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MACKERRAS and ERCOLE (1947) showed that after a gametocyte carrier of *P. falciparum* (New Guinea strain) was treated with a single dose of 300 mg. of proguanil, the crescents were so affected for a period of 8 days that subsequent development in the insect host was prevented. Likewise, a dose of 100 mg. prevented similar development for a period of 4 days and no infection of the gut or gland could be detected in the mosquitoes which were fed on the subject during this period. Thereafter, the sporogony cycle was found to be normal. Shute and Maryon (1948) working with a strain of *P. falciparum* observed that when a single dose of 800 mg. of proguanil was administered to patients with gametocytes, and mosquitoes were fed on them every day subsequently, there was no infection of the gut or gland for a period of 8 days. Working with two strains of *P. falciparum* (Panamanian p.f. 6 and South Carolina, U.S.A. p.f. 2) Ramakrishnan *et al.* (1952) had observed that when mosquitoes were fed daily on gametocyte carriers after they had been treated with a single dose of 300 mg. of proguanil, there was no infection of the salivary glands for a period of 3 days from the day following drug administration but oöcysts were detectable from the second day onwards. Thus it would appear that the conditions remained unfavourable for development of sporozoites in the mosquitoes for a maximum period of 6 days and not over a week as reported by the earlier workers.

It is evident from the foregoing that proguanil affects gametocytes in such a way as to inhibit their further growth in the insect host. The practical importance of this effect of proguanil lies in the duration of such activity in special

reference to clinical cure by single dose therapy in hyperendemic areas. It was considered that further studies on other plasmodia would throw more light on the subject.

The present study reports the effect of a single dose of proguanil on the sporogony cycle of *P. knowlesi* in *A. annularis* which has been conveniently used as a laboratory vector for transmission of this species of plasmodium (Jaswant Singh *et al.*, 1949, 1950).

MATERIALS AND METHODS.

Parasite.—A northern Indian *rhesus* monkey (*M. rhesus*) was experimentally infected with a strain of *P. knowlesi* (blood-induced) and daily parasite count was made after the date of inoculation. When gametocytes were detectable mosquitoes were fed before and after drug administration.

Blood smears were stained with J.S.B. stain (Jaswant Singh and Bhattacharji, 1944).

Insect host.—*A. annularis* hatched from larvæ collected in the vicinity of Delhi were kept overnight on dilute solution of glucose. Thirty mosquitoes were fed on the animal for 2 days prior to drug administration and served as the comparison group. On the day of treatment, a batch of 15 mosquitoes were fed at intervals of 4, 8 and 12 hours after the dose of proguanil. Fresh mosquitoes were continued to be fed every day thereafter for a period of 12 days. The technique adopted for feeding mosquitoes on the monkey and their maintenance subsequently has been described earlier by Jaswant Singh *et al.* (1950).

The insects were kept in Barraud's cages with raisins (soaked in water) on the top for subsequent nourishment after the infective blood meal.

The average temperatures and relative humidity during the period of observation in the insectarium varied from 74 to 78°F. and 66 to 75 per cent respectively.

Dissection.—Sample dissections of the batches of mosquitoes were commenced from the fourth day after the infective feed and continued up to the 12th day.

Drug administration.—Suspension of a single dose of 20 mg. of proguanil comparable to double the human dose of 300 mg. recommended by Afridi *et al.* (1947) was administered on empty stomach with the help of a Ryle's tube. The dose was calculated according to the body weight.

Results.—Dissection results showed infection of the gut and gland in both the batches of mosquitoes fed on 2 consecutive days prior to the administration of proguanil and the gland infection rates were 16 and 20 per cent respectively. Out of the three batches of mosquitoes fed on the day of treatment, those which had the infective blood meal 4 hours after drug administration showed oöcysts and sporozoite in one out of 11 mosquitoes while none was found infected amongst those fed 8 and 12 hours after. It will also be observed from Table I that no further infection of gut or gland was detectable in the subsequent batches of mosquitoes for the following 5 days.

TABLE I.

Dissection results.

Batch number.	Infective feed.		Number dissected.	Number showing infections of		Sporozoite rate, per cent.	Gametocytes per c.mm.	Remarks.
	Date : May 1951.	Number fed.		Gut.	Gland.			
1	7	30	25	2	4	16·0	4,000	
2	8	30	25	3	5	20·0	3,500	
3	9	...	...	...	...	...	...	
	A	15	11	1*	1	9·0	3,500	4 hours.
	B	15	11	...	...	...	...	8 ,,
	C	15	11	...	...	...	...	12 ,, after treatment.
4	10	30	23	...	...	...	2,000	24 ,, ,, ,,
5	11	30	23	...	...	...	1,500	48 ,,
6	12	30	19	...	...	...	1,800	72 ,,
7	13	30	17	...	...	...	1,200	96 ,,
8	14	30	22	...	...	...	1,000	120 ,,
9	15	30	24	1	5	20·8	800	144 ,,
10	16	30	24	1	4	16·6	800	
11	17	30	25	1	2	8·0	800	

* The same mosquito had infection of both gut and gland.

TABLE I—*contd.*

Batch number.	Infective feed.		Number dissected.	Number showing infections of		Sporozoite rate, per cent.	Gametocytes per c.mm.	Remarks.
	Date : May 1931.	Number fed.		Gut.	Gland.			
12	18	30	26	...	2	7·6	600	
13	19	30	24	...	1	4·1	600	
14	20	30	20	1	1	5·0	300	
15	21	30	19	...	1	5·2	300	

However, infection of both gut and gland was again detectable from the sixth day of drug administration throughout the period of observation. The sporozoite rate on the first day after the 5-day interruption appeared to be higher than those observed on the subsequent days and well comparable to the results of the comparison group.

From the day to day records the number of gametocytes appeared to decrease progressively throughout and the drop was quite significant on the day following administration of proguanil.

DISCUSSION.

Use of antimalarials purely for their effect on gametocytes have not met much enthusiasm in recent years, though in the past gametocyte 'blockage' was considered to be a prophylactic measure. Clemesha (1933) had reported that 'It is possible to control entirely the spread of malaria by careful anti-gametocyte work'. Obviously, this was with the idea that the drugs would cut off the source of supply of gametocytes from the peripheral blood and would thus make mosquito transmission impossible. Pamaquin (plasmoquin, plasmochin) either alone or in combination with other drugs had been used extensively by workers for gametocyte prophylaxis (Cordes, 1928; Green, 1929; Kligler and Mer, 1931; Kingsbury and Amies, 1931; Clemesha, 1933; Dupoux *et al.*, 1937; Genevray *et al.*, 1939; and others).

By experience, gametocyte prophylaxis has not shown such satisfactory results as had been anticipated earlier (Senior White and Adhikari, 1937; Field, 1939).

This is mainly attributable to the lack of a suitable drug which in non-toxic dosage could be used for a prolonged period and would affect equally the gametocytes of all the three plasmodia. Although antimalarials like quinine, mepacrine, and chloroquine have some degree of action against the gametocytes of *P. vivax* and *P. malariæ*, none of these has any action on the crescents (Findlay, 1951).

Pamaquin on the other hand is considered to be a polyvalent gametocytocida drug and it has been shown that even minute doses, too small to destroy the crescents directly, may still injure them in such a way that they become incapable of further development in the mosquitoes (Green, 1929). Amies (1930) had observed that two doses of 20 mg. of pamaquin administered 16 hours apart render crescent carriers non-infective for at least 3 days. Likewise, Jerace and Giovannola (1933) had observed that while 32 per cent of the mosquitoes became infective when they were fed 24 hours before drug administration, only 3 per cent became so when fed 24 hours after treatment. Mosquitoes fed subsequently from the third to the seventh day did not show any infection of the gut or gland. Chopra and Basu (1937) in laboratory experiments had shown that treatment with pamaquin alone prevented crescents from development in *A. stephensi*.

But the hazards of the toxic effects of the drugs of the 8-aminoquinoline series when used for a prolonged period are well known and thus render them unsuitable for such purposes. Proguanil, on the other hand, has proved an efficient suppressant which can be continued to be taken in weekly or twice weekly doses for a long period without any adverse side-effects. Besides, being a true causal prophylactic for *P. falciparum*, the drug affects the gametocytes of all the three plasmodia, though not morphologically but in such a way that subsequent development in mosquitoes does not occur during treatment and for several days thereafter (Black, 1946; Shute and Maryon, 1948). Some believe that the drug affects the macro-gametocytes most of which are either 'destroyed or sterilized before fertilization or before they can penetrate the gut wall' (Findlay, 1951). Others may, however, penetrate the gut wall subsequent to fertilization and after a stage of encystment they die (Black, 1946; Findlay, 1951).

Thus it would appear that during efficient mass suppression in a community in an endemic area, proguanil would not only cause a concomitant interference with the transmission of the disease, but would also prevent overt attacks of malaria due to *P. vivax* and *P. malariæ* and effect radical cure of *P. falciparum* at the same time. Therefore, it would seem that the reports of Mackerras and Ercole (1947) and Shute and Maryon (1948) that development in the mosquitoes does not occur for a period of 8 to 10 days after they had fed on a gametocyte carrier treated with proguanil are of special significance. Thus, regular administration with a weekly dose of proguanil either as a suppressive measure or after a single dosage therapy as recommended by Afridi *et al.* (*loc. cit.*), Viswanathan and Bailey (1947) and others, would automatically ensure cutting of mosquito transmission.

But the observations, that the sporogony cycle could commence earlier than 8 to 10 days, as shown by Ramakrishnan and his colleagues mentioned earlier, and during the present studies, would mean reconsideration of the question of recommendation of weekly or twice weekly suppressive regime. Working in a hyperendemic area, Ray (1948) has shown that a weekly suppressive regime with a dose of 300 mg. is almost as good as a twice weekly regime with 100 mg. of proguanil,

though the latter would present more administrative difficulties on account of more frequent drug administration that would be needed.

In the light of these observations it is obvious that the question of instituting suppressive treatment in a community living in an endemic area and the proguanil regime to be employed need deeper consideration.

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COMPARATIVE STUDIES ON CHLOROQUINE AND RESOCHIN AGAINST SIMIAN AND HUMAN MALARIA.

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STUDIES on synthetic antimalarials of the 4-aminoquinoline series were first started in Germany prior to the last World War. Of the two effective antimalarials, sontochin and resochin (Bayer), the former was captured by the Allies in North Africa in 1943 from the Germans (Kikuth and Mudrow-Reichenow, 1949) and it was later found to be identical to (SN 6911) one of the 14,000 compounds synthesized by Messrs. Winthrops & Co., U.S.A. Systematic investigations under the Board of Co-ordination for malaria studies in U.S.A. have shown that another highly active compound of the series named chloroquine (Winthrops) (synonyms SN 7618, Aralen, Nivaquine B) is identical to 'resochin', and that it is superior to sontochin 'though the German workers found it otherwise' (Wiselogle, 1946; Kenyon *et al.*, 1949).

Extensive trials with chloroquine both in India and abroad have shown that it is a powerful schizonticide and that clinical response to its treatment is striking (Most *et al.*, 1946; Halwani *et al.*, 1948; Chaudhuri *et al.*, 1948; Spicknall *et al.*, 1949). Koenig (1951) reported that excellent results were obtained with resochin in Thailand and also against West African strain of *P. falciparum*. In laboratory studies, Schmidt *et al.* (1948) and Genther *et al.* (1948) showed that chloroquine proved equally effective against simian malaria. In a comparative study with other antimalarials, Jaswant Singh *et al.* (1949) observed that this compound proved to be highly effective against blood-induced *P. knowlesi* infections.

In view of the claims made and the earlier controversies regarding the relative merits of resochin and chloroquine as compared to sontochin, it was considered

desirable that comparative studies should be undertaken and the present report records some of the observations made on the relative action of the two compounds in simian and human malaria. Laboratory trials to determine these activities against *P. knowlesi* infection in North Indian *rhesus* monkeys, and field and hospital trials in human malaria were undertaken during 1950-51. Resochin was received through the courtesy of Bayer Laboratories in 1950 and chloroquine had been received from Winthrop in 1946.

LABORATORY STUDIES.

Materials and methods.—Twenty-four monkeys were obtained from the neighbourhood of Delhi and were subjected to routine procedures of blood examinations and tuberculin tests prior to experimental blood inoculations with *P. knowlesi* (Jaswant Singh *et al.*, 1951).

