(NEW SERIES.)

No. 2.

# SCIENTIFIC MEMOIRS

BY

## OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

## GOVERNMENT OF INDIA.

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## MALARIA IN INDIA

BY

CAPTAIN S. P. JAMES, M.B. (LOND.), I.M.S.
*(On Special Duty with the Royal Society's Commission on Malaria).*

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ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT OF INDIA, SIMLA.

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## PART I.



## PART II.

# NOTE.

THE work recorded in this report includes the greater part of that done conjointly by Dr. J. W. W. Stephens, Mr. S. R. Christophers, M.B., and myself, during the time I was associated with them in India, as well as some of the work I have done since their departure. It may happen that, as I have not yet seen their reports, my conclusions may, in some cases, differ from those of Drs. Stephens and Christophers. It should be noted, therefore, that only my own opinions are expressed in this report.

S. P. JAMES,
Captain, I. M. S.

# MALARIA IN INDIA.



## PART I.

### METHODS OF INVESTIGATION.

I PROPOSE first to describe, under the following headings, some methods we have employed in our investigations :—

(1) Methods for the examination of the blood.

(2) Methods for the examination of the malaria parasite in mosquitoes.

(3) Methods for the investigation of malaria in hospitals and jails.

(4) Methods for the investigation of malaria among the general population.

(5) Methods for the study of mosquitoes.

### I.—The examination of the blood.

The diagnostic evidence of malarial infection which can be obtained from a microscopic examination of the blood is as follows :—

(*a*) The detection of malaria parasites.

(*b*) The detection of malarial pigment in the leucocytes.

(*c*) The detection of a change in the proportion of the large mononuclear leucocytes.

The two last are valuable when from any cause—such as the previous administration of quinine—parasites cannot be found in the peripheral circulation.

For the detection of parasites, the blood may be examined in fresh preparations, or in dried and stained films.

When the object is to study carefully any individual case of malaria in a hospital, it is necessary to examine both fresh and stained preparations of blood taken at the same time.

As a rule, however,—and especially in cases where only one specimen of blood can be obtained,—far more information is gained by the examination of stained films than from wet preparations, and for routine work we have usually employed this method.

In taking blood films for staining purposes the film of blood should be spread on a glass slide with the surgical needle with which the finger has been pricked. This method offers great advantages over any other, for not only does it produce an extensive and evenly spread film of blood, but the ease and rapidity with which a large number of specimens can be taken by this method renders it the only practicable one for taking specimens of blood from village children. During such operations the crowd and dust are usually so great that an attempt to take specimens on cover-glasses or by any other method could only end in failure.

A straight surgical needle, the eye of which has been previously removed with a pair of forceps, should be used for this method of taking films. We thus have a needle with a good point and a straight shaft. The finger is pricked and a clean glass slide is lowered on to the drop of blood that exudes, so that it touches the drop about half an inch from one end of the slide. Care should be taken to allow the slide only to just touch the drop of blood—if it touches the finger as well, dirt and numerous skin organisms will be transferred in addition to the blood. The slide is then held between the finger and thumb of the left hand, and the shaft of the needle (which is held by the point) is placed crosswise on the slide over the drop of blood. (Fig. 1.)

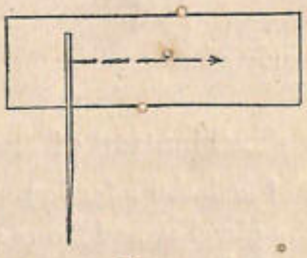

Fig. 1.

A slight to and fro motion of the needle, across the slide assists the blood to run along the under surface of the needle, and when this has occurred, it is drawn evenly along the slide to the end, thus making a broad even film of blood. The drop of blood taken should not be large enough to make the film cover the whole slide, and a good film should end in a number of "tags" about an inch from the end of the slide. (Fig. 2.)

Fig. 2.

The films taken in this way dry almost immediately in the air, but in the damp heat of Southern India it is well to warm the slides over a spirit lamp before taking specimens of blood, lest the moisture on them or on the patient's finger should cause the formation of vacuoles in the blood corpuscles. When dry, the films are fixed by immersion from ten minutes to half an hour in absolute alcohol. For this and for staining purposes the glass stain-pots shown in Fig. 3

Fig. 3.

will be found very useful. A large number of slides can be fixed in one of these pots at the same time ; but before placing each slide in the alcohol, a number by which it will be known should be written upon it with Indian ink or a "glass pencil."

For staining blood films we have usually employed either alcoholic hæmatin or Romanowsky's stain, and other stains than these are seldom necessary.

The alcoholic hæmatin stain possesses the advantages of being very easily worked, of being permanent, and of showing up the pigment in malaria parasites better than Romanowsky's stain. With a little practice, however, very beautiful results can be obtained by Romanowsky's method, and as, in a slide stained by this method, it is almost impossible to overlook even the smallest ring forms, it is to be recommended even for routine work.

The alcoholic hæmatin stain is made as follows :*—

| | |
|---|---|
| Hæmatein purissimus | 2 grammes. |
| Alcohol (90 %) | 50 C. C. |
| Alum | 50 grammes. |
| Distilled water | 1,000 C. C. |

The fixed and dried films are left in this solution for from ten to twenty minutes or longer, until the blood film has a faint brown hue. They are then washed in water, allowed to dry in the air, and examined with the oil-immersion lens without applying a cover-glass. The parasites show clearly against the grey brown colour of the red cells.

Romanowsky's stain is made as follows :—

*Stock solution, A :—*

| | |
|---|---|
| Pure medicinal methylene blue (Grübler) | 1 gramme. |
| Pure sodium carbonate | ½ gramme. |
| Distilled water | 100 C. C. |

Grind up the methylene blue in a pestle and mortar and add it gradually to the water. When the solution has been made, add the sodium carbonate.

* Reports to the Malaria Committee of the Royal Society, 3rd Series, Stephens and Christophers "The Malarial Fevers of British Central Africa."

This solution should be allowed to stand, in a corked bottle in a hot sun for one or two days (or in an incubator for a week), until the blue has acquired a red tinge when looked at through the bottle held up to the light.

*Stock solution, B :—*

| | |
|---|---|
| Eosin, extra, B. A. (Grübler) . . . . . | $\frac{1}{10}$th gramme. |
| Distilled water . . . . . . . . | 100 C. C. |

This solution should be kept in a dark place; if exposed to the sun it will fade.

A and B are stock solutions. For use each of them has to be diluted twenty-five times, that is, 4 C. C. of each should be added to 100 C. C. of distilled water. These diluted solutions (C and D) are kept in separate bottles.

The slides to be stained are placed, after previous fixing in absolute alcohol, in a flat porcelain or glass dish, film surface upwards. Equal parts of the dilute solutions (C and D) are taken (10 C. C. of each are usually sufficient for two or three slides in a flat dish), and poured simultaneously over the slides so that they mix at once. The dish is then rocked backwards and forwards, as is done in the development of photographic plates, for from fifteen minutes to one hour, during which time a red precipitate will form on the sides of the porcelain dish. The time required for staining varies greatly with different solutions, but a few trials will indicate the time which any particular solution takes.

In order to ascertain when the films are sufficiently stained they should be removed from the solution from time to time and examined while wet (without previous washing in water) with a $\frac{1}{4}$th or $\frac{1}{6}$th inch objective. If the blood platelets and the nuclei of the leucocytes are a deep ruby red, the slides are sufficiently stained, and the parasites will show up well. They are then washed for a few moments in distilled water and allowed to dry. Too much washing decolourizes the film. When dry, the slides are examined under the oil-immersion lens without applying a cover-glass.*

In well stained films the red blood corpuscles should be a transparent pink colour, the body of the parasites a light blue, and the chromatin a deep red. To obtain correct staining it is sometimes necessary to alter the proportions of the stains ; for example, better results are often obtained by using three or even four parts of the eosin solution to two of the methylene blue, but these details can be easily worked out by a few trials.

It happens, however, with some solutions that in spite of alterations in the relative proportions of the stains, and alterations in the time of staining, the red blood corpuscles remain stained a more or less deep blue colour. This, however, is of little or no consequence, provided that the nuclei of the leucocytes

* An exceedingly useful and easily worked "Romanowsky" stain in a single solution is that recommended by Major W. B. Leishman, R.A.M.C., in the "British Medical Journal" for September 21st, 1901, page 757.

and the blood platelets are correctly stained, for the parasites will be found to show up quite plainly in the bluish corpuscles.

A useful property of Romanowsky's stain is its power of causing the red cells which have been invaded by simple Tertian parasites to become stippled with red dots, thus making cells containing these parasites very easily recognisable. This stippling, which is probably the result of degeneration in the corpuscle, does not, of course, occur in every corpuscle that is invaded by a simple Tertian parasite, but in every correctly stained slide of simple Tertian parasites, there will be a large proportion of stippled cells, and on the whole, it is a very characteristic sign. A pseudo-stippling sometimes occurs in blood corpuscles invaded with malignant Tertian parasites, but this pseudo-stippling is readily distinguished from the characteristic appearance presented by corpuscles invaded with the simple Tertian parasite.

After a slide has been examined the oil should be removed with a few drops of xylol: the slide can then be wrapped in paper and kept indefinitely.

It is somewhat difficult to prepare good specimens of flagella bodies, and it is usually preferable to observe them in the fresh state. In cases, however, where flagella bodies are fairly numerous, I have found the following method give good results.

A moist chamber is first made by cutting a round hole the size of a large cover slip in a thick piece of blotting-paper, and applying this over the cell of a hollow ground glass slide. The blotting-paper is well moistened with water. The finger should be pricked and a large drop of blood squeezed out. During this operation it is well to breathe continuously on the drop of blood as it exudes, and while it is resting on the finger. The drop of blood is then transferred to the middle of a clean glass slide and inverted over the moist chamber cell: the blood should not be spread out on the slide but should remain as a hanging drop.

A number of similar hanging drops of blood are prepared and placed in moist chambers. At varying periods of time after their preparation,—from five minutes to half an hour or more,—the slides should be removed from the moist chambers, and thin films made from the drops of blood on other slides, in the manner before described for the preparation of ordinary blood films.

These films are dried in the air, fixed, and stained by Romanowsky's method.

With these thin films it is of course unnecessary to dissolve out the hæmoglobin of the red blood corpuscles in order to see the flagella bodies.

It is not necessary to describe the method of taking fresh specimens of blood. Absolute cleanliness of slides and cover-glasses is essential, and it may be noted that the best way to put the final cleaning touches on to slides is to heat them over a spirit lamp. As a rule it is preferable not to ring the cover-glasses of fresh specimens of blood with vaseline.

Fresh specimens should be examined with the oil-immersion lens and with carefully arranged light: if too much light passes through the specimens the parasites are difficult to see.

To those who are unfamiliar with malaria parasites the examination of fresh specimens of blood is full of pitfalls. Anything which seems to have the appearance of a parasite, but which is not actually inside a red blood corpuscle, and especially such things as "free pigment," "free spores" (unless the latter are actually seen breaking away from a segmenting parasite), should be disregarded.

In cases where there is any doubt and especially in cases where only unpigmented forms appear to be present, it is better to take films and stain them by Romanowsky's method.

In stained films, unless scrupulous attention has been paid to the cleanliness of the patient's finger, we should be on the watch for skin parasites, which often present a superficial resemblance to malaria parasites.

It is unnecessary, however, to enumerate all the things that may be mistaken for malaria parasites, and experience has shown that no advantage is gained by doing so:* it is sufficient to say that the malaria parasite is so definite an organism that anything about which, after careful examination, it is possible to have the least doubt is almost certainly not a parasite.

*The detection of pigmented leucocytes.*—It not infrequently happens in cases of malarial fever, and especially in cases where the patient has been taking quinine, that parasites are absent from the peripheral circulation. In such cases it has been pointed out by Stephens and Christophers that the diagnostic value of an examination for pigmented leucocytes and a differential count of the leucocytes is very great.

For both these examinations stained films should be employed. In an examination for the detection of pigment in leucocytes, special attention should be paid to the *large mononuclear elements*, for it is in these that the characteristic appearances are chiefly found. The pigment may vary from a number of large blocks or clumps to one or two fine spicules only, which may be difficult to detect, but which, when recognised, are as absolutely diagnostic of recent malarial infection as the presence of parasites. A common appearance is that of one or two large blocks of pigment and a number of fine grains or spicules scattered through the cell. Appearances as of pigment in any other elements

* A great deal of very careful practice is necessary to become an adept at finding parasites, and I have repeatedly seen men who could at once recognise a parasite when it was pointed out to them, spend an hour or more over a film containing a fair number of parasites without being able to find one for themselves. Even the Burman prisoners at Nagpur, who were well practised in examining slides, when examining malignant Tertian cases, on several occasions passed over very obvious simple Tertian parasites which were present in the films as well. On the other hand, it is even more common for men to think they see parasites in almost every field when none are present in the film at all.

than the large mononuclear cells are, in the absence of parasites, of less diagnostic value.

*The differential count of leucocytes.*—It has been shown that in malarial fevers a variation occurs in the relative proportions of the leucocytes, this variation consisting chiefly in an increase in the large mononuclear cells accompanied by a decrease in the polynuclear and small mononuclear cells. As a diagnostic sign of recent malarial infection this increase in the proportion of the large mononuclear cells is of especial value in cases where no parasites can be found in the peripheral circulation, *e.g.*, where quinine has been taken.

To make this differential count of the leucocytes is a somewhat difficult task and one requiring much time and patience: it is essential also to have made a good many counts of normal blood slides before attempting a differential count of malarial blood films.

For making the count, a mechanical stage and well-spread even films prepared by the method before described are necessary. The films may be stained by Romanowsky's method or with Ehrlich's triple stain. In a well-spread film nearly all the leucocytes will be found distributed along the edges and in the tags at the end of the film. If these parts of the slide are examined first, practically all the leucocytes in the film can be counted. One thousand leucocytes in all should be counted and, unless the observer is very practised in making blood counts, results obtained from counts of less than this number cannot be relied upon.

In making the count, the following forms should be noted separately:—

(1) Large mononuclear leucocytes.
(2) "Transitional" forms of leucocytes.
(3) Small mononuclear leucocytes (lymphocytes).
(4) Polymorphonuclear leucocytes.
(5) Eosinophile leucocytes.

The chief difficulty is in deciding definitely the form of leucocyte to be included under the term large mononuclear, and on this decision will depend the accuracy or the reverse of the results obtained.

The large mononuclear leucocytes are sharply distinguished from the lymphocytes.* They are large cells about twice to three times the size of the red blood corpuscles. They possess a large oval nucleus as a rule eccentrically situated, and a relatively abundant protoplasm which is free from granulations and stains very faintly.

In normal blood of natives from 7 to 10 per cent. of all the leucocytes are of the large mononuclear variety.

"Transitional" forms, which should not, as a rule, be counted along with

* "Histology of the Blood," by Ehrlich and Lazarus, translated by W. Myers. Cambridge University Press.

the large mononuclears in malarial films, are distinguished from these by the presence of a deeply notched nucleus and by the presence of scanty granulations in the protoplasm.

In suspected cases of malaria, slides for making differential counts should be taken, if possible, during an apyretic interval, as it is at such times that the difference in the proportions of the different varieties of leucocytes is most marked.

When all possible care to place each variety of leucocyte met with under its proper heading in the count has been taken, a result which gives the number of large mononuclear elements as over 15 per cent. of all the leucocytes may be regarded as a proof of recent malarial infection.

## II.—Methods for the examination of malaria parasites in mosquitoes.

The forms of the malaria parasite which it is important to be able to recognise in the tissues of *anopheles* mosquitoes are zygotes, in the stomach wall, and sporozoites, in the salivary glands.

*To dissect out the salivary glands for the demonstration of sporozoites.*— The salivary glands consist of six lobes, three on each side, and the ducts of the three lobes of each side unite to form a single duct which, after joining with its fellow of the opposite side, passes forwards and ends in the first portion of the alimentary canal close to the base of the proboscis.

The object in a dissection of the salivary glands is to remove the six lobes cleanly and entirely from the tissues which surround them. This requires careful dissection and cannot be done,—except occasionally by chance,—by fixing the mosquito's head with one needle and the thorax with another and pulling the needles apart, as is usually recommended. The position of the salivary glands in an *anopheles* is shown in the figure (Fig. 4).

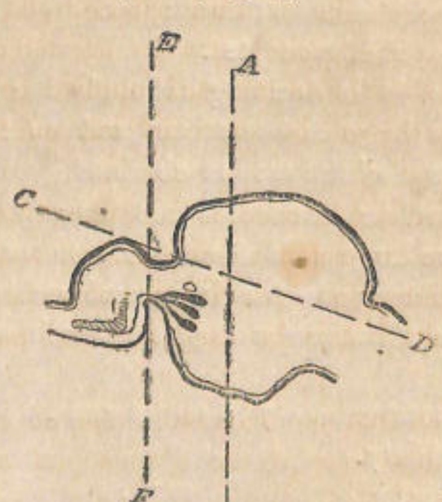


Fig. 4.

The mosquitoes to be dissected are killed with chloroform and the species of each carefully identified. They are then placed in a normal saline solution in watch glasses—one watch glass for the members of each species. This is necessary because it is of course impossible to identify an *anopheles* after it has been some little time in the salt solution.

The importance of exactly identifying each *anopheles* before dissection is very great, for the finding of sporozoites in any particular mosquito is of little value unless we know the exact species.

For dissection, a mosquito is taken out of the watch glass, its legs and wings are removed, and it is laid on its side in a drop of salt solution on a glass slide.

With a sharp triangular needle or a fine entomological knife, the thorax is cut across just behind the glands in the direction shown in the figure (AB, Fig. 4), and the hind part of the thorax with the abdomen attached is discarded. The top of the fore-part of the thorax is then removed by a clean cut in the direction (CD, Fig. 4).

If now the head of the mosquito is held, as near the neck as possible with the point of one needle, and the remaining tissues surrounding the gland with the other, and the needles are gently and carefully separated, the glands will remain attached to the head and almost clear of all surrounding tissues. The preparation is now examined with a low power of the microscope (¼th inch objective) to see whether the gland is really attached to the head as it should be.

The dissection is completed by a clean cut across the neck at its point of junction with the head (EF, Fig. 4), and by this cut, and perhaps one or two final touches with the needles, the six lobes will be freed of all outside tissues—a most important point if sporozoites are to be detected. A cover-glass is now placed on the glands and firm pressure applied to it with a needle. By this operation the sporozoites will be squeezed out and will be seen with greater ease.

The ⅙th inch objective is now used, and if sporozoites are present, they will be seen in large numbers as transparent sickle-shaped bodies floating about in the salt solution. The oil-immersion lens is not necessary for the detection of sporozoites, but if in doubt, it may be used and each part of the crushed gland thoroughly searched.

It is a mistake to think that sporozoites in infected mosquitoes are present only in the so-called middle lobe of the glands: as a rule they are equally numerous in all the lobes.

*To stain sporozoites.*—The sporozoites that have been demonstrated by the above method may be easily stained. The excess of salt solution is removed with blotting-paper and the cover-glass slipped off the slide in the same manner as is done when making a cover slip preparation of blood. The slide and cover-slip are then dried in the air, fixed by immersion in absolute alcohol for half an hour, and stained by Romanowsky's method like an ordinary blood film.

Examination with the oil-immersion lens will show the sporozoites singly and in groups as sickle-shaped bodies, pointed towards the ends, and with one or more brilliant red chromatin dots at or near the centre of each.

It would be of little or no advantage to enumerate the appearances and structures that may be mistaken for sporozoites. The best way to avoid error is to ensure that no other structures except the glands are included under the cover-glass. If in doubt, the specimen may be fixed and stained as above described.

In making dissections of a large number of mosquitoes caught in a village, several other parasites besides sporozoites will almost certainly be found. The most interesting of these are the bodies which for want of a better name we have called the "sausage bodies." They are small oval or crescent-shaped bodies, which are occasionally found in great numbers in the tissues of the head surrounding the salivary glands, but not as a rule in the glands themselves. When stained with Romanowsky's stain they present a red capsule, blue body substance, and two round chromatin patches in the centre surrounded by a clear area. Their nature is unknown.

*To dissect out the stomach to demonstrate zygotes.*—In order to demonstrate zygotes successfully, the stomach of the mosquito must be *quite empty of blood*. The method of dissection is as follows:—

The mosquito is divided into two by a cut across the thorax as for the dissection of the salivary glands, and the head and fore-part of the thorax discarded.

The abdomen is laid in a drop of salt solution on a slide, and while the point of one needle holds the thorax to the slide, the pointed end of the other needle is laid across the abdomen near the seventh or eighth segment. The needles are then gently separated, and the stomach with the malpighian tubes attached comes away with the hind segments. The malpighian tubes, which will probably be lying over the stomach after it has been pulled out, are carefully drawn away, and a cover-glass applied. The weight of the cover-glass is usually sufficient to flatten out the stomach, and it is inadvisable to use any pressure on it. The preparation is now examined with a ⅙th inch objective, and if the zygotes are fairly large they can easily be seen under this power. It will be found as a rule that most of the zygotes are situated within the posterior third of the stomach (nearest the origin of the malpighian tubes) although in some cases they are more uniformly spread over the whole stomach. The number of zygotes present varies greatly; sometimes only ten or fifteen can be counted, at other times as many as four or five hundred, so that the whole surface of the organ is studded with them.

Very young zygotes can only be made sure of by examining the whole of the specimen with the oil-immersion lens: they can then be recognised by the presence of grains of malarial pigment the appearance of which is very characteristic. These grains of pigment may be arranged in little groups or in a line across the zygote.

There are so many cells in and around the stomach and malpighian tubes that bear a superficial resemblance to zygotes that their detection presents more difficulty to those who are unfamiliar with their appearance than does the detection of sporozoites. In this case,—as indeed in the detection of malaria parasites in the blood,—the difficulty lies not so much in recognising them when they are really present, but in not mistaking other structures for them when they are absent.

Those, however, who are unfamiliar with the appearance of zygotes can study them easily by feeding a few of the common Indian culex (*C. Fatigans*) on a sparrow with proteosoma parasites in its blood. In a few days almost all the culex will have zygotes in large numbers, developing in the stomach wall.

*To make permanent preparations of zygotes.*—Fairly good preparations of zygotes may be made by simply mounting the fresh stomach in a 5 per cent. solution of formalin and ringing the cover-glass thickly with balsam. Such specimens keep good for some months at any rate.

The staining of zygotes in order to show any definite structure in them is a difficult matter and requires special methods. Moderately good specimens may, however, be obtained by allowing the stomach to dry as flatly as possible on the slide, fixing it first in formalin and then in alcohol, and staining very lightly with methylene blue. The specimen can then be permanently mounted in xylol balsam.

*To carry out feeding experiments.*—The chief difficulties in carrying out feeding experiments arise from two causes: first, the difficulty in getting a suitable case on which to feed the mosquitoes, and second, the difficulty in making the *anopheles* feed and in keeping them alive sufficiently long for the parasites to develop.

The first of these difficulties is the more important, and is the one which has probably been responsible for the many negative results in feeding experiments that have been recorded. Thus Dr. Daniels, working with *A. Fuliginosus*

(Giles) and *A. Rossii* (Giles), states that neither of these species carry the malignant Tertian (crescent) variety of the malaria parasite.

Positive results have, however, been obtained by us with both those species of *anopheles* for simple Tertian, malignant Tertian and Quartan parasites. We have also shown that zygotes and sporozoites will readily develop in six other species of Indian *anopheles* with which we have experimented (*viz.*, *A. Culicifacies*, *A. Fluviatilis*, *A. Stephensii*, *A. Theobaldi*, *A. Turkhudi*, *A. Barbirostris*), so that we may infer that almost certainly all the species of Indian *anopheles* are capable of harbouring the malaria parasite under experimental conditions, and *negative results in feeding experiments with* anopheles *are more likely to be due to failure in carrying out the details of the experiment than to the fact that any species of* anopheles *is hostile to the parasite.*

The most important thing is, as we have said, to obtain a suitable case for the *anopheles* to feed upon. By this is meant a case in which a large number of gametes are ripe for flagellation at the time of the experiment. *It is practically useless to carry out a feeding experiment on a case in which, by the examination of fresh specimens of blood, a fair number of flagellating bodies have not been seen shortly before the experiment.*

Having procured a good case on which to carry out the experiment, the best method of feeding the mosquitoes and keeping them alive can be easily devised after a few trials. We have found that *anopheles* do not, however, live so well in the mosquito netting boxes that are usually recommended for feeding experiments as they do in glass bottles, but if the latter are used a rough piece of cardboard or stiff paper must be placed in the bottles for the *anopheles* to rest comfortably upon. This detail, which is due to the ingenuity of Dr. Christophers, is very important.

The following is the plan we have found most successful :—

A number of large wide-mouthed glass jars are obtained—glass prune jars or crystalized fruit bottles do well—and thoroughly cleaned and dried. Into each of these bottles is fixed lengthwise a piece of rough cardboard on which the *anopheles* can rest easily. The mouth of the jar is closed by a removable cover of mosquito netting. Ten or twelve female *anopheles* (preferably including several species) are placed in each bottle. The patient upon whom it is intended to feed the mosquitoes should sit on the ground, and the bottles (closed by their mosquito netting covers) be arranged on a low stool or box in front of him, so that he may be able with comfort to rest both his forearms on the mouths of the bottles placed upright on the box. In this manner he must remain until the *anopheles* have fed. When using mosquitoes bred from larvæ it is often difficult to get them to feed on the first night, but once having fed there will be no difficulty on subsequent nights.

Fig. 5.—To show method of feeding mosquitoes in bottles.

After the mosquitoes have fed, the bottles should be inverted—the cover having been removed—over a small tin of water in which a piece of paper is floating for the mosquitoes to rest upon when laying their eggs. With care this manipulation can be easily performed without letting any escape.

The bottles should be labelled and kept in a dark cupboard until the next night when the mosquitoes must be again allowed to feed.

Each morning the water in the tin should be renewed and any mosquitoes that have died removed from it. If they are fed each night the mosquitoes will keep alive for from twelve to twenty days or more, and no other food than blood is necessary.

When it is desired to examine any of the mosquitoes, they should be kept without being fed until they have got rid of all the blood they have ingested. This usually takes about two days, but the longer they can be kept alive without food the more satisfactory will be the examination of the stomach.

If the weather is cool the bottles may with advantage be kept in an incubator at a temperature of between 70°—80°F., by which means the parasites will develop more readily.

It has been proved by Dr. Stephens that fertilization is not essential for the development of zygotes, so that it is not absolutely necessary to place any males in the bottles with the females, although in order to imitate natural conditions more closely it is possibly of advantage to do so.

Either mosquitoes bred from larvæ, or those caught in a village may be used; the advantage of employing the latter is that they will usually feed more readily on the first night.

For infecting a healthy person, all that is necessary is to allow the *anopheles* which have previously fed on the case of malaria to bite the healthy man in the same manner as they did the malaria case. It is of course useless to attempt to infect a healthy person with malaria unless sporozoites have been detected in the glands of some of the *anopheles* of the same batch as those which are being used in the infecting experiment, and experiments which simply consist in feeding a number of *anopheles* for some days on a case of malaria, and then on a healthy man, without simultaneous dissection of the mosquitoes, are of little if any value; and when negative results are obtained, such experiments are liable to throw discredit on the fact that malaria is conveyed only by mosquitoes.

## III.—The investigation of malaria in hospitals and jails.

There are many opportunities for the study of individual cases of malaria in the hospitals of India, but as a rule it is difficult to take advantage of them owing to the fact that the great majority of the cases have been treated with quinine before admission. For this reason it is quite exceptional to be able to find malarial parasites, or even to see a typical Tertian or Quartan chart in hospital cases; for although irregular fever may continue for some time afterwards, yet the administration of even small doses of quinine almost invariably causes the disappearance of parasites from the peripheral circulation.

On one occasion we visited the out-patient department of one of the large hospitals and examined the blood of all the patients who presented themselves complaining of "fever." On that day we did not find parasites or other indication of malaria in any of the cases, but in spite of this they were all diagnosed as "ague" by the native assistant physician in charge and given ten grains of quinine twice a day.

Possibly some of these cases were in reality cases of malaria who would therefore have benefited by the quinine, but at any rate it would appear to be preferable in all cases to wait a few days before giving a drug which, once given, lessens the chances of an accurate diagnosis of the case being afterwards made by an examination of the blood, or, in the absence of this, by an inspection of the temperature chart.

