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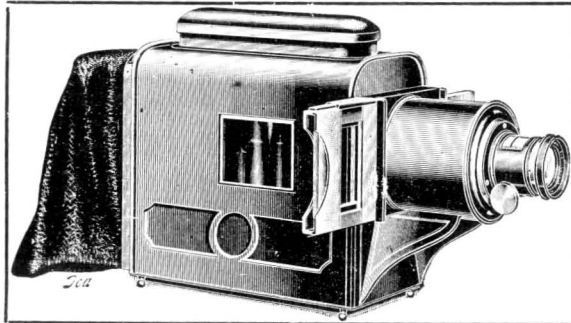
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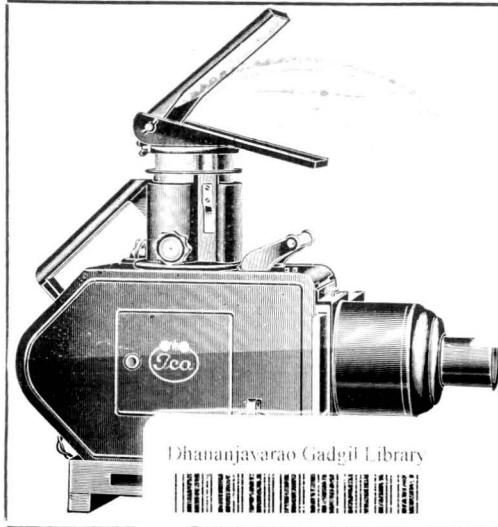


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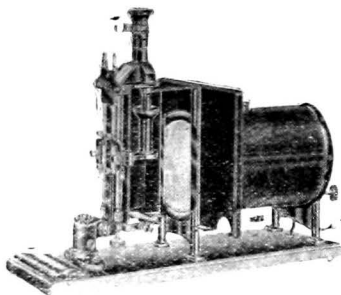


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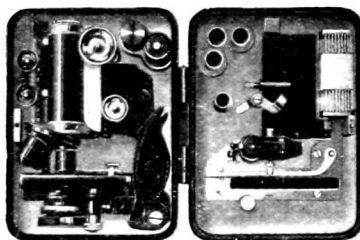
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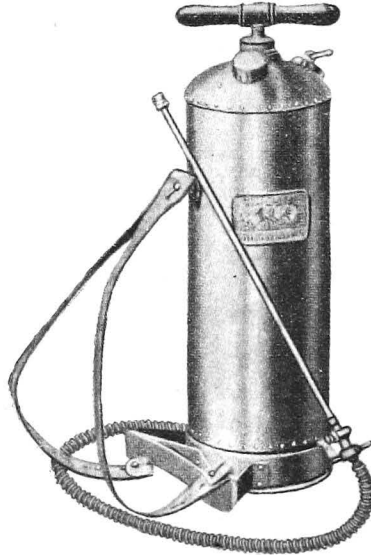
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**M A L A R I A**  
**Its Investigation and Control**

# MALARIA

## Its Investigation and Control

with special reference to Indian conditions

BY

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MAJOR, INDIAN MEDICAL SERVICE

*Professor of Protozoology,  
Calcutta School of Tropical Medicine*

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MALARIA RESEARCH OFFICER

*Central Malaria Bureau, Government of India;  
formerly*

*Malariologist, The Kepitigalla Rubber Estates, Ltd.,  
Ceylon, and*

*Malariologist, Bengal-Nagpur Railway Co., Ltd.*

*'Go where Death's pickets hide—  
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That is the work of a spy.'—Kipling.*



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**TO**  
**THE WIVES OF RESEARCH WORKERS**  
**(IN RECOGNITION OF THEIR SUFFERINGS IN THE CAUSE OF SCIENCE)**

## PREFACE

NUMEROUS as are the existing books on malaria, there at present exists in India no suitable handbook which can be put into the hands of medical men of the assistant surgeon class to help them in their malarial problems. Stephens and Christophers' *Practical Study of Malaria* has long been out of print: James' *Malaria at Home and Abroad* is now out of date with regard to its entomological side, whilst it contains much clinical matter which is beyond the purview of the malariologist engaged in the practical eradication of the disease; whilst the little book recently published on *Mosquito Reduction and Malarial Prevention* by Crawford and Chalam, although giving the layman an excellent idea of what can be done by the expert, is, in our opinion, rather too elementary for those actually engaged in anti-malarial campaigns. The preparation of the present volume was suggested to the authors by Colonel Martin-Leake, V.C., F.R.C.S., Chief Medical Officer, Bengal-Nagpur Railway; and it is hoped that in it the worker in the field will find the practical instructions that he may require; how to diagnose malaria in the laboratory; how to treat his patients; when, where, and how to look for his mosquitoes; how to set about and carry out a malaria survey; and what steps to take as the result of the knowledge gained by such a survey. The chapters on the life-cycles of the malaria parasites in man and in the mosquito, on the practical laboratory diagnosis of malaria, and on treatment are by the senior author; the chapters on malaria survey work, the design, construction and maintenance of anti-mosquito measures, and the appendices are by the junior author; whilst both authors have collaborated in the final putting together of the book. The Governing Body of the Endowment Fund of the Calcutta School of Tropical Medicine has very generously financed its publication, and the authors' gratitude is due to that body for making its publication possible.

To the crime of piracy the senior author at once pleads guilty. Much of Chapters I and II is taken from Wenyon's splendid *Protozoology*, together with several of the illustrations. The warmest thanks of both authors are due to

Messrs. Baillière, Tindall and Cox for permission to reproduce several of Dr. Wenyon's illustrations. The authors' warmest thanks are also due to Dr. C. Strickland, M.A., B.Ch., Professor of Entomology, Calcutta School of Tropical Medicine, for the loan of lantern slides and for his very kind permission to reproduce many of his illustrations. In the appendices the authors have further borrowed figures from James and Liston's *Anopheline Mosquitoes of India*, James' *Malaria at Home and Abroad*, from the monograph by Mr. H. F. Carter on the Anopheline Mosquitoes of Ceylon in the *Ceylon Journal of Science*, Patton and Cragg's *Text-book of Medical Entomology*, and papers by Iyengar, Bains Prashad, Edwards and Kirkpatrick. A special word of thanks is also due to Mr. H. M. Roy and Mr. J. K. Mullick, artists of the Calcutta School of Tropical Medicine, for their admirable execution of the illustrations.

When the publication of this book was first proposed, it was suggested to the authors that advertisements by reputable firms might be included in order to reduce the cost of publication. To this the authors consented: in the first place it was felt that this might help to place the book financially within the reach of the class of readers for whom it has been written; in the second place the inclusion of advertisements of microscopes and accessories, stains, cinchona derivatives, oil sprays, etc., might be of use to the reader in informing him where to buy materials. It would have been better for such material to have been relegated to the back of the book, but, by a misunderstanding, a number of advertisements have been inserted to precede the text. This is unavoidable as far as the present edition goes, but should a second edition be called for, it will not be repeated.

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## CHAPTER I.

### THE MALARIA PARASITES. CYCLE IN MAN.

THE malaria parasites belong to the Protozoa. The Protozoa may be defined as animals whose tissues are non-cellular. The Animal Kingdom in general is divided into two main phyla ; the Protozoa, a word derived from the Greek words *protos* = first, and *zoon* = a living animal, and so called because it is clear that in the course of evolution the Protozoa came into existence before the Metazoa ; and the Metazoa—from *meta* = after—which came afterwards.

If we take a section of any tissue from a metazoal animal, we find that it is composed of cells, separated from one another by cell walls. Each cell leads an independent existence, but the tissue as a whole acts and functions together. Some of the Protozoa reach a very high degree of differentiation of structure ; yet in them the tissue structure is not cellular, and the body of the animal acts and functions as a whole, reacting in many diverse ways to all sorts of external and internal stimuli.

The phylum Protozoa is divided into two main sub-phyla. The first sub-phylum, the Plasmodroma, are characterised by the fact that when male and female sex cells unite, there is complete fusion of their entire bodies : their nuclear matter unites, and so also does their non-nuclear matter or cytoplasm. The malarial parasites belong to this sub-phylum. In the second main sub-phylum when the sex cells unite, a very peculiar process of conjugation takes place : two cells which are apparently similar come together ; each exchanges nuclear matter with the other ; and the two individuals then separate again. These Protozoa constitute the sub-phylum Ciliophora, and are motile by virtue of a fine hair-like coating of vibratile cilia, by the incessant beating of which these animals are rowed about, just as is a boat by oars.

The sub-phylum Plasmodroma is divided into four main classes, as follows :—

1. *The class Rhizopoda.*—In these the animal possesses no outer limiting cell membrane, and movement is effected by a creeping, amœboid movement, rather recalling that of a snail. To this class *Entamœba histolytica*, the parasite of amœbic dysentery in man, belongs.

2. *The class Mastigophora.*—In these movement is effected by the possession of certain long, whip- or lash-like organs known as flagella, by the lashing action

of which the animal is pulled along. To this class belong the trypanosomes which cause sleeping sickness of man, and the parasites of kala-azar and oriental sore.

3. *The class Cnidosporidia.*—These are parasitic Protozoa which occur chiefly in fish, insects and worms. They produce spores which possess a peculiar explosive structure known as a polar capsule. On ingestion of the spore into the gut the polar capsule explodes, setting free the spore, which parasitises in the first instance as a rule the intestinal epithelium. There are no human parasites in this class.

4. *The class Sporozoa.*—These are parasitic Protozoa, and are characterised by possessing two life-cycles. During the first, asexual life-cycle—to which the term schizogony is applied—the parasites multiply by dividing into a number of daughter individuals, the nucleus and then the cytoplasm dividing in turn. After this the parasites pass into their sexual or sporogony cycle—as it is termed—in the course of which sexual conjugation takes place and is followed by multiplication of the parasite. We have thus a regular alternation of generations consisting of schizogony followed by sporogony, followed again by schizogony, and so on.

The malarial parasites belong to this class. Further, in addition to this alternation of generations, and together with it, goes an alternation of hosts. The schizogony cycle takes place in the blood stream of man; the sporogony cycle takes place in the tissues of the mosquito. In the course of evolution we must suppose that the malarial parasites were at first intestinal parasites of the mosquito, and multiplied in the mosquito by sporogony. Later, as mosquitoes took to biting man, malarial parasites were introduced into man, and, as man was not acclimatised to them—so to speak—in man they cause disease. Insects are immeasurably older in the scale of life than is man, and the mosquito has become so acclimatised to the malarial parasite that it (or rather she) suffers no inconvenience from being infected with it.

It is absolutely essential for the student, before commencing the study of malarial parasites, to grasp once and for all the correct terminology in connection with their life-cycles. Text-books on tropical medicine are apt to be very remiss in this matter; and not only does such confusion muddle the student, it may also lead to serious mistakes on the part of research workers. Thus Aragão (1907, 1908) in studying the development of *Hæmoproteus columbæ* of the pigeon in the transmitting fly *Lynchia maura*, made the suggestion that the oökinete which forms in the midgut of the fly on conjugation of the gametes, was inoculated by the fly into the pigeon. Such a procedure would be most unlikely to happen: the function of the oökinete of one of the blood-inhabiting Sporozoa is not to be inoculated into the vertebrate host by the invertebrate transmitting agent, but to settle in the tissues of the latter and develop to the formation of sporozoites, which are the final and infective products of the sporogony cycle in the invertebrate host.

In the general life-history of the Sporozoa, infection of the vertebrate host—i.e., man in the case of the malarial parasites—is brought about by the ingestion or inoculation—inoculation in the case of the malarial parasites—of motile sporozoites, the final product of the sporogony cycle. The sporozoite finds its way to some tissue or blood cell, into which it enters and becomes a growing 'trophozoite' form (from *trophos*=nutrition). As the trophozoite grows up its nucleus sooner or later divides into a number of daughter nuclei; around each daughter nucleus the cytoplasm condenses, and a number of daughter individuals are thus formed. To this process of multiplication by fission of the parent body the term 'schizogony'—from *schizos*=splitting—is applied; and as soon as the nucleus of the trophozoite has commenced to divide, the parasite is known as a 'schizont.' As the schizont matures it ruptures, setting free a swarm of spores of the schizogony or asexual multiplicative cycle—which are known as 'merozoites.' The merozoites penetrate into fresh tissue cells and grow up into trophozoites and later schizonts, thus repeating the schizogony cycle. In general this asexual multiplicative cycle continues incessantly until the degree of infection of the vertebrate host is such that most of the nutrition available for the parasite is used up.

By degrees, as the intensity of the infection increases, conditions become unfavourable for the parasite, either because most of the available nutrition has been used up, or owing to the development of an increased resistance on the part of the vertebrate host. In order to conserve its own existence and pass into a fresh vertebrate host, the parasite now passes into its sporogony or sexual cycle. Certain trophozoite forms develop, which do not undergo schizogony, but develop into sexual mother cells; the female of which is known as the 'macrogametocyte,' owing to its larger size, and the male as the 'microgametocyte,' as it gives rise to small male sex elements, the 'microgametes.' The gametocytes develop into conjugating elements; the macrogametocyte into a 'macrogamete,' whilst the microgametocyte gives off a number of motile individuals which resemble spermatozoa in function, and which are known as 'microgametes.'

Fertilisation is accomplished by the complete union of one macrogamete with one microgamete, both the nuclear matter and cytoplasm of both individuals fusing; and to this process the term 'syngamy' is applied. The result is what corresponds to an egg among the Metazoa, and if such an egg is motile—as is the case with the malarial parasites—the term 'zygote' or 'oökinete' is applied to it, or it is sometimes referred to as the 'travelling vermicule' owing to its worm-like movements. This oökinete finally comes to rest, secretes a cyst wall around itself, and is now known as an 'oöcyst.' Within the oöcyst nuclear multiplication takes place and ultimately a number of spores of the sexual generation—or 'sporozoites' as they are termed—are formed. These constitute the fresh infective forms for the next vertebrate host.



In this way the schizogony and sporogony cycles alternate. They may be summarised as follows :—

Asexual, schizogony cycle : consisting of growing trophozoite, early schizont, mature schizont, which finally gives rise to merozoites, the spores of the asexual multiplicative cycle.

Sexual, sporogony cycle : consisting of growing trophozoites developing into macro- and microgametocytes, from which macro- and microgametes are produced. Fertilisation or syngamy then occurs, with the resulting production of a zygote or oökinete. This becomes transformed into an oöcyst inside which sporozoites—the spores of the sexual multiplicative cycle—are finally developed. These constitute the form infective to the vertebrate host.

The class Sporozoa are divided into two sub-classes : the Coccidiomorpha and the Gregarinina. In the former, to which the malarial parasites belong, when the sex cells unite by conjugation—or syngamy, as the process is termed—there is a very marked difference in size between the male cell, which is small and is known as the microgamete, and the female, which is much larger, and is known as the macrogamete. In the Gregarinina the uniting sex cells are usually equal in size.

The Coccidiomorpha consist of two Orders, the Coccidiida, and the Adeleida. In the former when fertilisation takes place the sperm mother cell—so to speak—of the male, or microgametocyte as it is termed, produces a large number of male sex cells or microgametes ; the female or macrogametocyte is not closely associated with the male, and the microgametes—which correspond to spermatozoa in function—have to travel a considerable distance to find the female. This is the case with the malarial parasites, which accordingly belong to this sub-class. In the Adeleida the uniting sex cells are in close apposition throughout.

The Coccidiida are divided into three sub-orders ; the Eimeriidea, which are mostly intestinal, but sometimes hepatic or renal parasites ; the Hæmosporidiidea, which are blood-inhabiting parasites, and to which sub-order the malarial parasites belong ; and the Piroplasmidea, which are also blood-inhabiting parasites, but whose true affinities are at present a little uncertain.

The Hæmosporidiidea are divided into two families ; the Hæmoproteidæ and the Plasmodiidæ. The Hæmoproteidæ are chiefly parasites of birds and cold-blooded animals, are at one stage parasitic in endothelial cells, at a later stage in the blood stream, and are transmitted from vertebrate host to host by a blood-sucking invertebrate host, in which the sporogony cycle occurs—usually a biting fly or a leech. They are very closely related to the malarial parasites and may be regarded, so to speak, as their first cousins. Thus the student may follow out what corresponds to the mosquito cycle of the malarial parasites of man in the *Anopheles* mosquito, by studying the exactly similar life-cycle of the common ' halteridium '

(*Hæmoproteus columbæ*) parasites of the pigeon in the biting fly, *Lynchia maura*, which feeds on the pigeon's blood.

The Plasmodiidæ consists of the single genus *Plasmodium*, and are parasitic within the red blood corpuscles of man, birds, and other vertebrate hosts. Their further definition will be given later. To this genus the malarial parasites belong, and the brothers and sisters of the malarial parasites—so to speak—are found in the red blood corpuscles of monkeys, squirrels, buffaloes, birds, bats, and cold-blooded animals. There are three species of malarial parasites of man: *Plasmodium vivax*, the parasite of benign tertian malaria—so called from the highly amœboid or vivacious character of its cytoplasm; *Plasmodium malaricæ*, the parasite of quartan malaria, and the first of the malarial parasites to be discovered; and *Plasmodium falciparum*, so called from the sickle-shaped character of its gametocytes, the well-known malarial 'crescents,' the parasite of malignant tertian or sub-tertian malaria.