The dose of inoculum was 50 million parasites per kg. body weight of the animals. Drug administration was commenced when the erythrocytic cell infestation reached 0·1 to 0·2 per cent, as determined by daily blood examinations. Ehrlich's eyepiece adjusted to include 100 erythrocytes per field under oil immersion objective was used for parasite counts. One hundred such fields were examined in each case. All blood smears were stained with J.S.B. stain (Jaswant Singh and Bhattacharji, 1944).

Drug administration.—Requisite amount of solution of each drug, calculated in terms of mg. base per kg. body weight of the animal, was introduced through a Ryle's tube on empty stomach. Six dosage schedules ranging from 2·5 to 80 mg. were administered as shown in Table I.

TABLE I.

Speed of clearance of asexual parasites in monkeys.

Schedule number.	Dosage in mg. base kg.	Regime I (Chloroquine).			Regime II (Resochin).		
		Number of monkeys.	Asexual parasite clearance in hours (average).	Recrudescence/cure (in days).	Number of monkeys.	Asexual parasite clearance in hours (average).	Recrudescence/cure (in days).
I ...	2·5	2	114	Cured	2	120	8 and 15
II ...	5·0	2	108	,,	2	120	Cured
III ...	10·0	2	72	,,	2	72	,,
IV ...	20·0	2	54	,,	2	60	,,
V ...	40·0	2	48	,,	2	48	,,
VI ...	80·0	2	42	1 cured 1 died*	2	48	1 cured 1 died*

* Animals died during the course of treatment or soon after its termination presumably due to toxic effects.

Follow up.—Sterilization of blood-induced infections was determined by daily blood examinations and splenectomy was performed in each case if no parasites were detectable for six weeks after termination of the course of treatment.

Results.—Two animals were placed under each schedule in regime I (chloroquine) and an equal number in the schedules under regime II (resochin).

From Table I it will be observed that complete parasite clearance was attained in all schedules under both regimes in 42–114 hours (chloroquine) and 48–120 hours (resochin). In schedule VI, one animal each under the two regimes died due to the toxic effect of the drug. Except under schedule I, regime II, complete sterilization of the blood-induced infection was obtained in all cases.

FIELD AND HOSPITAL STUDIES

(*a*) *Field trials.*—Kosi Kalan, a small town in the district of Mathura in U.P., and the two neighbouring villages Azizpur and Barhana were selected for field trials. Overall spleen and parasite rates were 29 and 8 per cent respectively.

Blood smears of all fever cases were collected at the local dispensary and also by domiciliary visits. Fifty-four cases of malaria (*P. vivax* 3, *P. falciparum* 47, *P. malariæ* 3, mixed 1), microscopically diagnosed, were placed under chloroquine and resochin by rotation. Thirty of these cases were from 10 months to 12 years old while the remaining 24 were adults. Both sexes were included. Drug was administered orally in all cases.

(*b*) *Hospital studies.*—Police Hospital, Civil Lines, Delhi. Blood from all adult male fever cases reporting at the out-patients department of Police Hospital were examined at the laboratories of this Institute and 40 malaria cases* diagnosed amongst the Delhi Police Force who had contracted malaria during their visits either on leave or on duty outside Delhi area in known malarious localities, were treated as in-patients with one or the other antimalarial. In addition 10 other cases were treated and followed up subsequently.

Dosage schedules.—The total adult dose under the two regimes was chloroquine 1·5 gm. of the base content and consisted of 6 tablets (0·25 gm. base per tablet) and resochin 10 tablets (0·15 gm. base per tablet). Each drug was administered orally in 5 doses, 0·6 gm. on the first day (in two divided dosages) and 0·3 gm. on the three subsequent mornings as recommended by Jaswant Singh (1950). Children received proportionately smaller doses calculated according to age.

Distribution of cases.—Table II shows that out of a total number of 104 cases, 49 were placed under chloroquine regime while 55 received resochin. The total number of cases with *P. vivax* was 39, of which 14 were under chloroquine and 25 under resochin treatment. Out of the 59 cases suffering from *P. falciparum* infections, 32 and 27 cases were placed under regimes I and II respectively. Three out of 4 cases of *P. malariæ* infection were treated with chloroquine and the remaining one was under resochin as also the two cases of mixed infections (*P. vivax* and *P. falciparum*).

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* *P. vivax* 36, *P. falciparum* 12, *P. malariæ* 1, mixed 1.

TABLE II.

Distribution of cases according to type of infection and regime.

Species.	Chloroquine.	Resochin.	TOTAL.
B.T. ...	14	25	39
M.T. ...	32	27	59
Q.T. ...	3	1	4
Mixed ...	...	2	2
TOTAL ...	49	55	104

Results.—In *P. vivax* infection asexual parasite clearance was obtained within 72 hours in cases under both regimes, and in *P. falciparum* infection in 96 hours (Table III). In *P. malariæ* infection parasite clearance was attained in 48 hours and within 24 hours in the two cases with mixed infection (B.T. and M.T.).

TABLE III.

Asexual parasite clearance.

Species.	REGIME I (CHLOROQUINE). Parasite clearance within hours.					REGIME II (RESOCHIN). Parasite clearance within hours.				
	24	48	72	96	Total.	24	48	72	96	Total.
B.T. ...	10 / 71·4	3 / 21·4	1 / 7·2	...	14	16 / 64·0	8 / 32·0	1 / 4·0	...	25
M.T. ...	13 / 40·6	18 / 56·2	1 / 3·2	...	32	13 / 48·1	12 / 44·4	1 / 3·7	1 / 3·7	27
Q.T. ...	2 / 66·6	1 / 33·3	...	...	3	1	...	...	...	1
Mixed ..	...	...	...	...	...	2	...	...	...	2

Note.—The figures above represent number of cases while those below represent percentage of cases.

Clearance of gametocytes.—Out of 104 cases, gametocytes were detected only in 40, 20 each under chloroquine and resochin (Table IV). Out of these, 19 cases were with *P. vivax* infection and 20 with *P. falciparum*. There was only one case of *P. malariæ*.

TABLE IV.

Gametocyte clearance.

Species.	Regime I (Chloroquine).						Regime II (Resochin).					
	Gametocyte clearance in hours.						Gametocyte clearance in hours.					
	24	48	72	96	120 or over.	Total.	24	48	72	96	120 or over.	Total.
B.T. ...	4 / 57·1	2 / 28·5	1 / 14·4	...	...	7	3 / 25·0	8 / 66·6	1 / 8·4	...	...	12
M.T. ...	...	1 / 8·4	...	3 / 25·0	8 / 66·6	12	1 / 12·5	...	1 / 12·5	...	6 / 75·0	8
Q.T. ...	...	1	...	...	...	1	...	...	..	...	...	...
Mixed ...	...	...	...	...	...	...	...	...	...	...	...	...
TOTAL ...	...	...	...	...	...	20	...	...	...	...	...	20

Note.—The figures above represent number of cases while those below represent percentage of cases.

As shown in Table IV gametocyte clearance in all cases of B.T. infection under both the regimes was attained within 72 hours whereas in most of the cases of *P. falciparum* infection, the crescents persisted for 120 hours or longer.

Clearance of clinical symptoms.—Control of temperature was taken as the main criterion for the effect of each drug on clinical symptoms. Only those cases where the patients had fever at the commencement of treatment were considered. Forty-three such cases were treated with chloroquine and 46 with resochin (Table V). Out of the total 89, thirty-one had *P. vivax* and 54 *P. falciparum* infections. There were in addition three cases of *P. malariæ* and one with mixed infection.

TABLE V.

Clearance of clinical symptoms (fever).

Species.	Regime I (Chloroquine). Fever clearance within hours.				Regime II (Resochin). Fever clearance within hours.			
	24	48	72	Total.	24	48	72	Total.
B.T. ...	9 / 81·8	1 / 9·09	1 / 9·09	11	15 / 75·00	5 / 25·00	...	20
M.T. ...	16 / 53·3	14 / 46·7	...	30	15 / 62·5	7 / 29·1	2 / 8·3	24
Q.T. ...	2	...	...	2	1	...	...	1
Mixed ...	...	...	...	...	1	...	...	1
TOTAL ...	...	...	...	43	...	...	...	46

Note.—The figures above represent number of cases while those below represent percentage of cases.

All cases became afebrile within 72 hours and in most within 48 hours irrespective whether it was *P. vivax* or *P. falciparum* infection. In the three *P. malariæ* infections, the patients became free from fever within 24 hours. One of the patients suffering from *falciparum* malaria showed heavy parasitæmia and was admitted to the hospital in a semicomatose condition. As he could still swallow the tablets, the first dose of chloroquine was administered orally, though quinine hydrochloride was kept in readiness for parenteral administration in case of worsening of his condition. On the following day he became fully conscious and was free from fever within 48 hours. Parasite clearance occurred within 72 hours from the commencement of the treatment.

Relapses.—None of the cases treated in the Police Hospital had relapsed during an observation period of two to three months.

Side-effects.—Eleven out of the 104 cases (10·5 per cent) showed some mild untoward symptoms which could possibly but not definitely be attributed to the drug. Eight had *P. falciparum* infection and out of these six had vomiting—2 under chloroquine and 4 under resochin treatment. One case under the former regime had only nausea while the other under resochin had abdominal pain. One of the 3 *P. vivax* cases under chloroquine had vomiting only, one vomiting and urticaria, and the third had pruritus. Thus it would appear that the cases were more or less equally distributed under both the regimes.

Amongst the 11 cases, seven were between 2 and 13 years of age while the others were between 18 and 40 years.

In none of these cases, the symptoms were of such severity that medication had to be discontinued.

TABLE VI.

Toxic symptoms.

Species.	Nausea.		Vomiting.		Abdominal pain.		Pruritus.	
	C.	R.	C.	R.	C.	R.	C.	R.
B.T. ...	...	...	2 2, 5	...	...	...	1 25	...
M.T. ...	1 18	...	2 11, 26	4 3, 12, 13, 40	...	1 10	...	...

C = chloroquine. R = resochin.

Note.—Figures above represent number of cases and those below represent age of patients.

DISCUSSION.

Working with *P. cynomolgi* in *rhesus* monkeys, Genther *et al.* (1948) observed that blood-induced infection could be controlled with a small dose of 2·5 mg./kg. of chloroquine. Similarly in the present investigations, low doses of 2·5 to 5·0 mg./kg. of both chloroquine and resochin proved effective in clearing asexual parasites, although comparatively the results were somewhat slower than with higher doses ranging from 10 to 40 mg./kg. A further increase to 80 mg./kg. however proved of no material advantage as one monkey under each regime died due to toxic effects. Thus it would appear that schedule V (40 mg./kg.) showed the quickest result and yet was not toxic. Jaswant Singh *et al.* (1949) made somewhat similar observations during their preliminary investigations on the chemotherapeutic activity of different antimalarials.

Though there was no significant difference in the time for asexual parasite clearance under schedule V (40 mg./kg.) under both the regimes, comparatively slightly better results were obtained with chloroquine than resochin in the lower dosage schedules like I, II and IV. Further, overall average time for parasite clearance under all schedules under chloroquine and resochin was 72 and 78 hours respectively—a difference of only 6 hours. Thus it would appear that though antimalarial activities of both are not altogether dissimilar, chloroquine yielded somewhat quicker results.

Investigations in human malaria have shown that asexual parasite clearance occurred promptly in all cases. Out of 104 cases, parasite clearance was attained within 48 hours in over 90 per cent of the patients and in 99 per cent (103 out of 104) within 72 hours. These results are more or less comparable to the records published by the United States Army Medical Department in 1947 (Findlay, 1951) wherein

it has been reported that out of 700 cases treated with chloroquine, disappearance of parasites occurred in 88·7 per cent of the cases within 48 hours and in 90 per cent within 72 hours.

Although the rate of parasite clearance within 48 hours is more or less similar under both regimes in *P. vivax* and *P. falciparum* infections, it is obvious that within 24 hours parasite clearance is obtained in more cases with *P. vivax* (64 per cent) than with *P. falciparum* infection (44 per cent). Chaudhuri (1948) made similar observations and reported that chloroquine was less effective in malignant tertian than benign tertian malaria. In the present series, action of chloroquine against *P. malariæ* infection appeared to be somewhat similar to that observed against *P. vivax*, though parasite clearance occurred slightly earlier in the latter type of infection. Although the number of *P. malariæ* cases were too small, observation seems to be in conformity with those made by Young and Eyles (1948) and Lucena (1948) in whose series parasite clearance was attained earlier in *P. vivax* than *P. malariæ* and the same in *P. malariæ* earlier than in *P. falciparum* infections.