In all ordinary cases of malaria it is quite unnecessary to begin quinine treatment immediately, and the advantage which accrues in the treatment of a patient from the knowledge of whether his case is one of simple Tertian, of Quartan, or of malignant Tertian fever far outweighs any remote danger there may be in withholding quinine for a few days until the diagnosis can be accurately made. I say "for a few days" advisedly, because in a first attack of malaria it sometimes happens that during the first or even during the second day of fever, parasites may be absent from the peripheral circulation. During the first day or two also the temperature may be continuous and give us no clue as to the nature of the fever. If we give quinine during this time we do so empirically and without the least knowledge of the nature of the case, and in doing so we do away at once with almost all chance of afterwards finding out (should the fever not yield to quinine) whether the case is one of malaria or not. In very severe cases of malaria, as, for instance, where patients are brought into hospital in a semicomatose condition, parasites can usually be found by a few minutes examination, and we are then in a position to treat the case with appropriate vigour.

This question of the routine administration of quinine during the first few days in nearly all cases of fever has an important bearing on the question of the differentiation of Indian fevers. Unless we can absolutely exclude malaria by repeated examinations of the blood *before quinine has been taken*, it is impossible to say definitely that a case of fever is not malaria.

It is quite possible that indefinite cases of fever which cannot be classed as enteric fever, Malta fever, relapsing fever, or malarial fever occur in India, but if in such cases quinine has been given early in the disease it is impossible to definitely exclude malaria, because a malarial fever may continue in spite of the administration of quinine in ordinary doses, and in spite of the absence of parasites from the peripheral circulation. If, however, in such a case we have refrained from giving quinine, we are able, by repeated examinations of the blood, to definitely exclude malaria and can therefore, with confidence, search for some other cause of the fever.

It appears, therefore, that until the plan of not beginning quinine treatment until an accurate diagnosis of every case of fever has been made, is adopted systematically for a year or so in one of the large hospitals of India, our knowledge of the non-malarial fevers will remain no further advanced than it has been for many years past. This, together with a close study of individual cases of malaria, is the kind of work which can be best carried out in hospitals and jails.

The large class of cases which are characterised by the presence of greatly enlarged spleens and much anæmia, accompanied frequently by long continued irregular fever, are also very common in Indian hospitals and offer excellent material for study, but although it is generally considered that these cases are hardly, if at all, amenable to quinine, it is quite exceptional to find in any hospital a case of this nature which is not being treated more or less vigorously with this drug, so that to prove definitely the connection between such cases and malaria becomes a difficult matter.

### IV.—The investigation of malaria among the general population.

It is certain that an examination of the statistics of admissions into hospitals for malaria gives us no real indication of the amount of malaria prevailing in a district at the time, and from a study of hospital cases we can learn nothing regarding the conditions under which the disease prevails. The method of investigation by which, above all others, the most valuable advances in our knowledge of malaria are likely to be made, is that of the investigation of the disease as it occurs among the general population of a place or district. The study of cases in hospitals and a detailed study of the structure of parasites is of little or no importance compared to a comparative study of the conditions under which malaria prevails in different places, and as this is a method of investigation which has received little attention in India up to the present, special importance should be attached to it.

An outline of the principles upon which such investigations have been carried out by us is as follows:—

Having selected a village or series of villages in any district for investigation, our first object is to find out, as accurately as possible, the amount of malaria and the liability to infection existing at the time of the investigation.

We have found in India, as was previously found in Africa by Koch and by Stephens and Christophers, that in any place which is more or less malarious, a certain number of the young children will have malaria parasites in their blood; and the percentage of young children so affected affords the most accurate test of the amount of malaria and the liability to infection existing in the place under examination.

The term "Endemic Index" has been given to this percentage of infected children, and the first thing to do on arriving at a village is to determine this endemic index.

For this purpose films of blood must be taken from at least thirty or forty children, none of whom should be over ten years of age. As may be imagined, this is often a difficult matter in Indian towns and villages, but with tact and patience it can be done, and except in one or two villages in Bengal we have never failed to get the required number.

The blood films should be taken by the method previously described, and any other method is practically impossible under the circumstances. At the same time as the films are being taken, the spleen of each child may be examined* in order to ascertain the percentage of children with enlarged spleens.

In the examination of the films the variety of parasite met with in each slide should be noted, in order that the proportion of each variety present in the village and district may be ascertained. The number of "large infections" should also be noted, as if there are a good number of heavy infections there will be more likelihood of finding infected *anopheles*.

Having ascertained the endemic index, the next thing is to search for adult *anopheles* in the houses, out-houses and stables, and to find out by dissection—

(1) What species of *anopheles* are carrying malaria at the time.

(2) The percentage of infection with sporozoites of the malaria-carrying species.

It will generally be found that only two or three species of *anopheles* are present in sufficient numbers in the houses to be easily caught, and in order to ascertain the two points noted above, at least one hundred *anopheles* of each of these species should be dissected.

It may happen that a fairly high percentage of infection (5—10 per cent.) will be found in one species, and no infection at all in another, but before it can be definitely stated that only one species of *anopheles* is carrying malaria in any place, at least 300 or 400 of any other species than the one in which infection was found should be examined.

I have already described* the method of catching adult *anopheles* and the best places in which to look for them, so it is unnecessary to do so here. I may mention, however, that in places where *anopheles* are plentiful, a muslin net will sometimes be found useful.

We have proved that it makes no difference in the sporozoite rate whether the *anopheles* in a village are caught in the stables and out-houses, or in the houses where people are actually living, and sporozoites are as likely to be found in mosquitoes caught in stables as in those caught in inhabited rooms. This is a point of some importance, because it is far easier to catch *anopheles* in stables and empty houses than in inhabited rooms.

If desired, the *anopheles* which are caught may be kept in bottles until they have got rid of their blood, and they may then be examined for zygotes, but there is no particular object in determining the zygote rate as it gives us little help in determining the liability to infection, for some at least of the mosquitoes which are infected with zygotes will die before they reach the stage when sporozoites are in the glands.

* "The Causation and Prevention of Malarial Fevers; a statement of the results of researches, drawn up for the use of Assistant Surgeons and Hospital Assistants." G. C. Press, Simla.

Having determined the percentage of infected *anopheles*, we can turn to the simpler but very important questions of—

(i) The exact identification of all the species of *anopheles* that are present in the village.

(ii) An estimation of the abundance of *anopheles* as a whole in the houses and the relative abundance of each species.

(iii) An examination of breeding grounds.

The breeding grounds of each species should be ascertained separately, and special attention should be paid to the breeding grounds of the species which we have found infected. Every pool, stream and collection of water of any kind within a radius of half a mile of the village should be thoroughly searched for larvæ.

Very important points to ascertain are—

(*a*) the extent of the breeding grounds as a whole and the extent of the breeding grounds of each species; and

(*b*) the distance of the nearest breeding grounds from the village and the distance of the nearest breeding grounds from the village of the infected species.

When all the points noted above have been determined, we may be said to have an accurate knowledge of the conditions determining the prevalence of malaria in the village or town under examination, and are able to compare our results with those obtained from the examination of other villages where the conditions may be different.

We are also in a position to formulate a definite system of prophylaxis for the place we have examined, for if there is one thing more certain than another with regard to the prophylaxis of malaria, it is that no general system of prophylaxis, which can be applied to any and every place, will ever meet with success. Each place must be studied by itself on the lines laid down above and independently of every other place.

It will be profitable, I think, to conclude this section with an example from one of the places we have examined, in order to show the kind of information which can be obtained from an investigation on these lines.

I shall select, as an example, the village of Ennur, which is situated on the coast about ten miles from Madras. The village was formerly used as a health resort by Europeans, but has been deserted by them for many years past on account of the fact that it is scarcely possible to pass even one night there without getting fever. It contains a number of European bungalows, now falling into decay.

The following were the results of our malarial survey of this place.

*I.—The determination of the source of infection and the Endemic Index.*

TABLE I.—SHOWING THE ENDEMICITY OF MALARIA AT ENNUR.

| Date of Investigation. | Number of children examined. | Percentage of children with enlarged spleen. | Percentage of children with malarial parasites in their blood (endemic index). |
|---|---|---|---|
| February 1902 . | 40 | 95 | 55 |

Thus in February, which is considered almost the healthiest time of the year, 55 per cent. of the native children have malaria parasites in their blood and they form therefore the *source of infection* at Ennur.

*II.—The variety of the infection.*

TABLE II.—VARIETY OF PARASITES FOUND.

| Number of infected children. | Percentage infected with Quartan parasites only. | Percentage infected with simple Tertian parasites only. | Percentage with a mixed infection of simple Tertian and Quartan. |
|---|---|---|---|
| 22 | 81 | 5 | 14 |

No malignant Tertian parasites were found at this place.

*III.—The carrier of the infection.*

Only two species of *anopheles* were found in the houses, *viz.*, *A. Rossii* (Giles), and *A. Culicifacies* (Giles).

The results of our dissections were as follow.

TABLE SHOWING INFECTION OF ANOPHELES AT ENNUR.

| Species of *anopheles.* | Number dissected. | Number in which sporozoites were found. | Percentage of infected *anopheles.* |
|---|---|---|---|
| *A. Culicifacies* . | 69 | 6 | 8·7 |
| *A. Rossii* . | 240 | *nil.* | *nil.* |

The carrier of infection at Ennur is therefore *A. Culicifacies*, and the high infection rate of this species indicates great liability to infection of any one residing at this place. As a matter of fact, this liability was also shown in another way by the fact that four out of the five servants who accompanied us developed malarial fever between 14 and 18 days after we had left the place. In the blood of three of these servants Quartan parasites were found, and in that of the fourth simple Tertian parasites.

*IV.—Conditions determining the prevalence and liability to infection.*

*Species of* anopheles *present and their abundance.—A. Rossii* and *A. Culicifacies* were present in the houses, *A. Rossii* being in great abundance and *A. Culicifacies* moderately so. A third species not present in the houses and of which only the larvæ could be found was *A. Fuliginosus* (Giles).

*Nature, extent and distance of breeding places.*—The breeding places were very extensive and surrounded the village. The nearest were within ten to twenty yards of the houses.

*A. Culicifacies* was found to be breeding almost exclusively in the "borrow pits" by the side of the railway and in the tanks in the compounds of the European bungalows.

*A. Rossii* was found to be breeding chiefly in the rice fields and in the pits dug for irrigating purposes.

*The condition of the European bungalows :—*

(i) Each bungalow has a main source of infection within fifty yards of it, *viz.*, the infected children in the village.

(ii) Each bungalow has an additional source of infection within ten to twenty yards of it, *viz.*, the servants' children who live in the compounds and who were also found to be infected with parasites.

(iii) Each bungalow has its own tank in which *anopheles* are breeding.

Under conditions such as these, unless very careful precautions were taken to avoid being bitten, it would be almost impossible for any one living in the bungalows to escape getting malaria.

The example given above refers to the month of February, but it would not be difficult to carry out a similar investigation, showing the variations in malarial endemicity, in the species of *anopheles* and in their infection, and in the other conditions noted above, *month by month throughout a period of a year*, and such an investigation would yield valuable results.

## V.—Methods for the study of mosquitoes.

The study of mosquitoes—and especially the study of mosquitoes of the *anopheles* genus—is inseparably associated with the investigation of malaria, and as comparatively little is at present known regarding even the identification of the various species of this genus, a great deal of study might be profitably spent on this subject. It has hitherto been considered sufficient to study the *anopheles* genus more or less as a whole, so that an exact identification of each species was not of great importance, but at the present time general statements regarding the characters and habits of the genus *anopheles* as a whole are of little or no value, and it is as necessary to study the different species separately as it is to study separately the different varieties of the parasites of malaria. In order to do this it is necessary to be able to identify each species correctly, and it is also of great advantage to be able to identify the larva of each species without the necessity of breeding out each batch of larvæ that have been caught.

The following are the chief points to be aimed at in a study of *anopheles* :—

(i) An exact and detailed description of the male and female adults of each species of such a nature as to prevent the possibility of confusing one species with another.

(ii) An exact description of the eggs and larvæ of each species with the points of difference between the eggs and larvæ of different species carefully noted.

(iii) An account of the breeding places, habits, prevalence and life history of each species separately.

(iv) An account of the pathological significance of each species separately.

The methods of collecting adult *anopheles* and their larvæ I have already described elsewhere,* and the method of mounting adult *anopheles* for examination is probably well known to all.† It is unnecessary, therefore, to refer to these points here.

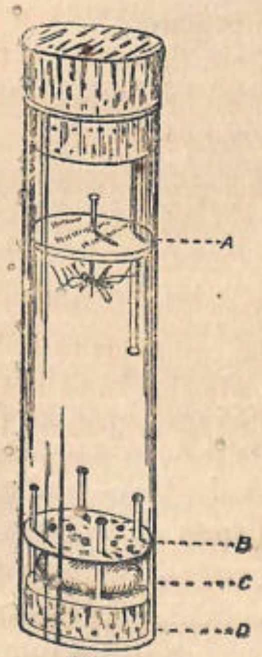

Fig. 6.

To keep mosquitoes permanently in India is a very difficult matter on account of the fact that the specimens either become very quickly mildewed, or destroyed by insects. For this reason I have devised the "mosquito tube" shown in the drawing (Fig. 6), and mosquitoes kept in these tubes remain in good preservation for almost any length of time.

For the identification of adult mosquitoes, an examination under a low power of the microscope ($\frac{1}{2}$ or $\frac{2}{3}$ inch objective) is almost a necessity, especially for the identification of closely allied species.

For this purpose the mosquitoes, which have already been mounted on card discs in the usual manner, are fixed by a pin thrust through the edge of the card disc to a flat piece of cork about an inch square.

A. Disc carrying the mosquito. This disc is pinned to the cork in the mouth of the tube.
B. Perforated cardboard disc pinned to the cork D in order to keep the bag of napthalene C in place.
C. Muslin bag containing napthalene.
D. Cork firmly fixed in bottom of the tube.



* See "The Causation and Prevention of Malarial Fevers; a statement of the results of recent researches." G. C. Press, Simla.

† For the methods of mounting mosquitoes, see "A Handbook of the Gnats or Mosquitoes," by Lieutenant-Colonel Giles, I.M.S., 2nd edit. Bale Sons and Danielsson, London, 1902; or "A Monograph of the Culicidæ of the World," by F. V. Theobald, F.E.S. British Museum (Natural History), London, 1901.

This cork, carrying the mosquito to be examined, is placed on the microscope stage and can be moved about on the stage at will.

The angle at which the pin is fixed in the cork should be such that the part of the mosquito which is being examined is always as nearly as possible parallel to the microscope stage.

It is best to begin with an examination of the palpi and to work backwards, noting every marking and character that presents itself, and the position of the disc carrying the mosquito will of course have to be frequently altered by removing the pin from the cork and readjusting it at a different angle. The palpi, the wings, and the legs are the structures to which chief attention should be directed, and diagrammatic drawings should be made showing clearly every marking on these structures. In addition, it is important to note the characters of the scales on the head, thorax, and abdomen.

In order to ascertain the arrangement of the transverse veins of the wings, and the detailed shape of the wing scales (an important point to note), one or more wings must be mounted separately on a slide and covered by a cover slip. The wing can then be examined if necessary under a higher power of the microscope. The tips of the male legs, especially the fore legs, and the male genitalia should be mounted on slides in xylol balsam. The characters of the fore ungues in the male and the male genitalia are of use in identification.

In order to obtain eggs for examination, a few female *anopheles* that have been caught in a village should be transferred to a glass jar similar to those already described for use in feeding experiments. The jar should be inverted over a small tin of water on which a piece of paper is floating for the mosquitoes to rest upon. If this is done in the early evening, by the next morning one or two of the *anopheles* will almost certainly have laid their eggs. Some of the eggs will almost always be found to have been deposited on the pieces of damp paper floating on the water in the tin, so that all that one has to do is to remove the paper carrying the eggs—which can be easily seen by the naked eye—and to place it on the microscope stage over the hole in the centre of the stage. The individual eggs can then be examined by reflected light with a ½ or ⅔ inch objective.

The differences that can be made out between the eggs of different species will be referred to later. For the measurement of the eggs and other structures in connection with mosquitoes an eye piece micrometer will be found useful.

The characters necessary for the identification of larvæ can be made out either by examining fresh larvæ or in permanently mounted specimens.

In order to examine a larva when it is alive, it is caught in a small spoon—care being taken not to injure it—and transferred with a small drop of water on to a glass slide. A cover-glass is then dropped very gently on to it. This has the effect of preventing the larva from continually wriggling out of the field of the microscope, and if carefully done it does not break any of the larval hairs or injure it in any way, so that at end of the examination the larva can be returned to the bottle and allowed to develop, in order that by an examination of the adult insect we may test the correctness of our observations on the larva.

After some practice it is of course possible to make out all the structures in the intervals of rest which follow each effort to wriggle off the slide, without the necessity of applying a cover-glass, but as the resting intervals are usually very brief, it is best at first to examine the larvæ imprisoned by the method just described.

Permanent specimens of larvæ may be obtained either (1) by mounting them simply in a solution of formalin in hollow ground glass slides—the deeper the hollow of the slide the better,—applying a cover-glass and ringing it with thick Canada balsam; or (2) by the following more permanent method:—

(*a*) Kill the larvæ by immersing them in strong formalin solution, and allow them to remain in this solution for 12 hours. This partially fixes them without causing any shrinking of the larvæ.

(*b*) Complete the fixing and dehydration by immersing the larvæ for ten or fifteen minutes in absolute alcohol.

(*c*) Remove the alcohol by immersing the larvæ for half an hour or more in oil of cloves.

(*d*) Clear with xylol and mount on an ordinary flat slide under a cover in Canada balsam.

During the above process great care must be taken to handle the larvæ very carefully so as not to break any of the finer hairs. They are best removed from one dish to another by lifting them up very gently on the edge of a piece of stiff paper.

The general anatomy of the larva is well described in Dr. Nuttall's paper in the Journal of Hygiène, "The Structure and Biology of Anopheles"—Journal of Hygiene, Volume I, No. 1, Jan. 1901, and need not be referred to here. I shall note therefore only the structures we have found of use in the identification of the larvæ of different species.*

These structures are the following:—

(1) The "*frontal hairs*" (Figure 7, I-A and II). These are four fine hairs which are attached to the dorsal surface of the head anteriorly. They may be called the external and the median frontal hairs. The external hairs are placed one at each corner of the dorsal chitinous end of the head and exactly overhang the prominent so called whorl organs or "shaving brushes." When the whorl organs are pushed out the external (corner) hairs are difficult to see, but when the whorl organs are drawn in (as often happens permanently when larvæ are killed with formalin solution), they stand out prominently.

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* The initiation of the work on the differentiation of eggs and larvæ and the chief part of the results obtained are due to Dr. Christophers.

The drawings of larvæ reproduced here are copied from "A Monograph of the Anopheles of India," by Capt. S. P. James and Capt. W. Glen-Liston. Thacker, Spink and Co., Calcutta (in the press).

The median hairs arise close together near the middle line and project forward in front of the head. They are not difficult to see with a $\frac{1}{2}$ or $\frac{1}{4}$ inch objective. A very important difference between the larvæ of different species depends upon the fact that the characters of these external and median frontal hairs differ in different species.

Thus in some species both the external and median hairs are simple and entirely unbranched (see figure 7, Larva of *A. Rossii*), in other species both pairs of hairs are slightly branched (*e.g.*, *A. Superpictus*, Grassi), in others the median hairs are slightly branched or frayed, while the external are very much branched ("like a small tree," Grassi, *e.g. A. Fuliginosus*), and again the median may be simple or slightly branched and the external undergo dichotomous branching so that it forms a very prominent and distinct "cocade" (*A. Nigerrimus, A. Barbirostris*).

Further details regarding these hairs are given under the descriptions of the different *anopheles* of India.

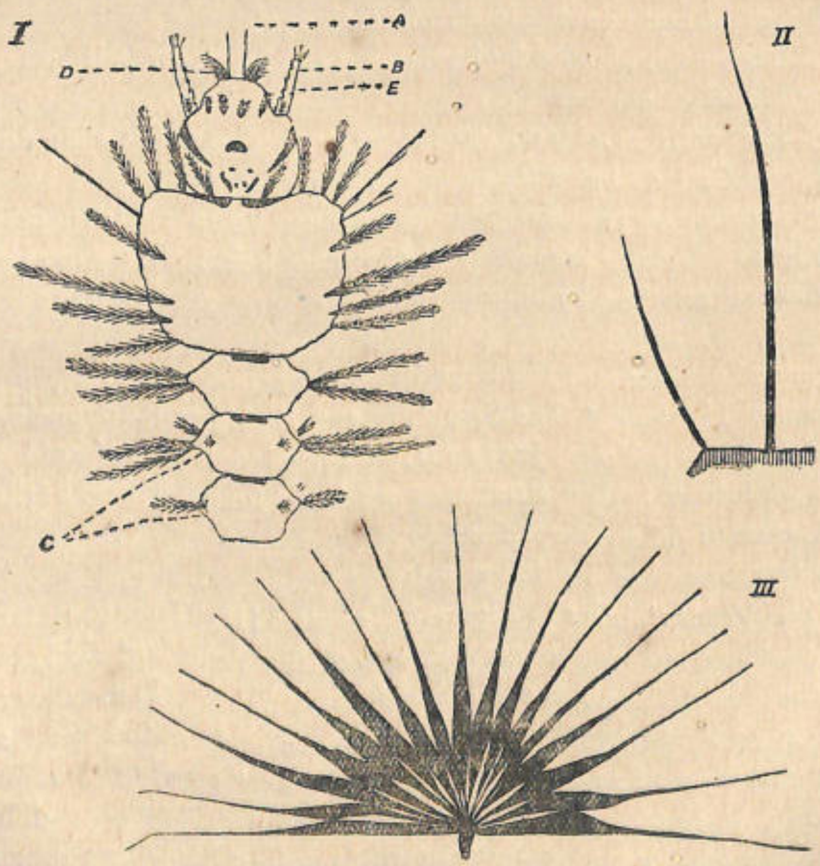


Fig. 7.—The larval characters of *A. Rossii*.

I. A. Frontal hairs.
B. Antenna.
C. Palmate hairs.
D. Whorl organs or "shaving brushes."
E. External hair on antenna.

II. Frontal hairs enlarged.

III. Palmate hair enlarged.

(2) The "*palmate hairs.*" (Figure 7, C.)

If the dorsal surface of the abdomen of the larva be examined at about the third or fourth segment, a pair of prominent small conical or fan-shaped hairs will be seen. The hairs are arranged in pairs—one pair to each segment of the abdomen—and their function is to keep the larva floating in a horizontal position at the surface of the water. Each palmate hair consists of a stalk, to the free end of which is attached a conical bundle of about fifteen to eighteen hairs, arranged round the stalk, like the petals of a flower of which a portion of the circle is incomplete. At the surface of the water these leaflets spread out and form a series of little cup-shaped organs which keep the larva afloat.

Both the number and the shape of the palmate hairs of different species of larvæ vary considerably, so that in this fact we have a second means of distinguishing between the larvæ of different species of mosquitoes.

In some species, for example, the larvæ are provided with a pair of palmate hairs *on the thorax and on all the segments of the abdomen except the last two, e.g., A. Fluviatilis, A. Culicifacies.*

In other species there are no palmate hairs at all on the thorax and only rudimentary ones on the first and second abdominal segments, the true palmate hairs being present from only the third to the seventh segments of the abdomen (*e.g., A. Rossii, A. Stephensii*). In other species again, the first three abdominal segments are devoid of true palmate hairs (rudimentary ones may be present on the third segment), *e.g., A. Turkhudi.* As regards the shape of the palmate hairs, considerable differences exist, which are useful points of distinction between larvæ.

Thus, in *A. Rossii* larvæ each leaflet of the palmate hair is provided with a very long terminal filament (see Fig. 7), in the larva of *A. Fluviatilis* and *A. Theobaldi*, on the contrary, the terminal filament is so short as to form only short spine.

A reference to the figures of the different larvæ will show other differences in the shape of the palmate hairs by which the larvæ can be recognised.

(3) The *Antennæ.* (Fig. 7, B.)

These structures are of course very easily recognised, and in the larvæ of the majority of Indian species the characters of the antennæ differ but little. A very small hair (E, Fig. 7) should be noted on the outer side of the antenna in the majority of Indian species. Two of the Indian species, however (*viz., A. Nigerrimus* and *A. Barbirostris*), are easily distinguished from all other larvæ by the fact that their larvæ possess a stout branching hair on the inner side of the antenna, quite distinct from the small external hair referred to above.

(4) The pattern on the *head.*

Although the pattern on the dorsal surface of the head of *anopheles* larvæ is formed by dots of pigment only, and is therefore liable to considerable variation, yet in some species the head pattern is sufficiently constant to be of assistance in the identification of larvæ. The inverted triangular area enclosing four dots of pigment on the dorsal surface of the head of the larva of *A. Rossii*, for example, is fairly characteristic, as is also the complete absence of any pattern on the head of *A. Stephensii* larvæ. The usual markings on the heads of these and other larvæ are given in the diagrams.

The above are the chief characteristics by which we have differentiated the larvæ of the Indian species of *anopheles*. Further work would doubtless show that other structures might be used for the same purpose, such for example as the spines on the maxillæ or the varying length of the caudal papillæ. It would also be interesting to discover the characters which distinguish the male and female larvæ from each other if in this stage of the mosquito's existence any marked difference exists.

# PART II.

## I.—OBSERVATIONS ON THE CHARACTERS AND HABITS OF ANOPHELES MOSQUITOES AND THEIR LARVÆ.

AT an early stage in our investigations it became evident that the number of species of *anopheles* mosquitoes that exist in India was much larger than had been anticipated, and that the descriptions of the *anopheles* which had been recorded, both from India and from other countries, were so inadequate as to render their identification difficult, if not, in many cases, impossible.

Until recently, however, this was a matter of small importance from a medical point of view, because the fact that some species of *anopheles* might be more important than others in connection with the transmission of malaria had not been generally recognised by observers, the majority of whom had therefore been content with a study of the *anopheles* genus as a whole, without recognising the importance of a study of each species separately.

Our observations, however, have shown that the different species of *anopheles* differ very markedly not only in their habits and breeding places, but also in their relation to the prevalence of malaria in different places, so much so, indeed, that whereas the presence of one species appears to be almost always associated with a considerable amount of malaria, the presence of another species appears to exert no influence whatever on the prevalence of the disease.

For these reasons, if for no other, it is certainly of the greatest practical importance to be able to identify the different species of *anopheles* correctly, and to study the habits, life history, and pathological significance of each species separately.

Unfortunately, however, the classification of *anopheles* is at present in an early stage, and the difficulties of identification of any particular species are enhanced by the fact that a considerable number of *anopheles* mosquitoes have been lately described as new species, which differ in only very minute respects from existing species.

It is doubtful whether all these so called new species are really distinct, and it becomes necessary to enquire whether the characters by which entomologists separate closely allied species are sufficiently constant to be relied upon, or whether the large number of species which differ but slightly from each other are in reality only variations of one or two existing species.

*A. Culicifacies* (Giles), for example, may be taken as a type of the group which I have called the group of "small brown *anopheles* with unbanded legs." The five following mosquitoes in India which resemble *A. Culicifacies* closely have been described as new and distinct species:—*A. Fluviatilis* (re-named *A. Christophersi* by Mr. Theobold), *A. Listonii* (Giles), *A. Listoni* (Liston), *A. Indica* (Theobald), and *A. Jeyporiensis*, as well as the African species

*A. Funestus*, *A. Minimus*, *A. Rhodesiensis*, and the Italian species *A. Superpictus* (Grassi).

We thus have at least nine closely allied species of this one group which, although considered by entomologists to be quite distinct, are nevertheless very difficult to identify and to separate from each other. The points relied upon by Mr. Theobald* for separating all the above mosquitoes from each other as distinct species are as follow:—

(1) Differences in the characters of the wing markings.

(2) Differences in the relative position of the transverse veins of the wings.

(3) Differences in the character of the male genitalia and of the fore ungues of the male.

(1) The wing markings of the nine species mentioned above are admitted to be almost identical, and in forming an estimate of the value to be attached to slight differences in wing markings we must remember that if only a small number of any one species of *anopheles* are examined, considerable variations in the wing markings will be found. Indeed, it is quite possible that in some species at any rate a seasonal variation in these markings may occur. These variations have not previously been taken note of by entomologists in England, owing to the limited supply of specimens to which they have access, and as several of the so-called new species (*e.g.*, *A. Indica*, and *A. Minimus*) have been described from only a single specimen, it might easily happen that such a specimen was a variation from the type of an already described species.