The systematic position of the malarial parasites is thus as follows:—

Animal Kingdom.

Phylum: Protozoa.

Sub-Phylum: Plasmodroma.

Class: Sporozoa.

Sub-Class: Coccidiomorpha.

Order: Coccidiida.

Sub-Order: Hæmosporidiidea.

Family: Plasmodiidæ.

Genus: *Plasmodium*.

Species: three in number—*Plasmodium vivax*, *Plasmodium malaricæ* and *Plasmodium falciparum*.

The members of the family PLASMODIIDÆ (Mesnil, 1903) are pigment-producing parasites which live in the red blood corpuscles of vertebrate animals, in which they have their schizogony cycle. Gametocytes or sexual forms are also produced in these cells. These are ingested by the transmitting invertebrate host in whom the sporogony cycle develops. This group is of enormous importance in tropical medicine since it includes the malarial parasites of man, as well as the plasmodia of birds and other hosts, such as monkeys.

Prior to Laveran's great discovery (November 6th, 1880) of the malarial parasite, certain pigmented bodies had been noted in the blood of malarial patients by Virchow (1849), and others. Laveran, however, discovered the phenomenon of 'flagellation'—i.e., production of microgametes—in the microgametocytes of the parasite of quartan malaria, and concluded that in malaria the red blood corpuscles are invaded by pigment-producing parasites. This was confirmed by Richard (1882), and the asexual schizogony cycles of the three species of malarial parasites were worked out especially by Golgi (1886, 1886a, 1889), and the Italian

workers—notably Marchiafava, Celli, Canalis, Grassi, Feletti, Bignami, Bastianelli, Sanfelice and Manneberg.

The sole genus in the family Plasmodiidae is the genus *Plasmodium*, to which the malarial parasites of man belong. There is considerable discussion to-day as to the exact number of species of malarial parasites, yet we may deal first with the three species which are universally admitted. These are:—

1. *Plasmodium vivax* (Grassi and Feletti, 1890); the parasite of benign tertian malaria. As the work of Acton (1920), and others has shown, this is essentially

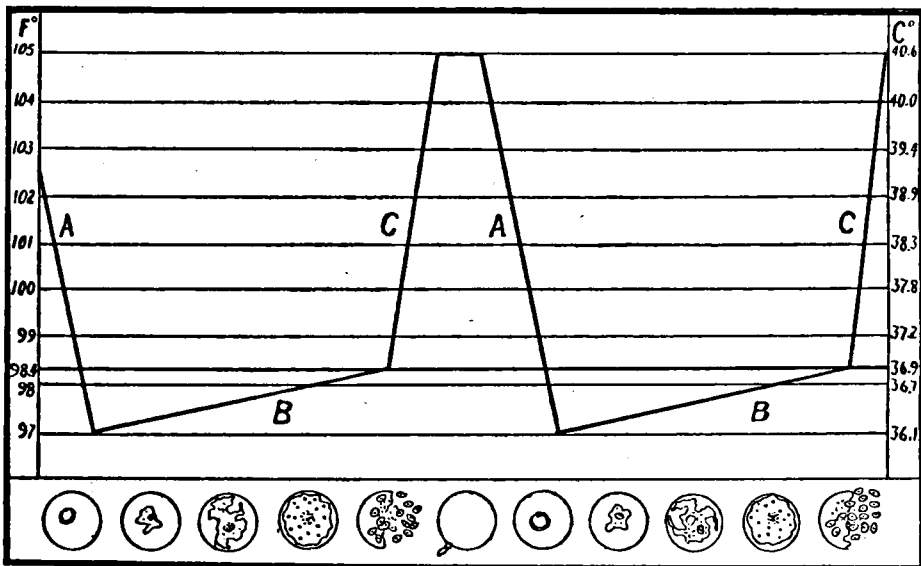


FIG. 1.—Chart of temperature in malaria, showing relation to growth of and schizogony of the parasite. (After Wenyon, 1926.)

The temperature (A) falls to subnormal when the merozoites have entered the red blood corpuscles; B gradually rises to normal as the parasite grows; and C suddenly rises to produce fever when schizogony occurs and the merozoites and toxins are liberated into the plasma. The interval A to A, B to B, or C to C is 48 hours for *P. vivax*, 72 hours for *P. malariae*, and 48 hours for *P. falciparum*; the interval C to A varies from 6 to 12 hours.

the parasite of relapsing and chronic malaria, and is more resistant to quinine than is *P. falciparum*.

2. *Plasmodium malariae* (Laveran, 1881); the parasite of quartan malaria. This parasite is also associated with relapsing malaria, and according to some authors is the most difficult to eradicate of all three malarial infections. In India the distribution of quartan malaria is very clearly defined. It occurs especially in Assam, Bengal and the Dooars; but only rarely on the western side of India.

PLATE I.

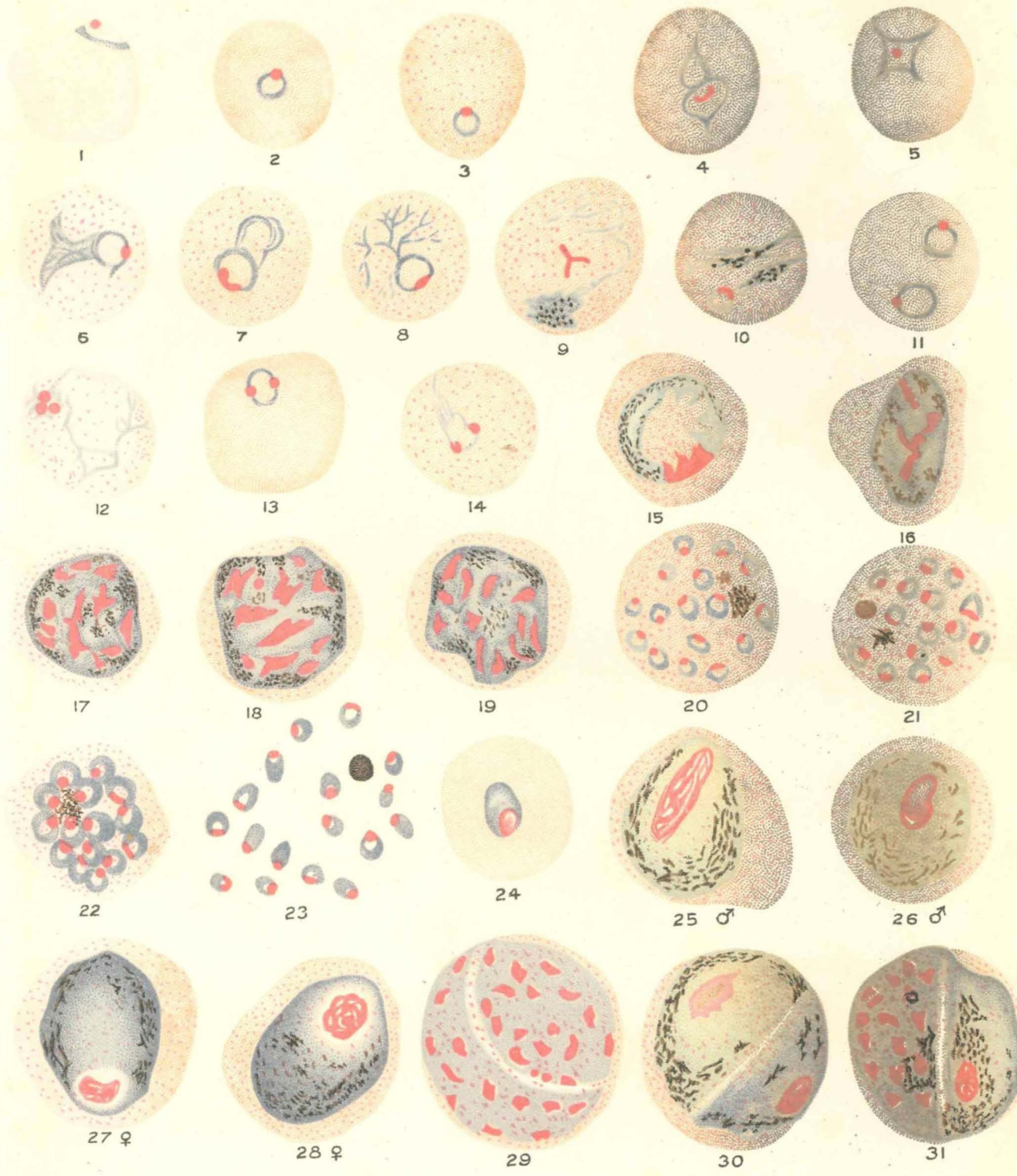


PLATE I.

Forms of *Plasmodium vivax*, the parasite of benign tertian malaria, as seen in dried blood-films stained by Leishman's stain.

(Partly original and partly after Acton, Curjel and Dewey, 1921.)

- Fig. 1. Marginal or accolé young 'ring' form.
- Figs. 2—3. Young trophozoite 'ring' forms.
- " 4—10. Growing trophozoite forms.
- Fig. 11. Double infection of a cell.
- Figs. 12—14. Early schizont forms.
- " 15—19. Growth of schizont, enlargement of red cell, formation of Schüffner's dots, and development of hæmozoin pigment in the parasite.
- " 20—22. Mature schizonts or 'rosettes.'
- Fig. 23. Rupture of mature schizont, with merozoites and pigment liberated.
- " 24. Young compact form, probably a pre-gametocyte.
- Figs. 25—26. Mature microgametocytes.
- " 27—28. Mature macrogametocytes.
- Fig. 29. Schizont plus schizont within the same erythrocyte.
- " 30. Microgametocyte plus macrogametocyte within the same erythrocyte.
- " 31. Macrogametocyte plus schizont within the same erythrocyte.

3. *Plasmodium falciparum* (Welch, 1897); the parasite of malignant or subtertian malaria. This parasite is especially associated with epidemic malaria. On the other hand it is less resistant to quinine treatment than are *P. vivax* and *P. malariae*.

The relationship of the different phases of the schizogony cycle of the malarial parasites to the patient's temperature chart is shewn in Fig. 1.

We may deal first with the schizogony cycles of these three parasites in man. These are illustrated in Plates I, II and III.

*Plasmodium vivax* (Grassi and Feletti, 1890), (Plate I).

The life-cycle may be traced from the moment when the parasite appears as a small 'ring'—i.e., young trophozoite—on the surface of the red corpuscle. This ring-like appearance is due to the development inside the little amœbula of a vacuole—possibly digestive in function. The young ring forms tend to lie flat on the discoid surface of the red corpuscle, and they are rounded or oval, with a marked vacuole, a flimsy cytoplasmic ring around the vacuole, and the chromatin present as a rounded dot, blob or granule, either in one thinner aspect of the ring, or just within the vacuole, close to the thinner side of the ring. At this stage the ring has a diameter of about  $2\frac{1}{2} \mu$  to  $3 \mu$  and is about a quarter to a third of the diameter of the red corpuscle in size (Plate I, figs. 2, 3, 11).

In addition to such forms on the surface of the corpuscle a certain small percentage of parasites are attached to the margin of the red blood corpuscle—the so-called accolé or marginal forms. Accolé forms are not as characteristic of *P. vivax* as of *P. falciparum*, but they do occur; perhaps one per cent or so is about the average incidence; they are more often seen in heavy than in light infections. In the accolé forms, a crescentic bridge of cytoplasm is arched over part of the margin of the red blood corpuscle, and contains within it a rounded or blob-like chromatin dot (Plate I, fig. 1).

The most characteristic feature of *P. vivax* is the highly amœboid or 'vivacious' character of its cytoplasm; whence the name *vivax*. Sooner or later, the parasite penetrates into the interior of the cell, inside which it passes the rest of its existence (at least in the writer's opinion; but see p. 28). Within the red corpuscle the parasite is extremely amœboid, throwing out pseudopodia in every possible direction and then withdrawing them. The ring form of the parasite is in reality its resting phase; but so great is the amœboid activity of the parasite that it is not at all uncommon to find rings which have become somewhat distorted by amœboid activity. At this stage hæmozoin pigment is produced in the cytoplasm of the parasite in the form of yellowish-brown granules, rodlets, or angular particles.

Together with the development of the trophozoite into its amœboid form, certain changes take place in the infected red blood corpuscle. It becomes markedly increased in size; its hæmogoblin is used up by the parasite and it becomes

increasingly paler and paler. In the limiting membrane of the red corpuscle there begins to appear a fine granule-like stippling, the so-called Schüffner's dots. Exactly what Schüffner's dots are, it is difficult to say; they may be points of the erythrocyte membrane to which pseudopodial processes of the parasite have been attached, which have subsequently been withdrawn. The appearance of Schüffner's dots varies a good deal. The stippling is fine and almost uniformly distributed, innumerable little dots being present all over the surface of the red cell. They are probably always present in a benign tertian infection, but in poorly-stained films they may not be seen. In deeply-stained films they are often very prominent, and they appear to become more and more prominent in the red cell, as the contained parasite within it matures (Plate I, figs. 3, 6, 7, 8, 14, etc.).

At this—the growing trophozoite phase—the appearances seen are characterised by:—(1) the enlargement and pallor of the infected red corpuscle, and the presence of Schüffner's dots; (2) the yellow-brown hæmozoin pigment of the parasite; and (3) the fact that, owing to the greatly amoeboid activity of the parasite, no two consecutive forms encountered are alike; all sorts of shapes are met with, angular forms, oval forms, tennis racquet shaped forms, and quite irregular forms. Further, so delicate is the cytoplasm that in spreading the film thin cytoplasmic bridges of the parasite may be broken, so that in the stained film apparently detached masses of parasite cytoplasm may be seen within the red cell (Plate I, figs. 5, 6, 7, 8, 9, 10, 12, 14).

In most benign tertian malarial infections one may occasionally encounter curious pale, enlarged red blood corpuscles showing what are apparently Schüffner's dots, but with no included parasite. These may possibly be cells to which parasites have been attached, but from which they have fallen off. A second appearance not infrequently encountered in benign tertian infections is the presence of demi-lune red corpuscles. These are very pale remnants of red corpuscles, in shape resembling the crescent moon, with finely drawn out horns. Sometimes a thin circular line may be traceable from one tip to the other—the former outline of the cell. Demi-lune red corpuscles may be present in any severe anæmia, but they seem to be most often seen in blood films from patients suffering from benign tertian malaria or from kala-azar.

Sooner or later, the single nucleus of the parasite commences to divide; in other words the trophozoite becomes a schizont. As to the period when this occurs, there are diverse views. Usually the parasite grows until it occupies about two-thirds of the infected cell before schizogony sets in. On the other hand it is not unusual to come across ring forms showing two quite distinct blobs of chromatin, one on each side of the ring (e.g., Plate I, figs. 13 and 14). Thomson and Woodcock (1922) consider that this is due to rupture of the chromatin of the ring in spreading the film; but in the writer's opinion it is far too regular an occurrence to be so accounted for. Further, the two chromatin blobs are often about equal

in size; and it would seem that some *P. vivax* parasites may commence division earlier than others.

At about 36 hours of age the parasites occupying the infected red cells are in the form of a coarsely irregular meshwork which may assume almost any conceivable shape. Numerous yellowish-brown pigment particles are scattered throughout the cytoplasm. The parasites occupy about two-thirds or a little more of the infected cells.

At this stage, as schizogony sets in, the amœboid activity of the parasite becomes less marked, and it assumes a more globular outline. The nucleus divides—(by mitosis, according to Schaudinn, 1902)—into two, then four, eight, sixteen or more daughter nuclei. The number of daughter nuclei produced varies from 12 to 24; in a series of 200 mature schizonts carefully examined in Calcutta, the mean number was 14; but most text-books place it a little higher—at about 16 to 18 daughter nuclei (Plate I, figs. 15 to 19).

Around these daughter nuclei the cytoplasm condenses and thus merozoites are differentiated in the growing schizont, each with its own delicate cell membrane. This process is really one of budding, so that one may come across schizonts in one portion of which the merozoites have become fully differentiated, whilst in the other the individual merozoites have not yet become differentiated.

The 'rosette'—i.e., mature schizont—of *P. vivax* lies in a much enlarged and pale red corpuscle, some  $9\ \mu$  to  $10\ \mu^*$  in diameter, and showing Schüffner's dots—if well stained. It consists of a grape-like cluster of about 16 to 18 merozoites, gathered around an excentrically situated residual body—which contains the aggregated hæmozoin pigment of the parasite, together with a little residual cytoplasm. The schizont is mature at the 48th hour of age of the parasite (Plate I, figs. 20, 21 and 22).

The infected cell now ruptures, setting free a swarm of little merozoites (Plate I, fig. 23). The merozoites are little oval bodies, about  $2.5\ \mu$  long by  $1.5\ \mu$  broad, with a single nucleus, but with no vacuole. These apply themselves to the surface of fresh red corpuscles, to initiate again the schizogony cycle. On rupture of the infected cells the pigment is set free and is phagocytosed by the macrophages in the blood stream, or carried to the internal viscera and ingested by the endothelial cells of the capillaries.