As regards comparative action of the two compounds, asexual parasite clearance occurred in *P. vivax* infection within 24 hours under chloroquine regime, i.e, 71·4 per cent as against 64·0 per cent under resochin treatment. On the other hand in *falciparum* infection there was complete clearance of parasites in 48·1 per cent within 24 hours under resochin as against 40·6 per cent under chloroquine.

Against the sexual forms of *P. vivax* both chloroquine and resochin were effective and gametocyte clearance occurred in most cases within 48 hours, and in 100 per cent of the cases within 72 hours. However, the difference in the action of chloroquine and resochin becomes apparent when the clearance rates within 24 hours are compared. With chloroquine, the clearance occurred in 57·1 per cent of the cases as compared to 25 per cent under resochin. Against the crescents, both the drugs responded poorly. In majority of the cases (75 per cent) the gametocytes of *P. falciparum* persisted for over 120 hours under both the regimes. Van der Walle (1949) observed similar results and reported that gametocyte clearance in *P. vivax* and *P. malariæ* infections occurred in all cases within 72 hours but the crescents did not disappear within 6 to 10 days.

Thus it would appear that in *P. vivax* infection both asexual parasites and gametocytes disappeared within 24 hours in a larger percentage of cases under chloroquine than under resochin. In this type of infection, therefore, the former seems to be slightly superior to the latter.

With regard to their efficacy in the control of clinical symptoms, 90 to 100 per cent of the cases with *P. vivax* or *P. falciparum* infections became afebrile within 48 hours. These observations are in conformity with those reported by Loeb *et al.* (1946), Chaudhuri *et al.* (1948), Coatney *et al.* (1949) and Butts (1950).

Disappearance of clinical symptoms within 24 hours occurred in a larger percentage of cases with *P. vivax* (75 to 81 per cent) than *P. falciparum* infections (53 to 62 per cent). Thus, like parasite clearance, disappearance of clinical symptoms under the two regimes occurred more rapidly in *P. vivax* than *P. falciparum* infection. All the three cases with *P. malariæ* were afebrile within 24 hours.

As compared to resochin in *P. vivax* infection, 81·8 per cent of the cases became afebrile within 24 hours under chloroquine and 75·0 per cent under resochin. On the other hand, in *P. falciparum* infection the latter proved slightly better than the former in that clinical symptoms disappeared within 24 hours in more cases (62·5 per cent) under resochin than chloroquine (53·3 per cent).

Therefore, both in regard to the clearance of parasites and clinical symptoms in *P. vivax* infection the results shown under chloroquine regime appear to be slightly better than those under resochin treatment. However, in *P. falciparum* infection resochin treatment gave slightly better results than chloroquine.

From all available reports it is noted that chloroquine treatment causes certain side-effects like nausea, vomiting, pruritus, etc., in a number of cases. As a rule they are of such a minor nature that interruption of treatment is not considered necessary. In their series, Gordon *et al.* (1947) encountered toxic manifestations in 22·9 per cent of the total number of 236 cases treated with chloroquine whereas out of 1,277 patients treated by the United States Army Medical Department, as high as 27·8 per cent showed various types of mild toxic reactions. But in the present series untoward side-effects were observed in 11 out of 104 (10·5 per cent) cases and the toxic manifestations were of a minor nature. This is in conformity with that reported by Chaudhuri (1948) who had noted such minor symptoms in 5 out of 50 (10 per cent) cases.

Although untoward side-effects like nausea, vomiting, etc., appeared to be equally distributed under both the regimes, such manifestations were more common in *P. falciparum* (8 out of 11) than in *P. vivax* infection. As these symptoms are not uncommon in untreated cases of malignant tertian malaria it is difficult to assess as to how much of the effect was due to toxic reaction of the drug.

As compared to the observations by Most *et al.* (1946) who had reported pruritus in 20 per cent of 284 cases, dermal manifestations in the form of pruritus in one case and urticaria (associated with vomiting) in another during the present investigations, appear to be less important. Alving *et al.* (1948) did not encounter a single case of pruritus in his series of 20 patients though others have reported about cutaneous eruptions and itching after chloroquine treatment (Gordon *et al.*, 1947; Chaudhuri, 1948; Brito and Cotrim, 1950).

SUMMARY.

Chloroquine and resochin were found effective against *P. knowlesi* infection in *rhesus* monkeys though average time for parasite clearance under all schedules seems slightly earlier under the former regime.

In 90 to 100 per cent of cases, under both regimes and with all types of human malaria, clearance of asexual parasites and clinical symptoms occurred within 48 hours. Both were significantly effective against the sexual forms of *P. vivax* but response against crescents was poor.

Comparing the results obtained within 24 hours in *P. vivax* infections, chloroquine appeared to be slightly superior to resochin. On the other hand in *P. falciparum* infections reverse was the case.

Neither appeared to be more toxic than the other.

Both the drugs are highly effective against all types of human malaria but the choice of treatment in real emergency cases must fall on parenteral administration of quinine, at least, until such time as comparative efficacy of chloroquine or resochin parenteral treatment is thoroughly tested.

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LABORATORY AND FIELD STUDIES ON THE RESIDUAL TOXICITY OF CHLORDAN, ALDRIN AND DIELDRIN AGAINST MOSQUITOES.

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INTRODUCTION.

SINCE the discovery of D.D.T., several new synthetic compounds, such as analogues of D.D.T., B.H.C., chlordan, toxaphene, aldrin and dieldrin, etc., with similar insecticidal properties, have been developed. The utility of some of the newer compounds in malaria control has been investigated and the results have been compared with D.D.T. application. Chlordan, aldrin and dieldrin are the three insecticides which were selected for this purpose because of the extraordinary toxic properties attributed to them.

The insecticidal properties of chlordan (1, 2, 4, 5, 6, 7, 8, 8-octachloro-3a, 4, 7, 7a tetrahydro-4, 7-methanoindane empirical formula $C_{10}H_6Cl_8$) were discovered in the U.S.A. by a commercial firm during World War II (Kearns *et al.*, 1945). The commercial product is probably a mixture of isomers but these have not yet been defined. It is produced as a brown odourless liquid which is miscible with mineral oils. It can be formulated as a solution, emulsion, dispersible powder or dust. It has been found quite effective for the control of flies resistant to D.D.T. (Mosna, 1949, 1950; Missiroli, 1950).

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Both aldrin and dieldrin are of recent origin. Aldrin has the empirical formula $C_{12}H_8Cl_6$ and the chemical name 1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 8, 8a-hexahydro-1, 4, 5, 8-dimethanonaphthalene. It is a white crystalline solid with a melting point of 100° to 102°C. It is highly soluble in most organic solvents but is insoluble in water. It can be formulated as a solution, emulsion, dispersible powder or dust.

Dieldrin is an oxygenated derivative of aldrin with the empirical formula $C_{12}H_8OCl_6$ and the chemical name 1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4, 5, 8-dimethanonaphthalene. It is also a white crystalline solid with a melting point of 172° to 175°C. It is moderately soluble in most of the organic solvents but is insoluble in most of the aliphatic petroleum solvents and water. Like aldrin it can also be readily formulated as a solution, emulsion, dispersible powder or dust. Both aldrin and dieldrin have been used against agricultural pests with encouraging results by various workers (Chandler, 1950; Rings, 1950; Bobb, 1950; Mitchener, 1950; Medler and Chamberlin, 1950; Dominick, 1950; Ivy and Scales, 1950; Wilcox and Howland, 1950; Eide and Stitt, 1950; Dean, 1951; Michelbacher *et al.*, 1951; Rings and Gould, 1951). Recently, dieldrin has also been tried as an aerial spray against adult salt marsh mosquitoes resistant to D.D.T. (Deonier *et al.*, 1950).

LABORATORY STUDIES AS LARVICIDES.

These insecticides were tested in the laboratory against mosquito larvæ by the method described by Pal (1951), whereby mosquito larvæ can be exposed to a treated water surface for a limited period of time and removed with a minimum disturbance of the surface film.

Large photographic trays with glass partitions are used for this purpose. One-half of the tray is treated with the insecticide under test whilst the other side is not treated. A known number of larvæ (usually ten) are introduced from under the glass barrier towards the treated side without disturbing the surface film. Larvæ are removed in the same manner as they are introduced after an exposure of 30 minutes. They are washed with tap water and then transferred to an enamelled bowl containing larval food and held for 24 hours. Number of larvæ immediately knocked down and dead after 24 hours is recorded. For every three trays treated with the insecticide one is left untreated for purposes of comparison.

Tests were repeated every day till the larval mortality after 24 hours was below 50 per cent when the larvicidal effect was considered as having ceased. Larvicidal action of chlordan, aldrin and dieldrin was determined in each case with five different dosages, i.e. 0·5 mg., 1 mg., 10 mg., 20 mg., and 40 mg. per sq. ft. of water surface. One per cent solution of the insecticide in acetone was used for these tests. The required amount of acetone solution was squirted by means of a pipette. Acetone evaporated quickly leaving a thin film of the insecticide on the water surface.

For comparison D.D.T. 2 mg. per sq. ft. of water surface (the usual dosage recommended for larvicidal purposes : Puri and Pal, 1947) was also tested in a similar manner. Fourth instar larvæ of *A. stephensi* and *C. fatigans* were used for these tests.

EXPERIMENTAL DATA.

Chlordan at dosages of 0·5 mg. and 1 mg. per sq. ft. of water surface was found to be ineffective against *A. stephensi* larvæ whereas at dosages of 10 mg., 20 mg. and 40 mg. per sq. ft. of water surface it was effective for 4, 11 and 12 days respectively. It was also ineffective at dosages of 0·5 mg. and 1 mg. against *C. fatigans* but at dosages of 10 mg., 20 mg. and 40 mg. per sq. ft. of water surface it was effective for 4, 8 and 12 days respectively.

Aldrin at dosages of 0·5 mg. and 1 mg. per sq. ft. of water surface was effective for one day only against *A. stephensi* larvæ whilst at dosages of 10 mg., 20 mg. and 40 mg. per sq. ft. of water surface it was effective for 7, 10 and 36 days respectively. At dosages of 0·5 mg. and 1 mg. per sq. ft. of water surface it was effective on the day of application only against *C. fatigans* and at dosages of 10 mg., 20 mg. and 40 mg. per sq. ft. of water surface it was effective for 7, 10 and 17 days respectively.

Dieldrin at dosages of 0·5 mg., 1 mg., 10 mg., 20 mg. and 40 mg. per sq. ft. of water surface was effective for 3, 3, 15, 33 and 56 days respectively against *A. stephensi* larvæ. Whereas at dosages of 0·5 mg. and 1 mg. per sq. ft. of water surface it was effective against *C. fatigans* larvæ on the day of application only and at dosages of 10 mg., 20 mg. and 40 mg. per sq. ft. of water surface it was effective for 8, 24 and 47 days respectively.

D.D.T. at a dosage of 2 mg. per sq. ft. of water surface was effective for about 7 days against *A. stephensi* larvæ. D.D.T. was not effective against *C. fatigans* at this dosage. The result of the larvicidal tests are summarized in Table I.

TABLE I.

Effectiveness of chlordan, aldrin, dieldrin and D.D.T. as larvicides (average of five replicate tests).

Insecticide.	Dosage in mg. per sq. ft. of water surface.	Number of days effective against *A. stephensi.*	Number of days effective against *C. fatigans.*
Chlordan ...	0·5	Not effective.	Not effective.
	1·0	" "	" "
	10·0	4	4
	20·0	11	8
	40·0	12	12
Aldrin ...	0·5	1	On the day of application.
	1·0	1	" " "
	10·0	7	7
	20·0	10	10
	40·0	36	17

TABLE I—*contd.*

Insecticide.	Dosage in mg. per sq. ft. of water surface.	Number of days effective against *A. stephensi.*	Number of days effective against *C. fatigans.*
Dieldrin ...	0·5	3	On the day of application.
	1·0	3	" " "
	10·0	15	8
	20·0	33	24
	40·0	56	47
D.D.T. ...	2·0	7	Not effective.

Field studies were not carried out, because larvicides have limited application in rural areas. Moreover, these insecticides are highly toxic to fish and other aquatic life and until definite observations are obtained on their toxicity to warm-blooded animals it was not considered safe to use them in the villages.

LABORATORY STUDIES AS ADULTICIDES.