Some of the variations that occur in wing markings will be mentioned in the notes on the different species, and on the whole I am of opinion that such slight differences as are recorded between the wing markings of the species enumerated above, are of little or no value as a means of separating them into distinct species.

(2) Great stress is laid by Mr. Theobald on the value of the relative positions of the cross veins of the wings as a means of distinguishing species, and a reference to the monograph will show that this is the chief character by which he separates nearly all the species of this group. Thus of *A. Funestus* he says, "It is clearly related to *A. Rhodesiensis* and *A. Superpictus*, but it can be readily told by the positions of the cross veins." Of *A. Indica* he says, "It is seemingly related to Grassi's *A. Superpictus*, the wings, etc., being almost identical ......... From *A. Funestus* it differs in the position of the cross veins." Of *A. Rhodesiensis* he says, "At first sight one would take it for Grassi's *A. Superpictus*, but the position of the cross veins at once separates them." Of *A. Minimus* he says, "Very like *A. Funestus*, *A. Rhodesiensis*, and *A. Superpictus*, but from *Funestus* it can be at once told by the disposition of

* "A Monograph of the Culicidæ of the World," by F. V. Theobald, Esq., M.A., F.E.S. British Museum (Natural History), 1901.

the cross veins and the darker scaled wings, from *Rhodesiensis*, by the ornamented wing fringe and the cross veins, and from *Superpictus* by its darker hue and ornamented fringe."

Before, however, it would be possible to place such reliance as this on the value of this character as a means of distinguishing species, it must be shown that the relative position of the cross veins to each other in any species is always constant. If it can be shown that in any species the relative position of the cross veins is not constant, then of course its value as a distinguishing character is very much lessened.

In order, therefore, to test this point I examined a number of specimens of two well known species of *anopheles, viz.*, *A. Culicifacies* and *A. Rossii*, to ascertain whether any marked variations occur in the relative positions of the cross veins. The results of my examinations are given in the diagrams below (Fig. 8), and it will be seen that in the same batch of both these species very marked variations are found, so that, in my opinion, this character does not appear to be sufficiently constant to enable it to be used for the separation of species.

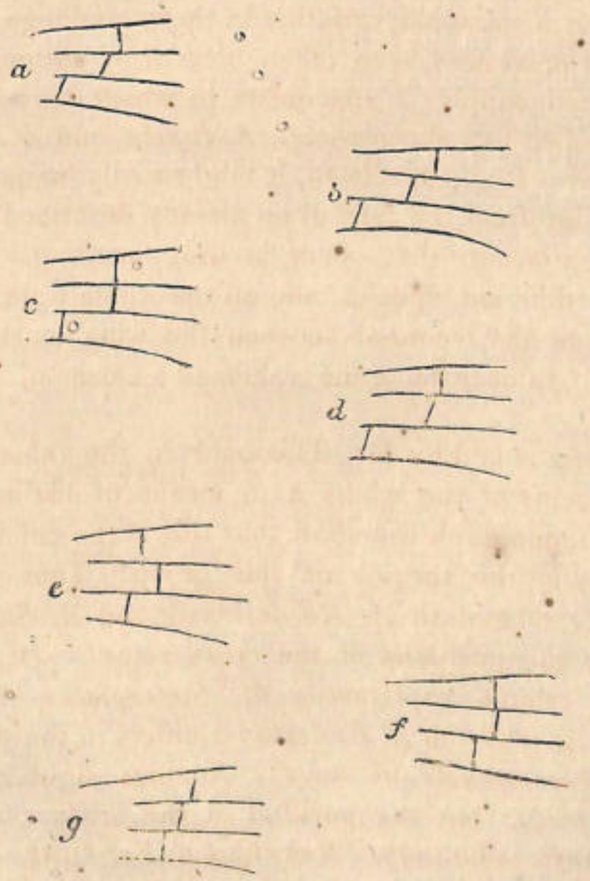


Fig. 8.—To show variation in the relative positions of the transverse veins of the wing.
*a, b, c, d.* *A. Rossii* (20 examined). | *e, f, g.* *A. Culicifacies* (15 examined).
The apex of the wing is towards the right side in each figure.

(3) The other characters upon which Mr. Theobald relies, depend upon differences in the *males*. If, however, two species which can be separated from each other only by the characters of the males, occur in the same locality, we shall be unable to say to which species any female specimens we may catch belong, and if only one of the two species happens to be carrying malaria at the time, we shall be unable to say which species is to blame.

None of the above methods of separating closely allied species therefore can be considered entirely satisfactory, and for the most part they are liable to lead us into the error of considering as a new species any particular specimen which varies slightly from the type of an already known species. A good deal of our time has been spent on the question of the distinctions between the different Indian species of *anopheles*, and it appears probable that our work on the differences between the eggs and larvæ of different species of *anopheles*, will point the way to a definite and final system of classification; for it will, I think, be admitted by all, that if a definite difference between the eggs or larvæ of any two species of *anopheles* can be shown to exist these species must be certainly distinct—whatever the characters of the adult may be.

I purpose, therefore, in the following notes on the Indian species, to include as distinct species only those in which a difference between the eggs or larvæ can be shown to exist.

We have found that, to a certain extent, the various species can be divided into groups based on the following characteristics :—

(i) the general appearance of the adult insect;
(ii) the characters of the eggs and larvæ;
(iii) the habits of the adult insects and their larvæ.

I have therefore arranged the different species in four groups as follows :—

GROUP I.

1. *A. Fluviatilis* (sp. n.) — Indian species.
2. *A. Jeyporiensis* (sp. n.) — Indian species.
3. *A. Culicifacies* (Giles) — Indian species.
4. *A. Immaculata* (sp. n.) — Indian species.
[5. *A. Superpictus* (Grassi) ... Italian species.]
[6. *A. Funestus* (Giles) ... African species.] and its allies.

*Group characteristics.*—The *adults* of this group are characterised by their small size, by their being generally of a dull brown colour, and by the fact that their legs are uniformly coloured without any banding at the joints.

The *eggs* are characterised by their small size, by the upper surface being very narrow, and by the lateral floats being near together on this surface. The *larvæ* are characterised by possessing a pair of well developed palmate hairs on all the abdominal segments *and on the thorax.*

In habits this group is chiefly characterised by the fact that they breed almost exclusively in running water. They may in fact be called the "stream breeding mosquitoes." They are further characterised by the very important fact that their members appear to be the chief malaria-carrying species in India and Africa.

### 1. A. Fluviatilis, sp. n.

Synonyms—*A. Christophersi* (Theobald), *A. Listoni* (Liston), Fig. 9.

Fig. 9.—Wing of *A. Fluviatilis.*

This mosquito has been re-named *A. Christophersi* (though not as yet described) by Mr. Theobald. It has also been accurately described by Captain Glen-Liston, I.M.S., in the *Indian Medical Gazette* (Oct. 1901) under the name *A. Listoni*. The description of *A. Listonii* (Giles) in Colonel Giles' Hand-book of Mosquitoes* does not, I think, apply to this species, and his description is possibly from a specimen of *A. Culicifacies.*

It is a small mosquito of a generally brown colour. The palpi are marked with three white bands—one broad band which includes the tips of the palps and two narrow bands nearer the base.

The legs are unbanded.

The wing markings are detailed in the diagram, and it is therefore not necessary to enumerate the number of black and white spots on each vein here. The following points should be specially noted: (1) The third longitudinal vein is white scaled nearly throughout its length. (2) Including the white apical spot there are six white patches on the wing fringe opposite the terminations of the longitudinal veins, except the sixth.

The wing markings in this species may, however, vary a good deal, and it may even be possible to find specimens in which the third longitudinal vein is black scaled almost throughout, thus making it difficult to distinguish this species from *A. Culicifacies.*

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* A Hand-book of the Gnats or Mosquitoes, 2nd edition, 1902. John Bale Sons and Danielsson.

One specimen which I received from Sukchar, which might possibly deserve to be considered a new species, has two equal broad white bands and one narrow one on the palpi, but in the absence of more specimens and of its larvæ it is, for the present, best placed as a variety only.

The adults can be distinguished from the other members of this group by a careful comparison of the wing markings (see figures).

*Characters of larva.*—These are shown in the drawing (Fig. 10). The "Frontal hairs" are simple and unbranched. The "Palmate hairs" occur on all the abdominal segments and very prominently on the thorax. The terminal filament of each leaflet is short.

The "head pattern" is shown in the figure.

Fig. 10.—Larval characters of *A. Fluviatilis.*

*Habitat and observations.—A. Fluviatilis* was first found by us in the Duars where it was present in fair numbers in the native huts on the tea plantations. We have also found it in the Central Provinces (Nagpur) and in the Jeypur State. It has also been found in the Berars (Liston) and in the Goa Province (Aitken), so it appears to be fairly widely distributed in India. It does not, I think, occur in the Punjab, but it has lately been found in Kashmir.

We have usually found its larvæ in clear streamlets with grassy edges.

This species is the carrier of malaria in the Duars (6·25% infected with sporozoites at the time of examination). Experimentally we have proved that the parasites of simple Tertian, Quartan, and malignant Tertian will develop in it.

## 2. A. Jeyporiensis, sp. n.

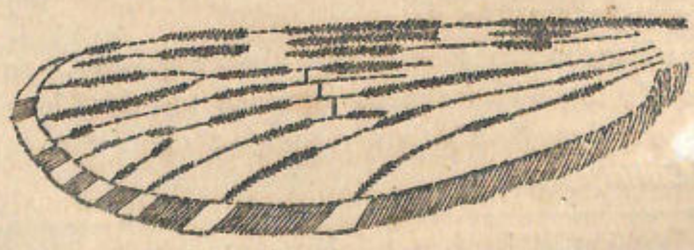

Fig. 11.—Wing of *A. Jeyporiensis.*

This species resembles the preceding one very closely in the adult stage, but the differences of the larvæ at once separates them as distinct species.

The markings of the palpi are the same as those of *A. Fluviatilis* and the legs are unbanded. The wing markings are shown in the figure, and it will be seen that they differ in certain points from those of *A. Fluviatilis*, thus *A. Jeyporiensis* has three black scaled areas on the sixth longitudinal vein and seven pale patches on the wing fringe (including the apical one), whereas *A. Fluviatilis* has only two dark spots on the sixth longitudinal vein and only six white patches on the wing fringe.

*Characters of the larva.*—These are shown in the figure (Fig. 12.). Both the median and external frontal hairs are thickly branched, whereas those of *A. Fluviatilis* and *A. Culicifacies* are simple.

Fig. 12.—Larval characters of *A. Jeyporiensis.*

*Habitat.*—The Jeypur State, the Central Provinces (Nagpur).

### 3. A. Culicifacies (Giles).

Fig. 13.—Wing of *A. Culicifacies.*

This mosquito has probably been described by Mr. Theobald under the name *A. Indica*. Colonel Giles' description of *A. Listonii* is also possibly taken from a specimen of this species.

The palpi resemble those of the other members of this group. The legs may be described as unbanded, although the apices of some of the segments may occasionally show small white markings.

The wing markings are shown in the diagram. It should be noted that the third longitudinal vein is black scaled throughout its length, and that, including the white apical spot, there are only three white patches on the wing fringe.

*Characters of the larva* (Fig. 14).—Both the median and external frontal hairs are simple and unbranched. There is a pair of palmate hairs on all the abdominal segments and on the thorax, but the thoracic pair are not so well developed as in *A. Fluviatilis* or *A. Jeyporiensis*, and the terminal filament of each leaflet is longer than in either of those larvæ.

The head pattern also differs from that of both the above larvæ (see figure).

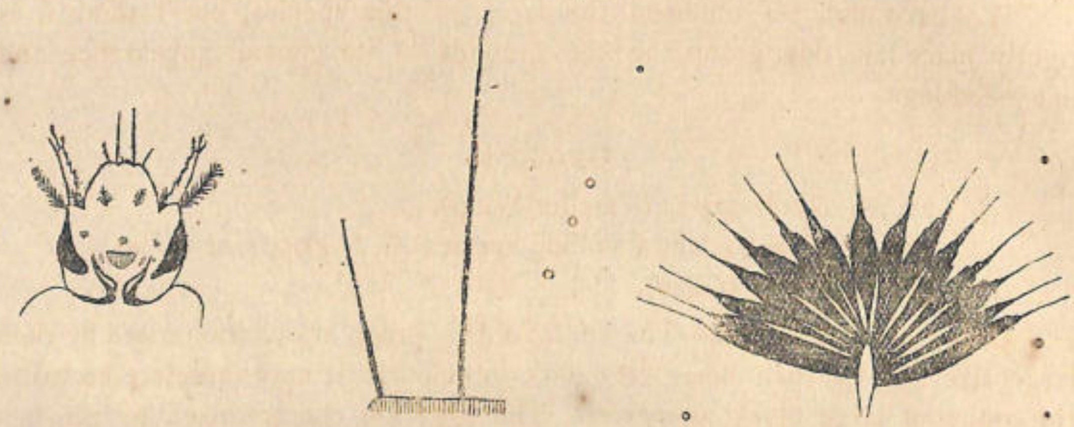

Fig. 14.—Larval characters of *A. Culicifacies*.

*Habitat and observations.*—This mosquito is widely distributed in India. We have found it in the Punjab (Lahore, Mian Mir, etc.), Madras and several places on the East Coast (Ennur, Armagaon, etc.), the Central Provinces (Nagpur, etc.) and in the Jeypur State. It has also been recorded from the Berars (Ellichpur) and from other parts of India.

In the Punjab it appears to have no special seasonal prevalence, and its larvæ can be found in the irrigation canals throughout the year. The adults are found in the houses as soon as the weather become swarm enough for the larvæ to develop. Its prevalence in fact coincides rather with the changes which occur in the flow or stoppage of the irrigation canals (in which it breeds almost exclusively in the Punjab), than with any particular season.

At Ennur, near Madras, I found it breeding chiefly in the clear pools by the side of the railway "borrow pits," and it has also been found breeding in pools in the bed of a river.

The adults are peculiar in that they do not adopt the characteristic attitude of other *anopheles* when resting on a wall or roof, but sit more or less like a culex with the body parallel to the wall and the back hunched (the proboscis, head and body not being in one line). For this reason it is easily mistaken for a culex. We have proved this species to be the malaria carrier in Mian Mir and in Ennur (Madras). Experimentally also we have shown that the parasites of the three varieties of malarial fever can readily develop in this species.

### 4. A. Immaculata, sp. n.

This is a small mosquito resembling in general characters the other members of this group, except that *its wings are entirely unspotted.* It is apparently a rare species, for we only obtained one specimen from Ennur. Several specimens, however, have since been obtained by Mr. Aitken from the Goa Province.

The legs are unbanded but are darker coloured towards the tarsi.

We have not yet obtained the larva of this species, but I think it is rightly placed in this group, on the grounds of its general appearance and unbanded legs.

### Group II.

1. *A. Barbirostris* (Van der Wulp).
2. *A. Sinensis* and its allied species *A. Nigerrimus* (Giles).
3. *A. Gigas* (Giles).

*Group characteristics.*—The *adults* of this group are characterised by their large size and by their being very dark in colour. It may therefore be called the group of large black *anopheles*. The *eggs* are characterised by the fact that the lower surface is marked off by light coloured lines into polygonal areas, by the fact that their upper surface is very narrow, and that the floats do not reach the margin of the upper surface.

The *larvæ* are characterised by the fact that the external pair of frontal hairs are developed into large and prominent "cocades" and the antennæ possess a large branching hair on their inner surface which is absent in the larvæ of the other groups.

In *habits* this group may well be termed the group of "wild" mosquitoes. The adults are very seldom found in houses, and the larvæ are usually found in deep natural weed-grown pools in the jungle or under trees.

### 1. A. Barbirostris (Van der Wulp).

This mosquito is characterised by its very large size and black colour. Its wing veins are covered for the most part with dense black scales, interrupted on the costa by two small white spots. There is almost always one pale patch on the wing fringe opposite the lower branch of the fifth longitudinal vein. The thick black densely-scaled palpi which are ***unbanded*** will serve to distinguish this species from any other of the Indian *anopheles.*

*Characters of the larva.*—Its larvæ are easily identified by the naked eye, from their large size, black colour, and the fact that when resting at the surface, they are nearly always bent more or less in the shape of an S. Their microscopical characters are shown in the figure. The external frontal hairs are converted into a large tuft which is easily seen lying over the so-called whorl-organs or "shaving brushes," and on the inner side of each antenna is a large branching hair.

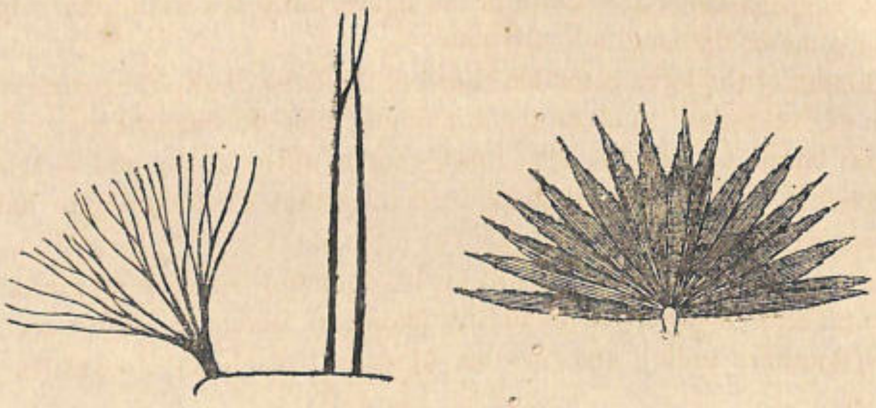

Fig. 15.—Larval characters of *A. Barbirostris.*

*Habitat and observations.*—We have found this mosquito in several places in the plains of Bengal, in the Central Provinces, and in the Punjab. It occurs also in Assam and in the United Provinces as well as in several places in the south of India.

Its larvæ are found almost exclusively in deep dark pools with much acquatic vegetation, and they can only be caught separately one at a time by dipping with a cup or tin. In Lahore they occur in the lily ponds in the public gardens.

Experimentally we have proved that the zygotes and sporozoites of the human malaria parasites can develope in this species, but it is doubtful whether it ever acts as a carrier in nature owing to its rarity in houses.

## 2. A. Sinensis, sub.-species Nigerrimus.

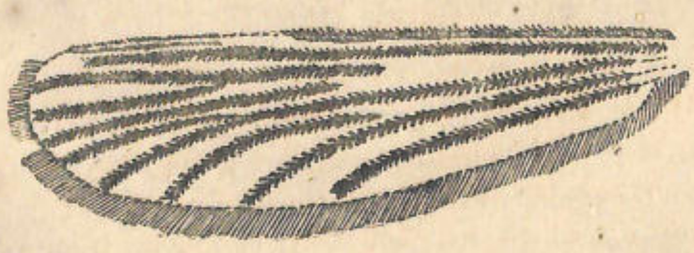

Fig. 16.—Wing of *A Nigerrimus.*

This mosquito may be considered as a type of several species of *anopheles* which are regarded by Mr. Theobald as distinct, *viz.*:—

*A. Sinensis* (Wiedmann), *A. Indiensis* (Theobald), *A. Annularis* (Van der Wulp), *A. Psuedopictus* (Grassi).

Colonel Giles, however, regards all these as one moderately variable species, and with this view I am inclined to agree.

The palpi of *A. Nigerrimus* are marked with four white bands, the terminal of which includes the tip. The tibiæ and tarsal segments of the legs are apically white banded.

The chief wing markings are shown in the figure, but a few additional white spots occur on some of the longitudinal veins.

The characters of the larva resemble those of the larva of *A. Barbirostris* in the possession of "cocades" and of the branching hair on the antenna. Palmate hairs are borne by the second, third, fourth, fifth, sixth, and seventh abdominal segments. Their general shape resembles that of the palmate hairs of *A. Barbirostris* (Fig. 15).

*Habitat and observations.*—This is a fairly common species in the outskirts of Calcutta and in other places in the plains of Bengal. It also occurs in Travancore (Southern India) and in the Madras Presidency (outskirts of Madras).

We have not carried out any feeding experiments on favourable cases of malaria with this species.

I have shown that the embryos of *Filaria Sanguinis Hominis* can develop in this species, as well as in *A. Rossii*, and this observation has been lately confirmed as regards *A. Nigerrimus*, in Calcutta.

At Jalpaiguri (Bengal) we found its larvæ chiefly in deep natural pools on swampy ground at some distance from houses.

### 3. A. Gigas (Giles).

We have not yet met with this new species which is, however, probably related to the above mosquitoes. Colonel Giles states that it has up to the present been found only at an elevation of 5,000 feet in Conoor (Madras Presidency).

### Group III.

| | |
|---|---|
| 1. *A. Fuliginosus* (Giles). | 3. *A. Theobaldi* (Giles). |
| 2. *A. Jamesii* (Theobald). | 4. *A. Lindesayii* (Giles). |

*Group characters.*—The mosquitoes of this group may be considered as intermediate between those of Group II and those of Group IV.

The adults are of medium size and vary in colour from very dark (*A. Jamesii*, *A. Lindesayii*) to a light grey or brown (*A. Fuliginosus*, *A. Theobaldi*).

The larvæ are characterised by the fact that although the external frontal hairs are very distinctly branched (except *A. Lindesayii*), they are not developed into the actual cocades which characterise the larvæ of Group II. They are, in fact, intermediate between the larvæ of Group II and Group IV in which the frontal hairs are simple and unbranched.

In habits the members of this group are intermediate between the typically "wild" mosquitoes of Group II, and the typically "domestic" mosquitoes of Group IV.

They are all occasionally found in houses in small numbers, and exceptionally *A. Fuliginosus* may be found in stables and out-houses in large numbers.

The breeding places of *A. Fuliginosus* and *A. Jamesii* are similar to those of Group II, except that they appear to prefer shallower, clearer water with more sunlight.

### 1. A. Fuliginosus (Giles).

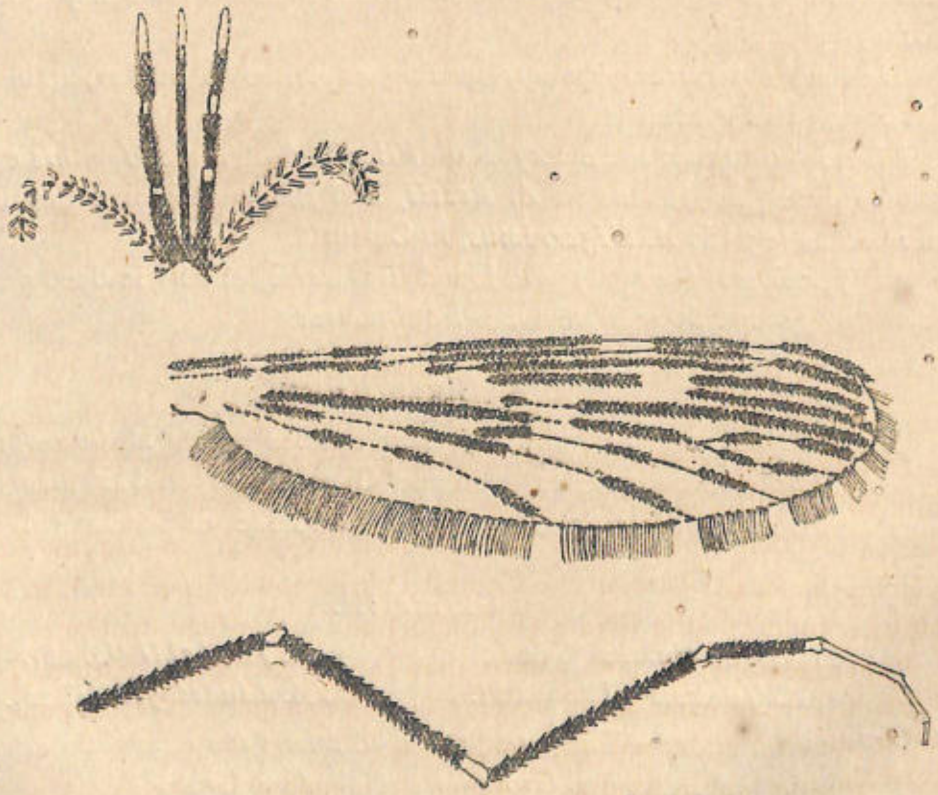

Fig. 17.—*A. Fuliginosus.*

The markings on the wings, palpi, and hind legs of this mosquito are shown in the figure (Fig. 17).

In the fore legs there are apical white bands to the femora, tibiæ and tarsi except the fourth tarsus, the joint between this and the fifth tarsus being black. At the joints of the other legs, there are apical white bands.

Near the apex of the femur of each middle leg is a large white patch placed on one side and not forming a complete ring round the femur. This white patch is a useful character in identification.

*Characters of the larva* (Fig. 18).—The median frontal hairs are slightly branched, the external frontal hairs are much branched so as to form a small tuft in front of the whorl-organs or shaving brushes.

There is no branching hair on the antenna as in the larvæ of Group II.

The palmate hairs and the head pattern are of the shape and character shown in the figure.

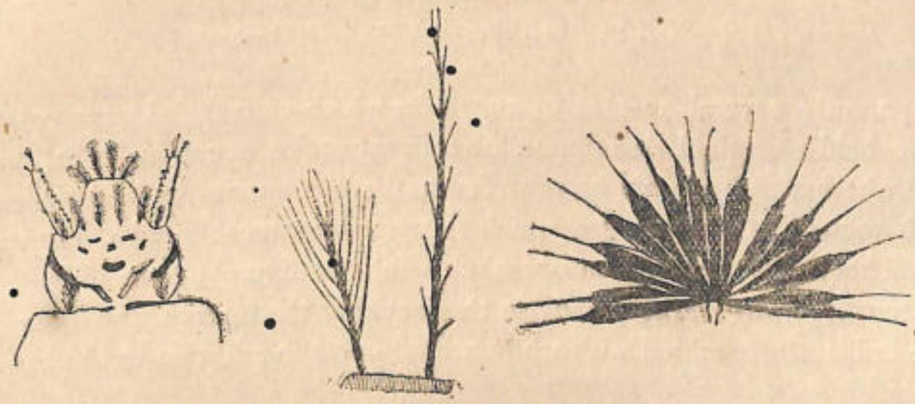

Fig. 18.—Larval characters of *A. Fuliginosus.*

*Habitat and observations.*—A widely distributed species in India. It occurs in Calcutta, and many places in the plains of Bengal, in Kurseong (at an elevation of 5,000 feet), in the Punjab (Lahore, etc.), in Madras and several places on the East Coast, in the Central Provinces (Nagpur, etc.), in Travancore (Southern India), in the Berars (Ellichpur) and in the Goa Province.

Experimentally we have shown that the parasites of Quartan, Tertian and malignant Tertian malaria will develop in this mosquito. We have not, however, found it infected under natural conditions.

In Nagpur and in Madras we found it breeding in the large natural tanks with grass and weed at the edges. In other places we have found its larvæ along with the larvæ of *A. Nigerrimus* in shady weed-grown pools.

## 2. A. Jamesii (Theobald) and its allied species.

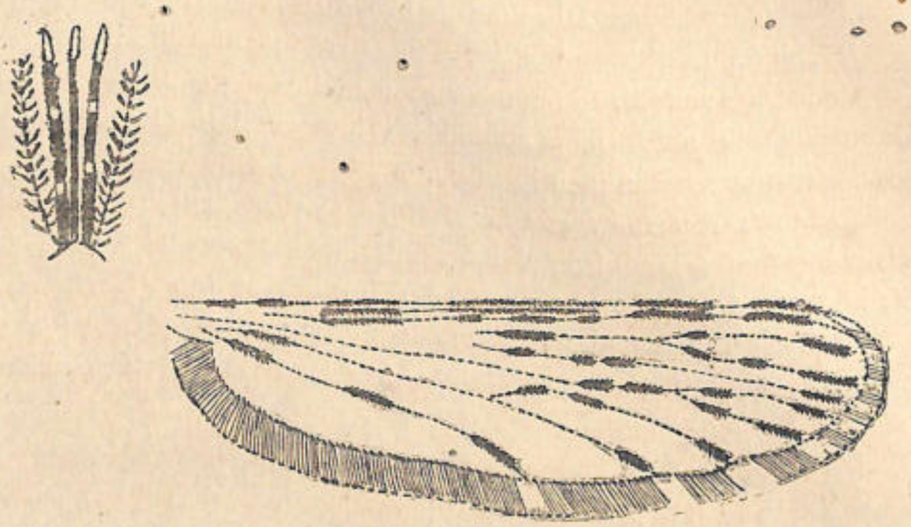

Fig. 19.—Palpi and wing of A. *Jamesii* (Theobald).