The schizogony cycle is repeated every 48 hours, and the infection becomes heavier and heavier. Sooner or later, however—probably owing to conditions commencing to become unfavourable for the parasite, and to a gradually increasing resistance on the part of the host—the parasite prepares to pass into its sexual, sporogony cycle, and gametocytes begin to appear in blood films of the peripheral

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\* The micron, for which the Greek letter  $\mu$  is used, is the unit of measurement of size of microscopical objects. One  $\mu$  = the one-thousandth part of a millimetre. The average diameter of a red blood corpuscle is  $7.5\ \mu$  and this fact is often useful for rough measurements.



blood. Bastianelli and Bignami (1899) were able to infect mosquitoes from a case of malaria—experimentally induced—on the 5th day of illness; but, as a rule, it is not until the 10th to the 12th day after the first rigor that mature gametocytes begin to appear in films of the peripheral blood.

The gametocytes develop from young trophozoite 'ring' forms. These rings, however, show but little trace of a vacuole, and remain more solid and compact than do the pre-schizont forms (Plate I, fig. 24). They have but little amœboid activity. As they grow, however, the infected red corpuscle becomes pale and enlarged and shows Schüffner's dots. Young gametocyte forms are but rarely encountered in blood films, and it would appear that the gametocytes tend to be developed in the internal blood circulation, only appearing—in order to be ingested by the mosquito—in the peripheral blood as they mature. They grow more slowly than do the schizont forms, and take longer to mature, maturing at about the 96th hour of age.

The full-grown macrogametocyte of *P. vivax* is a very striking object as seen in Romanowsky-stained films (Plate I, figs. 27 and 28). It is a large globular body lying in an enlarged red corpuscle showing Schüffner's dots, some 12  $\mu$  to 14  $\mu$  in diameter. With the Romanowsky stains its cytoplasm stains a deep 'Oxford' blue, and is denser than that of the male. It shows a considerable amount of yellow-brown hæmozoin pigment. To one side or other—and not infrequently near the margin of the parasite—is the nucleus, small, consisting of compact and deeply-staining chromatin. The nucleus often appears as if it had a halo-like clear zone around it, where the cytoplasm of the parasite is less dense than elsewhere. The pigment grains are scattered throughout the cytoplasm.

The full-grown microgametocyte (Plate I, figs. 25 and 26) is a slightly smaller globular, compact body, lying in an enlarged and pale red corpuscle showing Schüffner's dots. Its cytoplasm is less dense than that of the female, and with the Romanowsky stains tends to stain a faint greyish-green colour, sometimes however a rather pale blue. The hæmozoin particles are scattered throughout the cytoplasm. The nucleus is large and diffuse and stains badly. It may lie as a diffuse mass laterally near one margin of the parasite; or, more usually, it tends to stream half way or more across the equator of the parasite, like a cholera belt. The further development of the gametocytes occurs in the mosquito and will be dealt with later (*vide* Chapter II).

The appearances seen in blood films from a patient with benign tertian malaria vary with the duration and the intensity of the attack. James (1926) in an admirable memoir has made a most careful study of experimentally induced primary benign tertian malaria in general paralytics who had never previously had malaria. After the bite of the infected mosquito, he found that the incubation period to the first symptoms of illness was from 7 to 23 days and that the patient then developed

the fever of the 'initial stage,' which lasts from 2 to 5 days. This is at first a gradually increasing fever, at first sub-continuous or irregularly remittent, but towards the end of this stage always intermittent. There are no rigors during this period, and its termination is sometimes shown by an intermission lasting 24 or 48 hours.

The initial stage is followed by the developed stage; in 80 to 90 per cent of cases this is not a fever of tertian periodicity, but a fever of *quotidian* periodicity with a daily rigor. This lasts for ten days, or often for longer. It is followed by the terminal stage. In this the type of fever changes again, and becomes the typical benign tertian fever of the text-books with tertian periodicity and a rigor every 48 hours. At a future date, should the patient have a relapse, or again contract benign tertian malaria, the initial stage and the stage of daily rigors are not shown; the fever is a typically tertian one.

Such a state of affairs can be explained only by a lag or delay in development of some of the parasites. In *P. vivax* infections most of the schizonts mature in 48 hours, but some may mature a little earlier, some a little later, and a further lag may lead to daily rigors. In films taken from a patient in his first attack all the parasites seen may be at the same stage; e.g., they may all be young ring forms, or half-grown schizonts, or mature schizonts. But, as the infection establishes itself, one tends to get all phases of development seen simultaneously in the same blood film; early growing ring forms, developing trophozoites, half mature and mature schizonts, and, if the infection has persisted for some 10 days or longer, gametocytes. Further, multiple infection of the red corpuscles—although not as common as in infections with *P. falciparum*—is also seen in *P. vivax* infections (Plate I, fig. 11). Thus two, or even three or four, rings may be seen inside the same red corpuscle. Later, as the infection dies down, the number of asexual forms becomes less, until finally only scanty gametocytes are present. Schaudinn (1902) believed that the macrogametocytes of *P. vivax* were forms with a very prolonged life, and that they persisted in the blood stream unaltered for months or even years, and attributed relapses to parthenogenesis on the part of these forms (*vide* p. 44). The present evidence, however, all goes to show that this is not the case, and it is probable that the life of the gametocytes does not exceed some 8 or 9 days at most. If they are not taken in by an anopheline mosquito, they die off in the blood stream.

There seems to be but little relationship between the number of gametocytes which may be present and other forms. In some *P. vivax* infections gametocytes are very scanty, as compared with asexual forms; in others they are comparatively numerous. As a rule, in an established infection, the number of asexual forms greatly exceeds that of the gametocytes, whilst most of the latter are females. The proportion of the two sexes, however, again appears to be subject to great variation.

*Plasmodium malariae* (Laveran, 1881), (Plate II).

The schizogony cycle of this parasite—which is responsible for quartan malaria—takes 72 hours; whilst it is the most deeply pigmented of the three species. The infected red blood corpuscles are not altered in size and staining reaction, and do not show any Schüffner's dots or other stippling. It may be possible indeed that—if anything—the infected red corpuscle is a little shrunken in size, and slightly smaller than normal. The parasite is much less amoeboid than *P. vivax*, whilst if infected blood be examined in the fresh state the parasites have a specially opaque, white look with very prominent pigment.

The youngest ring forms may show the typical 'signet ring' appearance described in text-books on tropical medicine (Plate II, fig. 2). They are rather denser and contain a little more cytoplasm than those of *P. vivax*, whilst hæmozoin pigment may be present in the ring forms. They are about one-third of the diameter of the infected red corpuscle. On the other hand, the young ring forms of *P. malariae* appear to lie obliquely to the discoid surface of the red corpuscle; and when such forms are seen they are viewed from one side laterally; hence 'bird's-eye' forms (Plate II, figs. 1 and 3) are common in such infections.

The vacuole is present in the ring phase, but quickly disappears as the parasite grows. The growing trophozoite forms are characterised by (a) their very slight amoeboid activity, so that they tend to remain compact and globular, and often assume angular shapes; (b) the early formation of hæmozoin pigment, present in the form of dark brown-black granules, rodlets, and chunks. Pigment is, indeed, the most prominent feature of the parasite. As the trophozoite grows, certain shapes are commonly seen. Of these one may mention (a) the egg-shaped form (Plate II, figs. 7 and 8). In this the chromatin is placed at the narrower pole of the egg-shaped form, with the cytoplasm at the broader pole, considerable in amount and deeply pigmented, with the remains of the vacuole between the chromatin and the cytoplasmic mass. (b) The comet form (Plate II, figs. 4 and 6). In this the cytoplasm tends to stream out in a dispersed manner like the tail of a comet and is deeply pigmented, whilst the chromatin extends as a deeply staining ribbon-like lower border to the comet's tail. (c) The equatorial form (Plate II, figs. 5 and 9). In this the deeply pigmented cytoplasm extends across the middle of the infected red corpuscle, like an equatorial belt, with the chromatin, deeply staining, in a ribbon-like shape along one border of the parasite. The equatorial form is especially characteristic of *P. malariae*.

At some period or other the growing trophozoite enters into the red corpuscle—probably at about 20th to the 24th hour of growth. Schizogony sets in later than in *P. vivax*, and commences at about the 48th hour of growth, when the parasite fills about two-thirds of the infected red corpuscle. The nucleus divides into 2, 4, 8, etc. Usually from 6 to 12—on an average 9—merozoites are produced (Plate II, figs. 10, 11, 12, 13 and 14). The mature schizont does not quite fill

PLATE II.



1



2



3



4



5



6



7



8



9



10



11



12



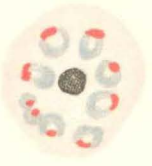
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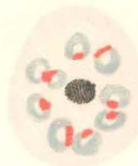
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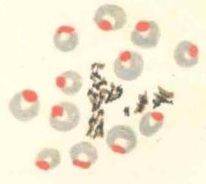
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16



17



18



19 ♂



20 ♂



21 ♀



22 ♀

PLATE II.

Forms of *Plasmodium malariae*, the parasite of quartan malaria, as seen in dried blood-films stained by Leishman's stain.

(Partly original and partly after Acton, Curjel and Dewey, 1921.)

- Fig. 1. Young 'bird's-eye ring' form.  
" 2. Young 'signet ring' form.  
" 3. Early trophozoite form.  
Figs. 4—9. Growing trophozoite forms. Note the intensity of pigmentation present.  
5. A 'band-like' form. 6. 'Comet-like' form. 7. 'Egg-shaped' form.  
" 10—14. Growth of schizont.  
" 15—17. Mature ripe schizonts, 'rosettes.'  
Fig. 18. Rupture of the ripe schizont, with scattering of the merozoites and liberation of pigment.  
Figs. 19—20. Mature microgametocytes.  
" 21—22. Mature macrogametocytes.

the infected red corpuscle, and is about  $6.5 \mu$  in diameter. As schizogony occurs, the pigment collects together—with a little residual cytoplasm—into a dense mass. At first the merozoites are rather irregularly arranged with this pigment mass excentric in position (Plate II, figs. 14 and 15). As maturity is attained, however, the dense pigment mass comes to occupy the exact centre of the red corpuscle, with the merozoites arranged symmetrically around it, thus giving the typical 'daisy-like' appearance characteristic of the mature schizont of *P. malariae* (Plate II, figs. 16 and 17). It should be noted, however, that the mature 'rosette' is not always quite as regular and symmetrical as the text-books sometimes state.

As with *P. vivax*, so with *P. malariae*, after some days gametocytes are produced. The proportion of gametocytes produced appears to be definitely less than in *P. vivax*, and they are relatively scanty in blood films. On the other hand, they are not quite so rare as some of the text-books state; and in an established infection they may be relatively numerous. It is practically impossible to say with certainty whether a young ring form is going to develop into a schizont or into a gametocyte, owing to the fact that the parasite has so little amœboid activity. The gametocytes are developed from globular ring forms. The mature macrogametocyte is a very striking object (Plate II, figs. 21 and 22). It is a globular body, some  $7 \mu$  in diameter, almost completely filling the infected red corpuscle. Its cytoplasm stains deeply blue with the Romanowsky stains, and shows an abundance of deep brown-black pigment distributed throughout its cytoplasm as granules, rodlets and bars. The nucleus is compact, stains deeply red, and lies to one side of the parasite, often near one margin. It does not tend to show the same halo-like appearance around it that occurs in the macrogametocyte of *P. vivax*.

The microgametocyte of *P. malariae* is the most deeply pigmented form of malarial parasite known (Plate II, figs. 19 and 20). It is a compact globular body, occupying almost the whole of the infected red corpuscle. Its cytoplasm stains a dirty greyish-green with the Romanowsky stains—sometimes, however, with a rather faint pinkish tinge. Abundant and conspicuous pigment is present in three situations: (a) and (b) peripherally, where the lateral margins of the parasite are deeply pigmented, the pigment shading off towards the centre of the parasite; (c) a large and dense mass of pigment a little to one side of the centre of the gametocyte. The chromatin of the nucleus is diffuse and stains badly; it is usually present as a more or less diffused equatorial band stretching across the equator of the parasite and lying over the excentric cluster of pigment. Sometimes, however, the distribution of the pigment and chromatin is less regular, and the diffuse nucleus may be more lateral in position.

As with *P. vivax*, so with *P. malariae*, in an established infection one tends to find all forms present simultaneously in a single blood film; young ring forms, growing and mature schizonts, and scanty gametocytes. The merozoites of

*P. malariae* are smaller than those of *P. vivax* and about 1.75  $\mu$  in diameter. Double infection of the same red corpuscle with two ring forms does occur, but is rare. The infections with *P. malariae* as a rule are less intense than those with *P. vivax*, and parasites are scantier in films.

In an early case of *P. malariae* infection, where only ring forms are present, it may sometimes be difficult to decide whether the rings are those of *P. vivax* or of *P. malariae*. The student should always remember, however, that in examining blood films from a suspected case of malaria, he is not called upon to diagnose which species of malarial parasite every ring form encountered belongs to; but is called upon to make a diagnosis of what species of parasite is present on a general study of the film. If there is any doubt, films taken a few hours later, when schizogony has begun, will clear the diagnosis.

*Plasmodium falciparum* (Welch, 1897), (Plate III).

This is the parasite of malignant or sub-tertian malaria, and is especially associated with epidemic malaria. Golgi (1886, 1889) was the first to differentiate it from the other species, whilst Marchiafava and Celli (1889) were the first to give a clear account of its differential characters.

There has been a good deal of discussion as to the correct name of this parasite. Owing to the crescentic character of its gametocytes—the well known ‘malarial crescents’—Grassi and Feletti (1890) proposed to place it in a separate genus, and to call it *Laverania malariae*. Schaudinn (1902), however, pointed out that mere difference in the shape of a gametocyte is not sufficient to justify giving it different generic status. The schizogony cycle of the parasite of malignant tertian malaria is less regular than that of *P. vivax*, and merozoites tend to be liberated over a period of several hours, instead of almost all simultaneously as in the case of *P. vivax*. Further, schizogony is confined to the blood stream in the internal viscera, and—except in very heavy infections—does not take place in the peripheral blood, as with *P. vivax*. J. D. Thomson and Woodcock (1922) state that the gametocytes of the parasite of malignant tertian malaria have a special capsule of their own, apart from the investing membrane of the red corpuscle; and on this, and the above mentioned grounds, adhere to the view that the parasite should have separate generic status as *Laverania malariae*. It is very difficult, however, to make out such a capsule, if indeed it be present at all; it is true that one occasionally encounters a ‘crescent’ in which the whole body stains a dirty deep-reddish colour with the Romanowsky stains, and no detail can be made out, suggesting that the stain has deposited in the capsule, thus obscuring the details of the internal structure. Such forms, however, may be degenerating ones, and not encapsuled ones. On the whole, it is probably wiser to adopt the more conservative view, and to follow Wenyon (1926) in adhering to the name *Plasmodium falciparum* rather than *L. malariae*.

PLATE III.



1



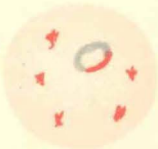
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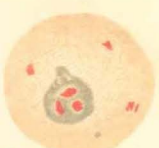
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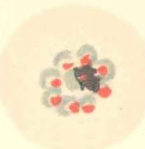
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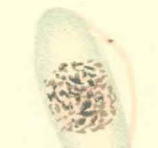
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16 ♂



17 ♀



18



19 ♀



20 ♀



21



22



23



24



### PLATE III.

Forms of *Plasmodium falciparum*, the parasite of malignant tertian malaria, as seen in dried blood-films stained by Leishman's stain.

(Partly original and partly after Acton, Curjel and Dewey, 1921.)

- Fig. 1. Double infection of red cell, marginal or accolé 'ring' form, plus a second 'ring' form on the surface of the cell.
- " 2. Marginal or accolé 'ring' form.
- " 3. 'Ring' form, with chromatin in the vacuole. Cells shew Stephens' and Christophers' dots.
- " 4. Irregular amoeboid young form.
- " 5. Young 'ring' form. Red cell shews Stephens' and Christophers' dots.
- Figs. 6—9. Multiple infection of erythrocytes.
- " 10—11. Early division of the chromatin of the parasite.
- " 12—13. Growing schizonts.
- Fig. 14. Mature schizont 'rosette.'
- " 15. Young 'ring' form, possibly a pre-gametocyte as the chromatin lies in the vacuole.
- Figs. 16—17. Mature microgametocytes or male 'crescents.'
- Fig. 18. Young 'ring' form, possibly a pre-gametocyte.
- Figs. 19—20. Mature macrogametocytes, or female 'crescents.'
- " 21—24. *Tenuis* forms corresponding to *Plasmodium tenue* Stephens (1914). These were all encountered in films which also shewed 'ring' forms typical of *P. falciparum* infection.