Laboratory technique for testing the biological effectiveness of insecticidal deposits described by Pal (*loc. cit.*) was adopted to test the residual toxicity of these insecticides. Glass panels sprayed uniformly by a standardized technique are used for such tests. Sprayed panel is clamped in between two wooden boards. Five inches diameter area of the sprayed panel is exposed as the upper wooden board has a hole of this dimension. A clean glass panel is slid over the top of the superimposed board thus forming an enclosed space. Test insects are introduced in the space between the two glass panels by means of a glass tube fitted with a plunger arrangement.

Two separate experiments are carried out—firstly, to find out the knockdown time, i.e. the time in minutes required for 75 per cent and 100 per cent knockdown of test insects by constant contact with the sprayed surface, and secondly, the 24 hours' percentage mortality amongst the test insects after an exposure of 15 minutes.

Ten to 25 laboratory bred, two days' old *C. fatigans* ♀ ♀ mosquitoes were used in these tests.

Chlordan (74 per cent emulsifiable concentrate*), aldrin (aldrin, toluene and triton × 155† emulsifiable concentrate) and dieldrin (24 per cent emulsifiable concentrate*) were sprayed at the rate of 50 mg./sq. ft. The concentrates were diluted with water to obtain 2·5 per cent emulsions. For comparison similar tests were carried out with D.D.T.-aromex-soap emulsion† at a similar dosage.

* Supplied by Julius Hyman & Co., Denver 1, Colorado, U.S.A.

† Prepared at the Malaria Institute of India, Delhi.

Tests were repeated every week till the mortality results showed that the residual effectiveness had ceased. If the mortality amongst the test insects was less than 50 per cent, it was considered that the effect of the insecticide had worn off.

EXPERIMENTAL DATA.

Chlordan, aldrin and dieldrin were effective for 7, 7 and 8 weeks respectively, whereas D.D.T. was effective for 4 weeks only (Table II).

TABLE II.

24 hours' percentage mortality amongst the test insects C. fatigans ♀ ♀ *with different insecticides (average of five replicate tests).*

Weeks.	CHLORDAN 50 MG./SQ. FT.	ALDRIN 50 MG./SQ. FT.	DIELDRIN 50 MG./SQ. FT.	D.D.T. 50 MG./SQ. FT.
	24 hours' percentage mortality.	24 hours' percentage mortality.	24 hours' percentage mortality.	24 hours' percentage mortality
1	100	100	100	65
2	100	100	100	65
3	100	95	95	60
4	90	95	95	55
5	85	85	85	50
6	95	87	87	40
7	60	90	90	...
8	40	45	65	...
9	...	...	25	...
10	...	...	30	...

FIELD STUDIES.

Field studies were only confined to chlordan and dieldrin, as aldrin is mostly used against agricultural pests. Chlordan was used as a 74 per cent emulsifiable concentrate and dieldrin as 25 per cent dispersible powder. Both were diluted with water to a concentration of 2·5 per cent and sprayed at the rate of 2 c.c. per sq. ft. (50 mg./sq. ft.).

Three villages near Palwal, District Gurgaon, Punjab (India), were selected for this purpose. Two were treated with the insecticide and third was not treated for purposes of comparison. Particulars of the experimental villages, formulation and dosages of insecticides employed are shown in Table III. The houses in these villages are 'kutcha' with mud-plastered walls and thatched roofs. Spraying was carried out by experienced workers and under the direct supervision of one of the authors. Stirrup pump, fitted with nozzle of 3/64th inch orifice, giving a cone-shaped spray was used for spraying. In each village two suitable mosquito catching stations were established where routine collections were made to determine the density of mosquitoes. In 'treated' villages one of the catching stations was not sprayed with the insecticide. Total mosquito catches were made every week from these catching stations by spraying a weak solution of pyrethrum and collecting all the dead mosquitoes on a floor sheet.

TABLE III.

Particulars of the experimental villages, formulation and dosages of insecticides employed.

Name of village.	Number of houses.	Population.	Total area sprayed, sq. ft.	Insecticide and formulation used.	Dosage mg./sq. ft.	Quantity sprayed c.c./sq. ft.
Sarai ...	98	582	55,994	2·5 per cent chlordan emulsion.	50	2
Khatela ...	73	403	53,606	2·5 per cent dieldrin suspension.	50	2
Khusropur	102	511	Comparison village.	.....	...	...

ENTOMOLOGICAL DATA.

In Table IV are summarized the following entomological data:

(*a*) Number of mosquitoes collected after pyrethrum spray once a week.

(*b*) Percentage mosquito density as compared to the density before the start of the experiment.

(*c*) The ratio of mosquitoes caught from sprayed and unsprayed catching stations (s/us).

TABLE IV.

Total mosquito catch, percentage mosquito density as compared to pre-spraying density and ratio of mosquitoes caught from sprayed and unsprayed catching stations.

Weeks.	VILLAGE SPRAYED WITH CHLORDAN 50 MG./SQ. FT.				VILLAGE SPRAYED WITH DIELDRIN 50 MG./SQ. FT.				UNTREATED COMPARISON VILLAGE.	
	TOTAL MOSQUITO CATCH.		PERCENTAGE MOSQUITO DENSITY AS COMPARED TO PRE-SPRAYING DENSITY.	Ratio of mosquitoes collected from sprayed and unsprayed catching stations (s/us).	TOTAL MOSQUITO CATCH.		PERCENTAGE MOSQUITO DENSITY AS COMPARED TO PRE-SPRAYING DENSITY.	Ratio of mosquitoes collected from sprayed and unsprayed catching stations (s/us).	Total mosquito catch (average of 2 catching stations).	Percentage mosquito density as compared to prespraying density.
	Sprayed catching station.	Unsprayed catching station.	Sprayed catching station.		Sprayed catching station.	Unsprayed catching station.	Sprayed catching station.			
0	358	537	100	0·66	827	630	100	1·31	1,630	100
1	33	206	9·2	0·16	3	221	0·3	0·01	1,630	100
2	66	157	18·4	0·42	106	296	12·8	0·35	1,915	117·4
3	103	199	28·7	0·51	26	441	3·1	0·05	3,824	234·6
4	47	208	13·1	0·22	52	262	6·2	0·19	3,621·5	222·1
5	83	254	23·1	0·32	33	268	3·9	0·12	3,133·5	192·1
6	117	296	32·6	0·39	51	175	6·1	0·29	3,563	218·5
7	89	272	24·8	0·32	77	146	9·3	0·52	1,526·5	93·6
8	63	214	17·5	0·29	67	151	8·1	0·44	662	40·6

It would be seen from the data presented that there was a significant reduction in the number of mosquitoes both in the sprayed and unsprayed catching stations of treated villages. But after 3 weeks the number of mosquitoes gradually increased though not to the extent of 50 per cent of the pre-spraying density. During the 6th week the mosquito density was 32·6 per cent in the catching station treated with chlordan, whereas in the catching station treated with dieldrin the mosquito density remained much below the 50 per cent level throughout the period of observation which extended to 8 weeks after spraying. It cannot be definitely stated, however, that dieldrin was effective for eight weeks or more because during the 7th week, there was a decline in the mosquito population in that area due to natural causes.

Residual toxicity of sprayed surfaces was also determined by 'wall cage' test described by Simmons *et al.* (1945) by using wall cages made of cellophane paper. *C. fatigans* ♀ ♀ mosquitoes were exposed to the sprayed surface in the cages for 15 minutes and percentage mortality amongst them was noted after 24 hours (Table V). With chlordan and dieldrin more than 50 per cent of the mosquitoes survived during the 3rd and 6th weeks respectively.

TABLE V.

24 hours' percentage mortality in C. fatigans ♀ ♀ *on exposure to wall surfaces sprayed with chlordan and dieldrin for 15 minutes (average of five replicate tests).*

Weeks.	Chlordan 50 mg./sq. ft. Percentage mortality in mosquitoes.	Dieldrin 50 mg./sq. ft. Percentage mortality in mosquitoes.
1	80	100
2	65	95
3	25	90
4	35	95
5	30	95
6	25	35
7	25	45

DISCUSSION.

Laboratory studies showed that chlordan applied at the rate of 0·5 mg. and 1 mg. per sq. ft. of water surface was ineffective against *A. stephensi* and *C. fatigans* larvæ. At dosages of 10 to 40 mg./sq. ft. of water surface it was effective for 4 to 12 days. D.D.T. applied at the rate of 2 mg. per sq. ft. of water surface was effective only against *A. stephensi* larvæ for about a week. Both aldrin and dieldrin were found to be effective for a longer period than chlordan and D.D.T. Residual

larvicidal effect of aldrin and dieldrin was well marked against both *A. stephensi* and *C. fatigans* specially at dosages of 10 mg. or more per sq. ft. of water surface. Dieldrin was more toxic to larvæ than aldrin. Keller *et al.* (1951) and Stage (1951) have also pointed out that dieldrin is more toxic to mosquito larvæ than D.D.T., chlordan and aldrin.

Laboratory tests showed that chlordan, aldrin and dieldrin exhibited residual toxicity for 7, 7 and 8 weeks respectively against *C. fatigans* when applied at the rate of 50 mg./sq. ft., while D.D.T. at the same dosage (50 mg./sq. ft.) was effective for 4 weeks only. Field studies on the residual toxicity of these insecticides are inconclusive because of the sudden drop in the mosquito population in this area due to natural causes during the period of observation. Preliminary data, however, indicate that dieldrin was effective for at least seven weeks. D.D.T. under similar conditions and dosage was found to be effective for about five weeks (Jaswant Singh *et al.*, 1951). Further trials in the field are, however, necessary to determine the superiority or otherwise of these insecticides as compared to D.D.T.

SUMMARY AND CONCLUSIONS.

1. Laboratory and field studies on the residual toxicity of chlordan, aldrin and dieldrin against larvæ and adult mosquitoes have been described.

2. Tests were carried out in the laboratory against *A. stephensi* and *C. fatigans* larvæ. The dosages employed were 0·5 mg., 1 mg., 10 mg., 20 mg. and 40 mg. per sq. ft. of water surface.

3. These insecticides were only effective for a couple of days when applied at smaller dosages but at dosages of 10 mg. or more per sq. ft. of water surface they exhibited prolonged residual toxicity. Dieldrin was found to be more toxic to mosquito larvæ than either aldrin or chlordan. Results were compared with D.D.T. application.

4. In the laboratory, chlordan, aldrin, dieldrin and D.D.T. (each at 50 mg./sq. ft.) were found to be effective for 7, 7, 8 and 4 weeks respectively against *C. fatigans* ♀ ♀ mosquitoes.

5. In the field, chlordan and dieldrin (each 50 mg./sq. ft.) were found to be effective against mosquitoes for at least 6 to 7 weeks.

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OBSERVATIONS ON ANOPHELINE POPULATIONS IN HUMAN DWELLINGS IN SOUTHERN TAIWAN (FORMOSA).

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INTRODUCTION.

THE prospect of an extensive malaria control programme in Taiwan, employing residual spraying methods, has emphasized the lack of specific information regarding the house-frequenting habits of potential vector anophelines. The studies of Chow, Watson and Chang (1950) on natural infectivity of Formosan mosquitoes reported dissections of 4,256 females collected from houses, but included no information regarding specific resting places where the specimens were collected. The present study was undertaken to

(*a*) confirm the use of houses as daytime resting places of local anophelines, and

(*b*) determine, based on typical house construction patterns, specific resting places requiring coverage in residual spraying operations.

FIELD AND HOUSING CONDITIONS.

The general features of Southern Formosan topography and meteorology have been described by Chang, Watson and Chow (1950).

Houses in rural areas are aggregated in distinct villages, and are clustered along streets or paths of somewhat irregular pattern. House design is stereotyped, and resembles that of South China (Plate IV, fig. 1).

One, two or three houses may be built around a level court area of packed earth or of concrete. Construction materials include brick, adobe-like brick ('to-kat'), bamboo and plaster, bamboo and mud, or combinations in the same dwelling. Roofs are generally thatched, with tile often used on houses of brick construction. The irregularities in junction of roof and walls usually include gaps measuring more than 3 by 25 cm. Ceilings, with rare exceptions, consist of the underside of the sloping roof. Floors are generally of packed earth, although concrete may be used in houses of more expensive construction.

The typical rural house is rectangular in outline, measuring approximately 5 by 13·5 metres. It consists of five rooms, including a central sitting room, reached through an outside door, two adjacent bed rooms with entrances from the sitting room, and two end rooms intended as kitchen and bed room, and reached through outside doors. Number of occupants per house may vary from three to more than 20, and the store room is frequently modified to provide an additional sleeping room.