Apparently several species of *anopheles* which correspond more or less with Mr. Theobald's original description of this mosquito occur in India, and it is doubtful whether they should be regarded as distinct species or as varieties. The main characters of the species which I regard as most nearly corresponding to Mr. Theobald's description are shown in the figure above (Fig. 19). It differs from *A. Fuliginosus* chiefly in the fact that the femora and tibiæ of the legs are brilliantly speckled with white scaled spots in addition to the banding at the joints.

As in *A. Fuliginosus* the last $3\frac{1}{4}$ hind tarsal segments are pure white. The first tarsal segment of the fore leg has an indistinct median band.

The differences in wing markings between this species and *A. Fuliginosus* are shown in the drawings.

*Characters of the larva.*—Both the median and external frontal hairs are branched in the same manner as is shown under the figure of the larvæ of *A. Theobaldi* (Fig. 21), but somewhat more thickly than in that species.

There is a pair of palmate hairs on each segment of the abdomen from the first to the seventh, and a very rudimentary pair on the thorax. The shape of the leaflets is that shown under the figure of *A. Jamesii* allied species 1 (Fig. 20).

*Habitat and observations.*—This is not a common species in India, and the majority of specimens we have obtained have been reared from larvæ. We found it first at Nagpur (Central Provinces), but this or its allied species have been found in the Berars (Ellichpur) and in other parts of India, and the original specimen from which Mr. Theobald's description was taken was sent from Quilon (Southern India).

## 2a. A. Jamesii, allied species 1.*

Under this name I refer to a very distinct species which I found at Nagpur (Central Provinces). It is possibly the same species which Colonel Giles describes under the name *A. Maculipalpis*, an African species.

It differs from the mosquito above described as *A. Jamesii* in the following particulars :—

(1) It has two equal broad white bands and one narrow one on the palpi, instead of one broad and two narrow bands as in *A. Jamesii.*

(2) The palpi are brilliantly speckled with white scales in addition to the white bands.

(3) The wing markings differ in certain minor particulars.

(4) The last $3\frac{1}{4}$ hind tarsal segments are pure white as in *A. Jamesii*, but the legs are much more brilliantly speckled with white spots than in that species, and the markings at some of the joints of the fore and mid legs differ from the markings in that species.

*Characters of the larva* (Fig. 20).—The median and external frontal hairs are more thickly branched than in the larva of *A. Jamesii*, and the branches of the external frontal hairs are arranged like the veins of a leaf (see figure). In addition to a pair of palmate hairs on each abdominal segment it possesses a very prominent pair on the thorax. This pair is absent, or very rudimentary, in the larva of *A. Jamesii*.

Fig. 20.—Larval characters of *A. Jamesii*, allied species 1 ( = *A. Maculipalpis*, Giles).

* This is *A. Maculipalpis* (Giles).

*Habitat and observations.*—Nagpur (Central Provinces), Goa, Quilon.

The only specimens of the mosquito which I have, were reared from larvæ found along with those of *A. Jamesii* and *A. Fuliginosus.**

### 2b. A. Jamesii, allied species 2.

One specimen of a very distinct species was found by Dr. Stephens at Nagpur, and although we have not obtained its larvæ the characters of the adult are sufficiently distinct for it to be regarded as a new species.

Its main characteristics are as follow:—

(1) The palpi are marked with three narrow white bands, the terminal of which is some distance below the tip, so that ***the tips of the palpi are black.*** The base of the palpi is densly black scaled.

(2) In the fore leg there are no white bands at the apices of the femur and tibia, but there is a small white band at the apex of each tarsal segment. In the middle leg there are no white bands at any joints. In the hind leg there is a faint white spot at the apex of the tibia, and a white spot at the apices of the first and second tarsal segments.

The last $2\frac{2}{3}$ tarsal segments of the hind leg are pure white.

(3) There is no speckling of the legs or palpi.

### 2c. A. Jamesii, allied species 3.

Under the title of *A. Jamesii* (Theobald), Colonel Giles describes a species with speckled legs and palpi, but of which only the last *two* hind tarsal segments and the apex of third are pure white. We have not, however, seen this species.

### 3. A. Theobaldi (Giles).

This is a distinct and not very uncommon species which was first described by Captain Glen-Liston, I.M.S., from Ellichpur in the Berars. The palpi have two equal broad bands near the apex and one narrow one near the base. The tips of the palpi are white.

The wing markings resemble those of *A. Jamesii* except in some of the smaller spots.

The legs are brilliantly speckled with white spots.

The chief distinguishing characters lie in the markings of the hind legs (see Fig. 25). The last two hind tarsal segments and the apex of the third are pure white. Then comes a black band across the middle of the third tarsal segment, and above this a broad white band which involves the base of the third tarsal segment, the joint, and the apex of the second tarsal segment.

*Characters of the larva* (Fig. 21).—The frontal hairs are slightly

* M Aitken has recently sent me several specimens of this species from Goa.

branched or frayed. There are palmate hairs on the abdominal segments and a rudimentary pair on the thorax. The shape of each leaflet is characteristic and the terminal filaments are very short and blunt.

The head pattern is shown in the figure.

Fig. 21.—Larval characters of *A. Theobaldi.*

*Habitat and observations.*—The Berars (Ellichpur), the Central Provinces (Nagpur), the Punjab (Lahore), the Jeypur State. Experimentally we have proved that the human malaria parasites develop readily in this species.

## 4. A. Lindesayii (Giles).

This is a rare species which apparently only occurs at a considerable elevation.

It is a small black mosquito easily identified by the following characters:—

The palpi are unbanded.

The wings have the veins black scaled almost throughout except for a large white spot near the apex involving the costa, the first, second, and the tip of the third longitudinal veins.

On some of the other veins there are minute white interruptions.

The legs are uniformly black except for a beautiful broad white band on femora of the hind legs—a very distinctive character.

*Characters of the larva.*—I have not examined the larva of this species. Dr. Christophers states that the antennæ are provided with a small rudimentary branched hair somewhat like, but very much smaller than, the branched hair on the antennæ of *A. Barbirostris* and *A. Nigerrimus.* The frontal hairs are simple and unbranched.

The palmate hairs are large and the terminal filament is long. There are no thoracic palmate hairs.

*Habitat and observations.*—A few specimens of this species were obtained at Reneghat (Bengal) at an elevation of about 4,000 feet. It has been recorded by Colonel Giles as occurring at Bakloh (4,585 feet) in the Punjab and in Naini Tal (6,400 feet).

We found it breeding in natural pools along with *A. Maculata.*

GROUP IV.

1. *A. Rossii* (Giles).
2. *A. Stephensii*, sp. n., re-named *A. Metaboles* by Mr. Theobald.
3. *A. Maculata* (Theobald).

*Group characteristics.*—The *adults* of this group are light brown in colour and of moderate size, with dark brown or black spots on the light-coloured wings. The tarsal joints are banded.

The *larvæ* are characterised by the fact that the frontal hairs are simple and unbranched, and that there are no palmate hairs on the thorax.

The *eggs* have a very broad upper surface and the lateral floats touch the margin of this surface.

In *habits* the members of this group are essentially "domestic" mosquitoes (especially *A. Rossii* and *A. Stephensii*). Neither *A. Rossii* nor *A. Stephensii* are ever found at any distance from houses, and *A. Rossii* is usually found breeding in some shallow muddy pool or puddle among native huts. At Mian Mir *A. Stephensii* was found breeding exclusively in the tins and *gumlahs* kept in the lines of the native troops. In Calcutta I found it breeding along with *A. Rossii. A. Maculata* may be found further from houses, but it is also I think essentially a domestic mosquito, and in China, where I found it breeding in the pools and puddles on the granite soil of Kowloon, it is common in the houses.

## 1. A. Rossii (Giles).

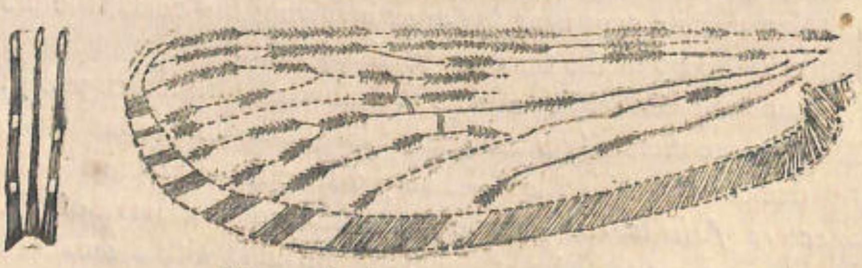

Fig. 22.—Palpi and wing of *A. Rossii.*

This is almost certainly the commonest species in India.

The palpi are marked with one broad and two narrow white bands. The tips of the palpi are white.

The tarsal joints of all the legs are banded with yellowish white bands.

The wing markings are shown in the figure. The large T-shaped middle spot on the costa is characteristic, but this and the other wing spots vary considerably.

*Characters of the larva* (Fig. 7, page 24).—Both the external and median frontal hairs are simple and unbranched.

Palmate hairs are present from the second to the seventh abdominal segments. The terminal filament of each leaflet is very long (the same length or longer than the body of the leaflet). The head pattern is constant and characteristic.

*Habitat and observations.*—It is common throughout India, and we have found it at elevations above 5,000 feet. Its breeding place is usually a muddy pool or shallow tank among native huts, and after the rains both larvæ and adults are usually present in very large numbers. In the Punjab (Lahore, Mian Mir), before the rains begin it is practically absent, owing probably to the fact that there are no suitable breeding places, for it will not breed in the irrigation canals as *A. Culicifacies* does. In October and November enormous numbers can be caught in native huts.

We have occasionally found its larvæ in very foul water, and at Ennur near Madras, I found it breeding readily in water containing 2·8 per cent. of salt.

In the Madras Presidency, where suitable breeding places are present throughout the year, it has no special seasonal prevalence. In that Presidency it breeds largely in the irrigated rice fields.

Experimentally we have proved that the three species of the human malaria parasites will develop in this mosquito, but although we have examined between seven and eight hundred individuals caught in houses in different parts of India, we have never yet found it infected in nature.

## 2. A. Stephensii, sp. n.

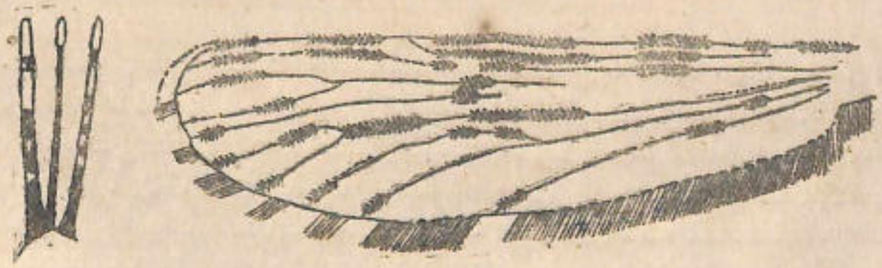

Fig. 23.—Palpi and wing of *A. Stephensii.*

This mosquito has been re-named *A. Metaboles* by Mr. Theobald. It had, however, been previously described under the above name by Captain

Glen-Liston, I.M.S. (*Indian Medical Gazette*, November 1901), so that the name *A. Metaboles* must be regarded as a synonym.

The palpi are marked with two broad and one narrow band, and with a few white speckles between the bands.

The legs are marked with white bands at the tarsal joints as in *A. Rossii*, but they are in addition brilliantly speckled with white spots. The wing markings are shown in the figure, but as in *A. Rossii*, they vary considerably.

*Characters of the larva* (Fig. 24).—The frontal hairs are simple and unbranched. The palmate hairs are present from the first to the seventh abdominal segments. The filaments of the leaflets are shorter than those of *A. Rossii*, and this character and the complete absence of any head pattern in the larva of this species, serve to distinguish it.

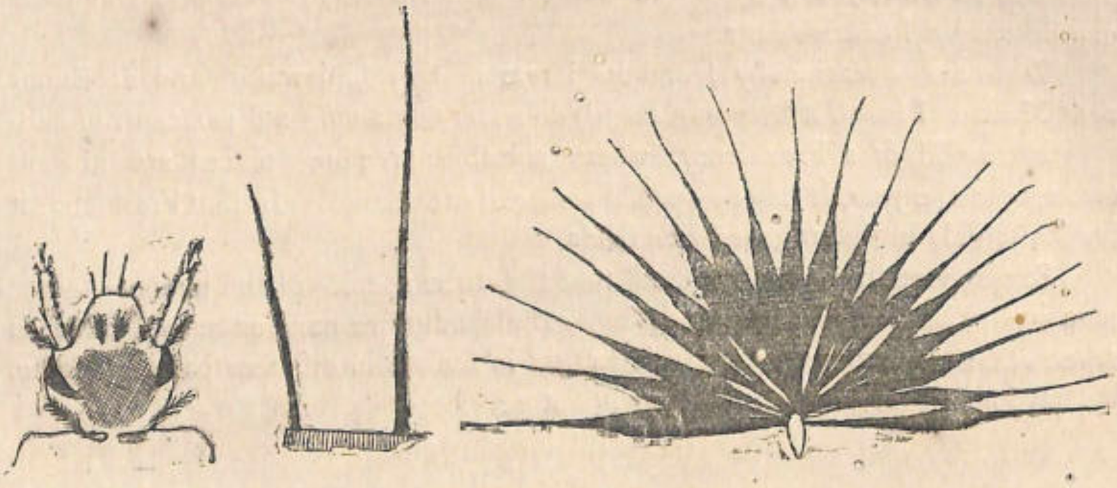

Fig. 24.—Larval characters of *A. Stephensii.*

*Habitat and observations.*—I first found this species along with *A. Rossii* in the native huts in Calcutta. In Mian Mir (Punjab) we found it in the barracks of the native troops, and here it was breeding exclusively in the *gumlahs* of water which are kept in the lines in case of fire. In Madras City I found it in the native houses. Here it was breeding exclusively in the wells which, since the introduction of a water-pipe supply, have been for the most part unused.

It occurs also at Ellichpur in the Berars and in the Central Provinces (Nagpur).

Experimentally we have proved that the human malaria parasites can develop in this species.

## 3. A. Maculata (Theobald).

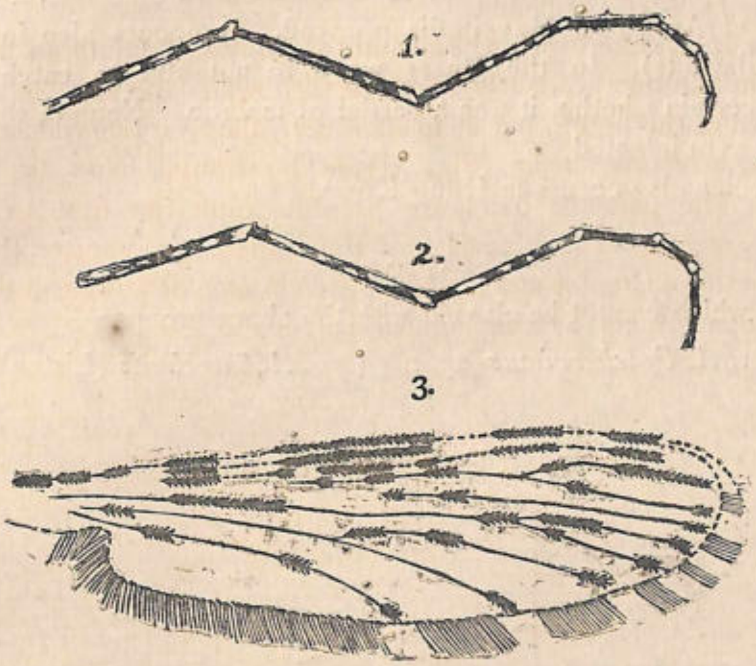


Fig. 25.—1. Hind leg of *A. Theobaldi* (Giles).
2. Hind leg of *A. Maculata*.
3. Wing of *A. Maculata*.

This species bears some resemblance to the following mosquitoes : *A Jamesii* (Theobald), *A. Stephensii* and *A. Theobaldi*. An examination of the markings of the hind legs will, however, serve to distinguish between them.

In *A. Maculata* only the last (fifth) tarsal segment of the hind leg is pure white.

In *A. Stephensii* none of the tarsal segments of the hind leg are white.

In *A. Theobaldi* the last two (fourth and fifth) and the apex of the third tarsal segments of the hind leg are white.

In *A. Jamesii* the last three (third, fourth and fifth) and the apex of the second tarsal segments of the hind leg are pure white.

The legs of *A. Maculata* are speckled with white and there are white bands at the tarsal joints as in the species above mentioned.

The wing markings are shown in the figure.

*Characters of the larva.*—The frontal hairs are simple and unbranched. The palmate hairs are borne by the second to the seventh segments. The terminal filaments of the leaflets are very short.

*Habitat and observations.*—This species was first found by us in the Duars.

It was also present at Kurseong (5,000 feet). At Sam Sing in the Duars it was breeding in clear pools on the rice fields.

In Lahore (Punjab) I found it breeding in a pool near one of the irrigation canals in March, and some of the larvæ hatched out in that month. During the hot weather of April and May it disappeared. It occurs also in the Jeypur State (hill districts). In the Duars we were not able to catch a sufficient number to ascertain whether it was a carrier of malaria. None of the specimens we dissected were infected.

This species also occurs in Hong Kong.

### GROUP V.

Species which cannot be classed with the above groups :—

1. *A. Pulcherrimus*, sp. n. | 2. *A. Turkhudi* (Liston).

### 1. A. Pulcherrimus, sp. n.

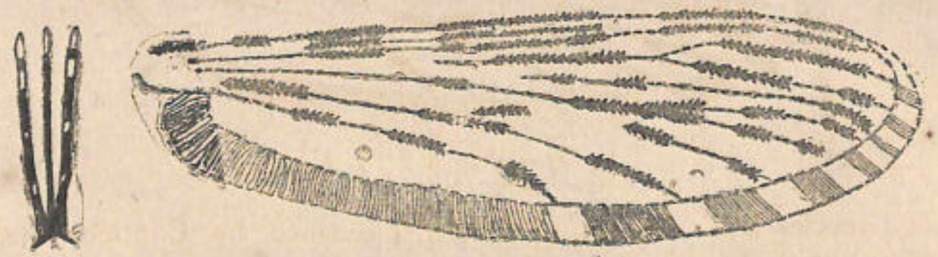

Fig. 26.—Palpi and wing of *A. Pulcherrimus*.

This is a very beautiful species which we found first in the barracks of the native troops at Mian Mir.

The palpi are marked with four white bands, two equal ones near the tip and two others nearer the base. The tips of the palpi are white.

In the hind legs the last $3\frac{3}{4}$ hind tarsal segments are pure white. There are also apical white bands to the first tarsal segment and to the tibia and femur.

In the mid leg there are apical white bands on the tibia, and on all the tarsal segments except the fourth; that is, the last tarsal joint has no band. The femur has a few white scales at the apex and above these a white band. The rest of the femur is brilliantly spotted with black and white scales.

In the fore leg the femur and tibia are brilliantly speckled with black and white scales, and there are apical white bands at the tarsal joints except the last.

The wings are very beautifully spotted with white and black scaled areas, and there is a white interruption of the wing fringe opposite each longitudinal vein.

The abdomen is densely scaled with black and white scales which form lateral tufts on the hinder border of each segment.

The eggs are of the same type as those of *A. Rossii.*

The larvæ have the external pair of frontal hairs branched.

The palmate hairs are present from the second to the seventh abdominal segments and the terminal filaments of the leaflets are long.

*Habitat and observations.*—This is a somewhat rare species. It occurs in the Punjab (Mian Mir, Ferozepore, etc.).

In Mian Mir we occasionally found it in the houses in very small numbers and there it was breeding in an overflow pool of an irrigation canal (September).

## 2. A. Turkhudi (Liston).

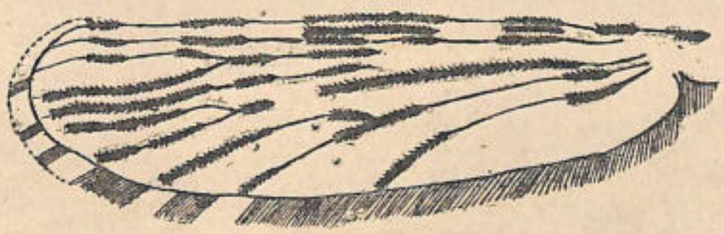

Fig. 27.—Wing of *A. Turkhudi.*

The species was first recorded and described by Captain Glen-Liston, I.M.S., in Ellichpur in the Berars.*

The palpi are marked with three narrow white bands. The tips of the palpi are *black.* This character serves to distinguish it from all the other lighter coloured Indian *anopheles.*

The legs are dark brown with indistinct yellowish apical spots on the tibiæ and femora and on all but the last tarsal joints.

The wing markings are shown in the figure.

*Characters of the larva and eggs* (Fig. 28).—The eggs of this species are very peculiar in that the lateral floats are exceedingly rudimentary and sometimes can scarcely be made out at all. According to Dr. Christophers the eggs sometimes sink in water from this cause.

The larvæ also are peculiar, in that there are as a rule no palmate hairs (or only very rudimentary ones) on the first three abdominal segments, these hairs being borne only by the fourth to the seventh segments.

For this reason the larvæ rest at the surface in an oblique attitude more or less like the larvæ of culex mosquitoes.

The shape of the palmate hairs is shown in the figure. In addition to the

* *Indian Medical Gazette* December 1901.

two pairs of unbranched frontal hairs a third pair of simple hairs projects over the mouth parts behind and between these.

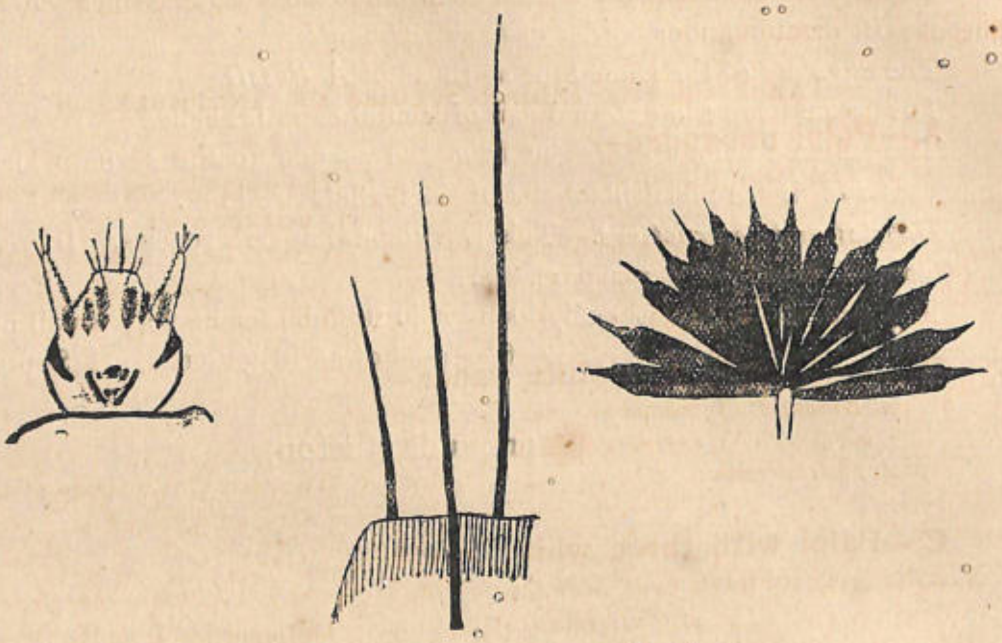

Fig. 28.—Larval characters of *A. Turkhudi.*

*Habitat and observations.*—The Berars (Ellichpur), the Central Provinces (Nagpur), Kashmir.

In respect to the structure of the eggs and larvæ this mosquito would seem to be intermediate between the genus culex and that of *anopheles.*

We have proved, however, that under experimental conditions, the human malaria parasites will develop in this species, as in all the other *anopheles* with which we have experimented.

The above notes include all the Indian species, which I regard as distinct, that we have met with. Were all the species which have been recorded as new species to be included, the list might be increased, but our desire has been as far as possible to reduce rather than to multiply the number of species, and it is interesting to note that already several species which were at first described by Mr. Theobald as distinct are now regarded by him to be identical. The question whether the well known African species *A. Funestus* is the same as the mosquito I have noted above under the name *A. Fluviatilis*(=*A. Christophersi*, Theobald) is an important and very interesting one, which, I believe, will be answered in the affirmative. At the present time Mr. Theobald, I believe, also inclines to this view, but the question cannot be finally settled until the eggs and larvæ of *A. Funestus* are described and compared with those of *A. Fluviatilis.*

In consequence of the difficulty which is usually found in the identification of the different species of Indian *anopheles*, I append the following table which has been drawn up with the help of Captain Glen-Liston, I.M.S. Its main features are based on the palp markings which, although they may occasionally vary slightly, are sufficiently constant to afford a basis of classification for the purposes of identification.

### TABLE OF THE INDIAN SPECIES OF ANOPHELES.

**A.—Palpi unbanded—**

| | |
|---|---|
| *A. Lindesayii* (Giles) . . . . | The wing has one large white spot near the apex. |
| *A. Barbirostris* (Van der Wulp) . . . | A very large species with black densely scaled palpi. |
| *A. Gigas* (Giles) . . . . . | A hill species. |

**B.—Palpi with four white bands—**

| | |
|---|---|
| *A. Sinensis* (Wiedmann) and its allied species *A Nigerrimus* (Giles). | |
| *A. Pulcherrimus* . . . . | The last $3\frac{3}{4}$ segments of the hind tarsi are pure white. |

**C.—Palpi with three white bands—**

*A.—The terminal white band includes the tips of the palpi which are therefore white.*

| | | |
|---|---|---|
| One or more of the terminal segments of the hind legs are pure white. | *A. Fuliginosus* (Giles) . | Distinguished from the three following species by the fact that though the tarsi are banded the legs are not speckled with white dots in addition. |
| | *A. Jamesii* and its allied species (including *A. Maculipalpis*). | The last $3\frac{1}{2}$ hind tarsal segments are pure white. Legs with white speckling. |
| | *A. Theobaldi* (Giles) . | The last $2\frac{1}{2}$ hind tarsal segments only are pure white. |
| | *A. Maculata* (Theobald) | The last $1\frac{1}{2}$ hind tarsal segments only are pure white. |
| Legs uniformly coloured without bands or white segments. | 1. *A. Fluviatilis.* (= *A. Christophersi* (Theobald). = *A. Listonii.* ? = *A. Funestus* (Giles).) 2. *A. Jeyporiensis.* 3. *A. Culicifacies* (Giles). | For differences, see text. |
| | 4. *A. Immaculata* . . | Wings unspotted. |
| The tarsal joints are banded but there are no terminal white segments to hind legs. | 1. *A. Rossii* Giles) . . | Legs are not speckled. |
| | 2. *A. Stephensii* . . (= *A. Metabolis* (Theobald). | Legs are speckled in addition to the tarsal bands. |

*B.—The tips of the palpi are black.*

1. *A. Turkhudi* (Liston).
2. One of the species allied to *A. Jamesii* (see text).

## The breeding places of anopheles larvæ.

It is now well known that *anopheles* larvæ may occasionally be found in almost any collection of water of whatever nature it may be. This fact has an important application when any practical measures at extermination of larvæ are attempted, in that it leads us not to overlook even the most unlikely places in our search for larvæ; for unless we search systematically *every* collection of water, temporary or permanent, we may easily allow an important breeding place to remain, when we think all possible breeding places have been done away with.

Under favourable conditions, however—that is, abundance of breeding places of all kinds near at hand—*anopheles* will not, as a rule, lay their eggs in an unsuitable collection of water, and there is no doubt, I think, that under such circumstances, not only do *anopheles* select their breeding places, but that *each species* has a particular kind of breeding ground that it prefers over any other. This observation, which was first made by Dr. Christophers at Jalpaiguri in Bengal, is a very important one.