The youngest forms of *P. falciparum* are very easily overlooked; one has known even careful workers to overlook a moderately heavy infection with accolé forms. These consist of a tiny bead of chromatin, with a scarcely visible wisp of blue-staining cytoplasm adhering to the margin of the red corpuscle—the marginal or accolé form. The margin of the red blood corpuscle is frequently elevated at this point into a tiny nipple-like protuberance. Sometimes it can be seen that the accolé form is in reality a small oval ring, squashed up against the margin of the red corpuscle, as it were (Plate III, figs. 1 and 2).

In addition to the accolé forms, rings are found situated on the discoid surface of the red corpuscle. They are usually—but not invariably—very small; some  $1\frac{1}{4} \mu$  to  $1\frac{1}{2} \mu$  in diameter, and about one-sixth of the diameter of the infected corpuscle. They are characterised by being hair-like, thin, and fine. The chromatin is present either as a rounded and prominent blob or dot in the margin of the ring; or, not infrequently, as a tiny curved bar either in the margin of the ring or a little within it, inside the vacuole. It must be mentioned, however, that some rings are slightly more compact and globular in form. In the young ring form of *P. vivax* the volume of cytoplasm is definitely more than that of the chromatin; in the young ring forms of *P. falciparum* the chromatin is relatively abundant in amount compared to the volume of cytoplasm (Plate III, figs. 3, 5, 6, 7, 8, 9 and 10).

At this phase of a sub-tertian infection, the infection is characterised by (a) its intensity; in heavy infections as much as 25 per cent of the corpuscles may be found infected; in general the infection is more marked than in infections with *P. vivax* or *P. malarix*. (b) Multiple infection of the red corpuscles. This is a most marked feature of *P. falciparum* infections; as many as eight parasites have been encountered within a single erythrocyte, whilst two, three and four rings per corpuscle are quite common (Plate III, figs. 6, 7, 8 and 9). (c) The early onset of schizogony. The chromatin commences to divide at once, and two—or more rarely three—bits of chromatin are encountered in the same ring (Plate III, figs. 8, 9, 10 and 11). Thomson and Woodcock (1922) give the following points in connection with the differential diagnosis of *P. falciparum* from *P. vivax* infections:—

(1) If the marginal forms are found in any number, a malignant tertian infection is certainly present.

(2) If, as is sometimes the case, few of these forms occur, the more uniform appearance of the rings, the frequent appearance of parasites with two nuclear granules, and a fair proportion (relatively to the degree of infection) of doubly infected cells, taken together, are very suggestive of malignant tertian.

(3) In a benign tertian infection, especially if at all heavy, a few rings will usually be found, irregular in appearance, or slightly in advance of the others as regards growth, and beginning to send out pseudopodial processes. Indications of

enlargement of the cell, and a slight alteration in colour may also be noted here and there.

If any doubt remains, examination of another film, taken some hours subsequently, will settle the question. The possibility of a mixed infection, with both types, has to be remembered.'

The ring form of *P. falciparum*, as noted by Wenyon (1926, p. 935), when studied in the fresh state is rather more amoeboid than is usually supposed to be the case. It is not as actively amoeboid as is *P. vivax*; hence if a film be taken from a patient in hot weather and dries rapidly, considerable distortion of the rings may occur. Especially where two or more rings are present in the same corpuscle, their cytoplasms may be pulled into one another; whilst one may get rounded, rectangular, or flame-shaped parasites, sometimes merely streaks or wisps of blue-staining cytoplasm with a red-staining dot or dots of chromatin (Plate III, figs. 4, 6, 10 and 11).

The infected red corpuscle does not enlarge at all. Not infrequently in old-standing infections it shows a crenated margin, and a tendency to basophilic staining with the Romanowsky dyes. In many—but not in all—sub-tertian infections Maurer's—or as they should be more correctly called Stephens and Christophers'—dots occur. These dots are a coarse stippling of the infected corpuscle into which the red of the stain deposits. Stephens and Christophers' dots are larger and fewer in numbers than Schüffner's dots, and are not so readily demonstrated. They are best seen in films which have been deeply stained. They are stellate or crack-like, rather than dot-like, and give the impression of clefts or shrivellings of the membrane of the erythrocyte; they stain brick red, rather than the bright pink of Schüffner's dots, whilst the infected cells often have a basophilic mottled, or blotched appearance (Plate III, figs. 3, 5, 11 and 12).

The infected red corpuscles in infections with *P. falciparum* have a special tendency to clump together into masses as was clearly demonstrated by J. G. Thomson and D. Thomson (1913) in cultures of the parasite, and such clumps of infected red corpuscles have a special tendency to adhere to the membrane of the blood capillaries. Hence cells infected with growing ring forms tend to accumulate in the blood capillaries in the internal viscera, and schizont forms later than the early ring stage are not encountered in films of the peripheral blood. The latest phase encountered in peripheral blood films is usually the ring form with two chromatin dots. Occasionally—usually in fatal cases—schizogony forms may be seen, however, in the peripheral blood; thus the writer was one day with Lieutenant-Colonel E. C. Hodgson, I.M.S., in the Central Malaria Bureau at Kasauli, when the latter came across a magnificent blood film showing numerous schizont forms of *P. falciparum* in the peripheral blood. A telegram was immediately despatched to the civil surgeon who had posted the film from Lahore the day before, asking

him to take fifty slides from the same patient. The reply was received that the patient was dead. On the other hand, since adopting the routine use of thick blood film examination in cases of malaria (*vide* p. 74) the writer has not infrequently seen schizogony forms of *P. falciparum* in such thick films. The best way of studying the schizogony cycle, however, is either in culture (*vide* p. 85), or by carrying out spleen puncture during the acute phases of the attack (*vide* p. 84).

Sooner or later hæmozoin pigment appears in the growing trophozoite forms, the pigment being brown-black in colour and distributed like grains of pepper. The exact stage at which the young forms leave the peripheral blood appears to vary somewhat; in the majority of infections the trophozoite forms seen in the peripheral blood have not yet formed pigment, but occasionally later forms showing pigment may be present. It is probably this fact which has led certain observers to suggest that there are two species of parasite responsible for sub-tertian malaria; a pigmented and a non-pigmented species.

Within the internal capillaries schizogony proceeds. During growth the parasite remains more or less compact, with, at most, an irregular outline, and does not occupy more than half of the infected red cell. Scattered hæmozoin grains appear in the cytoplasm and Stephens and Christophers' dots—(Stephens and Christophers, 1900, 1903)—in the infected red cell (Plate III, fig. 13). As nuclear division sets in the pigment granules run together to form a dark, granular mass, which is usually excentric in position. The fully developed 'rosette' (Plate III, fig. 14) has a diameter of about 5  $\mu$ , and occupies about two-thirds of the infected red corpuscle. The number of merozoites formed varies from 8 to 24, but is usually about 14 to 16; they are grouped together in a small grape-like cluster in the infected red cell, the outlines of which are still well preserved, with the pigment in a dense granular mass, excentric in position. Spleen puncture films from a patient in the acute phase often show very pretty pictures of schizonts of *P. falciparum* at all phases of growth. On rupture of the infected red corpuscle the merozoites are scattered, and pass to infect fresh corpuscles. They are very small forms, only some 0.7  $\mu$  to 1  $\mu$  in diameter; they may be very numerous in spleen puncture films, and one has known students to mistake them for pyogenic cocci in such films.

Certain further points with regard to the schizogony cycle must be considered at this point. The distribution of the infection in the internal viscera is far from being a uniform one. Thus the brunt of the infection may in one patient fall upon the cerebral capillaries, leading to the cerebral and comatose type of malaria (Fig. 2); in another on the liver, in a third on the bone marrow, in a fourth on the intestinal capillaries. It is the occurrence of schizogony in the internal viscera, together with the tendency of the clumped infected red corpuscles to block the capillaries, that leads to the severity of the symptoms in malignant tertian malaria.

Secondly, the schizogony cycle is not as regular in *P. falciparum* as it is in *P. vivax*; instead of all the schizonts maturing and rupturing at about the same time, rupture of the schizonts and liberation of the merozoites is a more continuous process, extending over some hours; hence the temperature chart in malignant tertian malaria is considerably more irregular than it is in benign tertian or quartan malaria.

The gametocytes of *P. falciparum* are the well known 'malarial crescents.' They develop almost exclusively in the blood stream in the internal organs, and only the mature forms are seen in the peripheral blood as a rule. The younger

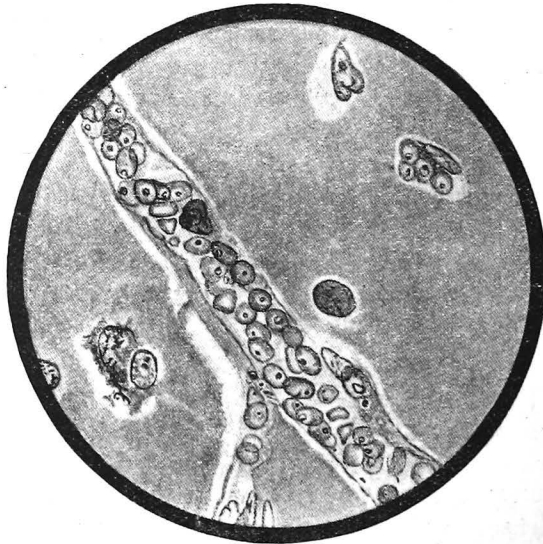


FIG. 2.—To illustrate blockage of the cerebral capillaries by agglomerated red blood corpuscles infected with the schizogony forms of *P. falciparum*.

(From a lantern slide kindly lent by the Wellcome Bureau of Scientific Research.)

forms are slightly elongated parasites with one side concave and the other convex. When fully grown the gametocytes are crescentic or sausage-shaped—hence the name 'crescent' which is used for them. They are about one and a half times the length and about half the breadth of a normal red corpuscle. They usually have rounded ends, but sometimes the ends are pointed, giving the crescent a sickle-shaped appearance. In deeply stained blood films the margin of the red corpuscle is seen to be closely applied to the convex side of the crescent, whilst it is stretched across the concave side, where it very often shows a bulge convexly outwards, the corpuscle retaining some of its original shape. In some cases, especially when the film is stained intensely, the crescent appears to be completely

surrounded by a somewhat irregular, red-staining border, which J. D. Thomson (1917) has interpreted as a definite capsule, in addition to the investing membrane of the red corpuscle, but Wenyon (1926) is very doubtful whether such a capsule really occurs.

The female crescent or macrogametocyte is about  $12\ \mu$  long by  $2\ \mu$  to  $3\ \mu$  broad (Plate III, figs. 19 and 20). It is thinner and longer and its ends more pointed than in the case of the male. Its cytoplasm stains deeply blue. The pigment is aggregated together into a cluster in the centre of the crescent, and in this pigment cluster the nucleus lies. Very frequently in drawing the film the nucleus is a little displaced so that one frequently encounters it peeping out below the pigment cluster. The nucleus is compact and deeply staining. The male crescent or microgametocyte is shorter, squatter, broader and more stumpy than the female, with more rounded poles (Plate III, figs. 16 and 17). Its cytoplasm stains a faint blue or a faint dirty pink colour with the Romanowsky stains. The poles are clear, but the hæmozoin pigment is scattered throughout the middle two-thirds of the crescent, and amid this the nucleus lies. The nucleus has diffuse chromatin and stains badly. In wet-fixed well stained films it is seen that in the female gametocyte the nucleus has a large karyosome, but in the case of the male gametocyte the chromatin is present as granules within the nuclear membrane.

The number of crescents present can be easily counted by the use of the hæmacytometer. It bears no relationship to the number of asexual forms present. Sometimes a single crescent will only be found after a prolonged search; at other times they are exceedingly numerous. Their numbers may run up to 50,000 to 150,000 per cm. of blood. According to D. Thomson (1911) the average duration of life of a crescent does not exceed a few days; usually 5 to 8 days, never more than 20 days. In one instance the writer—(Knowles, 1919)—noticed crescents only present, without asexual forms, in blood films taken from a convalescent malarial patient on 12 examinations during 19 days. Further, it is very common to come across crescents in blood films taken from persons living in an endemic area, who are quite free from all symptoms of malaria at the time. As an illustration the case of 12 Kuki villagers may be quoted (Knowles, Chopra, Gupta and Das Gupta, 1923). Twelve villagers from the Kuki Hills in Assam were admitted to hospital in Calcutta, all of them suffering from frambæsia. All except one were afebrile, and in this patient cinchona febrifuge did not affect the fever, which gradually came down to normal with 8 days' rest in bed. Yet blood examinations showed that 11 out of the 12 patients harboured malarial parasites, all three species being found amongst the different patients, both trophozoite and gametocyte forms being encountered.

*P. falciparum* is essentially the parasite of epidemic malaria, and hence the problems relating to gametocyte formation are of special importance in connection with this species. The whole subject has recently been the subject of an admirable

study by Sinton (1926), whose paper includes a valuable bibliography. He finds (a) that there is a marked seasonal variation in crescent production in patients. Over 2,000 Indian prisoners in the Lahore Central Jail in whose blood *P. falciparum* was found were studied in connection with crescent production over a period of eight months. The percentage showing crescents dropped from 4.2 per cent in July to 1.1 per cent in October, then suddenly rose to 15 per cent in November, and declined to nil in February. MacGilchrist (1915), dealing with 68 patients, had noted a rise in the proportion of crescent carriers in October. There appears, therefore, to be some ground for believing that the autumnal epidemic sub-tertian malaria which prevails in different parts of India may coincide with an increased crescent production by persons infected with *P. falciparum*. (b) Secondly, different localities show a different proportion of crescent carriers, and observers in Africa in general record a lower percentage than do those in India. Thus Statham (1915) records 1.4 per cent of 666 adult patients suffering from sub-tertian malaria in West Africa to have shown crescents in blood films; and Macfie and Ingram (1917), collecting observations by different authors, record a 5.4 per cent crescent carrier incidence in 683 infected persons observed in Africa. MacGilchrist (1915) records a 34.2 per cent incidence in 1,789 adult Indian patients suffering from sub-tertian malaria in Lahore Jail, on careful search of their blood. Christophers (1925) in a hyper-endemic area in Singhbhum, Bihar, found a proportion of 17.6 per cent of crescent carriers among children as compared with one of 4.4 per cent amongst adults; whilst Sinton (1926) found a 4.5 per cent incidence in Lahore in 1,535 Indian patients examined by the thick-film method.

Sinton's further conclusions are (c) that there seems to be a distinct correlation between the numerical prevalence of asexual forms in the peripheral circulation and the number of crescents which appear about ten days later; (d) that the development of crescents appears to be associated with a lowered immunity, possibly due to a change in the reaction of the pulp in the bone marrow and spleen; (e) that no marked correlation could be traced between the degree of splenic enlargement and the occurrence of crescents in the peripheral blood; (f) that the duration of life of a crescent may possibly be as long as 40 to 50 days in the peripheral blood, but the great majority of them disappear within three weeks after the asexual cycle has been destroyed; (g) that his method of treatment by combined alkalies and quinine gave a smaller percentage of crescent carriers than any other form of treatment tried; and (h) that the reduction in the number of crescent carriers by efficient treatment is an important factor in all anti-malarial campaigns.

The paper should be consulted in the original by those concerned in anti-malarial work; but it is a good example of what Sir Ronald Ross has long claimed, that future progress in our knowledge of malaria will only come about by the use of mathematical and statistical methods in the study of infections.

*Differential Diagnosis of the Malarial Parasites of Man.*

This subject is dealt with in tabular form in Table I. The student should remember four things: (1) that he is not called upon to identify the species of every ring form that he sees, but to determine upon a general examination of the slide what species of infection is present; (2) that aberrant forms may be encountered; (3) that everything depends upon technique, for the appearances in a deeply stained slide may be quite different from those in a lightly stained slide; (4) that mixed infections are very common.

The frequency of mixed infections has been strikingly demonstrated at the Calcutta School of Tropical Medicine during the last five years. It is customary for all out-patients suffering from fever to be sent to the Protozoology Department for blood examination, and whenever malarial parasites are found, 50 or 60 films are taken from the patient for the students' classes. Not infrequently where the diagnosis of a single species present has been given on examination of the first film, examination of some fifty films from the same patient by as many students has shown a second species present in scanty numbers. Recently a class was asked to examine films from a patient with benign tertian malaria in order to exclude malignant tertian malaria, as the patient's blood was to be used for treatment of a case of general paralysis of the insane. Ring forms suspicious of *P. falciparum* were found by three students, and finally a crescent. Even all three species may be encountered simultaneously; thus in one film asexual forms of *P. vivax* and *P. malariae*, with a few gametocytes of both species, and crescents, but no asexual forms of *P. falciparum*, were simultaneously present.

*Doubtful Species.*

The question as to the number of species of malarial parasites of man has been the subject of considerable controversy during recent years. Certain observers on the one hand have gone so far as to propound the view that all malarial parasites belong to one and the same species, which assumes the forms characteristic of *P. vivax* during the spring months, and those characteristic of *P. falciparum* during the autumn. This view cannot be entertained for a moment. The treatment of general paralysis of the insane and of tabes by inoculating the patient with malarial parasites, either by direct inoculation of infected blood or through the agency of infected mosquitoes, is now a well-established therapeutic procedure, and in every instance where a given strain has been given to a long succession of patients it has remained true to morphological type, without variation.\* Thus, Mühlens and

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\* S. P. James in a recent paper states that the British Government takes the view that there are 'serious objections' to direct inoculation of malaria blood. It is likely, therefore, that legislation will be introduced to make direct transmission illegal, and that in Great Britain transmission will have to be effected in future *via* the mosquito.