The typical bed room has a raised platform, 0·5 to 0·6 metre above the floor, occupying one-fourth to one-third of the room. The area beneath this sleeping platform is normally accessible, and may be used for storing small items (Plate IV, fig. 2). In some wealthier homes the sleeping platform is part of an elaborate structure including a wooden canopy, bedding shelf, and fixed or sliding panels shutting off the sub-platform space (Plate V, fig. 1).

Bed-nets are not in general use. When present, they are rolled compactly during the day. Closets are not present. A desk or cabinet may complete the bed-room furnishing. Clothing is normally hung from horizontal bamboo poles or on rope or wire lines.

The highly stereotyped sitting-room furniture includes a shelf-like table, 1·8 to 2·2 metres in length, on which are placed the utensils for observance of religious customs. In front of and at a slightly lower level than this structure is another table approximately one metre square.

This table or a counterpart may be used at meal time as a dining table. Other sitting-room furniture is usually limited to simple benches, a desk, and miscellaneous bamboo or earthenware vessels.

The store room, unless converted into sleeping quarters, houses a variety of household and agricultural implements, straw or burlap bags, bundles of fuel, and often a large woven bamboo granary for bulk storage of rice.

The kitchen is frequently of loose bamboo construction in less wealthy homes, as well as those more substantial houses where the original kitchen room has been modified for sleeping purposes, and an additional room has been built. Furniture is usually limited to a bamboo food cabinet, a small table, and the built up hearth where the cooking utensils are heated directly over an open fire. The fast-burning fuels commonly used produce much smoke, and no chimney is provided. Kitchens therefore undergo partial fumigation several times daily, and heavy soot deposits accumulate on the walls and ceiling.

PLATE IV.

Fig. 1.—Hsiang Tan (鉤潭), a foothill village with high populations of *A. minimas*. Note rural house of bamboo and plaster.

Fig. 2. Simple sleeping platform in bed room, showing under-bed harbourage.

PLATE V.

Fig. 1. Elaborate sleeping platform, with bed canopy, and panels enclosing under-bed area.

Fig. 2.—Native fibrous raincoat, a common daytime resting place of mosquitoes.

Enclosures sheltering water buffalo, cows, pigs or poultry frequently adjoin the houses or are located within 10 to 20 metres.

SPECIFIC VILLAGES STUDIED.

The three villages selected for study are characterized by considerable differences in topography and agricultural pattern. Li She is a plains village, of 216 houses (population 1,250) surrounded by ricefields irrigated from artesian wells. Hsiang Tan is a village of 197 houses (population 1,140) situated close to the mountains on rocky foothill terrain (Plate IV, fig. 1). Ricefields are limited. The area is relatively dry, with the exception of streams of moderate to rapid velocity. Wan Lung is a village of 209 houses (population 1,172) less proximate to the mountains than Hsiang Tan, and on similar rocky terrain. A limited irrigation system permits cultivation of rice and sugarcane.

METHODS.

Collections were made in houses selected at random in three villages during the period from September 22 to October 2. After preliminary instruction and practice collections to standardize technique, six mosquito technicians of the Malaria Institute assisted the authors in the study. Specimens were collected in test tubes, specific localities were noted, and females were identified and held 5 to 7 days in the insectary before dissection and examination of gut and salivary glands.

After establishment of arbitrary categories describing resting places, some interpretations and definitions were needed.

(*a*) *Walls* were examined for specimens resting from the floor up to one metre, from 1 to 2 metres, and above 2 metres.

(*b*) *Under-bed* specimens were those captured by laboriously crawling beneath the sleeping platforms. Specimens on walls under beds were considered ' under-bed '.

(*c*) *On clothes* specimens were those resting on or among the folds of garments, whether the clothing was hung from hooks on the wall, or from poles, wires or ropes remote from the walls.

(*d*) *In containers* specimens were those found in baskets, crocks, boxes, or similar resting places.

(*e*) *Under shelves* specimens were those under horizontal portions of cabinets, tables, mantels (shin-ui) and desks. Where window openings in thick walls afforded deep recesses, specimens resting therein were included as ' under shelves '.

(*f*) *On ceiling* specimens were those collected on the sloping lower side of the roof thatching, rafters supporting the thatching, or on the horizontal ceilings of those few houses with this structural refinement. In those bed rooms with wooden canopies above the beds, specimens collected under these false ceilings were considered as ' on ceiling '.

(*g*) *On cobwebs*, etc., specimens included those collected from spider-webs, strings, ropes, loose straw, or on the long fibres of the native raincoat (Plate V, fig. 2).

RESULTS AND DISCUSSION.

Systematic and painstaking collections from human habitations confirmed the extensive use of such daytime resting places by Formosan anophelines of local importance. Of the five species of female Anopheles collected (*A. minimus*, *A. ludlowii*, *A. hyrcanus* var. *sinensis*, *A. tessellatus*, and *A. subpictus* var. *indefinitus*), only the first three were found in significant numbers. These three species showed marked variations in proportions of total mosquito population among the three villages studied. The foothill village of Hsiang Tan yielded large populations of *A. minimus*, moderate numbers of *ludlowii*, and small numbers of *hyrcanus* var. *sinensis*. In Wan Lung, the *ludlowii* populations exceeded the *minimus*, while *hyrcanus* var. *sinensis* again held third place. The village characterized by extensive wet-rice cultivation (Li She) yielded maximum numbers of *hyrcanus* var. *sinensis*, limited numbers of *minimus*, and no *ludlowii*.

Two of the house areas included in the detailed collection routine ('kitchens' and 'under eaves') were relatively sterile. Specimens were rare in kitchens except when that room adjoined cow-stables. Eaves, usually exposed to moderate light intensities and wind action, harboured specimens only in exceptional instances where the overhanging eaves of adjacent houses afforded cave-like protection (Plate VI, fig. 1).

As shown in Tables I to III, the three house areas most interesting from the standpoint of population level and proportionate species distribution were the bed rooms, sitting rooms and store rooms.

In all villages, collections were highest from bed rooms, moderate from sitting rooms, and lowest from store rooms. On a specific basis, the populations of *minimus* showed the same trend in all villages, while *hyrcanus* var. *sinensis* showed variance only in Li She where the sitting room-store room relationship was reversed. This variance was attributable to abnormally high collections of 57 and 62 *hyrcanus* var. *sinensis* in store rooms of two houses located next to cow-stables.

Within bed rooms the favourite resting place was the under-bed area. Next in numbers of specimens harboured were the bed room ceilings, the lower metre of the walls, and the area under shelves (including desks).

In sitting rooms maximum collections were made under shelves (mantel and adjacent table), lower walls, and on cobwebs in the *hyrcanus* var. *sinensis* village Li She.

Store rooms were relatively low in anopheline populations except for Li She, where *hyrcanus* var. *sinensis* averages were raised by high recoveries in several store rooms near cowsheds.

The tabulations of the mosquito populations resting in various rooms and parts of rooms emphasized apparent mosquito preferences for some of these locations. The applicability of such mosquito population differences to residual control operations is rather obvious. Preliminary insecticidal trials preceding the extensive control operations may, on the basis of the present findings, give priority to spraying of bed rooms, and to limited portions of sitting rooms, such as the areas under shelves, desks and tables. Store rooms and kitchens adjacent to cow-stables may harbour anopheline population requiring spray treatment, although the low

PLATE VI.

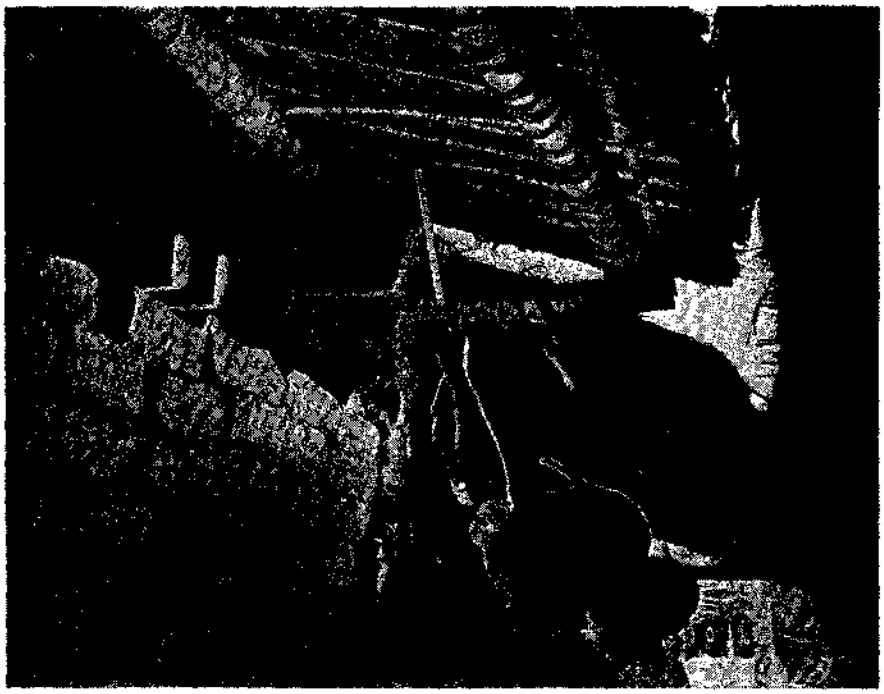

Fig. 1.—Mosquito harbourage formed by eaves of adjacent houses.

population in these rooms under normal circumstances may not justify insecticidal coverage. Preliminary trials should be run to determine whether selective residual spraying of favoured resting places will effectively reduce the number of mosquitoes in the houses, or merely alter their distribution. The possibilities of control through spraying only the bed rooms should be explored by field tests*. Precipitin tests of mosquito blood meals of specimens collected from store rooms, kitchens and sitting rooms would throw definite light on sources of such populations and the importance of including their resting places in the residual spraying operations.

Dissections and examinations of gut and salivary glands were made on 1,267 specimens (965 *hyrcanus* var. *sinensis*, 230 *minimus*, 66 *ludlowii*, 5 *tessellatus*, and 1 *subpictus* var. *indefinitus*). Of this series, 1 *hyrcanus* var. *sinensis* from Li She had an immature oöcyst on the gut. The present malaria incidence in the test villages is low (3 to 8 per cent); the probability of finding infested mosquitoes was relatively small. *A. minimus* has been previously proved an important malaria vector in Taiwan, particularly in foothill regions (Chow, Watson and Chang, 1950). The transmission rôle of *A. hyrcanus* var. *sinensis* needs further confirmation. Of the eight positive glands recorded for this species in the paper cited above, the single slide now retained at the Ch'ao Chow Branch Laboratory of the Taiwan Malaria Institute was re-examined and was found to consist of Crithidial forms of an unknown flagellate instead of the sporozoite identification previously reported.

SUMMARY.

1. Morning collections of mosquitoes resting in houses of three southern Taiwan villages were made to determine daytime resting places among various rooms and within individual rooms.

2. Features of house construction, room arrangement and furnishings were described.

3. Specimens from one plains village and two foothill villages were identified and were dissected after 5 to 7 days. One oöcyst was found on the gut of one among 965 *A. hyrcanus* var. *sinensis* specimens examined; 230 *A. minimus*, 66 *A. ludlowii*, and 6 other females being negative.

4. Only *A. minimus*, *A. ludlowii* and *A. hyrcanus* var. *sinensis* were found indoors in significant numbers, varying in proportion among the three villages.

5. Bed rooms, sitting rooms and store rooms yielded mosquito populations in declining order. Kitchens and caves were relatively sterile.

6. Maximum numbers of specimens were found under beds in sleeping quarters. Preferences for specific locations in various rooms were discussed.

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* Definite information regarding residual spraying in selected rooms in houses, or favoured resting places inside rooms, capable of effectively reducing the number of mosquitoes in houses or altering their distribution is required. The consensus of opinion seems to be that limited selective spraying may not be successful in certain cases. It has been rightly suggested by the authors that field trials should be undertaken.—*Editor.*

7. Use of the findings for guidance in residual spraying operations was discussed.

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TABLE I.

HSIANG TAN VILLAGE.