At Jalpaiguri two species of *anopheles* were common *viz.*:—*A. Rossii* (Giles), and *A. Sinensis* sub-species *Nigerrimus*. The breeding places of *A. Rossii* were invariably found to be small, shallow, muddy puddles and pools close to, and among the native huts. *A. Nigerrimus*, on the contrary, was breeding entirely at some distance from the village in the deep natural pools of a swampy marsh. The larvæ of *A. Rossii* were never found in these pools and those of *A. Nigerrimus* never in the shallow muddy pools near the huts.

In the Duars, the same selection of breeding places by *A. Rossii* and *A. Fluviatilis* was found to exist, the larvæ of *A. Rossii* being found only in the shallow muddy pools near the coolie huts, and the larvæ of *A. Fluviatilis* (the adults of which were nevertheless more common in the huts than those of *A. Rossii*) being found at some distance from the lines only in the small clear pools formed by a mountain stream, and in the water among the grass and weed at the edge of the stream itself.

This selection of particular breeding places extends to all the species of Indian *anopheles*, and in September of last year I found an excellent example of its application to the three species, *A. Culicifacies* (Giles), *A. Fuliginosus* (Giles) and *A. Rossii* in one of the isolated bazaars at Mian Mir. This bazaar is surrounded by an irrigation channel about four feet wide and three feet deep. At the upper end of this watercourse and about ten yards from it are a number of broad, shallow muddy pools. At the lower end of the watercourse and about thirty yards from the bazaar is a swampy piece of land covered with thick trees and shrubs and containing a number of deep clear pools in which water plants and weed have grown.

The adults of the three species, *A. Rossii, A. Culicifacies,* and *A. Fuliginosus,* were present in the houses of the bazaar, and we should have expected that the larvæ of all the species would have been found together in any and all of the breeding places. This, however, was not the case. In the irrigation channel no other larvæ than those of *A. Culicifacies* were ever found, in the shallow muddy pools no other larvæ than those of *A. Rossii*, and in the clear deep pools under the trees no other larvæ than those of *A. Fuliginosus*.

It is evident, therefore, that each of these species had selected a particular kind of breeding place.

In another part of Mian Mir an example of selection of breeding places by another species of *anopheles* was found. In this place several species of *anopheles* were present in the barracks of the native troops, and there were abundance of breeding places of different kinds near. The larvæ of only one species, however (*viz., A. Stephensii*), were found in the *gumlahs* of water kept in the lines in case of fire, and the larvæ of this species were never found in any of the other breeding places—a good example of selection of a breeding place by this species.

At Mian Mir the breeding grounds of the species of *anopheles* were in accordance with what we had found for the same species in other parts of India; and it is possible to divide the Indian *anopheles* as regards their breeding grounds into at least three groups, *viz.*, (1) those which breed almost exclusively in running water (the members of Group I, page 30), (2) those which breed in shallow muddy pools in the vicinity of dwellings and in tins and *gumlahs* of water (the members of Group IV, page 44) and (3) those which breed at some distance from houses in deep natural pools with much aquatic vegetation and in marshes (the members of Group II and some of those of Group III, page 35).

The favourite breeding grounds of some of the species of Indian *anopheles* may be summed up as follows :—

*Group I.—A. Fluviatilis.* The larvæ of this species are found most frequently in quickly running streamlets with grassy edges. Their presence in a stream can be detected only by dipping with a tin among the grass and vegetation at the edge of the stream and in the pools and backwaters.

*A. Culicifacies.* This species is essentially a breeder in slowly moving water. It will be found in canals, ditches and streams when quite absent from neighbouring shallow, stagnant pools. Irrigation canals are its favourite breeding place in the Punjab. In places where there is no running water it may be found in clear pools as at Ennur near Madras, where it was breeding in the borrow-pits by the side of the railway. It has also been found in rocky pools in the bed of a river.

*Group II.—A. Nigerrimus.* The larvæ of this species are found chiefly in deep dark natural waters with much aquatic vegetation. Favourite breeding places are deep overgrown canals, swamps in the jungle and ponds with water weeds. Their breeding places are not as a rule found near houses. The larvæ are generally scattered singly among the water plants at the edges of the pool and can be caught only by dipping with a tin or muslin net among the plants and weed.

*A. Barbirostris.* The breeding places of this species are practically the same as those of *A. Nigerrimus.* In Lahore the larvæ were present in the lily ponds in the public gardens until the end of November.

*Group III.—A. Fuliginosus* (Giles). The larvæ of this species are often found along with those of *A. Nigerrimus.* As a rule, however, they prefer more open water and more sunlight. In Madras and in the Central Provinces they were common in the large open tanks with grassy edges.

*A. Jamesii* (Theobald). In the Central Provinces the larvæ of this species were found among the grass and water weeds at the edge of a large open lake; and in Travancore (Quilon) in a large sandy tank.

*Group IV.—A. Rossii.* The breeding places of this species have already been sufficiently referred to. Its larvæ are never found at any distance from houses. Shallow muddy pools are its favourite breeding ground and hundreds of larvæ are usually present in the same small pool, and being easily seen the larvæ of this species are the ones which are most frequently found by observers. The larvæ of most other species have to be fished for with a tin or net. In Madras, the larvæ of *A. Rossii* are common in the irrigated rice fields and in the muddy tanks, as well as in pools made by digging for building purposes, in brick-fields, and in cultivated areas.

*A. Stephensii.* In Mian Mir (Punjab) this species was found to be breeding only in the earthenware *gumlahs* and tins kept full of water in the line of the native troops in case of fire. In Madras I found it breeding only in the disused wells in the courtyards of the Native and Eurasian houses. It is probable that since the introduction of a pipe-water-supply into Madras City the numbers of this species, at any rate, have largely increased, for its larvæ are only found in those wells which, since the introduction of the pipe-water-supply, have been unused.

*A. Maculata*. The larvæ of this species may be found further from houses than those of *A. Rossii*, and whereas the larvæ of *A. Rossii* are usually found in muddy water, the larvæ of *A. Maculata* prefer clear sandy or rocky pools. In the Duars its larvæ were found in clear pools on a rice field. In Hong-Kong (China) I found the larvæ of this mosquito in the small marshy pools on the granite soil of Kowloon.

Sufficient observations have not been made with regard to the remaining species of *anopheles* to enable their favourite breeding places to be stated with certainty.

Under unfavourable conditions the selection of suitable breeding places by the different species is not of course so apparent, and many instances might be given which show that if all the suitable breeding places near a village are dried up the *anopheles* will lay their eggs on any collection of water. The "test pool" experiment of Stephens and Christophers in Africa is sufficient to prove this, and the same expedient for finding out whether adult *anopheles* are really present in a place or not, when all the natural breeding places have disappeared, has been frequently resorted to by us in India.

Even without making artificial pools or placing large *gumlahs* of water in a village, many examples may be found in nature. Thus in one part of Calcutta (Hastings) in June, when no breeding place could be found anywhere near, and no adults could be caught in the houses, we were able to prove that adults were really present by finding their larvæ in the cisterns of water *on the roofs of the houses*—the only place where at that time we were able to find larvæ in this part of Calcutta. Again, during the hot weather in Mian Mir, when the irrigation channels had been stopped for a long time and there were no natural breeding places of any kind, I was able to find larvæ in very curious situations, as, for instance, in the water of the swimming bath, in the horse troughs, in tins of water which had been accidentally left about, in many of the small drains which take off the waste water of the standpipes, wash houses, etc., and in the small stone reservoirs in the gardens.

These facts point to the necessity of a very thorough search in every collection of water when any efforts at extermination of larvæ are attempted, and although under favourable conditions we may certainly say that we are more likely to find *anopheles* larvæ in certain collections of water than in others, no hard and fast rule, which might perhaps prevent us from searching a particular place, can be made.

**The enemies of larvæ and the effect of running water on larvæ.**

We have made a good many experiments which show that many small water animals and young fish feed voraciously on mosquito larvæ, but I do not think so much importance attaches to this subject as is generally supposed. It is of course true that a number of small water animals, larvæ, and young fish eat mosquito larvæ, but it is equally true that under natural conditions *anopheles* larvæ may always be found in the same pools in which the most voracious fish or water animals are living, and experiments on this subject carried out under artificial conditions are apt to be misleading. A kind of young fish, for example, is very common in the irrigation watercourses at Mian Mir: if a few specimens of this fish are put into a basin of water with twenty or thirty *anopheles* larvæ, in an hour or two the fish will have devoured all the larvæ. Under natural conditions, however, the number of larvæ in the watercourses does not appear to be lessened by the presence of the fish, and I have watched a natural pool of water containing these fish and *anopheles* larvæ for some days without the number of larvæ having appreciably diminished.

In other parts of India, too, it is not at all uncommon to find larvæ in pools containing fish and other water animals which will devour the larvæ readily if placed along with them in a bottle or basin.

Another point to which I am not inclined to attach much importance is the effect of running water on larvæ. If it were possible in all the streams and canals of India to make the water run as swiftly at the sides as it does in the centre they would of course afford poor breeding grounds, but however swiftly a stream runs in the centre there are almost always many small bays and backwaters where the larvæ can live sheltered by weeds and grass, so that it is possible to find *anopheles* larvæ by dipping at the edges of almost every stream, however swiftly it may appear to be running. The effect of running water on larvæ is not to kill them, but simply to carry them on with it until they find some sheltered bay or backwater where they can develop unmolested. This is the case in the more swiftly running irrigation channels at Mian Mir. If a length between two bridges of one of these water channels be examined, a large number of very young larvæ which are evidently being carried onwards with the stream will be found. At the bridges, however, where the water is collected into a comparatively still pool before it passes under the narrow bridge, full grown larvæ and pupæ will invariably be found. The inference is that the young larvæ have been carried down by the stream to develop in the comparatively still waters near the bridges.

## The distance of flight of anopheles mosquitoes.

At the present time practically all observers are agreed that *anopheles* do not fly to any great distance from their breeding places in search of food, and although it is of course possible to find *anopheles* larvæ in jungles several miles from any human habitation, the inference is that the adults of these larvæ feed either on the blood of animals or on vegetable juices.

The importance of ascertaining the usual distance of flight of those species which habitually feed on the blood of man is very great, because unless this is known it is impossible to state the safe distance for a residence from a focus of infection.

There are three groups of rain-formed pools in Mian Mir which afford a good opportunity for observation in connection with this important subject. One of these groups of pools is 450 yards away from any habitation, the second group is 750 yards away and the third is over three-quarters of a mile away. With each shower of rain these pools are filled and remain full for over a week as a rule.

On every occasion I have found the first group of pools to contain many larvæ within a week of a shower of rain: on three out of four occasions the second group of pools has contained larvæ, and on no occasion have larvæ been found in the pools of the third group. This shows, I think, that mosquitoes will readily fly to a breeding place a quarter of a mile away, that they will less readily fly to a breeding place half a mile away, and that they will never (except perhaps under exceptional circumstances) fly over three-quarters of a mile. In these instances the larvæ found were those of *A. Rossii.*

In an indirect way also the usual distance of flight of mosquitoes can be determined and the safe distance from a focus of infection ascertained.

In Mian Mir, for example, there are two groups of syce lines, one of which is 150 yards and the other 500 yards from the same and only breeding place of the malaria-carrying species of *anopheles*. In the nearer syce lines numerous adult *anopheles* were caught, and in the further syce lines we were not able, at the time of our examination, to catch any adult *anopheles*. The endemic index of the nearer syce lines was 56 per cent., and that of the further lines only 20 per cent. This is sufficient to prove that the majority of the *anopheles* did not fly the 500 yards to the further syce lines.

The following examples will show, however, that it is not unusual for *anopheles* to fly a quarter of a mile. In the village of Anjini in the Central Provinces three species of *anophcles* were abundant, the endemic index was 25 per cent., and the nearest breeding places were a quarter of a mile away. Three species were also present, though scanty, in the village of Takli, with the nearest breeding places the same distance away and an endemic index of 15 per cent. Above a quarter of a mile, however, from a breeding place the amount of malaria rapidly diminishes, and over half a mile away safety from *anopheles* is practically assured, as the following examples show :—

| Names of places. | Presence of adult *anopheles*. | Distance of nearest breeding grounds. | Endemic index. |
|---|---|---|---|
| Lawa . . . . . . | None found. | Half mile. | 5% |
| Wadi . . . . . . | None found. | Half mile. | 5% |
| Chintarapelly . . . . | None found. | Over half mile. | 0% |

We may say, therefore, that the usual distance of flight of *anopheles* mosquitoes rarely if ever exceeds half a mile, and that at this distance from a focus of infection we are practically safe from malaria.

## II.—The Malarial Infection of Native Children in India.

THE discovery by Koch, and independently by Stephens and Christophers, that a large percentage of native children in Africa constantly have malaria parasites in their blood, although at the time they may present none of the more evident clinical symptoms of malarial fever, is well known.*

These observers showed further that the infection in the children decreased rapidly with advancing age, and that the adult Negro was relatively quite immune to malaria.

Working on the same lines, we have been able to confirm and extend these observations for certain parts of India. Thus we have found that in the tea planting districts of the Duars between 65-75 per cent. of the children under ten years of age have malaria parasites in their blood, while no infection at all is found in the adults. At Mian Mir in the Punjab and at Ennur in Madras the same condition prevails, *viz.*, an infection of between 60-70 per cent. of the children, and no infection of adults. In such districts as these also a diminution of infection in the children at advancing age periods occurs.

This discovery of the infection of native children is of the greatest importance both from a scientific and practical point of view, and it provides us not only with abundant material for the study of individual cases of malaria, but also with a new method of investigation which, in its application to many of the vexed problems of malaria, will prove of the greatest service. As a matter of fact, the study of malaria in any place at the present time resolves itself largely into a study of malaria as it occurs among the native children, and it is necessary that this fact should be clearly recognised in India, in order that we may obtain an accurate knowledge of the nature of the malarial fevers in different places, the conditions which determine their prevalence, and the best methods of prophylaxis.

* Reports to the Malaria Committee of the Royal Society from Drs. Stephens and Christophers, West Coast of Africa. Third Series, 1900. Harrison and Sons, London.

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* Reports to the Malaria Committee of the Royal Society from Drs. Stephens and Christophers, West Coast of Africa. Third Series, 1900. Harrison and Sons, London.

The following table shows the percentage of infected children in the different places we have visited :—

TABLE I.—TO SHOW THE MALARIAL INFECTION OF NATIVE CHILDREN IN INDIA.

| District. | Name of Locality. | Percentage of infected children (=Endemic Index). |
|---|---|---|
| | Calcutta | 0 |
| | Madras | 5 |
| Plains of Bengal | Belgurriah | 7·3 |
| | Jalpaiguri | 16·1 |
| | Mainaiguri | 24·3 |
| The Duars | Rungamutty | 43 |
| | Naigaisurie | 55 |
| | Nagrakata | 72 |
| | Sam Sing | 28 |
| Darjeeling hills | Kurseong | 0 |
| | Seypudura | 0 |
| | Tonetta | 0 |
| Mian Mir (Punjab) | Infantry Bazaar | 52 |
| | Artillery Bazaar | 35 |
| | Syce lines A. | 56 |
| | Syce lines B. | 20 |
| | Cavalry Lines | 24 |
| Central Provinces | Phutala Village | 45 |
| | Sahadara ,, | 15 |
| | Didgoh ,, | 20 |
| | Seendra ,, | 20 |
| | Ambigheri ,, | 25 |
| | Angini ,, | 25 |
| | Takli ,, | 15 |
| | Telenkeri ,, | 10 |
| | Kamla ,, | 7 |
| | Sunegaon ,, | 8 |
| | Bighori ,, | 30 |
| | Lawa ,, | 5 |
| | Wadi ,, | 5 |
| Vizagapatam District. | Vizagapatam | 0 |
| | Tapara | 10 |
| | Chintarapalli | 0 |
| | Fishing village | 27 |
| Jeypur State | Sukni | 50 |
| | Patungi | 35 |
| | Koraput | 20 |
| | Bodawalasa | 86 |
| | Kanaputty | 50 |
| | Sisagoda | 70 |
| | Koomba | 60 |
| Madras | Ennur | 55 |

From the above table it will be seen that the proportion of infected children varies greatly in different places. In very malarious districts a large percentage of the young children have malaria parasites in their blood, in places which are less malarious a smaller proportion of infected children is found, and in places where malaria is rare or absent, few or none of the children are found infected. The percentage of infected children affords, in fact, a very true test of the endemicity of malaria in different places, and the figure representing this percentage may, therefore, be termed the "Malarial Endemicity Index," or shortly the "Endemic Index."

It would of course be possible to arrive at a rough estimate of the amount of malaria and the liability to infection at different places by other methods than this, as, for example, by an examination of the statistics of admissions into hospitals for malarial fever, or by an estimation of the number of people with enlarged spleens, but by neither of these methods can we arrive at an accurate result, for neither of them will give us any information regarding the prevalence of malarial parasites at the time of our examination. It is obvious that a knowledge of the prevalence of parasites among the general population, and a knowledge of the number of infected *anopheles* mosquitoes are the only reliable data on which we can form an estimate of the amount of malaria and the danger of infection.

The reasons for the great differences in the malarial endemicity of different places in India shown in the above table will be discussed later: at present it is necessary to refer to the significance of the infection of native children, and to the relative immunity of the adult native.

The great importance of the infection of native children lies in the fact that *they form undoubtedly the chief source from which anopheles mosquitoes become infected.* This is a point of the greatest practical importance and one which cannot be too clearly recognised. That this must be so, is easily understood when we remember that practically all the cases of malarial fever occurring in native children are untreated, and go on for many months without it being recognised by anyone that the children are suffering from malarial fever, and have malaria parasites in their blood. A further proof is given in the fact that the highest proportion of infected *anopheles* is found in the places where we find the largest number of infected children; and in places where there are no infected children it is difficult or impossible to find infected *anopheles*. This is shown in the following table.

TABLE II.—TO SHOW THE CONNECTION BETWEEN INFECTED CHILDREN AND INFECTED ANOPHELES MOSQUITOES.

| Locality. | Percentage of infected children. | Percentage of infected *anopheles*. |
|---|---|---|
| Calcutta . . . | *Nil* (140 examined) | *Nil* (352 examined). |
| Mian Mir . . . | 55-60 | 4·6 (one species). |
| The Duars (Rungamutty) | 45-50 | 6·25 (one species). |
| Ennur (Madras) . . | 60-65 | 8·8 (one species). |

It is apparent from the above table that the greatest liability to infection of any new comer exists in those places which contain the largest number of infected children, and in places, such as Calcutta, where there are no infected children, there is practically no danger of infection with malarial fever.

We have seen that two other important points were brought out by Koch, and Stephens and Christophers, in Africa, in connection with the infection of native children, *viz.*, (1) that a diminished infection of the children occurred with advancing age, and (2) that the adult negro in Africa was relatively immune to malarial infection.

For certain malarious districts of India these same rules will apply, as the following table shows:—

TABLE III.—TO SHOW DIMINISHED INFECTION WITH INCREASING AGE OF NATIVE CHILDREN IN MALARIOUS DISTRICTS OF INDIA.

| Locality. | Infected children up to 3 years of age. | Infected children up to 5 years of age. | Infected children up to 10 years of age. | Infected children over 10 years of age. | Infected children over 15 years and adults. |
|---|---|---|---|---|---|
| Mian Mir (Punjab) . | 80 % | 66 % | 50 % | ... | None in 25 examined. |
| Ennur (Madras) . | 65 % | 51 % | 46 % | 16% | None in 10 examined. |
| Rungamutty (Duars) | ... | 48·3% | 43 % | ... | None in 33 examined. |

It is evident, therefore, that natives living in such places as the above acquire a relative immunity with advancing age. This immunity is probably an acquired one, due to repeated infection and re-infection occurring during childhood, for, in less malarious districts than the above, the relative immunity enjoyed by the adult native is not so marked, and the diminished infection of the children at advancing age periods is not shown.

The following is an example of this:—

TABLE IV.—TO SHOW THAT IN LESS MALARIOUS PLACES VERY LITTLE, IF ANY, IMMUNITY OCCURS WITH ADVANCING AGE.

| Locality. | Infected children up to 5 years. | Infected children up to 10 years. | Infected children up to 15 years. | Infected children up to 18 years. | Infected adults. |
|---|---|---|---|---|---|
| Jalpaiguri (Bengal) . | Rare | 13·3 % | 15·2 % | 16 % | Not uncommon. |

It is probable, therefore, that in Africa, as well as in India, it is only in the very malarious parts that the adult acquires a marked immunity. In Africa, however, malaria appears to be almost equally prevalent in all the parts that have been examined, and at the same time to be almost equally prevalent throughout the whole year. In India it is easy to find almost every condition of prevalence, so that whereas in Africa a very large proportion of the natives have the opportunity of becoming relatively immune by repeated infections during childhood, in India only those natives who have lived all their lives in such places as the Duars enjoy an immunity.

This, of course, includes but a very small proportion of the whole Indian community, and the fact that the average adult native of India enjoys no immunity is shown by the certainty with which he contracts malarial fever when exposed to infection.

It probably requires ten or twelve years of constant infection and reinfection before an immunity is established, and it is possible also that, even then, the relative immunity only applies to the variety of parasite with which the individual has been infected.

### The nature of the infection in native children.

The work done by Drs. Stephens and Christophers in Africa, on the infection of native children,* led them to the conclusion that the infection in such cases was different from an ordinary attack of malaria in an adult, in that (1) they did not observe any rise of temperature in the children, nor any of the usual symptoms of malarial fever, and (2) although asexual forms of the parasites were not uncommonly seen, the chief characteristic of the infections appeared to them to consist in the presence of a large number of sexual forms having characters differing from those of the sexual forms usually found in cases of malarial fever occurring among Europeans. They had no opportunity of following out the cycle of the parasites in a case over a period of 24 or 48 hours.

This subject has not been worked at in India by Drs. Stephens and Christophers, but as it has always appeared to me that malaria must be one of commonest causes of death among native children in India, I thought it would be of advantage to study the question somewhat more closely, especially with the object of ascertaining whether the health of the children is really much affected by the presence of malaria parasites or not.†

Owing to difficulties which will be readily understood by any one who has attempted to take temperatures or blood specimens in an Indian village, the number of cases which I have examined is not large, but they are, I think, sufficient to draw conclusions from. The cases which follow were taken without selection from children who were playing about in the village, and presented, on a casual examination, none of the ordinary symptoms of malarial fever.

Freedom from symptoms, indeed, as was pointed out by Drs. Stephens and

* "The Malarial Infection of Native Children," by J. W. W. Stephens, M.D. Cantab., and S. R. Christophers, M.B. Victoria, in the Reports to the Malaria Committee of the Royal Society. Third Series, 1900.

† The statement of Drs. Stephens and Christophers on this point is as follows:—"It must be understood that in speaking of a case of malaria in children, we are referring only to the presence of parasites; the children are perfectly well, and present none of the characteristic signs observed in Europeans affected with malaria." (*Loc. cit.*, page 4.)

Christophers in Africa, is characteristic of the infection of native children, and if they are seen *in the mornings* (which is the usual time at which one goes out to take specimens of blood), it would certainly be difficult to say from their appearance that any of the children were suffering from malarial fever.

In the evenings one or two of the children may be lying down, others, however, though evidently suffering from fever, may be still playing about with the other children of the village, and the parents are seldom aware that their children are not in good health.

The following cases were first examined for a few days in February and again for a period of a week during March.

INFECTION OF NATIVE CHILDREN.

*Case I.*

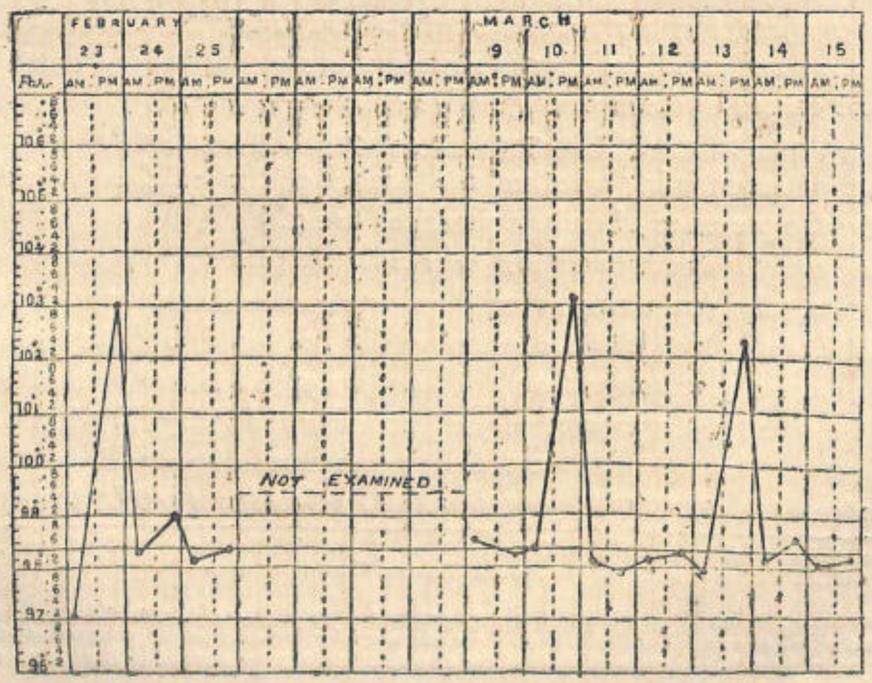
*Name.*—Palaythan. *Age.*—6 years.

First seen on the morning of February 23rd. He looks a moderately healthy child and his parents consider him to be quite well. His spleen, however, is enlarged one hand's breadth below the costal margin.

8 A.M., 23rd February 1902.—A few full-grown asexual Quartan parasites found.

5 P.M., on the same day (temperature 103°) two segmenting Quartan parasites and several pre-segmenting forms. No gametes. The child has apparently not felt any chilliness throughout the day, and though his skin feels hot, he is still playing about and is to all appearances little, if at all, inconvenienced by the fever.

DATES OF OBSERVATIONS.



24th February 1902.—Negative after a short examination.

25th February 1902.—A few asexual forms (Quartan) nearly filling the corpuscles. No evident symptoms.

9th March 1902.—The slide on this date contained a large number of nearly full-grown Quartan parasites. No gamete forms seen.

10th March 1902, 5 P.M. (temperature 103·4°).—A number of pre-segmenting and segmenting forms and in the same slide several forms which have the appearance of gametes. It is difficult, however, in a stained slide, to distinguish between a full-grown asexual Quartan and a gamete, so that, as I did not examine fresh specimens as well, I am unable to say whether gamete forms were really present in this case or not.

13th March 1902.—Segmenting forms were again seen in the slide at 5 P.M. on this date.

Throughout my examinations of this case, the child appeared to be very little affected by the fever, which lasted for only a few hours every fourth day.

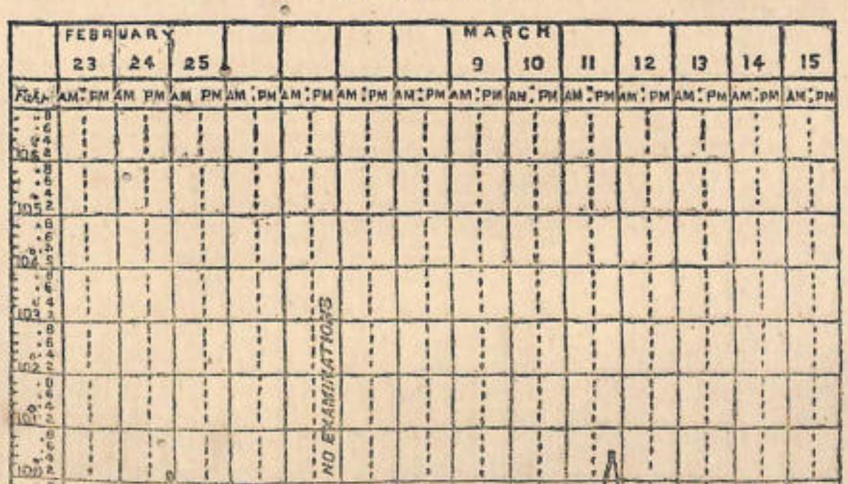
An examination of the chart and of the above notes will show, however, that this was an ordinary and very regular case of Quartan fever.

### *Case II.*

*Name.*—Mooragan. *Age.*—5 years.

This child is very thin and evidently weak. His spleen is enlarged more than one hand's breadth below the costal margin.

DATES OF OBSERVATIONS.