Kirschbaum (1921) passaged *P. vivax* in series through twenty patients, *P. falciparum* through four, and *P. malariae* through three. In a later paper Mühlens and Kirschbaum (1924) record still longer series of passage treatments, including a series of over forty passages with *P. vivax*. Yorke and Macfie (1924) inoculated seventy cases of general paralysis with malaria, including 23 direct passages with *P. vivax*, and 41 patients with the same parasite transmitted by *Anopheles maculipennis*. S. P. James (1926) inoculated 109 patients with *P. vivax* from infected *Anopheles maculipennis*. In every one of these instances the species of parasite concerned showed no morphological variation from type; *P. vivax*, for instance, always showed the characters of *P. vivax*, and never those of *P. falciparum*. The 'unity' theory of the species may therefore be said to be certainly wrong.

In addition to *P. vivax*, *P. malariae* and *P. falciparum*, however, certain authors have tried to prove that other and new species of malarial parasites of man exist. It is perhaps difficult for the writer, who has been to some extent involved in this controversy, to give an impartial view. Yet it must be recognised at the very outset of discussion on this matter that the morphological appearances of a given species may be profoundly affected by many different agencies.

The following quotation from Wenyon (1926, pp. 948, 949) seems very appropriate:—

'In many cases without any treatment the infections naturally abate as the result of some protective factor in the body. It is probable that some substance is produced which has a deleterious action on the parasite. A more rapid suppression of an infection is brought about by the administration of quinine. With these factors, and probably others of which there is at present no knowledge, acting upon the parasites, it is not to be wondered at that occasionally forms are seen which do not correspond in every way with the typical ones. Very commonly what are undoubtedly degenerating or damaged forms are seen after quinine treatment. The altered parasites may still grow and reproduce by schizogony. In the case of *P. vivax*, ragged-looking schizonts sometimes appear. They may be smaller and contain fewer nuclei than the normal schizonts, while the irregular staining indicates some degenerative process. In other cases, the departure from what may be considered the normal type is less marked, and consists of an unusual shape of the parasite, increase or diminution in the size of the nucleus or its chromatin element. It has to be remembered that the malarial parasites are almost invariably studied in dried films stained by the Romanowsky stains. In many respects these stains, though giving very beautiful pictures, are very unreliable, for they are subject to variations, which cause them to stain differently at different times. Thus, in the case of typical *P. vivax*, the absence of Schüffner's dots, which is sometimes noted, is almost invariably due to a poorly acting stain. The intensity of staining, depending on the quality of the stain, or the length of time it has been allowed to act, produces remarkable variations in the appearance of the parasites

and infected cells. When films are deeply stained, many more granules take a red coloration than when they are lightly stained, and it is certainly incorrect to regard all red-staining granules as chromatin. It has accordingly happened that new species of human malarial parasites have been described from time to time. Generally these have been seen in single or very few blood films taken from a case on one or two occasions. It has been impossible to follow the cycle of the parasite as has been done in the case of the three well-established species. Until this can be done, and the parasite has been proved to retain its characters, both in human and mosquito passages, it is quite unjustifiable to introduce specific names. Laveran (1914) remarked that he had long held that the descriptions which were usually given of the species of malarial parasites were too schematic, and that in practice intermediate and veritable transition forms were frequently met with. This remark is certainly correct, and if every slight variation from the normal is considered of specific importance, there is no limit to the number of species which might be created.'

With which remarks the present writer is in complete agreement. Nevertheless these supposed new species, or the most important of them, must be mentioned.

*Plasmodium vivax* var. *minuta* Emin, 1914. This was described by Emin (1914) from the blood of pilgrims at Camaran Island in the Red Sea. Ziemann (1915) came to the conclusion that it was a new species, and suggested the name *P. camarensis* for it. Emin gives the characters of this species as follows :—

(a) Marked amoeboid activity, so that the forms are very irregular in shape. (b) The red corpuscle is neither enlarged nor altered in colour. (c) Schüffner's dots are very well marked. (d) Nuclear division starts when the parasite is only half the size of the red cell. (e) The schizont is only two-thirds the size of the red cell. It occurs in the peripheral blood, and from four to ten merozoites are produced. (f) Very little pigment is produced. (g) The gametocytes are round, and reach a size of three-quarters of the red cell.

Craig (1909) had described a similar parasite, which in 1926 he concludes to be identical with the above; he notes that the band and ribbon-like (equatorial) forms so characteristic of *P. malariae* were not encountered in the infection. Stephens (1922) has described as *P. ovale* a parasite which also resembles the above (Fig. 3). In general it resembled *P. malariae* but band forms were absent, the infected corpuscles in many instances appeared oval in outline—whence the name suggested for the parasite—were slightly enlarged, pale, and stippled with Schüffner's dots.

'*P. ovale*' (Fig. 3) occurred in blood films from a British soldier in Liverpool, convalescent from malaria contracted in East Africa. These films were taken in 1918, stained for one hour with Leishman's stain, and the diagnosis at the time was (?) benign tertian, (?) quartan. The films were re-examined in 1922, when Professor Stephens suggested that this was a new species. It is worthy of note

that films taken from the same patient eight days previously had shown typical forms of *P. vivax*.

It seems to the present writer that, in connection with this supposed new species, a good deal depends on whether the patients concerned had or had not taken quinine; for quinine administration may markedly affect the morphological appearance of *P. vivax*. Further, Cragg and Naidu (1918) have drawn attention to the way in which atypical parasites tend to occur in cases where successive attacks of fever over a long period have reduced the patient's vitality to a serious degree. Recently the junior author showed the writer films from a fatal case of blackwater fever, taken the day before death occurred. A double infection with *P. vivax* and

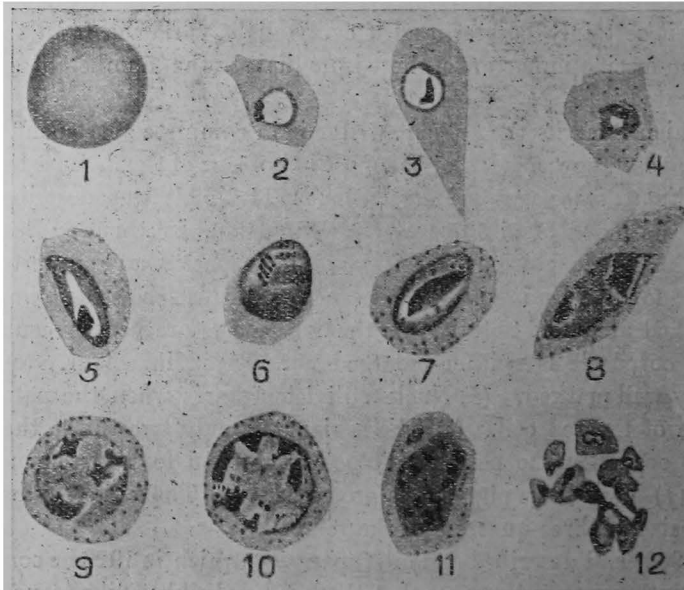


FIG. 3.—*Plasmodium ovale*. (From Wenyon, 1926, after Stephens, 1922.)

1. Normal red blood corpuscle.
- 2—3. Ring forms.
- 4—7. Partially grown forms.
- 8—12. Nuclear multiplication and schizogony.

*P. falciparum* was present, but many very aberrant forms of the former species were present.

The writer, personally, has not yet encountered *P. ovale*, and must admit that he does not expect to do so. If, however, the parasite should turn out to be a valid species, then as Wenyon (1926) remarks its correct name will be *P. minutum* Emin, 1914.

*Plasmodium tenue* Stephens, 1914 (Fig. 4). This is the name given to a malarial parasite seen by Professor Stephens in a single blood film posted from the Central Provinces to Liverpool. Only young forms were present, and they were characterised by their markedly amœboid form, by the large amount of chromatin present, and by its markedly erratic shape.

The publication of Stephen's paper was followed by a considerable controversy. Balfour and Wenyon (1914) pointed out that such amœboid forms were by no means uncommon in *P. falciparum* infections, and that there was no reason to regard *P. tenue* as a distinct species. The chief case for *P. tenue* as a valid species rests on

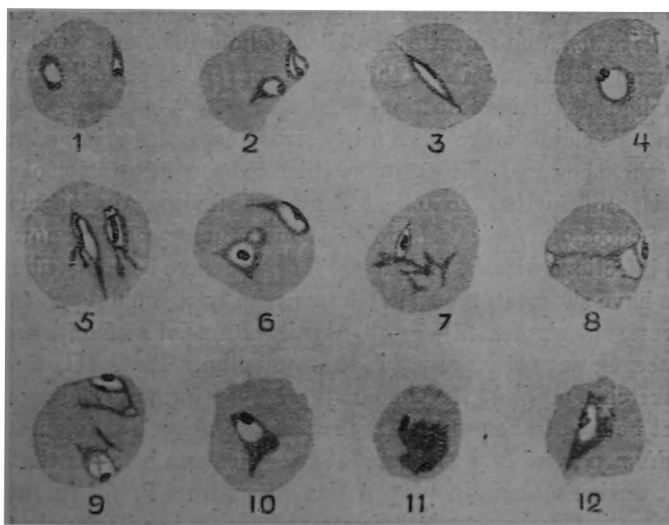


FIG. 4.—*Plasmodium tenue*. Stephens, 1914. (From Wenyon, 1926, after Sinton, 1922.)

- 1—2. Young forms probably varying in age from 3 to 8 hours.
- 3—6. Medium sized, irregular or oval-tailed forms, probably varying in age from 12 to 24 hours.
- 7—9. Forms during the 'tenue' phase, probably varying in age from 28 to 30 hours.
- 10—12. Large oval or polyhedral forms, probably varying in age from 36 to 40 hours.

Sinton's admirable paper (Sinton, 1922*b*) ; whilst Christophers (1925) in his study of malaria in Singhbhum notes that the parasites encountered in that district resembled *P. tenue* rather than *P. falciparum*.

Thanks to the kindness of Major Sinton, the writer has had the opportunity of examining some of his preparations. Major Sinton saw '*P. tenue*' infection first in the five cases in the Central Provinces mentioned in his 1922 paper ; still later in other cases in Lahore ; and he is practically convinced that the case for the

new species is a valid one. His claims for the existence of *P. tenue* as a valid species are as follows :—

(a) The smallest ring forms seen were never less than one-fifth of the diameter of the infected cell. (b) The chromatin dot does not appear to project as much externally as in the similar stage of *P. falciparum*. (c) The very varied forms of *P. tenue* differ very much from the pseudopodial forms of *P. falciparum*. (d) The irregularity and abundance of the chromatin differ from the more or less compact form seen in *P. falciparum*. (e) The largest forms of *P. tenue* seen in the peripheral blood were about half the diameter of the infected cell. (f) Accolé forms were rarer than in the early stages of *P. falciparum*. (g) In the infected cell the stippling seems to be of a more rounded character and more numerous than that seen in *P. falciparum* infections ; whilst finally (h) hæmozoin pigment appears later and is lighter in colour than in *P. falciparum*, and parasites as old as 36 hours of age are to be encountered in the peripheral blood. Clinically the fever is of tertian periodicity and unusually mild for a malignant tertian infection.

That Sinton's claims make out a fairly strong case, the writer admits ; but it is doubtful whether they are sufficient upon which to base the creation of a new species. The matter is still further complicated by the findings in the classes held at the Calcutta School of Tropical Medicine. Upon three occasions now to date films from typical infections with *P. falciparum* infection have been distributed to classes and students have shown to the writer forms exactly resembling *P. tenue*—(except that they were not pigmented)— whilst typical forms of *P. falciparum*, and in one instance crescents, were also present in the same film. Plate III, figs. 21, 22, 23 and 24 are sketches made at the time of these *tenue* forms. The case for *P. tenue* cannot as yet be held to be proved—at least in the writer's opinion.

*Plasmodium perniciosum* Ziemann, 1915. Ziemann believes that the parasite of malignant tertian malaria in West Africa differs from *P. falciparum* elsewhere ; that its pigment is more scanty in amount and darker in colour ; that the infected red cells do not show a 'brassy tint' ; that the infection shows a complete disappearance from the peripheral blood after rings have been formed ; the schizonts only occupy one-third to one-half of the infected red cell ; the number of merozoites is from 12 to 16 ; crescents are scantier in number and smaller and plumper in shape than in *P. falciparum*. Considering the variations which occur in undoubted *P. falciparum*, and that there may be some variation in the time of disappearance of parasites from the peripheral blood, however, the above claims are absolutely insufficient upon which to create a new species.

*Quotidian malaria.* Several observers have tried to establish the view that there is a separate species of malarial parasite responsible for quotidian malaria, with daily rigors, and with a schizogony cycle which occupies only twenty-four hours. Grassi and Feletti (1890) were the first workers to introduce this view, and they termed the parasite *P. immaculatum*, as it was supposed not to produce pigment. Marchiafava

and Bignami (1891, 1892) and Mannaberg (1893) adopted the same view and expanded it. The chief supporter of this view, however, is Craig (1909, 1926), who named the parasite *P. falciparum quotidianum* Craig, 1909. It is claimed that the ring forms are very minute,  $0.5 \mu$  in diameter; pigment is produced later than is the case with *P. falciparum*; when pigment is produced the ring form is lost and the plasmodium appears as a hyaline, spherical disc, with one or two pigment granules, only occupying one-fifth of the red corpuscle, which is shrunken and crenated in appearance and of a dark olive green colour. The number of merozoites is from 6 to 18 and they are very minute indeed, 'generally less than  $0.5 \mu$  in diameter.' The gametocytes are only a little over half as long as those of *P. falciparum* and the pigment in both sexes is very small in amount.

It is very doubtful indeed whether there is any malarial parasite with a 24-hour schizogony cycle. As Wenyon (1926, p. 952) remarks—'The occurrence of pigmented and unpigmented varieties of *P. falciparum* has been explained above by the fact that the young forms disappear from the peripheral circulation either before or after pigment has appeared in their cytoplasm. It seems not improbable that the time of disappearance of the growing forms of *P. falciparum* from the peripheral blood varies considerably. Very frequently the only forms found are the young minute rings which disappear into the internal organs after a few hours. In other cases the disappearance is delayed, and older forms with one or two granules of pigment may be found. A further delay of twenty-four hours or more will lead to the discovery in the films of still larger parasites, definitely pigmented, and sometimes markedly amoeboid in form, the larger forms retaining their irregularity during the drying process of film-making more easily than the younger ones, which contract to the definite ring more quickly.'

There are several alternative explanations of quotidian malaria, other than the necessity of postulating a different species of malarial parasite as its cause. If a patient is infected with two different strains of *P. vivax* or of *P. falciparum*, or with one strain of *P. vivax* and one of *P. falciparum*, and these strains undergo sporulation on alternate days, then he will have a daily rigor; inasmuch as strain A will sporulate on the 1st, 3rd, 5th, etc., days, and strain B on the 2nd, 4th, 6th days, etc. Even this is not necessary to produce quotidian malaria, however. It can result from the inoculation of a single dose of sporozoites, as has been emphatically shown by S. P. James (1926) for *P. vivax* infections. In any benign tertian infection it may happen that some parasites may lag in development, either through partial resistance on the part of the patient, or—not infrequently—owing to the administration of small doses of quinine; doses quite insufficient to stop the fever, but sufficient to somewhat affect the parasites. If two dominant strains of parasites are evolved, each of which comes to sporulate on days alternate to those for the other strain, fever with quotidian rigors will ensue. The patient's powers of resistance will then rise and overcome first the brood which is either less

numerous or less resistant, and the temperature chart will therefore revert to a tertian periodicity. Finally, the more resistant brood of parasites will be overcome, with resultant (clinical) cure.

*The Relationship of the Malarial Parasite to the Red Corpuscle.*

It is obvious from the above account of the schizogony cycles of the malarial parasites that the merozoites are liberated from the ruptured infected red corpuscles and pass in the plasma to the surface of fresh red corpuscles. The malarial parasites, therefore, in their earliest trophozoite phase are extra-cellular in position. It is held by most workers that early in the history of their development the trophozoites enter into the substance of the red blood corpuscle and that both the schizonts and gametocytes are intra-cellular in position. With this view, however, Lawson (1912—1919) does not agree. In a series of papers illustrated by beautiful microphotographs, she upholds the view that the malarial parasites are throughout their cycle in the human body applied to the surface of the erythrocytes and that they never penetrate into them; in fact she depicts the parasites as wandering from red cell to red cell in search of hæmoglobin—like bees to different flowers in search of honey. Sinton (1922c) justly points out that the study of parasites in air-dried films in which the red cells have been flattened cannot decide the point as to whether the parasites are actually within the cells or on their surface. He accordingly treated fresh blood containing infections with *P. vivax* and *P. falciparum* with hypertonic and with hypotonic saline. In hypotonic solution the red corpuscles swelled and in some instances the parasites appeared as if indenting the swollen margin of the erythrocyte; in hypertonic saline the red corpuscles became shrivelled and appearances resulted which suggested parasites applied to the shrivelled and crenated margins of the erythrocytes. Stephens and Gordon (1924a) have studied the relationship of the crescent to the red cell. They figure the different appearances encountered and conclude that in many cases the poles of the crescents project beyond the red cell, whilst they gathered the general impression that the crescents were extra-cellular and applied only to the surface of the red corpuscles.