Female Anopheles from morning collections, 20 houses, September 27 to October 1, 1951. Average number per house.*

	Bed rooms.			Sitting room.			Store room.			Totals.		
	sinensis.	*minimus.*	*ludlowi.*	*sinensis.*	*minimus.*	*ludlowi.*	*sinensis.*	*minimus.*	*ludlowi.*	*sinensis.*	*minimus.*	*ludlowi.*
0-1 metre ...	0·10	2·30	1·10	0·15	1·20	2·40	0·00	0·00	0·00	0·25	3·50	3·50
On walls 1-2 metres ...	0·00	0·65	0·10	0·00	0·40	0·55	0·00	0·00	0·00	0·00	1·05	0·65
above ...	0·00	0·05	0·10	0·00	0·00	0·05	0·00	0·00	0·00	0·00	0·05	0·15
Under bed(s) ...	0·20	6·55	0·80	...	...	...	...	...	...	0·20	6·55	0·80
On clothes	0·00	0·85	0·15	0·00	0·60	0·45	0·00	0·00	0·00	0·00	1·45	0·60
In containers ...	0·00	0·60	0·10	0·05	1·60	0·75	0·00	0·00	0·00	0·05	2·20	0·85
Under shelves ...	0·10	2·80	0·30	0·05	4·55	1·00	0·00	0·05	0·00	0·15	7·40	1·30
On ceiling	0·05	4·25	0·15	0·00	0·20	0·00	0·00	0·00	0·00	0·05	4·45	0·15
Cobwebs, etc. ...	0·35	1·70	0·55	0·25	0·50	0·15	0·00	0·00	0·00	0·60	2·20	0·70
TOTALS ...	0·80	19·75	3·35	0·50	9·05	5·35	0·00	0·05	0·00	1·30	28·85	8·70

* 3 *A. subpictus* var. *indefinitus* from bed rooms, and 4 *A. subpictus* var. *indefinitus* from sitting rooms have been omitted from these tabulations.

TABLE II.

WAN LUNG VILLAGE.

Female Anopheles from morning collections, 11 houses, October 1-2, 1951. Average number per house.

	Bed rooms.			Sitting room.			Store room.			Totals.		
	sinensis.	*minimus.*	*ludlowii.*	*sinensis.*	*minimus.*	*ludlowii.*	*sinensis.*	*minimus.*	*ludlowii.*	*sinensis.*	*minimus.*	*ludlowii.*
0-1 metre ...	0·09	0·36	1·27	0·09	0·00	3·00	0·00	0·18	0·55	0·18	0·54	4·82
On walls 1-2 metres ...	0·00	0·18	0·45	0·00	0·00	0·27	0·00	0·00	0·27	0·00	0·18	1·00
above ...	0·00	0·00	0·18	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·18
Under bed(s) ...	0·36	1·09	1·45	...	...	...	...	...	...	0·36	1·09	1·45
On clothes	0·18	0·27	0·55	0·00	0·00	0·00	0·00	0·00	0·00	0·18	0·27	0·55
In containers ...	0·00	0·00	0·18	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·18
Under shelves ...	0·00	0·09	0·00	0·00	0·18	1·00	0·00	0·09	0·09	0·00	0·36	1·09
On ceiling	0·55	0·64	1·00	0·00	0·00	0·18	0·00	0·00	0·09	0·55	0·64	1·27
Cobwebs, etc. ...	0·09	0·00	0·18	0·00	0·00	0·00	0·09	0·00	0·00	0·18	0·00	0·18
TOTALS ...	1·27	2·63	5·27	0·09	0·18	4·47	0·09	0·27	1·00	1·45	3·08	10·72

TABLE III.

LI SHE VILLAGE.

Femule Anopheles from morning collections, 41 houses, September 22 to October 2, 1951. Average number per house.*

	Bed rooms.			Sitting room.			Store room.			Totals.		
	sinensis.	*minimus.*	*ludlowii.*	*sinensis.*	*minimus.*	*ludlowii.*	*sinensis.*	*minimus.*	*ludlowii.*	*sinensis.*	*minimus.*	*ludlowii.*
0-1 metre ...	0·34	0·10	0·00	0·51	0·00	0·00	0·24	0·02	0·00	1·09	0·12	0·00
On walls 1-2 metres ...	0·34	0·02	0·00	0·00	0·00	0·00	0·17	0·00	0·00	0·51	0·02	0·00
above ...	0·10	0·02	0·00	0·02	0·00	0·00	0·02	0·00	0·00	0·14	0·02	0·00
Under bed(s) ...	0·54	0·24	0·00	...	...	...	...	...	...	0·54	0·24	0·00
On clothes	0·46	0·00	0·00	0·02	0·00	0·00	0·02	0·00	0·00	0·50	0·00	0·00
In containers ...	0·05	0·07	0·00	0·19	0·00	0·00	0·15	0·00	0·00	0·39	0·07	0·00
Under shelves ...	0·29	0·17	0·00	0·37	0·07	0·00	0·12	0·00	0·00	0·78	0·24	0·00
On ceiling	0·54	0·12	0·00	0·07	0·00	0·00	1·00	0·00	0·00	1·61	0·12	0·00
Cobwebs, etc. ...	1·85	0·10	0·00	0·68	0·00	0·00	2·75	0·00	0·00	5·28	0·10	0·00
TOTALS ...	4·51	0·84	0·00	1·86	0·07	0·00	4·47	0·02	0·00	10·84	0·93	0·00

* 2 *A. tessellatus* and 1 *A. subpictus* var. *indefinitus* from bed rooms, 1 *A. tessellatus* from sitting room, and 1 *A. subpictus* var. *indefinitus* from store room have been omitted from these tabulations.

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CONTROL OF MALARIA IN SARDA HYDEL POWER-HOUSE CONSTRUCTION.

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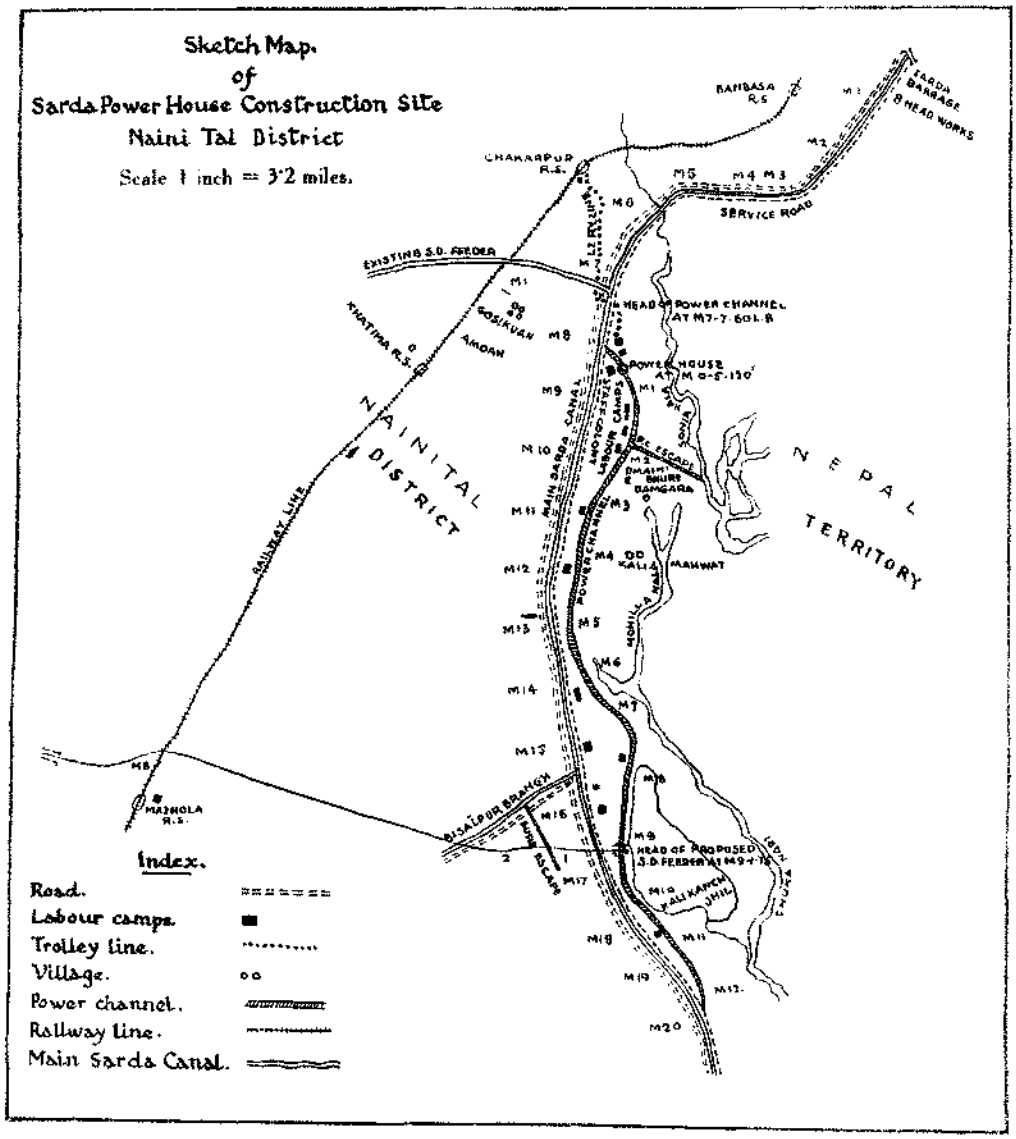
INTRODUCTION.

WITH a view to generate cheap power for agricultural and industrial development, the Government of Uttar Pradesh planned to construct a hydro-electric power-house, about 7 miles south of Sarda Canal Headworks at Banbassa, District Naini Tal, by diverting the main Sarda Canal into a power-channel on an adjacent cliff. Thus, approximately 12 miles length of the main canal will be abandoned after the completion of work. Further, to augment the supply of water in the power-channel, the present Sarda-Dehoa feeder which emerges from above the power-channel take-off will also be given up and a new feeder from the power-channel will be constructed lower down.

The magnitude of the project can be gauged to some extent from the fact that the work is estimated to cost approximately Rs. 7·5 crores and take about 7 years to complete. Another important feature of this work is that the foundations of the power-house have been laid about 90 feet below the sub-soil water-level, which at this site varies from 28 to 30 feet. The dug-out in which the foundations have been laid is kept dry by working several electrically-driven tube-wells constantly throughout the year and discharging the water through the power-channel escape ultimately into the River Sonia.

The work extends over an area of 10 square miles. This has been divided for administrative convenience into two divisions, namely, (1) the power-house


Sketch Map.
of
Sarda Power House Construction Site
Naini Tal District
Scale 1 inch = 3·2 miles.
BANBASA R.S.
SARDA BARRAGE & HEAD WORKS
CHAKARPUR R.S.
SERVICE ROAD
EXISTING S.D. FEEDER
HEAD OF POWER CHANNEL AT M7-7-60L.R
POWER HOUSE AT M 0-5-130'
KHATIMA R.S.
BOSIKUAN
AMOAN
NAINITAL DISTRICT
NEPAL TERRITORY
RAILWAY LINE
MAIN SARDA CANAL
POWER CHANNEL
LABOUR CAMPS
SONIA
KALI
MAHWAT
BAGARA
MAJHOLA R.S.
BISALPUR BRANCH
HEAD OF PROPOSED S.D. FEEDER AT M9-1-16
KALI KAPCH JHIL
Index.
Road.
Labour camps.
Trolley line.
Village.
Power channel.
Railway line.
Main Sarda Canal.

construction division, and (2) the power-channel construction division. Each of these is under the charge of a separate Executive Engineer.

The power-house site lies in the notoriously unhealthy belt of the Tarai of Naini Tal District. The nearest railway station is Chakrapur on Pilibhit-Tanakpur Section of the Oudh Tirhut Railway at a distance of about 3½ miles from power-house site and connected with it by means of a fair weather motorable road and a trolley line. A fairly big colony consisting of labourers' camps, staff quarters, offices, workshops, etc., has sprung up at the power-house site after clearing dense forests. The staff has been accommodated in temporary mosquito-proof brick-built quarters which are fairly comfortable and provided with piped water-supply, electricity, and sanitary conveniences. Waste water and sullage are disposed of into a natural watercourse outside the colony by means of a perimeter drain.

Contractors have provided their labourers with thatched huts and other sanitary conveniences. Bulk of the labour consists of adults as well as children imported from various parts of India. With the extension of works on the power-channel section, a second colony is also springing up about 3 miles south of the first colony. On completion of works, permanent residential accommodation will also be provided at the power-house site for a limited number of staff who will be stationed there.

WORKING SEASON.

In a work like this, it is of utmost importance that the working season should be extended to the maximum, as this enables the work to be carried out much more economically. This could only be possible by adopting measures to keep the labour healthy and fit to work. Speaking about the construction of the Sarda Canal Headworks at Banbassa, about 7 miles north of the present site of the power-house, Sir B. Darley, the Chief Engineer-in-Charge of the construction of Sarda Canal Headworks, says :—

'It is very difficult to estimate the economic value of the malaria staff at Banbassa in rupees, annas and pies. The work that has been done has certainly enabled the working season to be extended from April 15 to, say, June 15, i.e. two months each or 10 months in 5 years; in other words, work will thus be able to dispense with the services of about half the divisional staff a year earlier, a saving of Rs. 50,000/-.