23rd February 1902.—A scanty infection with small and large asexual Quartan parasites. The blood was not examined again until the 9th March, when scanty full-grown asexual forms were seen.

11th March 1902.—Two segmenting Quartan parasites and a few forms which were certainly gametes were seen.

14th March 1902.—The slide contains a few gamete forms. No asexual parasites seen.

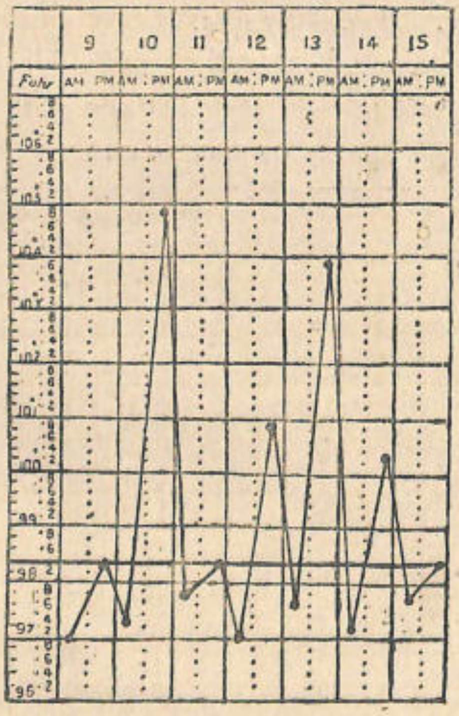
The infection in this case had evidently undermined the health of the child considerably. He had not, however, been sufficiently ill to prevent his walking about or taking food.

### *Case III.*

*Name.*—Migammah. *Age.*—4 years.

This case was first seen on March 9th. He appears a well-nourished child. Spleen enlarged two inches below the costal margin.

MARCH




This case shows very well the results of a mixed infection with Quartan and simple Tertian parasites

4 P. M., 10th March 1902.—A large number of full-grown simple Tertian

parasites, some of which were in the pre-segmenting stage, and a fair number of segmenting Quartan parasites were found.

11th March 1902.—A slide at 5 P.M. contained many medium sized-simple Tertian parasites, and Quartan parasites about $\frac{1}{4}$th the diameter of the corpuscles.

12th March 1902, 5 P.M.—One segmenting Tertian and a large number of nearly full-grown Quartan. A fair number of gamete forms.

13th March 1902, 4 P.M.—Several segmenting Quartan parasites and a few young simple Tertian parasites were seen. Many gamete forms.

Throughout the examinations of this case, gamete forms were present in fair numbers; and in an examination of fresh specimens on 14th March 1902, several flagellating bodies were seen.

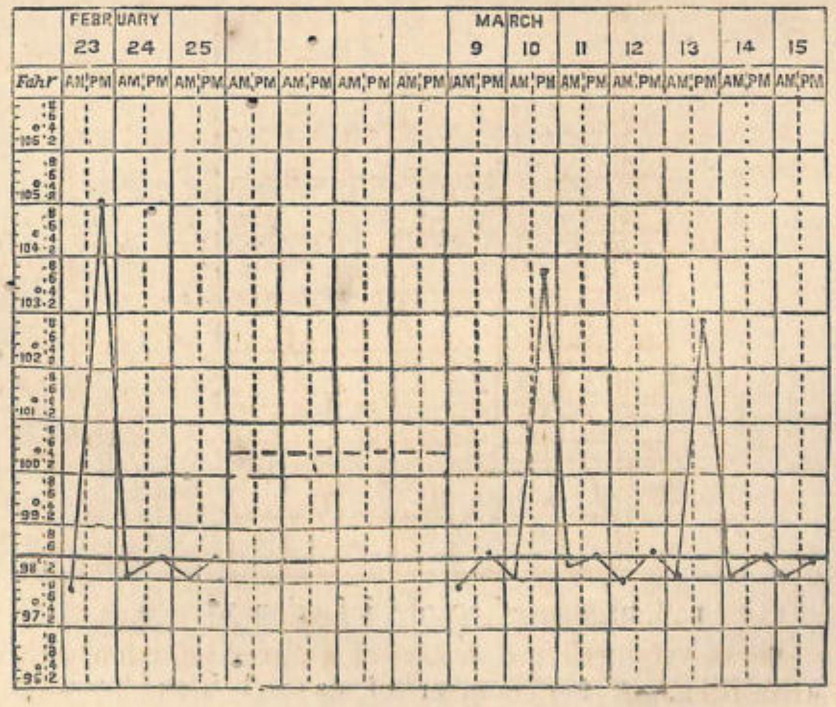
It is remarkable in this case that, in spite of the large infection and the high temperatures, the child appeared fairly well and, according to the statement of its parents, took its food heartily.

## *Case IV.*

*Name.*—Perumal. *Age.*—12 years.

A well-nourished boy whose parents said he had had fever every fourth day for over three months. Spleen enlarged to the level of the umbilicus.

DATES OF OBSERVATIONS.



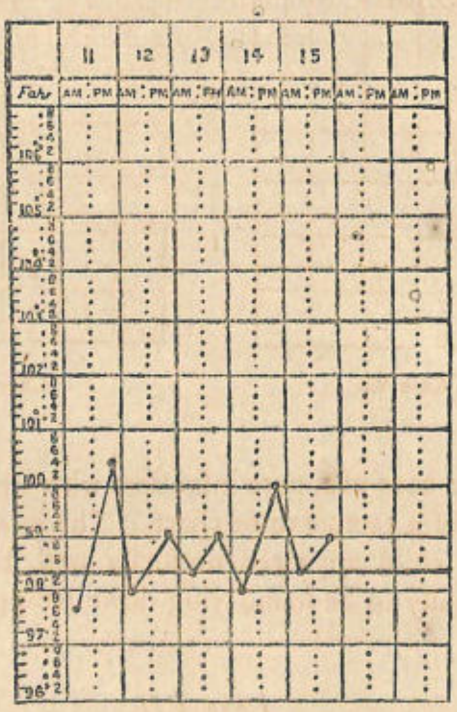
This was a case of a heavy infection with Quartan parasites, which went through their cycle with great regularity. The symptoms were more marked than in any of the other cases examined, but, except on his "fever days" he felt quite well and was accustomed to go out in the fishing boats with his father. The paroxysm begar with a chill and d slight shivering about three o'clock in the afternoon. By five o'clock the temperature had reached its height and the paroxysm ended with profuse perspiration between six and seven o'clock in the evening, after which he felt practically quite well for the next two days.

### *Case V.*

*Name.*—Vadyvaloo. *Age.*—5 years.

Although this child was able to run about with the other children, the physical signs were very marked. His face, body and legs were swollen and œdematous, his spleen was enlarged beyond the level of the umbilicus and his blood was so thin and "watery" as to make it difficult to prepare films from it.

MARCH.




The blood examinations showed scanty infection with at least two groups Quartan parasites.

11th March 1902.—A fair number of asexual forms from ¼th to ½ the diameter of the corpuscles together with a few segmenting forms were found.

13th March 1902.—In an examination of fresh specimens to-day, two flagellating bodies together with a few large asexual forms were seen.

## *Case VI.*

*Name.*—Chinnapan. *Age.*—7 years. Spleen palpable.

MARCH

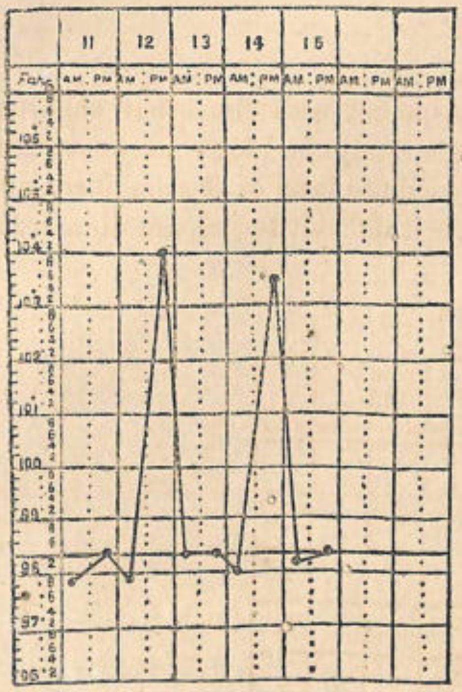


Chart for Case VI.

MARCH

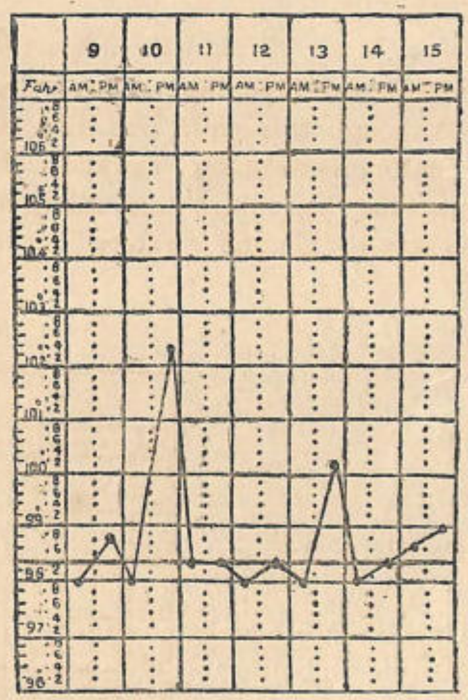


Chart for Case VII.

This was the only case of a pure infection with simple Tertian parasites of which I was able to get a temperature chart. The infection was a scanty one. In the mornings, the child appeared perfectly well, but on the evenings of his fever days he was usually to be found lying down wrapped up in a blanket.

## *Case VII.*

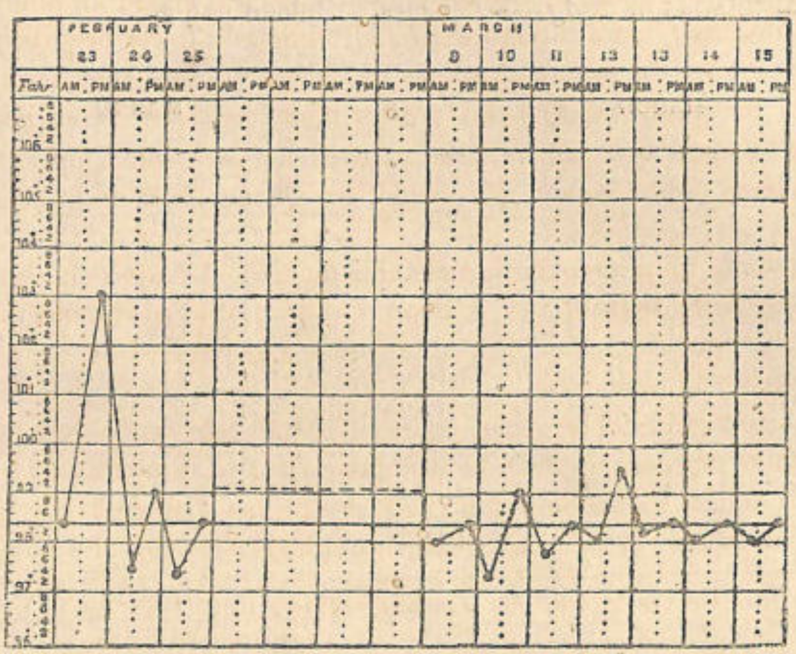
*Name.*— *Age.*—9 months. Spleen 1 inch below costal margin.

This case is interesting as showing infection in a young baby. A fair number of asexual Quartan parasites were found. The mother did not know that her child was suffering from fever.

## *Case VIII.*

*Name.*—Iyañcanoo. *Age.*—10 years. Spleen three inches below the costal margin.

DATES OF OBSERVATIONS.




On February 23rd a mixed infection with simple Tertian and Quartan parasites was found.

On March 9th only one medium-sized Quartan parasite was seen after a long search, and after this date parasites were absent from the peripheral circulation. A considerable increase in the proportion of large mononuclear leucocytes (an indication of recent infection) remained until the end of the examinations.

An evening rise of temperature was noted in four out of six other children whose temperatures were only taken once.

I have unfortunately not had an opportunity of following out any cases of malignant Tertian infection in children, but it may be noted that, although we have frequently observed crescents and occasionally crescent derived spheres, in the slides of blood from infected children, the spherical form of the malignant Tertian gamete, which was noted by Drs. Stephens and Christophers as characteristic of the infections of African natives, has never, so far as I am aware, been seen by any of us in India.*

* I have lately had an opportunity of following out several cases of infection with malignant Tertian parasites in babies and young children; during the periods when asexual forms of the parasites are present in the peripheral circulation the symptoms are more marked than in the case of Quartan and simple Tertian infections. The gametes are of the usual crescentic shape and character.

A consideration of the above cases will show, I think, that there is really no essential difference between child infections and those occurring in adults. The same febrile disturbance takes place about the time of segmentation of the parasites, and the asexual forms go through their cycle with the same regularity as is usually observed in an untreated case of malarial fever in an adult.

It is certain, too, that although the children appear fairly bright and well on a casual examination, the frequent attacks of fever and the continued infection with parasites for many months, undermine their health to a very great extent and result in the same after effects, *viz.*, marked anæmia, enlarged spleen, and sometimes œdema and dropsy, as would be observed in untreated cases in adults.

It is evident, therefore, that unless the malaria of native children in India differs markedly from that of the African children, the statement that native children may harbour the parasite in abundance and yet be free from fever and cachexia,* requires modification.

The well-recognised fact that young children are much less affected as regards clinical symptoms by even considerable rises of temperature than adults, is sufficient, I think, to account for their symptoms being easily overlooked, unless the children are carefully watched throughout a period of some days.

* "The Practitioner," Malaria number, March 1901. Page 259.

## III.—OBSERVATIONS ON THE CAUSES WHICH INFLUENCE THE PREVALENCE OF MALARIA IN DIFFERENT PARTS OF INDIA.*

WE have seen that the amount of malaria and the liability to infection (of which we have taken the "Endemic Index" to be the best test) varies greatly in different parts of India, and it becomes necessary to enquire therefore what are the causes which bring about such great differences in the endemicity of different places.

We may divide the districts we have examined into areas of high endemicity, such as the Bengal Duars, the Terai, and the Jeypur hill districts, and areas of low endemicity, such as Calcutta and the plains of Bengal.

1. We shall consider first the influence of *anopheles* mosquitoes on the endemicity of malaria.

Under this heading we may divide our observations as follows:—

(1) The influence of the presence of breeding grounds.

(*a*) The extent of the breeding grounds.

(*b*) The distance of the breeding grounds.

(2) The influence of the abundance of adult *anopheles*.

(3) The influence of the presence of particular species of *anopheles*.

(1) The influence of the presence of breeding grounds on the amount of malaria may be well illustrated from the examination of a number of villages in the Central Provinces. Here we were able to find several types of villages; one type, for example, with very extensive breeding grounds close at hand, another with extensive breeding grounds at a greater distance away, a third with no breeding places nearer than half a mile, and a fourth the nearest breeding places to which were at a greater distance than half a mile.

* I am indebted to Drs. Stephens and Christophers for the general plan and much of the material of this chapter.

The following table, therefore, shows the relation between the distance and extent of breeding grounds, the number of *anopheles* and the amount of malaria :—

TABLE I.

| Name of village. | Endemic index, per cent. | Spleen rate, per cent. | Species and abundance of *anopheles*. | Distance and extent of breeding grounds. |
|---|---|---|---|---|
| Phutala . . | 45 | 50 | *A. Culicifacies.* *A. Fuliginosus*, very abundant. | Less than 50 yards; extensive. |
| Sakadara . . | 15 | 15 | *A. Rossii.* *A. Fuliginosus*, not very abundant. | Less than 50 yards; not extensive. |
| Digdoh . . | 20 | 25 | *A. Culicifacies*, very abundant. | 100 yards. |
| Seendra . . | 20 | o | *A. Culicifacies.* *A. Fuliginosus*, abundant. | 200 yards. |
| Ambigheri. . | 25 | o | *A. Fuliginosus*, abundant. *A. Culicifacies*, scanty. | 200 yards. |
| Anjini . . | 25 | 40 | *A. Culicifacies.* *A. Stephensii.* *A. Fuliginosus*, very abundant. | One-fourth mile; very extensive. |

The above examples may be considered as type I where the breeding grounds are near, *anopheles* are fairly abundant and the endemic index is high—the variations in the amount of malaria (*e.g.*, Sakadara) being due chiefly to differences in the extent of the breeding grounds.

The following is an example of type II, where there are no breeding places within half a mile :—

| | | | | |
|---|---|---|---|---|
| Soonegaon. | 8 per cent. | o per cent. | *A. Stephensii*, scanty. | No breeding places within half a mile. |

The following are examples of type III, where the breeding places are over half a mile distant :—

| | | | | |
|---|---|---|---|---|
| Lawa. . . | 5 per cent. | o per cent. | *Nil* | Over half a mile. |
| Wadi . . | 5 per cent. | o per cent. | *Nil* | Ditto. |

In addition to the above types of villages in which the prevalence of malaria is easily explained by the varying distance and extent of breeding grounds, is possible to find villages with a considerable amount of malaria and yet a variable number of *anopheles*, sometimes indeed with much malaria and no *anopheles* at all, nor any breeding grounds near at the time of examination.

The cycle of events which has been gone through in such cases as this is as follows :—

Firstly, the presence of many small shallow rain-formed pools with numerous *anopheles*.

Secondly, the drying up of these pools and the consequent extinction of all breeding places, but yet with the continuance of the adult *anopheles* in the houses.

Thirdly, the disappearance of the adult *anopheles* but yet with continuance of the infection in the children.

It is at this third period when a good deal of malaria is found in the children of a village, and yet no adult *anopheles* can be found in the houses, and no breeding places are present, that, unless the above cycle of events is remembered, the presence of malaria seems difficult to explain.

That this explanation is the correct one is well shown in the following table :—

TABLE II.—To show continuance of Adult Anopheles after Breeding Grounds have dried up, and the continuance of Residual Malaria in the Children after disappearance of Anopheles.

| Place. | Date. | Endemic index, per cent. | Spleen rate, per cent. | *Anopheles* in the houses. | Breeding places at time of observation. | Signs of breeding grounds some time previously. |
|---|---|---|---|---|---|---|
| Mian Mir Bazaar . | September | 52 | 80 | Abundant *A. Culicifacies* and *A. Rossii* | Present | Yes. |
| Ditto . | October . | 35 | 75 | Scanty *A. Culicifacies*, abundant *A. Rossii*. | None | Yes. |
| Ditto . | December | 29 | 34 | None found | None | Yes. |
| Mian Mir, Syce Lines | October . | 56 | 48 | Abundant *A. Rossii* and *A. Culicifacies*. | Present | Yes. |
| Ditto . | December . | 12 | 30 | None found | None | Yes. |
| Tapara village . . | ... | 27 | 36 | Scanty | None | Yes. |
| Fishing village, Vizagapatam District. | ... | 10 | 17 | Very scanty | None | Yes. |

In places such as the following where no adult *anopheles* are found in the houses, no breeding places are present and there are no signs of breeding places having existed at some time previously, we find no malaria to be present in the children.

| Place. | Endemic index, per cent. | Spleen rate, per cent. | *Anopheles* in the houses. | Breeding places at time of observation. | Signs of breeding places at some time previously. |
|---|---|---|---|---|---|
| Vizagapatam Busty . . | 0 | 0 | None found | *Nil* | *Nil* |
| Chintarapelly . . . | 0 | 0 | None found | *Nil* | *Nil* |

(2) *The influence of the number of adult anopheles.*—Three types of villages may be found which show the influence of the abundance of adult *anopheles* on the amount of malaria. These are as follows:—

(1) Villages with no adult *anopheles* and no malaria. Examples of this type of village are Wadi, Lawa and Chintarapelly (the endemic indices, etc., of these villages have been given previously).

(2) Villages in which the *anopheles* are more or less abundant and the endemic index varies accordingly. Many examples of such places will be found in the tables already given.

(3) Villages in which the *anopheles* are scanty or absent and yet the endemic index is often high. The explanation of this and examples to illustrate it have already been given.

In addition to the above types of village the amount of malaria in which can be easily explained, there is another class of place of which Calcutta may be taken as the type in which there are abundance of *anopheles*, numerous breeding grounds close at hand and apparently every condition favourable to the development of malaria, and yet no infection is found in the children and no enlarged spleens. At Calcutta two districts which were reputed to be most unhealthy were chosen for work, *viz.*, the district of Hastings and that adjoining the Circular Road. In these districts all the conditions appeared most favourable for the development of malaria. The native population was crowded together in huts on low-lying flat ground which was, at the time of our visit, for the most part covered with water and surrounded by pools and tanks. The temperature was constantly high, and *anopheles* abounded. The Commissioners of the Royal Society are of opinion that not even in the worst fever districts of Africa have they met with *anopheles* in such abundance. In every house which we entered *anopheles* in large numbers could be caught with ease at any time. Under such conditions one would have expected much malaria to prevail.

On the contrary, however, we found that none of the 140 children examined by us had parasites in their blood, none of them had enlarged spleens, and not one of the 324 *anopheles* dissected was infected. At the end of September, which is considered to be the time at which fever is most prevalent in Calcutta, 42 children from the same districts were again examined with the same result, none of them showing parasites in their blood and none enlarged spleens.

One must conclude, therefore, that in these districts, in spite of the enormous number of *anopheles*, there is practically no endemic malaria, and the danger of infection is almost *nil*. In the Duars, on the other hand, the number of *anopheles* was not nearly so large—it was in fact often a difficult matter to catch a sufficient number to obtain the correct infection rate. The endemic index in this district, however, varied from 40 to 70 per cent. It is possible, therefore, to have a very large number of *anopheles* in a place without any malaria, while in another place with fewer *anopheles* there may be a large amount of malaria, so that it is unwise to make deductions regarding the prevalence of malaria in different places from observations on the relative number of *anopheles* in each place. Another place where *anopheles* were found in large numbers without the presence of any infection in the children and without any enlarged spleens was the village of Salur in the Madras Presidency. There is no satisfactory explanation for this condition of things, *but*, excluding these peculiar instances, *we may say that a direct relation exists between the extent and nearness of breeding grounds, the abundance of anopheles and the amount of malaria.*

(3) *The influence of the presence of particular species of anopheles.*—Practically the only species of *anopheles* which was present in the parts of Calcutta examined by us was *A. Rossii.* The mere fact that a species of *anopheles* could exist in such large numbers without producing any infection in the children was in itself suggestive that the species was not a good carrier of malaria.

A reference to the table of the Infection of Native Children (page 60) will show that as we proceeded from Calcutta through the plains of Bengal to the district known as the Duars, at the foot of the Himalayas, the endemic indices gradually increased from *nil* in Calcutta to as high a rate as 72 per cent. in the Duars. It was a significant fact that on reaching the areas of higher endemicity we encountered two new species of *anopheles* which we had not previously seen in India. One of these (*viz.*, *A. Maculata*, Theobald) was present only in scant numbers, the other (*viz.*, *A. Fluviatilis*=*A. Christophersi*, Theobald) was fairly common in the coolie huts of the tea plantations in the Duars. This species resembles in many respects and is possibly identical with the African species, *A. Funestus* (Giles), which has been proved to be responsible for so much of the malaria in Africa. Although we had not been able to find infection in any of the large number of

*A. Rossii* we had previously examined, infection with sporozoites to the extent of 6·25 per cent. was readily found in this new species. It was evident that this new species was the carrier of malaria in the Duars, and in none of the *A. Rossii* which we examined there did we find infection. Very few *A. Rossii*, however, were found in the Duars, and we were not able to obtain sufficient numbers to make the point certain that it was not also carrying malaria as well as *A. Fluviatilis*.

From Calcutta to the Duars the places were under practically identical conditions—similar climatic influences, a uniform high temperature, an abundant rainfall, and much surface water. In Calcutta, however, we had abundant *A. Rossii* and no malaria, and in the Duars a relatively small number of *A. Fluviatilis* and a large amount of malaria.

It appeared probable, therefore, that the question of species was an important one in connection with malaria, and that whereas *A. Fluviatilis* was an exceedingly good carrier of malaria, *A. Rossii* was a very inefficient one.

This probability received confirmation in a remarkable way in two other places which we visited, *viz.*, Mian Mir in the Punjab and Ennur in Madras.

In one of the bazaars at Mian Mir we found the children to be infected with parasites to the extent of 50 per cent. In this bazaar two species of *anopheles* were common, *viz.*, *A. Rossii* (Giles) and *A. Culicifacies* (Giles). *A. Culicifacies* is a species which belongs to the same class and resembles, in some respects, *A. Fluviatilis* of the Duars. Both species, *A. Rossii* and *A. Culicifacies*, were almost equally prevalent in the bazaar at Mian Mir, and we were thus able to catch a sufficient number of both species at the same time *and in the same house* to compare infection rates.

The following was the result of our dissections :—

| Species of *anopheles*. | Number dissected. | Percentage infected with sporozoites in the glands. |
|---|---|---|
| *A. Culicifacies* . . | 259 | 4·6 |
| *A. Rossii* . . . . | 496 | *Nil* |

It was practically certain therefore that in this bazaar *A. Culicifacies* was carrying malaria while *A. Rossii* was not.

The third place where we were able to show that *A. Rossii* was not carrying malaria under natural conditions, was at Ennur, a fishing village about ten miles from Madras. I visited this place in February and found the children to be infected with parasites to the extent of over 50 per cent. *A. Rossii* was present in very large numbers and *A. Culicifacies* somewhat scantily. In spite of its being present in fewer numbers, however, this was the species in which alone infection was found as the following table shows :—

| Species of *anopheles*. | Number dissected. | Percentage of infection with sporozoites in the glands. |
|---|---|---|
| *A. Culicifacies* | 69 | 8·7 |
| *A. Rossii* | 240 | *Nil* |

At this place, therefore, it was also certain that whereas *A. Culicifacies* was carrying malaria, ***A. Rossii*** was not.

The above facts afford a strong argument in favour of the view that whereas *A. Fluviatilis* and the species allied to it are very good carriers of malaria, *A. Rossii* is under natural conditions a very inefficient carrier, if indeed under such conditions it is able to exercise any influence whatever on the prevalence of malaria.

*Feeding Experiments.*—From some negative feeding experiments which we made at Calcutta with *A. Rossii*, added to the fact that working with this species in Calcutta Major Ross had previously been unable to cultivate human malaria in it, it seemed probable that even under experimental conditions *A. Rossii* did not carry malaria, but at Nagpur where we had an opportunity of feeding *anopheles* on some very suitable cases of malaria we were able to show that under experimental conditions both zygotes in the stomach and sporozoites in the salivary glands are readily developed in this species as in all the other species of *anopheles* (seven in number, *viz.* :—*A. Fluviatilis*, *A. Culicifacies*, *A. Turkhudi*, *A. Stephensii*, *A. Theobaldi*, *A. Barbirostris*, *A. Fuliginosus*) with which we experimented.

We have therefore the two facts that whereas *A. Rossii* is capable of carrying malaria under experimental conditions, it certainly was not doing so under natural conditions at any of the places we examined.

The explanation of these two apparently contradictory facts may possibly lie in a closer study of the habits of this species in nature—possibly in nature it habitually feeds not on the blood of man but on that of the cows, horses and other animals which are kept in every Indian village. The explanation certainly does not lie in the species of parasite which is present in the children, for in the Duars we were dealing almost entirely with Quartan parasites, in Ennur with Quartans and simple Tertians, and in Mian Mir chiefly with malignant Tertian parasites. Under experimental conditions we were able to show that the three species of parasite developed equally well in this mosquito.

Regarding the connection of other species of *anopheles* than those above referred to with malaria in India, the majority do not, I think, occur in sufficient numbers in houses to have very much effect on the amount of malaria. *A. Barbirostris* and *A. Sinensis* are certainly "wild" species very rarely found in houses. We have however shown that under experimental conditions the parasites of human malaria are capable of developing in *A. Barbirostris*. *A. Pulcherrimus* is a rare species which cannot, I think, exercise any influence on the amount of malaria. *A. Fuliginosus* is very common in houses and stables in some districts. Under experimental conditions, we have shown that the parasite of human malaria can develop in this species, and though we have never found it infected in nature, it is possible that it may occasionally have some influence. *A. Maculata, A. Stephensii, A Theobaldi, A. Jamesii,* and *A. Turkhudi* are all moderately frequent in some districts and may occasionally be responsible for some of the malaria, though we have never found any of these species infected in nature.