On the other hand, as Wenyon (1926, p. 913) points out, if the parasites are actually extra-cellular, it is not easy to understand how the marked changes which occur in the red cells in benign tertian and malignant tertian infections occur, nor how it is that the red cell is completely destroyed when sporulation takes place. Crescents are forms which are particularly liable to distortion in spreading films but when a typical crescent is seen—not distorted—the appearance of the outline of the red corpuscle around it and bulging on its concave side appears to be inconsistent with the view that the crescent is extra-cellular.

TABLE I.

*Morphological Characters of the Malaria Parasites. (Romanowsky Stains.)*

	<i>Plasmodium vivax.</i>	<i>Plasmodium malariae.</i>	<i>Plasmodium falciparum.</i>
Trophozoite, 'Ring' .. Early	Relatively large: occupies $\frac{1}{2}$ to $\frac{1}{3}$ vol. of R. B. C. Round or oval. Chromatin a round dot in thin part of ring or often appears as if in vacuole.	Smaller and denser than <i>P. vivax</i> . Compact, dense, little ring, round or oval. Often seen sideways. Cytoplasm deep blue. Large deep red chromatin dot. Ring often very narrow on each side of chromatin. 'Bird's-eye' forms common.	Smallest malarial form. Almost dots to begin with. Accolé forms very characteristic. Multiple infection of R. B. C.'s very common. Chromatin a deeply staining granule or bar. Cytoplasm very scanty. Rings thin and hair-like.
Growing trophozoite ..	Flimsy. Very amœboid. Fine streaming pseudopodia through R. B. C. Multiplicity of forms seen in same slide characteristic. Fine hæmozoin granules.	Compact with cytoplasm more collected together. Egg-shaped, equatorial-band, ribbon and comet-like forms streaming across the R. B. C. characteristic. Hæmozoin formed early in coarse, dense, black masses and chunks.	Typical but very small ring. Occupies up to $\frac{1}{4}$ of R. B. C. Chromatin much: cytoplasm little. Chromatin often rod-like and divides very early.
Hæmozoin pigment ..	Yellowish-brown: fine grains or tiny rods. Scanty.	Much. Coarse, dense rods, chunks and masses. Brown-black. <i>The pigmented parasite.</i>	Granular. Pepper-like. Brown.
Infected R. B. C. at post-ring stages.	Swollen, distended, and pale. Schüffner's dots.	Not enlarged: staining unaltered.	Unchanged or even shrunken in size. Staining reaction may be basophile. Outline may be crenated. Stephens' and Christophers' dots sometimes seen.
Schizont ..	In enlarged, pale R. B. C. shewing Schüffner's dots. Flimsy. Contour irregular. Fine hæmozoin granules, 2, 4, 8, etc., rounded chromatin masses.	Large round deeply pigmented body. Occupies most of unaltered R. B. C. Compact: globular: clear outline. Cytoplasm stains deeply blue. 6 to 8 chromatin masses.	Not seen in peripheral blood. If found, case very serious. Small. Only occupies $\frac{1}{4}$ to $\frac{1}{3}$ of R. B. C. 8 to 12 chromatin dots.



TABLE I.—*contd.*

	<i>Plasmodium vivax.</i>	<i>Plasmodium malariae.</i>	<i>Plasmodium falciparum.</i>
Sporulating Schizont : 'Rosette.'	Large form : $8\frac{1}{2}$ $\mu$ : almost fills the distended and pale R. B. C. 15 to 20 merozoites in an irregular grape-like cluster. Residual mass with hemozoin pigment excentrically situated.	Typical geometrically arranged 'daisy' rosette. 6 to 12 merozoites symmetrically arranged round deeply pigmented, central residual mass.	Not seen in peripheral blood except in very severe cases. Small : occupies $\frac{1}{4}$ to $\frac{3}{8}$ rds of R. B. C. 8 to 18 merozoites arranged in a grape-like cluster. Central residual mass with considerable pigment.
Microgametocyte (Male)	Large, round body, 10 to 12 $\mu$ . - Fills the distended, pale and almost invisible R. B. C. Stains badly. Cytoplasm pale grey-green or grey reddish colour. Pigment light and yellow-brown : fine grains. Large, diffuse nucleus, staining badly, and often extending partly across the equator of the parasite.	Round, spherical body. Fills the R. B. C. which is not altered in size. Stains badly, greyish green to pinkish. Central cluster of pigment chunks and masses. The most densely pigmented malarial form known. Chromatin diffuse, badly staining and arranged in zone-like bands.	Male 'crescent.' Sausage shaped. Shorter and stouter than female. Occupies whole R. B. C., traces of which may be seen on concavity of parasite. Clear pale blue cytoplasm. Hemozoin as scattered fine grains in middle $\frac{3}{8}$ rds. Large oval, central, diffuse nucleus stains badly, lying amongst pigment grains.
Macrogametocyte (Female).	Large round body, 12 to 14 $\mu$ . Fills enlarged, pale and almost invisible R. B. C. Cytoplasm stains deeply blue. Considerable granular hemozoin. Nucleus stains deeply red ; usually excentric in position, and frequently with a halo-like zone around it.	Almost fills R. B. C., which is not enlarged. Cytoplasm stains deeply blue. Abundant deep brown-black pigment. Nucleus stains deeply red and is excentric in position.	Female 'crescent.' Longer than male and with more pointed ends. Cytoplasm stains deeply. Central dense cluster of hemozoin grains. Nucleus stains intensely as compact mass amid the pigment cluster.
Peripheral Blood Films in an established infection.	Shew all phases of both schizogony and sporogony. Multiple infection of R. B. C.'s, not uncommon.	Shew all phases of both schizogony and sporogony. Multiple infection of R. B. C.'s very rare indeed.	Rings and crescents are the only forms usually seen in peripheral blood. Heavy multiple infection of R. B. C. is very characteristic : e. g., 2 to 8 rings or accolé forms per R. B. C. The infection is usually much more intense than those with <i>P. vivax</i> or <i>P. malariae</i> .

## CHAPTER II.

### THE MALARIA PARASITES. CYCLE IN THE MOSQUITO. MALARIA : MISCELLANEA. BLACKWATER FEVER. THE PLASMODIA OF MAMMALS, BIRDS AND LIZARDS.

IN the year 1895 Sir Ronald Ross—at that time Surgeon-Major Ross of the Indian Medical Service—determined to discover how malaria was transmitted from man to man.\* The conditions under which Ross worked are to-day almost incredible to the medical research worker in India. The story is fully told in his *Memoirs*, a volume which every worker imbued with the spirit of discovery should read. It is true that there had been great pioneers of medical research in India prior to Ross' day—D. D. Cunningham, Vandyke Carter and Timothy Lewis, to name but three; but in 1895 a military medical officer who was keen on research work was regarded as a somewhat unpleasant phenomenon—polo and pig-sticking being far more important. To-day, thanks to the re-organisation of the Indian Medical Research Department by the late Sir Pardey Lukis, conditions are utterly different; the problems are there, there is financial provision for their investigation, the only difficulty is to find men of the right type to investigate them; conditions such as were present in Ross' day can never recur.

Ross had been on furlough to England in 1894, where he came under the spell of the late Sir Patrick Manson, at that time Dr. Manson, who had retired from practice in China, and who was living in Queen Anne Street, Cavendish Square. Manson shewed him the malarial parasite, which had been discovered by Laveran in 1880, and drew his attention to the 'crescents' (gametocytes) of *Plasmodium falciparum*. Fedschenko in 1869, at the suggestion of the great German parasitologist Leuckart, had established the fact that the guinea-worm *Dracunculus medinensis* passes a phase of its life-cycle in the water-crustacean, *Cyclops*. Manson in 1877 had discovered that another filaria of man, *F. bancrofti*, has a similar cycle of development in the mosquito. King in 1883 had written a most remarkable paper in which he gave nineteen reasons why mosquitoes are likely to carry malaria; whilst the ancient Romans had used mosquito-nets as a

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\* Sir Ronald Ross himself has very kindly corrected the proofs of pp. 31 to 34. They were submitted to him in order to make certain that no incorrect statement was made.—R. K.

preventive measure against malaria—a practice which they appear to have borrowed from the Greeks, and which they in turn, as Herodotus remarks, appear to have borrowed from the ancient Egyptians. The ancient writings, including those of Susruta in India, contain many vague suggestions of the association of malaria with mosquitoes. To make a suggestion, however, is not equivalent to making a discovery. Manson suggested to Ross that malaria is conveyed by mosquitoes; he thought that the mosquito probably ingested the malarial crescents with the patient's blood; that they then developed further in the mosquito, which then fell into water, when it died; that the infected dead mosquito or its remains or parasites were swallowed by man in his drinking water or were carried into the air and that man thus acquired infection with malaria. The true facts were discovered by Ross, but from first to last Manson acted as sponsor and gave him every encouragement. Ross returned from furlough to Secunderabad in May 1895, determined to tackle the problem.

At that date there was practically no literature on mosquitoes, and Ross—not unnaturally—turned first to the 'grey' and 'brindled' mosquitoes, i.e., those of the genera *Culex* and *Aedes* (*Stegomyia*). The stage of flagellation of the microgametocyte he immediately discovered in mosquitoes, indeed it had been seen previously by Laveran in freshly drawn blood, and can be studied in fresh blood *in vitro* apart from the mosquito. In September of the same year he was suddenly transferred to Bangalore on special sanitary duty. To some extent, perhaps, Ross had himself to thank for the troubles that followed; he could tolerate neither fools nor authority patiently, whilst he sought not to unravel but to cut through red tape—an impossible procedure. Thus his views on his sudden transfer to Bangalore are given in the following stanza from his notebook at that date:—

' We cry, " God make us Kings,  
Poets or Prophets here "  
The scornful answer rings  
" First be My Soavenger ".'

In 1897, however, Ross reverted to Secunderabad, and at Sigur Ghat suddenly came across a mosquito which was neither 'grey' nor 'brindled' but which had spotted wings, i.e., an *Anopheles*. The discovery gave him furiously to think. He turned to this genus and on the 20th August, 1897, suddenly came across a malarial oöcyst in an infected anopheline mosquito, the first and most important event in his great discovery. His first publication of this discovery was despatched to the *British Medical Journal* in September, 1897, after he had further confirmed the existence of the oöcyst stage in infected anophelines, and was published in that journal on the 18th December, 1897. He had no sooner reached this very important stage in his discovery than he was transferred on military duty to Kherwara in Rajputana.

In the same year (1897) MacCallum discovered the process of fertilization in *Hæmoproteus columbæ*, the parasite of pigeon malaria, and the true nature of the malarial crescent now became clear. At Kherwara, however, cases of malaria and *Anopheles* mosquitoes were rare and Ross' work was interrupted. After insistent and persistent requests to be placed on special duty to investigate the problem, Ross was finally put on special duty for six months with instructions to investigate the transmission of both malaria and kala-azar within that period—a not inconsiderable task, seeing that the latter problem alone has taken twenty-three years of work, and the solution is not even now complete. With a comment of 'Victory or Westminster Abbey' Ross departed for Calcutta in March 1898.

In Calcutta cases of malaria were difficult to obtain owing to the plague scare, and Ross turned to the closely allied *Plasmodium præcox* parasite—or Proteosoma, as it is frequently termed—of birds. The stages of fertilization and of oöcyst formation of this parasite were immediately discovered, and in May 1898, Ross' first official report *Cultivation of Proteosoma, Labbé, in Grey Mosquitoes* was sent in to Simla. This report was not officially issued until October 1898, but in the meantime some thirty or forty copies of it were sent to Europe, America and elsewhere. In June and July of the same year Ross discovered the liberation of sporozoites into the cœlomic cavity of the mosquito and the invasion of the salivary glands and succeeded in infecting healthy birds from infected *Culex* mosquitoes. Sir Patrick Manson published an abstract of the work in the *British Medical Journal* of June 18th, 1898. In July of the same year Manson gave a full account of the whole discovery at the British Medical Association annual meeting at Edinburgh, and this address was published in the *Journal of Tropical Medicine* for August 1898, also in the *Lancet* in the same month, and in the *British Medical Journal* in September 1898.

The whole of the transmission cycle for *P. præcox* was thus discovered. In October 1898, Ross' *Preliminary Report on the infection of birds with Proteosoma by the bites of mosquitoes* was published from the Government Press, Calcutta. It now only remained to apply these results to man, and to work out the similar life-cycle of the human malarial parasites in *Anopheles* mosquitoes. Ross, however, had to leave Calcutta for Assam in August 1898 to take up the kala-azar investigation, with his malaria work unfinished.

He returned to Calcutta in November 1898, determined to finish the work if time allowed. Unfortunately he and C. W. Daniels, who had now joined him started work with *Anopheles rossi*—the commonest anopheline mosquito of Bengal, but not a true carrier of malaria in Nature.

It is to be noted that, up to this point, Ross had worked out the whole of the transmission cycle of *Plasmodium præcox* in *Culex* mosquitoes, had seen oöcysts of human malarial parasites in *Anopheles* mosquitoes, and had predicted that the

transmission cycle of human malarial parasites would be found to occur in *Anopheles* mosquitoes, and that it would be identical with that of *P. præcox* in *C. fatigans*. Also that advance copies of his first report had reached Europe and had been extensively circulated, and that the knowledge of his proteosoma discovery was widespread.

The Italian workers now entered the field. The question of priority with regard to the discovery of the sporogony cycle of the Plasmodiidae has been the subject of endless controversy since that date, but there can be little doubt that the Italian workers were familiar with Ross' proteosoma work. Thus Grassi in his first publication in October 1898 (*Policlinico*), refers directly to Ross' work; in his second paper in November 1898 (*Lincei*), he refers to Sir Patrick Manson's address to the British Medical Association in the previous July, and states that he had received some of Ross' proteosoma specimens and mosquitoes from Manson. Bignami succeeded first in experimentally infecting man with malaria from *Anopheles* mosquitoes on November 3rd, 1898, and in his first paper in November 1898 (*Bull. R. Accad. Med. d. Roma*), discussed Ross' work at length. The Italian workers deserve every possible credit for applying Ross' results with proteosoma to human malaria, but their work can hardly be regarded as having been 'independent' of Ross' great discovery.

The Italian workers were greatly helped by two further events, the introduction of the Romanowsky stains, and Ficalbi's work on Italian mosquitoes, which enabled them to identify the species of mosquitoes encountered and experimented with. Bastianelli, Bignami and Grassi (1898) observed the oöcysts of *P. falciparum* in anopheline mosquitoes just as Ross had done in 1897. They also succeeded in infecting man experimentally from mosquitoes. Later in the year Grassi, Bignami and Bastianelli (1898) observed the complete cycle of development of *P. falciparum* in *Anopheles maculipennis*, and the partial development of *P. vivax*. Early in 1899 Grassi, Bignami and Bastianelli reported the development of *P. malariae* in the same mosquito, and Bastianelli and Bignami (1899) that of *P. vivax*. Other papers followed by Grassi, Bignami and Bastianelli (1899a), Bastianelli and Bignami (1899), Grassi (1900), Ziemann (1900), and others. Ross himself left India on furlough in February 1899, and in the same year demonstrated the sporogony cycle of the three malarial parasites in *Anopheles* mosquitoes in Sierra Leone (Ross, 1899). In the meantime Ross' work on proteosoma had been confirmed—if it wanted any confirmation—by Koch (1899) and by Daniels (1899). Finally the whole sporogony cycle of the malarial parasites of man was described in full detail in a classical monograph by Grassi (1900). The full history of Ross' work and of his great discovery may be read in his *Memoirs* (London: John Murray, 1923); whilst Nuttall (1901) has published a full account of the history of the discovery of the malaria transmission cycle, dealing with the papers published in their chronological order.

*The Sporogony Cycle (Plate IV).*

The earliest phases of the sporogony cycle of the malarial parasites may be observed on a microscope slide, or in the stomach of any blood-sucking insect, but it is only in the genus *Anopheles* that the further development of the human malarial oökinetes takes place. When the mosquito feeds, both gametocytes and asexual forms are ingested with the blood. The latter die off in the stomach of the mosquito, whilst only a certain proportion of the gametocytes survive to complete the sporogony cycle.