'If, however, we take into account the value of delivering water one year earlier for irrigation, *the monetary value of the antimalarial work would probably run to half a crore of rupees.*

'Secondly, *by keeping the labour fit, it has been possible to inspire confidence, and good contractors have come forward to take up the work at lower rates* than was deemed possible at first.

'I have gone over the estimates and putting this saving at rupee one to rupees two per cubic foot of masonry, etc., this saving might be fairly estimated at Rs. 1,50,000/-, i.e. of course, assuming that any contractors would have taken up

the work at all under the adverse circumstances obtaining before the antimalarial staff got to work. *Indeed, it is very doubtful if the Sarda Canal Headworks could have been built at all without the expert help we have received from the medical department.*'

In 1947, the working season in the present project was about 7 months only. The notoriety of the area for extreme malariousness was responsible for the work to be closed down during the monsoon months. In 1948, the Antimalaria Organization was housed at the site of works permanently. This in conjunction with the benefits from malaria control measures inspired confidence of well-being amongst the entire population and, as a result, the working season was extended to 12 months in 1948 and thereafter. This enabled the engineers to carry on with construction work throughout the year and thus lay the foundations of the power-house at proper time. Had this continuity been disturbed, the progress once made in lowering the sub-soil water-level would have been nullified and the same process gone through over and over again with the possibility of never completing the laying of the foundations and a considerable loss to Government.

TOPOGRAPHICAL FEATURES.

The site on which the project has been launched is situated in the notoriously unhealthy Tarai tract of Naini Tal District and is covered by thick *sāl* jungles. It is intercepted with depressions, winding streamlets, springs and swamps. On the west of the cliff on which the power-channel runs, there is a small perennial rivulet called Chukanala. In its upper reaches, it is a small winding sluggish stream with shady banks, while in its lower curves it presents a huge swampy basin with small patches of cultivations seen here and there. Usually in between the watercourses, the land is undulating, rising as it leaves one and falling as it approaches the next. The communications in this tract are extremely poor.

HYDROGRAPHICAL FEATURES.

The soil consists of gangetic alluvial deposit, the top layer of which is covered with loam and is retentive of moisture. The sub-soil water-level varies from 6 feet in the proximity of the Chukanala to 30 feet on the ridge.

The drainage system of this tract has been rightly compared to ' a reticulation of leaf, the streamlets of the edge of moist country uniting to form a larger channel which in turn feeds the arterial line of drainage to eventually join a bigger mid-stream '. The streams are usually blocked in their courses with overhanging vegetation and trees to an inconceivable extent, thus diminishing their velocity and adding greatly to the already existing malariogenic conditions.

METEOROLOGICAL DATA.

Rainfall.—Rainy season starts earlier in the Tarai than in the plains. Table I shows monthwise rainfall for the area for the years 1948, 1949 and 1950. July, August and September are usually the wettest months of the year.

TABLE I.

Rainfall in inches.

Month.	Average for 1939–43.	Year.		
		1948.	1949.	1950.
Jan. ...	1·07	0·0	0·0	1·4
Feb.	1·08	1·6	0·0	0·9
Mar. ..	0·73	1·4	5·5	0·0
Apr.	0·16	0·0	0·2	0·0
May ..	0·56	0·2	0·6	1·8
Jun. .	7·52	0·0	0·2	22·4
Jul. ..	12·88	19·1	0·8	21·81
Aug.	13·09	31·8	27·5	26·73
Sep.	5·43	20·8	12·4	3·85
Oct. ...	0·33	4·5	10·4	0·0
Nov. .	0·00	0·0	3·6	0·0
Dec. ...	0·66	0·0	0·0	0·0
TOTAL ..	44·41	80·3	61·2	78·89

TEMPERATURE AND HUMIDITY.

The mean monthly dry-bulb temperature and mean monthly relative humidity for the years 1948, 1949 and 1950 are given in Table II. It will be seen therefrom that the temperature never goes up above 100°F. June is the hottest month and January and December the coldest. Humidity is high throughout the year.

TABLE II.

Temperature and humidity.

Month.	1948 (MONTHLY MEAN).		1949 (MONTHLY MEAN).		1950 (MONTHLY MEAN).	
	Dry bulb, °F.	Relative humidity percentage.	Dry bulb, °F.	Relative humidity percentage.	Dry bulb, °F.	Relative humidity percentage.
Jan. ...	55·5	90·5	59·4	90·0	55·9	88·7
Feb. ...	64·5	91·5	62·2	90·6	56·2	89·2
Mar. ...	67·2	90·5	69·1	83·4	65·5	85·5
Apr. ...	78·5	81·2	77·4	84·1	73·0	77·2
May ...	85·0	77·0	83·6	79·4	79·9	70·2
Jun. ...	89·5	72·0	86·5	74·7	85·0	63·0
Jul. ...	84·7	89·5	83·2	85·0	82·2	90·5
Aug. ...	83·0	90·2	82·2	92·0	81·9	92·0
Sep. ...	83·1	89·0	81·4	91·3	80·5	89·0
Oct. ...	76·5	91·3	76·4	86·4	73·0	76·0
Nov. ...	66·5	90·2	63·5	88·0	72·0	67·0
Dec. ...	59·0	90·3	54·5	89·2	59·8	80·4

Climate.—The climate of this tract is greatly influenced by the peculiar nature of the soil and presence of thick forests. High humidity ranging from 60 to 90 per cent and conspicuous variations in day and night temperatures which are as much as 30°F. are the marked features of the climate. The *District Gazetteer* of Naini Tal says 'from about the end of April the climate begins to be dangerous which in the rains is positively deadly; in the hot weather and the rains there has been history of peculiarly malignant type of malaria fever amongst the immigrants'.

The climate, on the whole, can be summarized as hot, muggy, enervating and malarious.

PEOPLE AND SOCIO-ECONOMIC CONDITIONS.

The life in this tract like that in the rest of the Tarai, on the whole, is one of continual struggle for existence against depredations of wild animals, rank and vigorous vegetation, enervating climate, bad drinking water and malaria with resulting high general and infantile mortality and low birth rate.

There are no habitations within half-a-mile perimeter of the power-house site but a number of villages are located along the Sarda Canal and the present Sarda-Dehoa feeder. These villages are inhabited by two different sets of races, locally known as Tharus and Bhuksas, and Desis, respectively. The Desis were lured to settle in this tract from the neighbouring over-populated districts of the plains due to highly fertile nature of the Tarai soil. The Tharus and Bhuksas being good cultivators are economically better off than the Desis. In addition to these two types of people, a third type of migratory population from the neighbouring hills visits Tarai in winter months for grazing cattle and selling milk products. They live in temporary hutments called '*gots*' along with their cattle and some of them work as labourers with the forest and P.W.D. contractors in this tract and go back to the hills on the onset of summer.

Agriculture is the main occupation of the people. Paddy is the chief crop. Houses are usually made of thick walls without any provision for light or ventilation. Cattlesheds are situated either inside the houses or just close by. The surroundings are usually insanitary and rooms overcrowded. Night watching of the fields to protect the crops against depredations of wild animals and hard labour, which one has to put in for cultivation of fields, are partly responsible for lowering the natural body resistance of the population against diseases, particularly at the harvest time in the months of October and November.

VITAL STATISTICS.

Efforts made to collect and study the statistical data from the registers maintained by village headmen did not meet with success as no proper records were maintained by them. Malaria is undoubtedly the chief cause of morbidity and mortality in the area.

Relative healthiness of Tharus and Bhuksas as compared to Desis is evidenced by a high birth rate and low mortality rate amongst them.

MALARIA PROBLEM.

As already stated, two different races of people, viz. Tharus and Bhuksas, and Desis, inhabit this tract, and the former thrive well and the latter do not. It is not easy to give a straight account of epidemiological features of this area in relation to malaria. The area has been known since very long to be notoriously unhealthy on account of highly endemic malaria. The spleen rate ranges from

70 to 100 per cent and the degree of splenic enlargement amongst the residents is abnormally high.

Table III gives an account of the studies of the spleen and blood examinations of both the races inhabiting this tract. These records are based on the observations made by Robertson (1909), Phillips (1924), Clyde (1931), Das (1930) and Diwan Chand (1944) from time to time in respect of this area.

TABLE III.

Summary of observations on malaria incidence in children and adults of different racial groups in Naini Tal Tarai.

Races.	Robertson (1909).		Phillips (1924).		Clyde (1931).		Das (1930).		Diwan Chand (1944).	
	Tharus and Bhuksas.	Desis.	Tharus and Bhuksas.	Desis.	Tharus and Bhuksas.	Desis.	Tharus and Bhuksas.	Desis.	Tharus and Bhuksas.	Desis.
(1) Spleen rate, per cent.										
Children ..	74·1	83·0	73·7	95·0	100·0	61·0	70·0	90·0	72·0	88·0
Adults ..	16·4	81·6	N.A.	N.A.	16·0	28·0	N.A.	N.A.	N.A.	N.A.
(2) Parasite rate, per cent.										
Children ...	N.A.	N.A.	10·0	24·0	85·0	N.A.	11·1	17·9	N.A.	18·1
Adults ...	N.A.	N.A.	N.A.	N.A.	9·0	N.A.	N.A.	25·8	N.A.	12·0

N.A. = not available.

In spite of high spleen rates prevailing amongst Tharu and Bhuksa children, their physical condition, when they become adults, is comparatively far better than that of Desis. The presence of small number of children amongst Tharus and Bhuksas bears evidence to the fact that they have suffered heavy sacrifices for the privilege of living in this unhealthy country. The explanations which could be offered in this respect is that soon after birth, children amongst Tharu and Bhuksa communities are exposed to malarial infections and reinfections by the local strains

of plasmodium resulting in a considerable degree of tolerance amongst those who survive. Phillips (1924) had observed that in Tharu and Bhuksa children the spleen does not attain the same enormous size as in Desi children and that the size begins to reduce from about 6 to 7 years of age. That the growing tolerance also appears to help in the disappearance of gametocytes from the infected individuals also seems to have been observed.

The history of Desi population, on the other hand, is a different story. The spleen and parasite rates are very high, which bear evidence to their poor physical state of health. The population is only kept up by constant influx of more people who are lured to this fertile area from the neighbouring over-populated plains.

It also seems probable that the relative degree of tolerance is slower to develop amongst Desis, than in Tharus and Bhuksas, because the former contract malarial infection on immigration to the Tarai at a comparatively advanced age and not from the very birth as is the case with the latter. The possibility of the new strains of malarial parasites being introduced into Tarai from the neighbouring districts through the immigrant Desi population cannot be ruled out altogether.

Why the Desi population, who have been resident in this part of the country for generations, has not acquired the same tolerance against malaria as in the case of Tharus and Bhuksas, also needs explanation. It is only within comparatively recent times that an attempt has been made by the Desi community to stay permanently in Tarai whereas the Tharus and Bhuksas have been living in this tract continuously for centuries. The immigrant nature of Desis and their tendency to intermarry with the new arrivals from the plains and their constant intermingling with their relatives and friends in their original homes outside Tarai, makes them liable to be infected and reinfected from strains of parasites other than those prevailing in the Tarai—a factor which seems to be responsible for a condition of acute endemicity.

It is generally conceded that the Tharus and Bhuksas, being economically better off than the Desis, live and eat better. Their houses are built of wattle with a very thin coating of mud which dries rapidly after the cessation of the rains while Desis live in houses made of thick and deep mud structures which become thoroughly saturated during the rains and take a long time to dry. Tharus and Bhuksas usually sleep indoors with doors closed all the year round and own a great number of cattle which are kept by them in cattlesheds located just adjacent to their individual huts. Desis, on the other hand, sleep in summer in front of their houses and possess a small number of cattle. These factors might also be partly responsible for the greater degree of protection afforded to Tharus and Bhuksas against malaria than in their brethren the Desis.

It will thus be observed that marked differences are discernible in the manner in which these two racial groups have reacted to their malarial infections. The question, however, needs further investigation.

DISPENSARY STATISTICS.

Some idea of the malaria morbidity can be had from the percentage of malaria attendance to total attendance from all causes. A study of such data from the

centrally situated dispensary at Khatima shows that the percentage of malaria attendance to total attendance for 5 years for the period 1939 to 1943 works out to 35·4 per cent. It was the highest (52 per cent) in the month of October and lowest (22·5 per cent) in March.