The group of *anopheles* which is certainly more important than any other from the point of view of malaria in India is the group of small brown *anopheles* comprising *A. Fluviatilis, A. Culicifacies* and their allies. The members of this group are the only ones that we have found infected in nature in India, and from this fact and the fact that in places of high endemicity one or other of the members of this group are always found to be present, while in places where there is little or no malaria the members of this group are absent, it is almost certain that this is by far the most important group of *anopheles* in India, and that in nature its members are better malaria carriers than any other *anopheles*.

II. The effect of the importation of non-immunised strangers into an immune district, and the effect of social status on endemicity.

Koch has shown that the importation of a number of non-immunised individuals into an immune district may bring about an increase in the fever rate, and that after a time the fever rate again declines as the new-comers become immune. This might partly account for the high endemicity of the Duars where coolies are imported for the tea plantations; but the parents of the children examined by us in this district had for the most part lived many years on the plantations and all the children had been born there, so that but little, if any, influence can be assigned to this cause. Moreover, no immigration occurs in many of the other areas of high endemicity which we examined, as, for example, at Mainaiguri in Bengal (endemic index, 25 per cent.) or at Ennur in Madras (endemic index, 54 per cent.).

As regards the influence of social status, children of low social status frequently showed a high rate of malarial infection, but on the other hand there are many instances (*e.g.*, Calcutta, and Vizagapatam village, endemic index, 0 per cent.), where people of very low social status showed little or no malaria, so that but little importance attaches to this factor.

III. The effect of Seasonal Variation.

In many parts of India the seasonal variation in the amount of malaria is very marked. In the south of India, where the temperature is fairly uniform all the year round and abundant *anopheles* are always present, this seasonal variation is not so marked as in the north where the climatic conditions vary extremely. Good examples of the effect of this cause may be taken from Mian Mir in the Punjab.

The malaria-carrying species of *anopheles* in Mian Mir breeds almost entirely in the irrigation watercourses, and the prevalence of this species (*A. Culcifacies*) throughout the year is influenced partly by the times at which the water in these watercourses is shut off and let in. The seasonal prevalence of this mosquito in Mian Mir is roughly as follows:—A few larvæ of *A. Culcifacies* may possibly be found if the water in the watercourses is turned on in March. No adults, however, will be found in the houses at this time. The water is usually shut off during the latter part of March and the beginning of April, and no adults or larvæ will be found at this time. During the latter half of April a few *Culicifacies* larvæ will be found in the watercourses, and from the beginning of May, when the water is usually turned on permanently, the number of larvæ in the watercourses gradually increases steadily month by month.

By careful search in the houses of the bazaars a few adult *Culicifacies* may be caught as early as May 15th, but until the beginning of June they are very rare. Throughout the month of June the number of adults increases gradually, but it is not until about the first week in July that they are sufficiently numerous to be caught with ease. In the beginning of this month, too, other species, and especially *A. Rossii*, which has previously been entirely absent, begin to appear in large numbers. The appearance of *A. Rossii* is dependent on the formation of pools by rain, whereas the prevalence of *A. Culicifacies* is, to a great extent, independent of the rainfall. From July until the end of October the numbers of larvæ and adults increase very rapidly, and during August and September especially very large numbers of adults can be caught with ease in any of the bazaars, in the harness rooms and in the barracks of the men. All the irrigation watercourses are at this time swarming with larvæ (chiefly *A. Culicifacies*), and in the numerous rain-formed pools abundant larvæ of *A. Rossii* are present.

During November the numbers of adults and larvæ gradually decrease, but a fair number of adults can still be caught in the bazaars as late as the 20th—25th of November.

By the beginning of December extremely few larvæ will be found in the watercourses, and the adults will have completely disappeared from the houses by December 10th. They will not be seen again until the end of April or beginning of May.

The above brief account refers to the years 1901 and 1902. A more detailed account of the seasonal prevalence of the mosquitoes and of malaria will be given in a later report.* The following are examples of the seasonal variation in the malaria at Mian Mir :—

TABLE III.—THE EFFECT OF SEASONAL VARIATION ON THE AMOUNT OF MALARIA.

| Name of place. | Date | Endemic index, per cent. | Spleen rate, per cent. |
|---|---|---|---|
| Mian Mir Infantry Bazaar . . | October 5th, 1901. | 52 | 80 |
| Ditto . . . | November 25th, 1901. | 20 | 38 |
| Mian Mir Artillery Bazaar . . | October 8th, 1901. | 35 | 75 |
| Ditto . . . | December 3rd, 1901. | 29 | 34 |
| Mian Mir Syce lines . . | October 30th, 1901. | 56 | 48 |
| Ditto . . . | December 22nd, 1901. | 15 | 30 |
| Mian Mir Cavalry Lines . . | November 1901. | 24 | 36 |
| Ditto . . . | December 10th, 1901. | 6 | 13 |

IV.—The effect of altitude.

We have found that under 4,000 feet altitude has no effect on the amount of malaria. If in a hill district at a lower height than this the slopes are very steep, so that there is no place where water can lodge and form breeding places ,there will be little or no malaria, but if, on the other hand, there are extensive plateaus among the hills and good breeding places, there will in all probability be a good deal of malaria, unless the hills are more than 4,000 feet high.

The following table summarises our observations in connection with this subject :—

TABLE IV.—MALARIA IN HILL DISTRICTS.

| District. | Name of Place. | Height. | Endemic index, per cent. | Spleen rate, per cent. | *Anopheles* present and breeding places. |
|---|---|---|---|---|---|
| DUARS . | Rangamutty . | 800 ft. | 43 | 83 | *A. Fluviatilis.* *A. Maculata.* *A. Rossii.* Streams, etc. |
| | Naigaisurie . | 1,200 ft. | 55 | 82 | Ditto. |
| | Sam Sing . . | 3,000 ft. | 28 | 7 | *A. Maculata.* Rice-fields. |
| DARJEELING . | Seypudura . . | 2,000 ft. | 0 | 12 | *A. Maculata*, scanty. Springs. |
| | Lower Singell Garden, Kurseong . | 4,000 ft. | 0 | 5 | *A. Rossii, A. Maculata, A. Fuliginosus.* |
| | Upper Singell Garden, Kurseong . | 4,400 ft. | 0 | 0 | *A. Maculata, A. Lindesayii.* |
| | Tonetta . . | 6,000 ft. | 0 | | *A. Lindesayii.* |
| JEYPUR HILLS | Sunki . . | 2,000 ft. | 50 | 55 | *A. Culicifacies*, Streams. |
| | Patungi . . | 2,800 ft. | 70 | 50 | *A. Jeyporiensis*, Marsh. |
| | Koraput . . | 2,000 ft. | 20 | 70 | *A. Jeyporiensis*, Marsh. |

* A Report on the Anti-Malarial Operations at Mian Mir, 1902, by Captain S. P. James, I.M.S.

V. There are doubtless other factors which influence malarial endemicity than those given above. The low endemicity of such places as Calcutta, Madras, and Salur is difficult to explain. The particular species of *anopheles* present may have some influence (as in Calcutta), although at Salur abundant *A. Culicifacies*, a good malaria carrier, were found and yet the endemicity was *nil*. This species is also present in small numbers in Madras. In some cases *special local conditions* doubtless affect the amount of malaria to a great extent. When all factors are considered, some regions, too, appear in themselves to be more or less malarious than others and some importance doubtless attaches to the "Regional Factor" (the expression used in this connection by Drs. Stephens and Christophers); thus, in the plains of Bengal we have a region of low endemicity, in the Duars and in the Jeypur hills regions of intense malarial endemicity, where Blackwater Fever is found, in the Punjab (Mian Mir) a region of high malarial endemicity, and in the Central Provinces a region of not very intense malarial endemicity with marked local variations.

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## IV.—The Relation between Enlarged Spleens and Malarial Infection in India.

THE prevalence of enlargement of the spleen is very great in India, and in almost every hospital a number of cases suffering from chronic enlargement of the spleen will be found.

In Africa, on the contrary, we are told that enlargement of the spleen among adult natives is very rare or almost entirely unknown.

As malaria is practically the only tropical disease which causes chronic enlargement of the spleen, one would naturally expect that the more malarious a country is, the greater would be the number of cases of enlarged spleen that we should find among the general population, and it comes therefore rather as a surprise to hear that enlarged spleen is rare among the inhabitants of Africa. When we enquire closely into the subject, however, the facts are not, I think, difficult of explanation.

But before going into the question of enlarged spleens in India, I must refer briefly to two points regarding malaria in India which have a bearing on this subject.

I. I have already indicated the fact that in all the parts of Africa which have been examined, there appears to be but one type of malarial region, *viz.*, that in which a high percentage of malarial infection is found in the children, this infection decreases rapidly with advancing age, and the adults are relatively quite immune to malarial infection.

In India, however, we may say that there are three types of malarial regions :—

(1) Regions of intense malarial prevalence exactly comparable to the malarial regions of Africa. The Duars, such places as Mian Mir, and the Jeypur hill districts, are examples of these regions. In these parts a very high rate of infection is found in the young children, this decreases with advancing age, and the adults are more or less immune.

(2) Regions of less intense malarial prevalence such as Jalpaiguri and other places in the plains of Bengal, and places in the Central Provinces. In these parts the liability to become infected with malaria is not so great. In the very young children no infection is, as a rule, found, in the children from five to ten years of age a moderate percentage of infection may be found, and the rate of infection increases instead of decreasing up to almost adult age.

Presumably the rate begins to decrease after 20 or 25 years of age, but very slight immunity is established in the adult.

(3) Regions of little or no malaria, such as the cities of Calcutta and Madras, and some of the places in the Central Provinces, and other parts of India. The liability to infection in these places is very slight. Even in children up to ten years of age very few cases may be found. With increasing age periods the percentage infected will be greater and the highest amount of malaria will be found in the adults. No immunity occurs in such places as these.

II. The length of time which infection, as shown by the presence of parasites in the peripheral circulation, lasts, when no reinfections occur.

Some light is thrown on this point by the results of the monthly examinations of children in Mian Mir.

TABLE TO SHOW THE GRADUAL DISAPPEARANCE OF PARASITES FROM THE PERIPHERAL CIRCULATION, IN THE ABSENCE OF REINFECTIONS.

| Mian Mir. | Endemic index, October 1901, per cent. | Endemic index, November 1901, per cent. | Endemic index, December 1901, per cent. | Endemic index, April 1902, per cent. | Endemic index, June 1902, per cent. |
|---|---|---|---|---|---|
| Artillery Bazaar . . | 35 | * | 29 | 20 | ·5 |
| Infantry Bazaar . . . . | 50 | 22 | * | * | 8·3 |
| Hospital Followers . . . . | 60 | * | * | * | *Nil* |

No new infections occurred from about the middle of November, and from that time until the end of June there had been a steady disappearance of parasites from the peripheral circulation. The asexual parasite sdisappear first, crescents and other gamete forms are the last to go; they probably last long enough to start the new series of infected mosquitoes of the following year.

We may say then that in the majority of cases of untreated malaria the parasites will, in the absence of reinfection, disappear from the peripheral circulation in seven or eight months.

Turning now to the question of enlarged spleens, we know that practically in every case of malaria the spleen becomes more or less enlarged, although the enlargement is not always sufficient to enable it to be detected by palpation. It is impossible, I think, to agree with Drs. Stephens and Christophers † that a high endemic index may exist without any appreciable spleen rate (Africa). One can easily understand that with a high endemic index there may exist no appreciable spleen rate *in the adults* and F. Plehn ‡ evidently referred to adults only when he

* Not examined.
† Reports to the Malaria Committee of the Royal Society, 6th Series, page 23.
‡ "Die Kamerun-Küste," page 97.

said "In the great majority of cases, it (the spleen) is not larger than in the cases of typhoid I have observed in Germany, often indeed smaller," but it is difficult to understand how a very high parasite rate could have existed *in the children* without a correspondingly high (or even higher) spleen rate. The sequence of events in India is, first, infection with parasites; secondly, enlargement of the spleen; thirdly, disappearance of the parasites; and fourthly, gradual reduction in size of the spleen. In all probability the same sequence occurs in Africa. As soon as the parasites disappear the spleen begins to reduce in size, but this always takes longer than does the disappearance of parasites, and for this reason it is quite possible to have *a high spleen rate in children, without a corresponding parasite infection*, but not, I think, the reverse. The reduction in the spleen rate with a reduction in the parasite rate is shown in the following table :—

| Mian Mir. | | October 1901, per cent. | November 1901, per cent. | December 1901, per cent. | April 1902, per cent. | June 1902, per cent. |
|---|---|---|---|---|---|---|
| Artillery Bazaar | Endemic index | 35 | ... | 29 | 20 | 5 |
| | Spleen rate | 75 | * | 34 | 31 | 33 |
| Infantry Bazaar | Endemic index | 50 | 22 | * | * | 8·3 |
| | Spleen rate | 82 | 38 | ... | ... | 26 |

The sudden decrease in the spleen rate in November and December represents the acute cases of enlargement which quickly disappear with the disappearance of parasites; the percentage of enlarged spleens which remain throughout the year, in spite of the absence of parasites, represents the chronic cases in which the spleen has become so enlarged as the result of former infections that it has not time to regain its normal size, before the new infections of the next season begin. In these cases we should not expect to find parasites, because we know that, without reinfection, the parasites disappear from the peripheral circulation in seven or eight months. When the series of new infections begins in the following year, some of these cases of chronically enlarged spleens may again become infected, and then parasites will be again found, the spleen will enlarge somewhat more, and after seven or eight months the parasites will disappear but the spleen remain enlarged.

From such cases as these we may pass on to the numerous cases of chronic enlargement of the spleen which are seen in Indian hospitals. At Calcutta we examined 80 of such cases. The prominent symptoms were the greatly enlarged spleen and marked anæmia. In some, there was irregular, more or less continued fever, between 99° and 101°, which went on for many weeks or months. In none of these cases did we find parasites or any indication

* Not examined.

Presumably the rate begins to decrease after 20 or 25 years of age, but very slight immunity is established in the adult.

(3) Regions of little or no malaria, such as the cities of Calcutta and Madras, and some of the places in the Central Provinces, and other parts of India. The liability to infection in these places is very slight. Even in children up to ten years of age very few cases may be found. With increasing age periods the percentage infected will be greater and the highest amount of malaria will be found in the adults. No immunity occurs in such places as these.

II. The length of time which infection, as shown by the presence of parasites in the peripheral circulation, lasts, when no reinfections occur.

Some light is thrown on this point by the results of the monthly examinations of children in Mian Mir.

TABLE TO SHOW THE GRADUAL DISAPPEARANCE OF PARASITES FROM THE PERIPHERAL CIRCULATION, IN THE ABSENCE OF REINFECTIONS.

| Mian Mir. | Endemic index, October 1901, per cent. | Endemic index, November 1901, per cent. | Endemic index, December 1901, per cent. | Endemic index, April 1902, per cent. | Endemic index, June 1902, per cent. |
|---|---|---|---|---|---|
| Artillery Bazaar . . | 35 | * | 29 | 20 | ·5 |
| Infantry Bazaar . . . | 50 | 22 | * | * | 8·3 |
| Hospital Followers . . . | 60 | * | * | * | *Nil* |

No new infections occurred from about the middle of November, and from that time until the end of June there had been a steady disappearance of parasites from the peripheral circulation. The asexual parasite sdisappear first, crescents and other gamete forms are the last to go; they probably last long enough to start the new series of infected mosquitoes of the following year.

We may say then that in the majority of cases of untreated malaria the parasites will, in the absence of reinfection, disappear from the peripheral circulation in seven or eight months.

Turning now to the question of enlarged spleens, we know that practically in every case of malaria the spleen becomes more or less enlarged, although the enlargement is not always sufficient to enable it to be detected by palpation. It is impossible, I think, to agree with Drs. Stephens and Christophers † that a high endemic index may exist without any appreciable spleen rate (Africa). One can easily understand that with a high endemic index there may exist no appreciable spleen rate *in the adults* and F. Plehn ‡ evidently referred to adults only when he

* Not examined.
† Reports to the Malaria Committee of the Royal Society, 6th Series, page 23.
‡ "Die Kamerun-Küste," page 97.

said "In the great majority of cases, it (the spleen) is not larger than in the cases of typhoid I have observed in Germany, often indeed smaller," but it is difficult to understand how a very high parasite rate could have existed *in the children* without a correspondingly high (or even higher) spleen rate. The sequence of events in India is, first, infection with parasites; secondly, enlargement of the spleen; thirdly, disappearance of the parasites; and fourthly, gradua reduction in size of the spleen. In all probability the same sequence occurs in Africa. As soon as the parasites disappear the spleen begins to reduce in size, but this always takes longer than does the disappearance of parasites, and for this reason it is quite possible to have *a high spleen rate in children, without a corresponding parasite infection*, but not, I think, the reverse. The reduction in the spleen rate with a reduction in the parasite rate is shown in the following table :—

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* Not examined.

whatever of a comparatively recent malarial infection. Nor indeed would one expect to find parasites in such cases, for few, if any, of them had been for some time in places where they would be very liable to contract a new infection, and I have already mentioned that parasites are not likely to be found in the peripheral circulation, unless a new infection has been received within seven or eight months of the examination of the case. The enlarged spleens are but the after-effect of former malaria which may have disappeared several years before. No parasites were found on puncture of the spleen or *post-mortem* in the organs of several cases which died.

The mere presence of an enlarged spleen has no influence in preventing the appearance of parasites in the peripheral circulation. If in any of these chronic cases a new infection with parasites occurs, the temperature chart at once assumes the ordinary malarial character of the particular species of parasite, and parasites are found in the peripheral circulation. This occurred in one of the Calcutta cases. A man who had an enormously enlarged spleen, and in whom no parasites had been found on repeated occasions, suddenly developed typical Quartan fever and Quartan parasites in abundance were found, which went through their cycle just as though there was no enlargement of the spleen at all.

It is doubtless on account of the large number of these chronically enlarged spleen cases in adults, which are treated in the hospitals of Calcutta and Madras, that these places have acquired the reputation of being very malarious, although, as a matter of fact, they are very slightly so. For the same reason, if one regarded the percentage of adults with enlarged spleens as a true test of the prevalance of malaria and the danger of infection in a district, many of the places in the plains of Bengal, which are in reality no more malarious than Calcutta or Madras, would be considered very much so, whereas such places as the Duars, Mian Mir, etc., which are really very malarious, to say nothing of the West Coast of Africa, would be regarded as being almost free from malaria.

In both Calcutta and Madras no enlarged spleens were found among the young children, but the cases in the hospitals show that it is a common disease among the adults. It is interesting, therefore, to enquire into the reason of this. I believe the reason is that in slightly malarious places a much larger number of cases of fever and enlarged spleen will be found among the adults than among the children; in fact the less liability to infection there is in a place the greater will be the proportion of infected adults to infected children.

I have already pointed out that there are at least three types of malarial regions in India.

The Duars or Mian Mir or Ennur may be taken as types of the first region (region of intense malaria).

In these regions there is very great liability to infection; so much so that even the young babies of a few months' old are frequently infected with malarial parasites, and any new-comer is usually down with fever in a few weeks. From repeated reinfection during childhood an immunity gradually becomes established, and by the time adult age is reached the immunity is more or less perfect.

In Mian Mir, for example, we may take the average endemicity as between 50 per cent. and 60 per cent. The average spleen rate of the same children (*i. e.*, those up to 10 years of age) is about 75 per cent. The spleen rate being higher than the parasite rate shows that, although in some of the children an immunity to parasites has become established, the spleens have not had time to regain their normal size. The spleens are of course much slower in reaching their normal size than the parasites are in disappearing, so that although the parasite rate may decrease rapidly with advancing age, the spleen rate may even show an increase up to a certain limit of age. Suppose, for example, it takes ten years of constant infection and reinfection to produce an immunity to new infections. During these ten years the spleen has been enlarged. At the end of the ten years parasites disappear and reinfection has no effect. Then the spleen begins to decrease in size, but it may take another ten years or more before it regains its normal size. Up to the age of twenty, therefore, enlargement of the spleen will be present not only in all those children who have parasites in their blood, but also in older children in whom an immunity to parasitic infection has been established, but whose spleens have not yet regained their normal size. After twenty, however, we may say that the spleens of the majority will have become of normal size, and in the adults, therefore, we shall not expect to find many enlarged spleens. This, as a matter of fact, is what we do find, as the following figures show:—

TABLE TO SHOW THAT IN VERY MALARIOUS PLACES A DECREASE IN THE SPLEEN RATE OF ADULTS OCCURS AS THE RESULT OF IMMUNITY.

| Place. | Age. | Number examined. | Number with enlarged spleens. | Percentage of enlarged spleens. |
|---|---|---|---|---|
| Mian Mir Syce lines | 0—5 | 9 | 7 | 77·2 |
| | 6—10 | 18 | 10 | 55·5 |
| | 11—15 | 16 | 4 | 25 |
| | 16—25 | 35 | 8 | 14·5 |
| | 26—40 | 74 | 6 | 8·1 |
| Duars Rungamutty | 0—5 | ... | ... | 77·4 |
| | 6—10 | ... | ... | 81·5 |
| | Young adults | ... | ... | 33 |
| Ennur | 0—5 | 15 | 14 | 93·3 |
| | 6—10 | 18 | 16 | 88·8 |

These places, therefore, are, I think, exactly comparable to malarious parts of Africa, where it is stated that enlargement of the spleen is very rare among the adults.

Secondly, we have the regions of less intense malaria, such as Jalpaiguri and other places in Bengal. Here the liability to infection was not so great. The endemic index was only 15 per cent. and in none of the children under five years of age did we find infection. The endemic index increased instead of decreasing with advancing age, and if immunity occurred in the adult it must have been at a comparatively late age. As would be expected from these facts, the spleen rate in the children under ten years of age was low; it increased rapidly with advancing age, and large spleens were very common among the adults.

TABLE TO SHOW THAT IN LESS MALARIOUS PLACES AN INCREASE IN THE SPLEEN RATE OCCURS WITH ADVANCING AGE.

| Place. | Age. | Number examined. | Percentage of enlarged spleen. |
|---|---|---|---|
| Jalpaiguri | 1—10 | 68 | 15 |
| | 11—15 | 24 | 62·5 |
| | 16—25 | 15 | 86 |

In this type of malarious region, therefore, there are certainly a larger number of cases of enlarged spleen among the adults than in the Duars or in Mian Mir. A number of moderately malarious places in the Central Provinces are exactly comparable to Jalpaiguri and other places in the plains of Bengal, and support the contention that in places which are not very malarious the spleen rate increases with advancing age. The following table shows the results of an examination of 1,134 children from different villages in the Central Provinces by Mr. Basak, the civil medical officer of Wardha :—

| Age of children. | Number examined. | Number with enlarged spleens. | Percentage of enlarged spleens. |
|---|---|---|---|
| 0—1 year | 439 | 10 | 2·2 |
| 2—5 years | 181 | 16 | 8·8 |
| 6—12 years | 514 | 78 | 15·3 |

Captain Rogers'* figures for spleen rates of places north of Calcutta also bear out this view. An examination of his results shows that in very malarious places the spleen rate of adults was lower than that of the children, in slightly malarious places it was much higher.

* "The relationship of the water-supply, water-logging and the distribution of *anopheles* mosquitoes respectively to the prevalence of malaria north of Calcutta." Journal of Asiatic Society of Bengal, Vol. LXIX, 1900.

Thirdly, we have places like Calcutta and Madras where the liability to malaria is much less than in either of the two types of places given above. No infection was found in any of the children under ten years of age in Calcutta, and only 5 per cent. in Madras. No enlarged spleens were found among the children of either place. Especially in Madras, however, a certain number of cases of fever do occur, and the large number of cases of enlarged spleens treated in the hospitals shows that enlargement of this organ is common among the adults. I think the above facts are sufficient to account for the general prevalence of enlargement of the spleen among the adults in the greater part of India, for the greater part consists of districts where the endemic index is only moderately high, although the liability to infection is sufficient to cause, during a number of years, a sufficient number of attacks of malaria to bring about (in the absence of continued treatment) a chronically enlarged spleen by the time 25—35 years of age is reached.

*The value of the "spleen test" as an indication of the prevalence of malaria.*

I propose to go only very briefly into this question which has lately been the subject of much discussion. My work during the present year has shown that even when applied to children the test is open to more serious errors as an indication of the prevalence of malaria than we supposed at first.

The truest test of the amount of malaria and the liability to infection existing in a place is the percentage of children (under ten years of age) infected with malarial parasites, and the best way of finding out what value can be assigned to an enumeration of enlarged spleens is to compare the results of our spleen counts with the endemic indices of the places under examination.

If we do this for various places in India we shall find that we can divide the places roughly into three types as follow :—

(1) Places in which the spleen rate in the children up to ten years of age is much higher than the parasite rate in the same children.

*Examples :—*

| Place. | Spleen rate, per cent. | Endemic index, per cent. |
|---|---|---|
| Rungamutty (Duars) . . . . . | 83 | 43 |
| Mian Mir (Artillery Bazaar) . . . | 75 | 35 |
| Ennur . . . . . . . . | 92 | 54 |

(2) Places in which the spleen rate in the children up to ten years of age is almost the same as the endemic index.

*Examples :—*

| Place. | Spleen rate, per cent. | Endemic index, per cent. |
|---|---|---|
| Jalpaiguri . . . . . . . | 17·7 | 16·1 |
| Sakadara . . . . . . . | 15 | 15 |
| Digdoh . . . . . . . . | 25 | 20 |
| Takli . . . . . . . . | 15 | 15 |

(3) Places in which the spleen rate in the children up to ten years of age is a good deal lower than the endemic index.

*Examples :—*

| Place. | Spleen rate, per cent. | Endemic index, per cent. |
|---|---|---|
| Seendra . . . . . . . . | 0 | 20 |
| Ambigheri . . . . . . . | 5 | 25 |
| Bighori . . . . . . . . | 0 | 30 |
| Soonegaon . . . . . . . | 0 | 8 |

Except in the second type of place, therefore, the spleen rate of the children up to ten years of age is not by any means an accurate indication of the endemic index, nor would it be possible to accurately compare the prevalence of malaria in these three types of places by taking the spleen rates of children *of the same age.* The age period at which the spleen rate most nearly approximates to the endemic index differs for each place according as the place is more or less malarious, and if we wished, for example, to accurately compare the prevalence of malaria in the Duars and in Jalpaiguri by the examination of spleens, we should probably have to examine the children up to two or three years of age in the Duars, and up to ten years of age in Jalpaiguri.

If we could exclude all the chronic cases of enlargement of the spleen from our counts, we should of course get fairly accurate results, because acute enlargements mean a comparatively recent infection with parasites : chronic enlargments are of course no indication of the prevalence of malaria at the time of examination, as the parasite infection which caused the enlargement may have occurred years before. For this reason little or nothing can be learnt regarding the prevalence of malaria from an examination of enlarged spleens in adults. By the time adult age is reached, too, immunity has, in very malarious places, come into play and affected the spleen rate. The spleen rate of the adults in Mian Mir, for example, is only 8·1 per cent., while it is over 50 per cent. in the certainly much less malarious places of Lower Bengal.* If, however, we depended only on a comparison of the spleen rates of adults for our results, we should be forced to the conclusion that these places were more malarious than Mian Mir or than the Duars.

The factor of *age* is, therefore, a very important one in connection with spleen rates.

Another factor which has to be taken into account is the season of the year at which the spleen counts are made. I have already pointed out how the parasite rate and the spleen rate decrease throughout part of the year in Mian Mir, and that there is a time when the prevalence of malaria is practically *nil*, although there are still a large number of enlarged spleens among the children. If at this time we depended on the spleen rate alone we should be misled into thinking that there was still a good deal of malaria and a moderate liability to infection when in reality there was none.

TABLE SHOWING THE PRESENCE OF A HIGH SPLEEN RATE IN CHILDREN WITHOUT A CORRESPONDING PARASITE INFECTION.

| Mian Mir. | Date. | Endemic index, per cent. | Spleen rate, per cent. |
|---|---|---|---|
| Artillery Bazaar | June 1902 | 5 | 33 |
| Infantry Bazaar | June 1902 | 8 | 26 |
| Hospital Followers | June 1902 | 0 | 50 |

Besides the factors of age, relative immunity, and season, a fourth factor *viz.*, the variety of parasite, may affect the spleen counts.