The first phase consists in the maturation of the gametocytes, a process studied by J. D. Thomson (1922), (Fig. 5). among the Metazoa cells is preceded by a 'reducing division' of the male and the female gametocytes, the process by which the number of chromosomes in the gametes is halved, so that the fertilised ovum has of chromosomes as many as the parent cell. In some of the Ciliophora a similar process has been observed; in malarial gametocytes the process consists in the condensation of chromatin which is the beginning of the sexual cycle. This has been studied by J. D. Thomson especially in the case of *P. falciparum*. According to this process the gametocytes of the par-



FIG. 5.—Development of the micro- and macro-gametes from the gametocytes of *Plasmodium falciparum*. (From Wenyon, 1926 after J. D. Thomson, 1917.)

1. Microgametocyte (male crescent) in blood film.
2. Spherical microgametocyte with two polar bodies.
3. Stage similar to 2.
4. Macrogametocyte (female crescent) in blood film.
5. Cytoplasm of macrogametocyte escaping through opening in capsule.
6. The macrogamete has completed its escape from the capsule, at one end of which a polar body is seen.

tertian malaria have a definite capsule of their own (Fig. 5); (though Wenyon, 1926, is very doubtful as to whether any distinct capsule exists, apart from the investing membrane of the red corpuscle). In the macrogametocyte a small pore is said to form in the concave aspect of this capsule, and through it the parasite flows out and becomes a rounded, motionless sphere lying in the fluid contents of the mosquito's stomach. As it emerges from the capsule one (or two) beads of chromatin—the so-called polar chromatin—are budded off, and are

of this cycle con-  
turation of the  
process especially  
Thomson (1917)  
and Woodcock  
As is well known,  
the union of sex  
by a 'reducing  
nucleus of both  
female cell; a  
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This has been  
Thomson especially  
*falciparum*. Ac-  
cording to the  
author the cres-  
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usually left behind, attached to the empty capsule. In the case of the male gametocyte, the limiting membrane gives way uniformly all round, polar chromatin is budded off and extruded from the parasite, and the gametocyte becomes transformed into a spherical, but motile body. Similar changes and a budding off of polar chromatin occur in the gametocytes of *P. vivax* and of *P. malariae*.

It is a little doubtful whether such a capsule occurs, and Wenyon's account of the maturation phenomenon is somewhat different. That polar chromatin is extruded, and that this process corresponds to the reduction division among the sex cells of the Metazoa is highly probable, though the process is said to be observable only in films fixed in osmic acid vapour. In either event, the gametocytes leave the red blood corpuscles which contain them, probably by a rupture of the membrane of the red corpuscles due to movements of expansion and contraction on the part of the gametocytes. The macrogametocyte becomes transformed directly into a single rounded, spherical macrogamete, lying motionless in the fluid contents of the mosquito's stomach (Plate IV, fig. 12). In the male gametocyte, after it has left the red corpuscle, a state of violent commotion sets in in its cytoplasm, and the pigment granules can be seen to be in violent movement, whilst the whole sphere commences to jerk about and to become more and more agitated. The nucleus divides into 2, 4, then into 6 to 8 daughter nuclei. These approach the periphery of the gametocyte. In front of each a long thin process of cytoplasm is extruded from the sphere, and into this the daughter nucleus passes. In this manner the microgametocyte comes to have from 4 to 8 such flagellum-like structures attached to it as it whirls about in the fluid contents of the mosquito's stomach. The whole process has been termed 'ex-flagellation'—a very bad term, since the flagellum-like structures are not flagella at all, but are the microgametes (Plate IV, fig. 12).

At varying intervals these microgametes break away from the microgametocyte, the remains of which constitute a residual body, in which all the pigment grains remain. The microgametes are liberated with explosive suddenness; they are actively motile, and by lashing movements swim about amongst the red blood corpuscles in the mosquito's stomach.

In the meantime certain changes have taken place in the macrogamete. Its pigment remains stationary, or is only slightly motile. After the extrusion of the polar chromatin, its nucleus moves towards the surface, where a slight elevation of the cytoplasm occurs. Through this elevation a microgamete enters, and male and female pro-nuclei unite; in other words syngamy occurs, and the macrogamete now becomes an oökinete—corresponding to the fertilised ovum of the Metazoa. The entire process of fertilisation takes place very rapidly, and may be studied in an ordinary wet blood preparation between a slide and

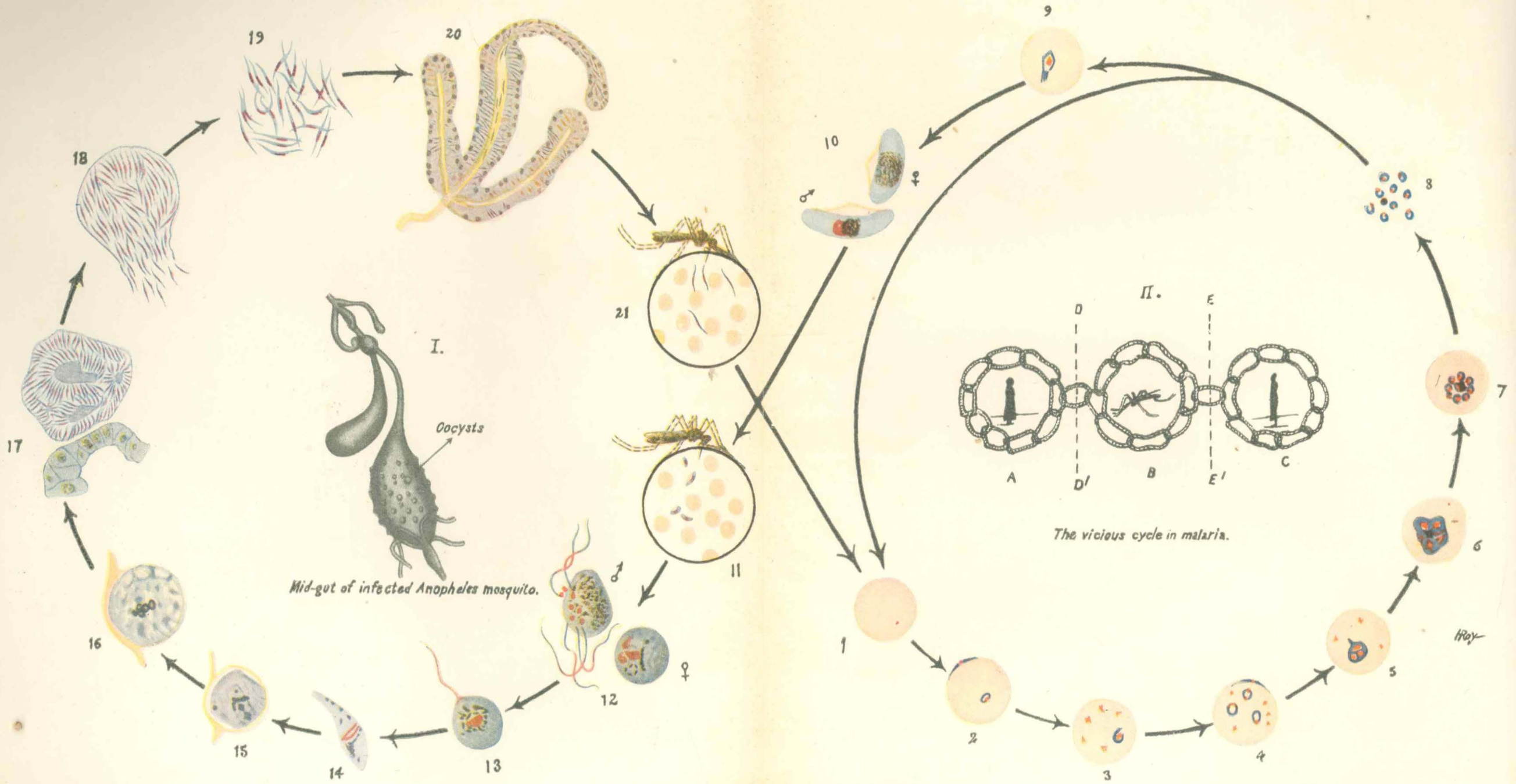




PLATE IV.

The Life Cycle of *Plasmodium falciparum* in Man and in the Mosquito.

(Partly original and partly from Wenyon, James, and other authors.)

- Fig. 1. Normal red blood corpuscle.  
Figs. 2—4. Early 'ring' forms.  
" 5—6. Growing schizonts.  
Fig. 7. Mature schizont, 'rosette.'  
" 8. Rupture of mature schizont.  
" 9. Young form, possibly a pre-gametocyte.  
" 10. Gametocytes of the two sexes. 'Crescents.'  
" 11. Female *Anopheles* mosquito ingesting gametocyte-containing blood.  
" 12. Maturation of macrogametocyte into macrogamete; and production of microgametes by microgametocyte—'ex-flagellation.'  
" 13. Fertilization of macrogamete or syngamy.  
" 14. Zygote or travelling vermicle, (oökinete).  
Figs. 15—16. Developing oöcysts, (encysted zygotes).  
Fig. 17. Mature oöcyst packed with sporozoites.  
" 18. Rupture of mature oöcyst and liberation of sporozoites into coelomic cavity of the mosquito.  
" 19. Free sporozoites.  
" 20. Invasion of salivary glands by sporozoites. Glands shewn in section; (after Wenyon, 1926).  
" 21. Infective mosquito injecting malarial sporozoites into the blood stream.  
INSET I. Midgut of infected mosquito shewing oöcysts *in situ*; (after Doflein, 1911).  
INSET II. The Vicious Circle in Malaria; (after James, 1920).  
A. Infected donor—gametocyte carrier.  
B. Female Anopheline mosquito, infected from A.  
C. Freshly infected human host, infected from B.

The circle can be broken;—

- (1) At D—D' by measures taken to eradicate malaria in man, such as quinine therapy.  
(2) At E—E' by anti-mosquito measures.

cover slip. It may be completed within a few minutes of making the preparation (Plate IV, fig. 13).

The oökinete—or zygote—is at first motionless. Soon, however, it elongates in form and becomes vermiform in shape; in the case of *P. vivax*, as studied by Schaudinn (1902) measuring from 18  $\mu$  to 24  $\mu$  in length by 3  $\mu$  to 5  $\mu$  in breadth (Plate IV, fig. 14). By movements of contraction, bending and gliding, with a worm-like, slow movement, the zygote—or, as it is often termed at this stage, the travelling vermicule—makes its way through the contents of the mosquito's stomach to the gut epithelium. It has an anterior—more pointed, and a posterior—more rounded end, a central nucleus, and pigment granules—derived from the pigment of the macrogamete—lying in its posterior half. Having reached the epithelium of the midgut, the zygote bores its way into an epithelial cell, through which it passes into the space between the epithelium and the tunica elastico-muscularis, which is a delicate membrane investing the outer surface of the stomach wall. Between this membrane and the epithelium the zygote comes to rest, and contracts into a small spherical body with a diameter rather less than that of a red blood corpuscle. A thin and delicate enveloping membrane is next secreted around the zygote; the exact origin of this delicate cyst wall is uncertain; it may be secreted by the parasite itself—as appears most likely—or it may be secreted in part by the cells with which the zygote is in contact (Plate IV, fig. 15; also Figs. 6 and 7 in the text).

With regard to the time intervals in the cycle thus far, fertilisation is usually completed within from 20 minutes to 2 hours of the ingestion of the blood meal by the mosquito; motile oökinetes are in evidence in from 12 to 24 hours after the feed, and early encysted oöcysts are visible in the gut wall from about the 40th hour after the feed onwards.

The encysted zygote—or as we may term it, the oöcyst—now grows in size, the rate of growth and of development depending on the atmospheric temperature, and finally attains a diameter of from 50  $\mu$  to 60  $\mu$ . The cyst wall does not become thinner as growth proceeds, and its thickness is apparently maintained by secretion by the parasite. The nucleus, at first single, divides again and again repeatedly until an enormous number of minute nuclei are present within the oöcyst. As this occurs, the cytoplasm of the oöcyst becomes progressively more and more vacuolated, until the whole of the cytoplasm is reduced to a sponge-work of numerous anastomosing septa. This change is undoubtedly adapted to bring about a great increase in the surface area of the cytoplasm, which is utilised by the nuclei in their subsequent development. The extent of vacuolation of the cytoplasm varies considerably, but a section of an oöcyst at this stage will resemble a section of a sponge; there are areas of cytoplasm which may or may not be connected with others by fine strands and bridges of cytoplasm (Plate IV, figs. 16 and 17).

When vacuolation is complete, the minute nuclei arrange themselves over the cytoplasmic surfaces within the oöcyst. Opposite each a finger-like elevation of cytoplasm is formed, which increases in length, and into which the tiny nucleus passes. In this way there are formed hundreds of little sporozoites within the oöcyst. At first the sporozoites are attached to irregular cytoplasmic masses within the oöcyst and are being formed at the expense of these cytoplasmic masses. Later, however, they break away from their attachments and form a tangled mass within the oöcyst, which also contains one or more residual masses of cytoplasm containing all the hæmozoin pigment within the oöcyst. The sporozoites themselves are not pigmented (Plate IV, fig. 17).

The mature oöcyst at this stage has a thin investing cyst wall, and contains hundreds of sporozoites within it. The sporozoites lie in tangled masses, and on focussing down on to the stomach wall of an infected mosquito the oöcysts are at first seen dimly, like glass globes. On further focussing, however, the oöcyst is suddenly seen very clearly in optical section, the hundreds of sporozoites lying within it in bundles giving it a finely striated appearance, whilst any pigment present will also be seen in the residual mass or masses of cytoplasm (Fig. 6). The number of oöcysts present on the stomach of any single mosquito varies with the number of gametocytes which it has ingested, and with the number present in the patient's blood on which the mosquito fed. Sometimes only 1 or 2 oöcysts are present. Very commonly 10 to 20 are found, not infrequently some 30 to 40, very exceptionally a very large number. The number of sporozoites within a mature oöcyst may vary from some hundreds to a thousand, and has been put down at as high a figure as 10,000 by Grassi. The oöcysts which develop from parasites ingested at one feed are all approximately at the same stage of development at one time. If, however, the mosquito feeds repeatedly, oöcysts at various stages of development will be present simultaneously.

The mature oöcyst now ruptures outwards into the body cavity or hæmocœle of the mosquito, liberating hundreds or thousands of free sporozoites. The sporozoites are about  $10\ \mu$  to  $12\ \mu$  in length; (Wenyon 1926, p. 921 gives  $15\ \mu$  as the average length, but the writer considers this figure as perhaps rather too large, although this is an impression merely); by about  $1\ \mu$  to  $1\frac{1}{2}\ \mu$  broad. They are sickle-shaped objects and they move (*a*) by forward progression in their own longitudinal axis; (*b*) by bending—tip to tail—and then straightening out; and (*c*) by waves of peristaltic contraction which pass along the body. There is nothing else in Nature which quite resembles the very characteristic movement of the sporozoites of the Plasmodiidæ and Hæmoproteidæ; it is a slow, unhesitating and continuous gliding, bending, deliberate, and never jerky progression (Plate IV, figs. 18 and 19).

Once liberated into the body cavity of the mosquito, the sporozoites wander throughout the body and may be found in any organ of the mosquito, except the

ovaries. Thus Mühlens (1921) for the malarial parasites of man, and Mayer (1920) working with *P. præcox* of birds have shewn that sporozoites may be encountered in masses in the aorta, between the muscle fibres, within the palps and scutellum, in the fat body, in the muscles of the wings, legs, neck and head—almost anywhere within the body of the mosquito except in the ovaries. The majority of the sporozoites, however, come in contact with the salivary glands, which occur at the anterior part of the body cavity. Accumulating around the posterior ends of the trefoil-shaped salivary glands, they penetrate into the cells of the glands, where they may be found in enormous numbers embedded in the cytoplasm. As the saliva is secreted into the salivary duct, sporozoites pass with it, and

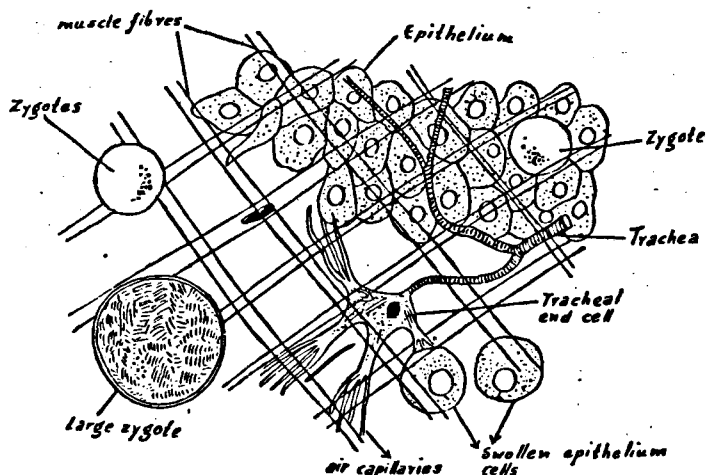


FIG. 6.—Microscopic appearance of the midgut of an infected *Anopheles* mosquito, shewing cell structure and zygotes. (After Stephens and Christophers, 1903.)

are finally injected by the mosquito into man at its next feed (Plate IV, figs. 20 and 21).