TABLE IV.

Khatima Dispensary, 1939–43.

Month.	Percentage of malaria cases to total cases, average of 5 years (1939–43).
Jan. ...	34·0
Feb. ...	27·5
Mar. ...	22·5
Apr. ...	26·5
May ...	34·3
Jun. ...	24·4
Jul. ...	23·7
Aug. ...	28·3
Sep. ...	35·3
Oct. ...	52·0
Nov. ...	50·3
Dec. ...	45·0

PRELIMINARY MALARIA SURVEY AND RECOMMENDATIONS.

Under the existing orders of the State Government, all major irrigation, water-works and drainage projects have to be examined by the Malariology Branch of the Office of the Director of Medical and Health Services, U.P., for scrutiny and advice so far as these concern the health of the population of the areas in which the schemes are to be launched. The present scheme was similarly examined on a reference from the Superintending Engineer, P.W.D. Project Circle, Irrigation Works, U.P., by the junior author, who undertook a detailed preliminary malaria survey of the areas in May 1944 and made recommendations summarized as follows :—

(*a*) Proper siting of the colony and camps ;

(*b*) Mosquito proofing of dwellings ;

(*c*) Deforestation of sites, cutting of elephant grass, jungle shrubs, weeds, etc.;

(*d*) Spray-killing of adult mosquitoes with pyrethrum-kerosene mixture (D.D.T. not available then);

(*e*) Weekly oiling of water-collections breeding mosquitoes within half-a-mile perimeter of the colony and camp with 'Malariol';

(*f*) Provision of drainage cuts where necessary;

(*g*) Filling up or draining of the disused water-collections, care in the construction of canal and road embankments so as to avoid obstruction to the natural drainage as far as possible;

(*h*) Control over excavations of earth and making of brick-kilns within a radius of 2 miles of the works;

(*i*) Regular medical inspection of all imported labour to detect malarial infections and exclude persons having enlarged spleens or parasitæmia;

(*j*) Prophylactic use of mepacrine hydrochloride 0·2 gm. weekly (paludrine being not then available) by the resident population;

(*k*) Provision of equipped hospital with indoor and out-patient accommodation and mobile medical aid;

(*l*) Provision of water-supply by fixing up hand-pumps and suitable sanitary conveniences.

Before it had been possible to analyse the results achieved due to the implementation of the programme submitted in 1944 for the protection of the local population, a complete reorganization of the control programme was undertaken in 1946 on account of the influx of the population connected with the new project. Moreover, availability of D.D.T. helped considerably in the reorientation of control measures. These measures consist mainly of :—

(*a*) Adult mosquito and larval control with D.D.T.;

(*b*) Prophylactic and therapeutic administration of paludrine;

(*c*) Proper siting of colony camps;

(*d*) Jungle clearance.

(*a*) *Antimosquito and antilarval measures.*—Systematic indoor residual spraying with D.D.T. is practised at six-weekly intervals. 2·5 per cent watery suspension of D.D.T. prepared in accordance with the Malaria Institute of India's formula (Puri, 1947) is used for spraying. The dose is 50 mg. of D.D.T. per sq. ft. in initial, and 25 mg. in subsequent sprayings. The sprayers in use are double action stirrup pumps fitted with special nozzles.

During the year 1949, 20,253 premises with a surface area of 6,742,000 sq. ft. were sprayed.

In addition to the above antimosquito measures, D.D.T. in Malariol was used to destroy mosquito larvæ in breeding grounds situated within half-a-mile radius of the residential colonies at weekly intervals.

(*b*) *Prophylactic and therapeutic administration of paludrine.*—Weekly administration of paludrine hydrochloride to the resident population and labour formed part of regular routine prophylaxis against malaria. For this purpose one tablet (300 mg.) of paludrine hydrochloride was administered at weekly intervals to each adult and proportionately smaller doses to children. Adequate treatment with paludrine was also administered to all malaria cases occurring in the area and found positive on microscopical examination of blood.

(*c*) *Proper siting of colony camps, etc.*—The staffs' colony and labourers' camps have been located at suitable sites near the works. During the year 1949, the colony had expanded considerably. Labourers' camps have been developed in concentrated units at convenient sites along the course of the power-channel where the work is to progress. Hand-pumps for water-supply have been provided in each of these camps.

(*d*) *Jungle clearance.*—The site has been cleared of forests and jungle. The ground has been levelled to permit proper drainage. Further deforestation will continue as the work proceeds in other directions. This measure has helped in drying up of the soil and discovering and draining of water-collections lying hidden in the forests in the neighbourhood of the works.

ANTIMALARIA ORGANIZATION.

Antimalaria staff.—The staff sanctioned for this organization is as under :—

Designation.	*Sanctioned strength.*
Medical officer-in-charge	1
Medical officers (subordinate cadre)	2
Sanitary inspectors	3
Compounders	4
Dresser	1
Hospital orderlies	4
Hospital kahar	1
Hospital cook	1
Hospital sweepers	2
Sanitation sweepers	20
Malaria field workers	60

MEDICAL AND SANITARY ARRANGEMENTS.

(*a*) *Hospital facilities.*—Necessary facilities have been provided for the treatment of common ailments amongst the population. A small hospital with 8-bedded indoor accommodation and an out-patient department has been provided at the site of works. Arrangement for isolation of infectious disease cases also exists in the hospital.

(*b*) *Sanitary arrangements.*—Temporary drains have been constructed to carry sullage water from the colony towards the natural line of drainage. Since the drainage conditions were not satisfactory, antilarval measures by oiling have also been carried out to control general mosquito nuisance in the colony.

Both public and private latrines have been provided for the convenience of the staff. Night-soil is disposed of in trenches which are regularly sprayed with D.D.T. As a result of this measure, fly nuisance in the colony is kept under control.

In spite of the provision of adequate latrines, the residents do not use them in preference to jungles and forests in the vicinity of the colony. Efforts are being made to check haphazard soiling of sites by flagging special areas for the purpose in the vicinity of the colony and picking up night-soil by sweepers and trenching it.

RESULT OF CONTROL MEASURES.

The control measures have proved to be successful as would appear from the study of :—

(*a*) Duration of working season ;
(*b*) Spleen rate ;
(*c*) Parasite rate ;
(*d*) Hospital attendance statistics.

(*a*) *Working season.*—Table V shows the average monthly labour population employed on the works and the percentage of sickness due to malaria amongst them. The duration of each year's working season has also been shown in Table V :—

TABLE V.

Year.	Average monthly labour population.	Percentage of sickness due to malaria in labour.	Duration of working season in the year.
1946-47 ...	3,500	0·11	7 months.
1948 ...	1,644	0·16	Throughout the year.
1949 .	1,702	0·15	do.
1950 ..	925	0·08	do.

It will be observed that sickness from malaria remained practically negligible and the working season could be extended from 7 months at the commencement of the scheme to the entire period of the year. This is a special achievement which could be possible only after enforcement of malaria control measures.

(*b*) *Spleen rate.*—Systematic examination of spleens of adults and children was carried out. Results of examination are given in Table VI. It will be seen therefrom that the spleen rate amongst the imported labour in this area remained below 15 per cent both in children and adults and the average enlarged spleen in children was reduced from 2·1 in 1946-47 to 1·8 in 1950, in spite of their continuous stay in this hyperendemic malarious area. This is also chiefly due to the effective enforcement of malaria control measures. The method adopted for determining the average enlarged spleen by the antimalaria officer was by measurements in finger-breadths.

TABLE VI.

Spleen rate in adults and children.

Year.		Number examined.	Number found with enlarged spleen.	Spleen rate, per cent.	Average enlarged spleen (children).
1946-47	Adults	3,331	361	10·8	2·1
	Children	630	60	9·5	
1948	Adults	3,600	390	10·5	2·0
	Children	205	29	14·1	
1949	Adults	2,545	248	9·7	2·0
	Children	104	15	14·4	
1950	Adults	2,070	177	8·5	1·8
	Children	83	9	10·8	

(*c*) *Parasite rate.*—Table VII shows that the malaria parasite rate ranged from 6·4 to 9·8 per cent. *Plasmodium falciparum* was responsible for more than 70 per cent of infections.

TABLE VII.

Parasite rate.

Year.	Number examined.	Number found positive for malaria parasite.	Parasite rate, per cent.	REMARKS.
1946-47	1,360	87	6·4	Malignant tertian was responsible for more than 70 per cent of infections.
1948	974	96	9·8	
1949	612	56	9·1	
1950	495	39	7·8	

(*d*) *Hospital statistics.*—Table VIII shows the total attendance from all causes and attendance from malaria as recorded in the hospital located at Lohia Head.

The fall in percentage of malaria attendance to total attendance from 10 per cent in 1946-47 to 1·3 per cent in 1950 is significant.

TABLE VIII.

Hospital statistics.

Year.	Attendance from all causes.	Attendance from malaria.	Percentage of malaria attendance.
1946-47 ...	13,722	1,370	10·0
1947 ...	16,967	643	3·8
1948 ...	20,591	989	4·7
1949 ...	32,409	905	2·7
1950 ...	35,074	460	1·3

MOSQUITO FAUNA.

Seepages along the Sarda Canal and springs and water recesses of Chukanala have been incriminated to be the chief sources of anopheline mosquito breeding. Species of anopheline found in the area were :—

Species.	Percentage.
A. culicifacies	10·0
A. fluviatilis	6·2
A. minimus	2·5
A. stephensi	2·5
A. annularis	12·5
A. pallidus	1·3
A. splendidus	21·3
A. maculatus	6·2
A. subpictus	36·3
A. vagus	1·2

The number of mosquitoes available for dissection was small. Only the following species were dissected for gland infection but none was found positive :—

A. culicifacies.
A. fluviatilis.
A. minimus.
A. stephensi.
A. splendidus.
A. maculatus.

FINANCIAL ASPECT.

Table IX shows the annual allotment and expenditure figures for the years 1945-46 to 1949-50.

TABLE IX.

Annual allotment and expenditure.

Year.	Allotment.	Expenditure.
1945-46 ...	Rs. 29,000/-	Rs. 20,659/5/3
1946-47 ...	„ 51,000/-	„ 45,625/2/-
1947-48 ...	„ 79,114/-	„ 64,284/-/-
1948-49 ...	„ 68,014/-	„ 47,923/15/6
1949-50 ...	„ 70,130/-	„ 47,475/4/-

SUMMARY.

The success of malaria control measures is evident from the fact that the spleen, parasite and malaria morbidity rates continued to remain low amongst the resident population in spite of their continuous stay. This also enabled the working season to be extended from 7 months to the whole year—a feature of great economic importance.

ACKNOWLEDGMENT.

The authors thank Dr. G. N. Mathur, M.B., B.S., Medical Officer-in-charge, Antimalaria Operations and Medical and Sanitary Arrangements, who supplied the necessary data for incorporation in this report and was responsible for the success of the scheme in the field.

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ABSTRACT.

IMPROVED TYPE OF BALL MILLS FOR PREPARING D.D.T. SUSPENSION.

BY

R. S. SRIVASTAVA, M.B., D.P.H.

(*Assistant Director, Malariology, Uttar Pradesh*).

[November 19, 1951.]

HAND driven, foot and hand driven, and electric driven (1 h.p. working at 220 volts D.C.) ball mills have been designed for preparing D.D.T. suspension. In all cases a strong and heavy stand has been provided to avoid shaking. The drums have been placed at the two ends of the axle and the lids covering them have been provided with a circular groove packed with asbestos to fit tightly on to the drum, and an inspection eye to watch the grinding. Ball bearing has been provided to enable the axle to move freely. The improved type of ball mills has been found to be very satisfactory for preparation of D.D.T. suspension in bulk.

J. S.

A copy of the original manuscript (3 pages and 3 photographs) 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. For detailed specifications the author may be approached direct.—*Editor.*

REVIEWS.

QUININE, QUINIDINE AND OTHER CINCHONA ALKALOIDS IN CLINICAL PRACTICE.

A SMALL and attractive booklet *Quinine, quinidine and other cinchona alkaloids in clinical practice* has been published by The London Cinchona Bureau. It emphasizes the place of quinine in the treatment of emergency cases, as also in the radical cure of *vivax* malaria in combination with 8-aminoquinoline group of drugs.

The booklet will be of use to all engaged in clinical practice. Free copies can be obtained on request to the Secretary, The London Cinchona Bureau, 10, Princes Street, London, S.W. 1.

J. S.

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