Assuming, however, that the object is to obtain a rough indication only of the prevalence of malaria in a place, the examination of the number of enlarged spleens among the children is certainly a quick and useful test, especially if the count is made during the "fever season." If, for example, in a village 90 per cent. of the young children have enlarged spleens, we could certainly infer that the place was very malarious, if only 20 per cent. to 30 per cent. have enlarged spleens we could infer that the place was only moderately malarious, and if only 5 per cent. to 8 per cent., that it was very slightly so.

The following conclusions may, I think, be drawn :—

(1) A high endemic index in India is always accompanied by a high (usually higher) spleen rate.

* Captain Rogers states that the percentage of enlarged spleens among adults near Calcutta is over 70 per cent., *vide Indian Medical Gazette*, October 1901.

(2) A high spleen rate may exist in adults ***and in children*** without a corresponding parasite infection. These are either, (*a*) chronic cases in which the parasites of the last new infection have gradually died out, but which will be liable to again become infected when opportunity occurs, or (*b*) cases which from repeated infections have become immune to new infections with parasites, but in which the spleen has not yet had time to diminish to its normal size.

(3) The spleen rate in adults is no indication of the prevalence of malaria. In places which are very malarious it may be very ow in places which are only slightly malarious it may be very high (Calcutta, etc.).

(4) Bearing in mind that the spleen is comparatively slow to enlarge and diminish in size, the spleen rate is entirely influenced by the parasite rate. In places where the parasite rate decreases at advancing age periods, the spleen rate also does so, although in some places an increase, up to a certain age, may occur owing to the time taken for individual spleens to diminish to their normal size. In places where the parasite rate goes on increasing with advancing age (*i.e.*, less malarious places where no immunity is established), the spleen rate also does so, so that while there may be no enlarged spleens among the children, there may be many among the adults (Madras, etc.).

## V.—A NOTE ON THE MALARIAL PARASITES AND FEVERS OF INDIA.

A FEW brief notes on this subject may be of interest.

The three forms of parasite—simple Tertian, Quartan, and malignant Tertian—with their corresponding fevers are prevalent in India. A fourth form of parasite, *viz.*, the malignant Quotidian parasite, possibly also occurs, though it would always be difficult to be certain of this on account of its resemblance to the ordinary malignant Tertian form.

It has generally been thought that Quartan fever is rare in India. We have, however, met with this form of parasite more frequently than with any of the others.

In some parts of India Quartan parasites are exceedingly prevalent; they are in fact almost the only form met with in certain districts. In the Duars, at least. 85 per cent. of the parasites found by us were Quartans, and both simple Tertians and malignant Tertians were rare in these parts of India. Again, at Ennur, near Madras, the percentage of children infected with Quartan parasites was 81 per cent., only 5 per cent. were infected with simple Tertian parasites and 14 per cent. with a mixed infection of simple Tertian and Quartan. No malignant Tertian parasites were found at this place. In the Jeypur hill districts, also, Quartan infections are very common.* It is interesting that in all these very malarious districts Quartan parasites should be so common, while malignant Tertian parasites, which one would have expected to be very prevalent in these regions (two of them, *viz.*, the Duars and the Jeypur State, being among the few districts of India where Blackwater Fever is met with), should be so rare.

In Mian Mir, another very malarious region, however, Quartan parasites are entirely absent. Simple Tertian and malignant Tertian infections are almost equally prevalent in Mian Mir, the former being the more common. In the plains of Bengal, and in the Central Provinces the three forms of parasites are more evenly distributed. At Jalpaiguri, malignant Tertian parasites were most common, then simple Tertian parasites and then Quartans. It was at this place also that we met with a few cases which were possibly infections with a distinct Quotidian parasite, although from lack of time we were unable to study a sufficient number of cases to definitely decide the question.

* Quartan infections are also common in Assam; *vide* "Report on the nature of Kala-Azar," by Major Ronald Ross, I.M.S. Calcutta, 1899.

At Calcutta we saw simple Tertian and malignant Tertian infections in the Military Hospital at Alipore, and one case of Quartan and one of simple Tertian in the General Hospital. The majority of the cases treated in the hospitals at Calcutta are, however, cases of malarial cachexia, characterised by marked anæmia, greatly enlarged spleens with sometimes enlargement of the liver, and irregular slight fever ranging from 99°F. to 101°F. continuing for many weeks or months. The same class of case is very common in many parts of India, and the diseases known under the names of kala-azar and kala-dukh would appear to be aggravated cases of the same nature. As already mentioned, no parasites were found by us in 80 of these cases examined in Calcutta, nor any other sign of recent malarial infection, and none of the cases appeared to be influenced by quinine. One case, sent as a typical case of kala-dukh, was examined by us in Jalpaiguri. No parasites or any signs of recent malarial infection were found. The man afterwards died of pneumonia. I have since examined slides sent from Assam of eleven cases of kala-azar without finding any parasites.

One of the first things that strike one in investigating malaria in India is the almost complete absence of typical malaria charts among the cases of "ague" under treatment in the hospitals. If one went by the charts alone, one would be led to believe that typical cases of simple Tertian and Quartan fevers were almost unknown in India. As a matter of fact, many medical officers have informed me that, though they have been a number of years in charge of hospitals in India, they have never seen typical temperature charts of simple Tertian or Quartan fever among the patients, and it appears, I think, to be the general opinion that the "malignant" forms of parasite, producing more on less irregular fever of varying duration, are much more prevalent in India than the "benign" forms. In reality this is not the case, and I believe that the reason why typical cases of ordinary malaria, and typical temperature charts, are not more frequently seen, is that in the majority of hospitals quinine is administered very early in almost all cases of fever. In order to diagnose a case of malaria correctly (including, of course, the form of parasite), it is, as a general rule, necessary to withhold quinine for a few days. In the commencement of nearly all cases of malaria the fever is more or less irregular and often practically continuous for the first two or even three days, until the parasites have settled down to their regular cycle, and intermittency becomes a marked feature. During these first two or even three days, also, it may be quite possible that parasites will not be found in the peripheral circulation. In cases of fever, if no parasites are found after careful search, it is, I think, unwise to give quinine, because by doing so we not only alter the true course of the temperature chart, but also do away with the chance of afterwards finding parasites. If the case is really one of malaria, to withhold quinine for a few days (in the absence of parasites from the peripheral circulation) will not endanger the case in any way, and if the case is not one of malaria, the administration of quinine, in doses suitable for a case of malaria, is certainly harmful. When quinine has not been given, if the case is one of malaria, the temperature chart quickly assumes its typical course, and even without an examination for parasites a correct diagnosis is usually assured.

A detailed account of the cases we have examined and of the characteristics of the parasites must be left for a later report.

I have notes of only two cases of "Comatose malaria" observed by us. One was a case in one of the hospitals at Calcutta which had been diagnosed as cerebro-spinal meningitis. Numerous ring forms of the malignant Tertian parasite were found on our first examination of this case. The second case occurred at Jalpaiguri. Almost every corpuscle in this case was infected with one or more malignant Tertian parasites, and all the large mononuclear leucocytes were crowded with pigment granules. This man died.

Blackwater Fever is chiefly prevalent in India in the Duars, in some parts of Assam, and in the Jeypur State. According to Drs. Stephens and Christophers, it is quite as prevalent in the Duars as in Africa. During our stay of only about a fortnight in the tea-planting districts of the Duars two cases were seen by Doctor Stephens, and a third case was reported to us on the day after our return to Jalpaiguri. A comparatively large number of the Europeans in the Duars had had the disease at some time or other, and the reports of a number of cases were received by Drs. Stephens and Christophers. No cases were actually seen in the Jeypur State, but reports of several cases were received and the disease is evidently not uncommon there. Notes of isolated cases of Blackwater Fever having occurred in other parts of India were also received, and it is certainly more prevalent in India than has generally been assumed to be the case.

From the examination of the cases seen in the Duars and the notes and slides of other cases, Drs. Stephens and Christophers have not, I believe, seen reason to alter the views expressed by them in Africa regarding the causation of Blackwater Fever. Their views have indeed been strengthened by the Indian cases.

Their opinions regarding its causation are as follows:—*

(1) There is no evidence whatever that Blackwater Fever is caused by a special parasite peculiar to itself.

(2) Blackwater Fever can rarely, if ever, occur except in a person who is suffering from, or has quite recently suffered from, a malarial

* See "Blackwater Fever" by J. W. W. Stephens, M.D., in "The *Lancet*" March 23, 1901, also "The Malarial and Blackwater Fevers of British Central Africa," by Drs. Stephens and Christophers, in the Reports to the Malaria Committee of the Royal Society, 1899-1900, also the Fifth Series of the same reports, 1901.

attack. It is malarial in origin, although in itself it cannot be considered as an attack of malaria.

(3) Quinine is, in the great majority of cases, the immediate cause of the Blackwater.

Although it is not common to find malaria parasites in cases of Blackwater Fever, Drs. Stephens and Christophers have been able to show (by the large mononuclear change, and by the presence of recent pigment) the existence of a malarial infection in all their cases. The administration of quinine alone would be sufficient in the majority of cases to account for the absence of parasites from the peripheral circulation, and, in addition, it is well known that many ordinary cases of undoubted malaria occur, in which, for several days after the fever has commenced, and occasionally practically throughout the case, parasites cannot be found in the peripheral circulation. In such cases the diagnosis can, as a rule, be accurately made by the finding of pigment in the large mononuclear leucocytes, or by a differential count of the leucocytes, and the diagnosis will usually be afterwards confirmed by the finding of one or two parasites.

In addition to proving the existence of a recent malarial infection in all their cases, Drs. Stephens and Christophers have been able to show that the onset of the Blackwater was, in all cases, preceded by one or more doses of quinine at an interval of from four to ten hours before the attack.

After a careful study of the evidence and cases which they have published, one can scarcely fail to come to the conclusion, with them, that Blackwater Fever is essentially a malarial infection, in which quinine is the most common immediate determining cause of the attack of Blackwater.

Blackwater Fever is therefore a preventible disease. The prophylaxis consists in the prevention of attacks of ordinary malaria. Complete protection from malaria may be ensured by any individual who is willing to take the trouble to pay scrupulous attention to the use of a good mosquito curtain at night, and to adequately protect himself from being bitten by mosquitoes during the evening hours. If these simple precautions are taken, it is quite unnecessary to use quinine as a prophylactic. No other precautions than the use of the mosquito net and the wearing of high boots or putties in the evenings have been used by any of us during our tours through some of the most malarious parts of India, and none of us have experienced a day's fever during this time. By the use of the same precautions also, and without taking any quinine, Dr. Stephens had previously passed two years in the most malarious parts of Africa without a single attack of malaria. If it were possible to induce the European planters, whose lives have to be spent in very malarious regions, to adopt the same simple precautions from the time of their first arrival in the districts, I have no doubt that Blackwater Fever would soon become unknown among them.

## VI.—Observations on Birds.

During our stay at Nagpur in January and February, I made the following observations on the infection of birds with Proteosoma and Halteridium parasites. So far as I am aware, none of my observations are new, but some of them confirm the work of previous observers. The study of the parasites of birds, moreover, is of great interest and has occupied but little attention in India.

The main objects I had in view were the following:—

(i) To determine whether any rise of temperature occurs in birds infected with Proteosoma and Halteridium parasites, respectively.

(ii) If any such rise of temperature occurred in birds infected with either form of parasite, to determine whether any marked periodicity, *e.g.*, a Quartan or Tertian periodicity, could be made out.

(iii) To conduct feeding experiments with *anopheles* and *culex* mosquitoes on birds infected with Proteosoma and Halteridium parasites, respectively.

The birds which were most easily available at Nagpur were sparrows and pigeons. The sparrows were very susceptible to both forms of parasite (Proteosoma and Halteridium), and it was very common to find both forms of parasite in the same bird.

Nothing but Halteridium infections were ever found in the pigeons.

It is sometimes rather difficult to obtain a drop of blood, sufficient to make good slides, from a bird, and much unnecessary pain may be caused unless the prick with the needle is made in the right place. The most satisfactory way is to draw the leg somewhat away from the body so that its under surface, where there are few or no feathers, is exposed, and then to look carefully for one of the surface veins which can be plainly seen through the white skin. The prick is then made into one of these veins, and a good sized drop of blood will immediately exude.

The temperatures of birds should always be taken in the cloaca: in small birds, such as sparrows, a correct temperature can never be obtained under the wing or leg. By using a small-bulbed thermometer I found the taking of temperatures in the rectum (cloaca) of both sparrows and pigeons a very easy matter.

Both *culex* and *anopheles* mosquitoes feed readily on sparrows and pigeons. All that is necessary is to confine a number of the mosquitoes in an oblong cage the sides of which are composed of mosquito netting. The head of the bird which is placed in the cage with the mosquitoes should be covered with a hood. This is quite sufficient to prevent the bird from flapping its wings and so driving the mosquitoes away. The voracity with which *culex* mosquitoes (*C. Fatigans*) will gorge themselves upon the blood of sparrows is extraordinary, and if too many mosquitoes are put in the cage with the same sparrow they will literally bleed it to death.

Bred mosquitoes will not, as a rule, feed on the first night after being hatched out: on the second or third night, however, they will almost certainly do so.

After many trials I came to the conclusion that the sparrows at Nagpur were not suitable birds on which to make observations regarding Proteosoma infection. In the first place, a large number died after being under observation one or two days. In the second, the temperature of a sparrow appeared to me to be affected by very slight causes. Even in sparrows not affected by any parasites I was unable to obtain anything in the nature of a regular chart. At intervals of half an hour or less the temperature would frequently vary as much as 1·5 or 2 degrees, and I can only conclude that the fright of being caught and held while the temperature was taken was sufficient to cause a rise or fall.

Again, it was almost impossible to find a sparrow which was not infected with both forms of parasite, and a bird which, on one day would present nothing but Proteosoma parasites, would, a day or two afterwards, show large numbers of both forms. A very careful daily examination was necessary to exclude either form of parasite, and even then it was not possible to be quite certain that a scanty infection (which would, in a few days, become a very large one) with the second form of parasite had not been overlooked, owing to the large number of parasites that were usually present in each field.

I shall not, therefore, reproduce here any of the charts of sparrows, as they do not present sufficiently marked characters to enable definite conclusions to be drawn from them. Taking the average of the results from a number of sparrows without parasites, I conclude, however, that the normal temperature taken in the rectum of a sparrow is between 106°F. and 107·5°F., and that the temperature in the evening is between 1° and 1·5° higher than that in the morning. It is not uncommon for the temperature of a normal sparrow to rise as high as 108·5°F. or even 109°.

The chief feature of the charts of sparrows infected with Proteosoma parasites was their irregularity. The temperature in the evenings frequently reached 109°F. and in the mornings fell as low as 103°F. I was unable to make out that a definite rise of temperature corresponding to particular stages of the parasites occurred, and from none of my charts could a definite periodicity be made out.

For the elucidation of the temperature of birds infected with Proteosoma, it would be necessary, I think, to study birds which are susceptible to this form of parasite only, and, if possible, larger and more robust birds than sparrows.

In sparrows affected with Halteridium parasites only, the temperature charts did not present any special features from which it could be decided that they differed from normal charts.

The temperature charts of the pigeons were much more regular and satisfactory than those of the sparrows.

Charts I and II are those of normal pigeons.

From these it will be seen that the normal temperature (taken in rectum) of a pigeon is between 107°F. and 108°F., and that there is a difference of about 1° between the morning and evening temperatures. Occasionally also the evening temperature rises as high as 109°F. without apparent cause.

PIGEON CHART NO. I. NO PARASITES.

(RECTAL TEMPERATURES.)

PIGEON CHART NO. II. NO PARASITES.

(RECTAL TEMPERATURES.)

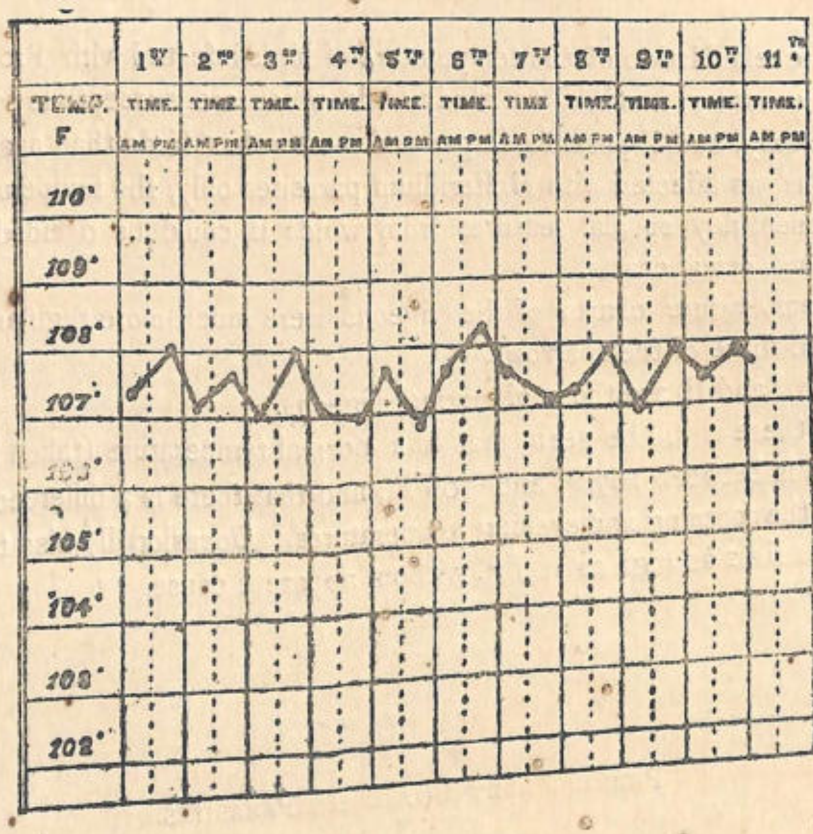
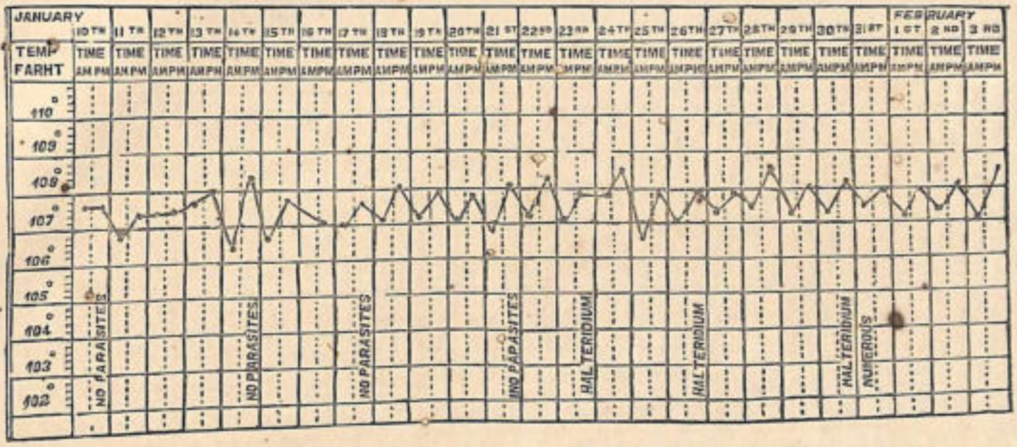



Chart III is that of a pigeon in which no parasites were found for the first fifteen days, on the 23rd January four Halteridium parasites were found in a slide, and after this date Halteridium parasites in increasing numbers were found at each examination.

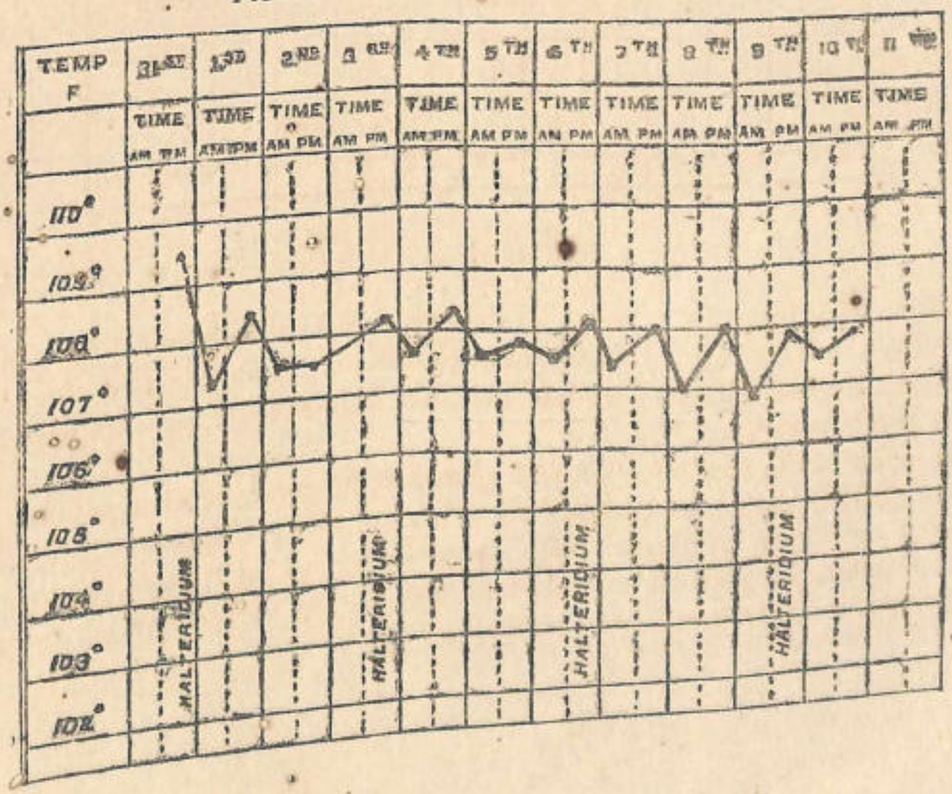
In spite of the new infection with parasites, however, no change in the temperature chart occurred, so that, taking this chart along with charts Nos. IV and V, which are those of pigeons with large infections of Halteridium parasites, we can, I think, infer, with some certainty, that no rise of temperature occurs in birds infected with Halteridium parasites.

## Pigeon Chart No. III.

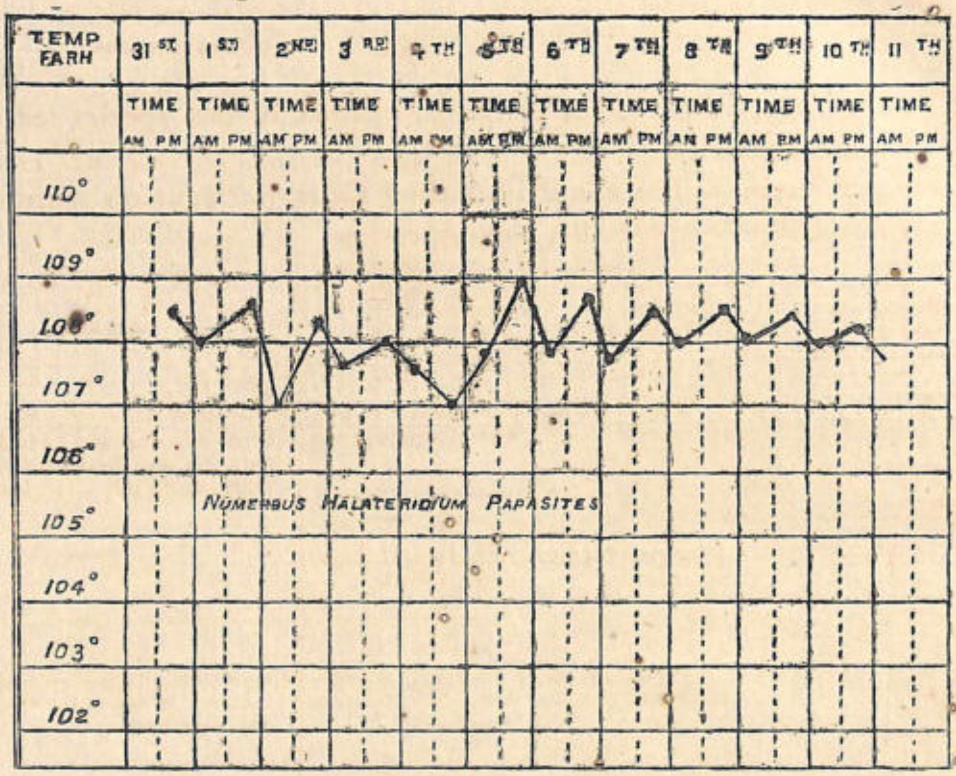
(Rectal Temperatures.)



## Pigeon Chart No. IV. (Halteridium.)



PIGEON CHART NO. V. (HALTERIDIUM.)



## Feeding experiments.

These may be summed up as follows :—

*1st experiment.*—The result of feeding *Culex Fatigans* on sparrows infected with Proteosoma.

| Total number fed and dissected. | Total number in which zygotes were found. |
|---|---|
| 26 | 23 |

That is, over 80 per cent. of the mosquitoes of this species fed on Proteosoma became infected.

*2nd experiment.*—The result of feeding *Culex Fatigans* on sparrows presumably infected only with Halteridium parasites.

| Number of experiment. | Particulars of experiment. | Result of dissections. | Remarks. |
|---|---|---|---|
| 1 | 6 *C. Fatigans* fed on 3 sparrows. | 5 negative, 1 with several small zygotes. | Experiment vitiated by the fact that Proteosoma as well as Halteridium parasites were found in one of the sparrows the day after feeding. |
| 2 | 5 *C. Fatigans* fed on 2 sparrows. | 4 dissected, all negative, 1 died. | ... |
| 3 | 8 *C. Fatigans* fed on 3 sparrows. | 8 dissected, 6 negative, 2 positive, (zygotes). | Experiment vitiated by finding Proteosoma in one of the sparrows the day after feeding. |

Besides showing the difficulty there was in excluding a concurrent infection with both forms of parasite in the sparrows, the large number of negative results in the above experiments, when taken in conjunction with the large number of positive results obtained by feeding this species of mosquito on Proteosoma, pointed to the conclusion that *Culex Fatigans* is a non-carrier of Halteridium parasites.

*3rd experiment.*—The result of feeding *Culex Fatigans* on pigeons infected with Halteridium parasites.

Owing to the difficulty of excluding a double infection in sparrows, I next fed a number of *Culex Fatigans* on pigeons, as these birds are not susceptible to Proteosoma infection.

Six separate experiments of feeding *Culex Fatigans* on pigeons infected with Halteridium were made.

The total number of mosquitoes afterwards dissected was 28. No zygotes were found in any of them. Two of the pigeons used were particularly favourable cases—in a fresh slide from one of them 15 flagella bodies and 95 asexual parasites had been counted just before feeding.

We can infer, with certainty, therefore, that the Halteridium parasite is not carried by *Culex Fatigans*, although this species is such a good carrier of Proteosoma.

*4th experiment.*—The result of feeding *anopheles* mosquitoes on sparrows infected with Proteosoma, and Halteridium.

(*a*) Six *anopheles* mosquitoes (2 *A. Fuliginosus*, 2 *A. Fluviatilis*, 2 *A. Rossii*) together with 3 *Culex Fatigans* as a control, were fed on a sparrow infected with Proteosoma parasites. Zygotes were afterwards found in the stomachs of the 3 *Culex* mosquitoes: all the *anopheles* were negative.

(*b*) Seven *anopheles* mosquitoes (*A. Fuliginosus*, *A. Culicifacies*, *A. Fluviatilis*, *A. Rossii*) were fed on a sparrow with Halteridium parasites. No zygotes were found in the stomachs of any of these *anopheles*.

*Anopheles* mosquitoes do not, therefore, appear to be capable of carrying either the Proteosoma or Halteridium parasites.

The foregoing observations may, therefore, be summed up as follows:—

(i) The normal temperature of a sparrow would appear to be between 106°F. and 107·5°.

(ii) The normal temperature of a pigeon is between 107°F. and 108°.

(iii) In healthy birds the temperature occasionally rises as high as 109°F. without apparent cause.

(iv) I was unable to decide from my own observations whether a rise of temperature occurs in sparrows infected with Proteosoma or not. No marked periodicity was shown in my Proteosoma charts of sparrows.

(v) No rise of temperature occurs in birds infected with Halteridium, and the character of the temperature chart does not change.

(vi) *Culex Fatigans* is a good carrier of Proteosoma infection. It does not carry Halteridium.

(vii) *Anopheles* mosquitoes do not appear to be capable of carrying Proteosoma or Halteridium.

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