Once the sporozoites are injected into man, they pass to the red blood corpuscles. Schaudinn (1902) by injecting malarial sporozoites into a small hæmatoma and then withdrawing samples, studied the process of actual infection, and has left in the literature a most theatrical picture of the entry of a malarial sporozoite into a red blood corpuscle. The exact mechanism of adherence of the sporozoites to the red blood corpuscles certainly requires further study. Presumably, however, an injected sporozoite attaches itself to a red blood corpuscle, becomes a trophozoite, and this grows up into a schizont. The patient is now infected,

and after an incubation period the degree of infection will rise above the febrile threshold, and he will develop malaria.

In his experimental work on the development of *P. præcox* in *Culex fatigans* in India, Ross noted that occasionally peculiar brown or black cysts were encountered on the stomachs of infected mosquitoes, the colour being due to certain dark bodies within the cysts (Fig. 7). Similar bodies were encountered by Grassi during his work on the development of the human malarial parasites in *Anopheles* mosquitoes. He noted that the bodies within the cysts were either sausage-shaped, rounded, oval, or irregularly lobed. Various suggestions have been made as to the nature of these 'black spores.' It seems probable that they are the result of death and degeneration of the oöcyst at various stages of its development.

Such being the sporogony cycle of the certain points with ent species remain to the oöcysts of *P.* present in the form of brown rodlets and in a curved line irregular clump. The shew dense blackish clump. The oöcysts like glass globes; well-defined contour highly refringent, blackish hæmozoin the form of percentage of gameto- is usually considerably higher than in *P. vivax* and *P. malariae*, and the stomachs of mosquitoes infected with *P. falciparum* may shew very numerous oöcysts, lying in contact with one another.

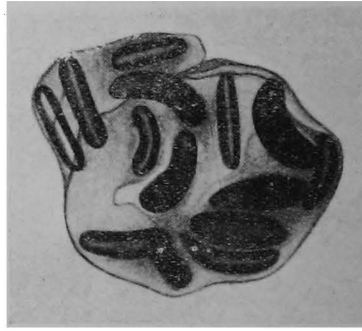


FIG. 7. Oöcyst of malarial parasite and the 'black spores' in *Anopheles maculipennis* after feeding on malarial cases. (After Wenyon, 1926), *P. vivax* infection.

general outline of the malarial parasites, regard to the difference noted (Fig. 8). In *vivax* the pigment is fine wispy yellowish-grains, often arranged instead of in an oöcysts of *P. malariae* pigment in a coarse of *P. falciparum* look they have a sharp, on focussing, and are with considerable pigment, present in like grains. The per-

cytes in *P. falciparum*

#### *Factors influencing the Sporogony Cycle.*

The factors which influence the sporogony cycle of the malarial parasites of man in *Anopheles* mosquitoes have been the subject of much study, but even further study of them is required, since, in a complete knowledge of them may lie many secrets of malaria control. In this connection an important paper by S. P. James (1926) is one which should be read in the original by all workers on malaria.

In the first place the cycle will only develop in mosquitoes of the genus *Anopheles*, and all attempts to experimentally infect *Culex* and *Aedes* mosquitoes have failed.

Further, it is only in certain species of *Anopheles* that development occurs under natural conditions. With regard to India, the most important transmitting species are: (a) *A. culicifacies*; found by Stephens and Christophers (1902) to have a 4 per cent sporozoite rate—i.e., 4 per cent showed sporozoites in their salivary glands—in an epidemic at Mian Mir, and an 8 per cent sporozoite rate in Ennur. (b) *A. funestus*; found to have from 3 per cent to 10 per cent sporozoite rate in different parts of Bombay. (c) *A. maculatus*; found both in the plains and in the foot-hills and especially associated with malaria in all foot-hill areas. Also *A. stephensi* in town wells, *A. ludlowi* in brackish swamps, and *A. willmori* in the Himalayan foot-hills. *A. rossi* constitutes a special difficulty. This species can be experimentally infected in the laboratory, yet numerous workers have failed to find it infected in Nature. Gill (1925) suggests that it may carry *P. vivax* infections, but not those with *P. falciparum*; on the other hand the majority of

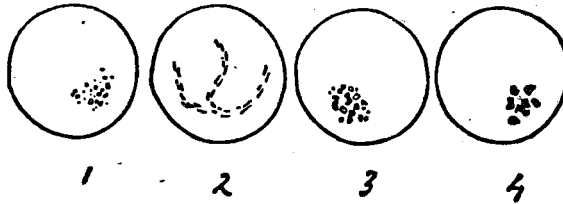


FIG. 8.—Appearance in the fresh state of oocysts of the malarial parasites. (After Stephens and Christophers, 1903.)

1. Oöcyst of *P. falciparum*.
2. Oöcyst of *P. vivax*.
3. Oöcyst of *P. malariae*.
4. Oöcyst of *P. præcox* in *Culex* mosquitoes.

workers have found that where one species of *Anopheles* transmits one species of *Plasmodium*, it will successfully transmit all three species.

Secondly, the question of 'dosage' is probably most important in malarial transmission. Mitzmain (1917) shewed that only a small percentage of gametocytes of *P. falciparum* develop in the mosquito's stomach, the majority passing out in the faeces. Hence, unless a certain number of gametocytes—a figure provisionally placed at 12 per c.mm. of blood by Darling (1909)—are present in the patient's blood, the mosquito will not contract the infection. Further, James (1926) has carried

important. At the first two daily feeds only 40 per cent of mosquitoes took ; by the fifth daily feed the proportion increased to 70 per cent infected ; 100 per cent infection only occurred in mosquitoes which had been given seven daily feeds. Further, it is not so much the number of gametocytes present in the donor's blood, as some quality of ripeness or maturity of the gametocytes that determines whether the mosquito will 'take' or not. Some persons are 'good infectors' of mosquitoes ; others are 'bad infectors.'

The actual development of the sporogony cycle in the mosquito is directly dependent upon temperature. The first workers to determine this point were Grassi, Bignami and Bastianelli (1899a). They noted that neither *P. vivax* nor *P. falciparum* would develop in mosquitoes kept at a temperature of from 15.5°C. to 17.5°C. The minimum temperatures for continuous development were found to be 17.5°C. for *P. vivax*, 16.5°C. for *P. malarie*, and 18°C. for *P. falciparum*. Jancsó (1904), Mitzmain (1917) and King (1917) have further studied the subject. The question as to whether a mosquito, infected in the late autumn, and hibernating over the winter, can transmit the infection in the next spring, has often been discussed ; the general trend of the evidence available is to shew that this can and does occur. Thus Wenyon (1921) in Macedonia found that partially developed oöcysts could be found in hibernating *A. superpictus* all through the winter. Mosquitoes were fed on cases of *P. falciparum* infection and partial development permitted at 22°C. Development was then completely arrested for 15 days by placing the mosquitoes at a temperature of 9.7°C. to 18.7°C. Twelve hours' exposure to 5.5°C. did not injure the oöcysts ; and in such mosquitoes subsequently exposed to favourable conditions the delayed cycle developed. James (1926) found that temperature profoundly affected the sporogony cycle in the mosquito. The optimum temperature was 22°C. At 26°C. the development of the cycle in the mosquito was much more rapid, but there was a heavy mortality amongst the infected mosquitoes. Mortality was also high amongst mosquitoes which were not given at least one blood meal every 48 hours. At 22°C. some 50 per cent of fed mosquitoes survived for a week to ten days. If, however, they were allowed to digest their blood meal at 22°C., and then transferred to an ice chest at 4°C. to 6°C., they would survive up to 2½ months. One very remarkable mosquito was caught as an adult on August 5th, 1925. It was finally dissected on November 16th, 1925, and shewed active sporozoites in its salivary glands. Between these two dates it had spent about three weeks in an incubator at 23°C. to 24°C., and about two and a half months in an ice chest at from 4°C. to 6°C. The rest of its days were passed in rooms, hospitals, railway trains, cabs, etc., at ordinary air temperature of the time of the year. It was continuously infective from August 17th till November 16th, when it was finally chloroformed and dissected, and during this interval of time successfully infected more than 40 mental patients with malaria.

In brief it is clear that infection may persist in mosquitoes which hibernate over the winter. Under such conditions the cycle is delayed, but not destroyed. With regard to optimum conditions Wenyon (1926, p. 960) gives the following figures; at from 18°C. to 25°C., *P. vivax* requires about 15 to 17 days to complete its sporogony cycle and *P. falciparum* about 19 days.

Humidity does not appear to affect the cycle, so long as there is sufficient moisture in the air for the mosquitoes to survive. Numerous workers, such as Bruce Mayne (1920), Bastianelli and Bignami (1900), Gualdi and Martirano (1901), have noted that the gametocytes of *P. falciparum* from a patient on full quinine treatment will still develop in the mosquito. This has been confirmed by Wenyon (1921), who found, however, that under similar conditions *P. vivax* only rarely developed. It appears that quinine affects the gametocytes of *P. vivax*, but not those of *P. falciparum*.

As to how long a mosquito once infected remains infective, there is considerable discussion. Given only a single feed on an infected person, it is clear that in the course of two to three weeks or so, all oöcysts will either have matured or died off, and the salivary glands will gradually clear of the infection. Yet Mitzmain (1916) succeeded in infecting nine individuals in fourteen days from one mosquito infected with *P. vivax*; and James—as noted above—infected more than 40 individuals from one mosquito over a period of three months. If the mosquito obtains repeated feeds upon malarial blood, as will usually be the case in Nature, successive crops of oöcysts will develop on its stomach wall, and successive swarms of sporozoites invade its salivary glands. Bruce Mayne (1920, 1922) has carried out careful investigations on the retention of infectivity by the mosquito. On feeding *A. punctipennis* on a case of malignant tertian malaria with crescents in the blood, he succeeded in transmitting the infection on the 55th day after the feed to a volunteer, but not on the 67th day; the mosquito was dissected on the 68th day, when active sporozoites were found, however; in a second experiment the mosquito failed to transmit at the 74th day, but sluggish sporozoites were found in the salivary glands some days later.

Turning to the other end of the cycle, it is obvious that the mosquito must inject a certain 'dose' of sporozoites into a man before the latter contracts malaria. Even in weakly individuals it is probable that a small injected 'dose' of sporozoites would be killed off and that infection would not result. There are numerous records in the literature on the treatment of general paralysis by induced malaria where infective mosquitoes have failed to infect the patients on whom they have been fed. These results have been criticised by James (1926), however, who found that in several such instances, the technique merely was at fault. Of 145 mental patients upon whom he fed infected mosquitoes, only 36 failed to contract the infection; and these failures all occurred with one batch of mosquitoes which failed to attain an infection rate of 100 per cent. He concludes that there are certain persons whose



blood is 'inhospitable' to the malarial parasite, and that a bio-chemical study of the conditions present in such persons is urgently needed.

Super-infection has been noted by several workers. The brothers Sargent (1907), and Whitmore (1918) working with proteosoma have noted that a sparrow suffering from a proteosoma infection due to the bite of mosquito A, could be successfully super-infected by the bite of infective mosquito B. 'One attack of malaria,' writes James (1926)—'induced either by blood inoculation or by mosquito bites—due to a strain of *P. vivax* does not confer immunity against a second infection with the same strain.' What usually happens in a hyper-endemic area is repeated infections until finally the patient acquires a certain measure of tolerance to the parasites—rather than any real immunity due to the development of immune bodies in the blood, or similar cause. A very striking and informative study of such conditions is that given by Christophers (1925) for the mining settlements in Singhbhum. During the first two years of life, the children born in this hyper-endemic area suffer from continuous, febrile malarial infestation, with an average parasite count of more than 10,000 parasites per c. mm. of blood. In children aged 2 to 5 years the proportion infected is still as high as ever, but the parasite count has fallen to an average of only 1,200 parasites per c. mm. of blood, and febrile attacks only occur about once in every 25 days. During the age period 6 to 10 years, 100 per cent of the children still shew infection, but the parasite counts are under 1,000 per c. mm., fever does not occur, and the condition present is one of afebrile infestation. In adults 50 per cent still shewed malarial infection on blood examination, but the spleen rate had dropped to 10 per cent, and these adults have to a large extent acquired full tolerance for the parasite.

The latent period of incubation of malaria in the person bitten by an infected mosquito will depend upon several factors; the dose of sporozoites injected, the species of parasite concerned, the patient's powers of resistance, and so forth. Approximately the incubation periods are from 14 to 18 days in *P. vivax* infections, 18 to 21 days in *P. malariae* and 9 to 12 days in *P. falciparum*.

#### *Malaria. Miscellaneous.*

*The Aetiology of Relapses.*—In 1902 Schaudinn had under observation at Hamburg a Jewess who suffered from chronic relapsing malaria, due to infection with *P. vivax*. On studying blood films taken from this patient just prior to a relapse, he came across certain puzzling appearances in some of the infected red corpuscles. These he interpreted as being macrogametocytes, which were producing merozoites. He concluded that the macrogametocyte of *P. vivax* has a very prolonged life in the blood, that it may persist for months or years unaffected; and that, if the patient's resistance be lowered from chill or other cause, such macrogametocytes give rise to merozoites by a process of parthenogenesis. These merozoites invade fresh red corpuscles, grow up into schizonts, the

asexual schizogony cycle becomes re-established, and the patient suffers from a relapse (Fig. 9).

Now parthenogenesis—i.e., the production of offspring by an unfertilised female parent—is a well-established fact in Nature. It has been shewn to occur among such insects as the saw fly, and the aphidæ on rose trees. In the case of the latter, males only appear in the autumn. These mate

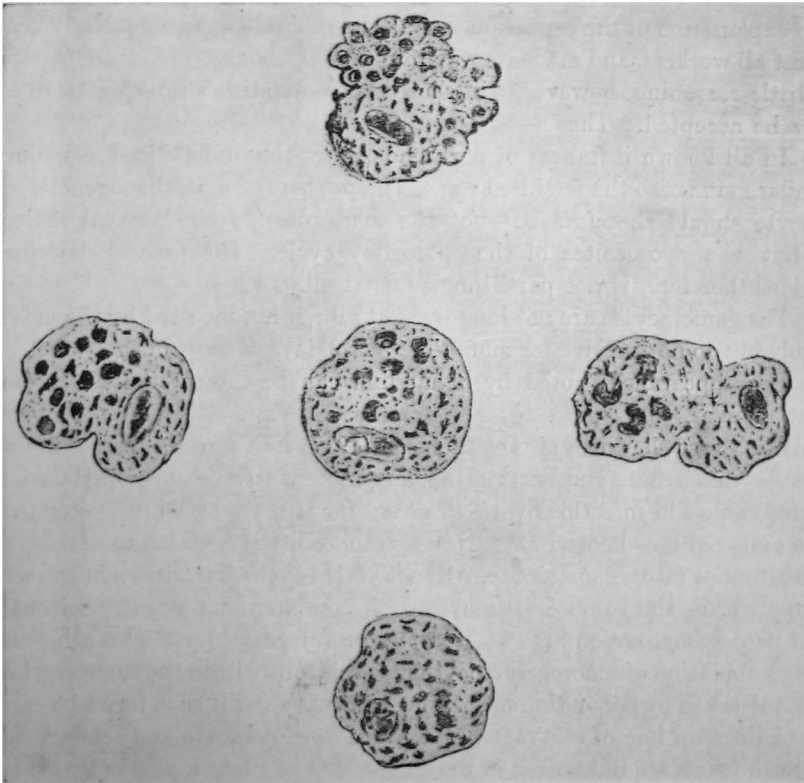


FIG. 9.—Tracings from Schaudinn's figures shewing 'parthenogenesis' in the macrogametocyte of *Plasmodium vivax*, as the cause of relapses in malaria. (From J. D. Thomson, 1917, after Schaudinn, 1902.)

with the females, and the latter lay fertilised eggs, which remain on the plant throughout the winter, and hatch in the spring into females (only). From these females brood after brood of young are born during the summer by parthenogenesis, the males only appearing again in the autumn. Further, Löeb and others have shewn that the unfertilised egg of the sea-urchin and of the frog can be made to develop by the application to them of suitable

physical or chemical stimuli; and frogs have been reared from unfertilised eggs in many laboratories.

There is therefore no inherent reason why parthenogenesis should not occur among the Protozoa. Indeed amphimixis—i.e., nuclear re-organisation—among the Ciliophora; and autogamy—fusion of nuclei within the same individual—which is said to occur in the cysts of many free-living Rhizopoda—may be interpreted as possible examples of it. And Schaudinn so dominated the protozoology of his day that his explanation of the causation of malarial relapses was immediately accepted by almost all workers, and made its way into the text-books.

A little reasoning, however, will shew that Schaudinn's interpretation cannot possibly be accepted. Thus :—

(a) In all known instances of parthenogenesis the unfertilised egg develops in a similar manner to the fertilised egg. The progeny of a parthenogenetic macrogametocyte should therefore be—not the merozoites of the asexual schizogony cycle—but the sporozoites of the sporogony cycle. The process described by Schaudinn, therefore, is not parthenogenesis at all.

(b) The gametocytes are not long-lived at all; if not ingested by the mosquito, they probably do not survive for more than 8 or 9 days at most in the blood.

(c) The appearances noted by Schaudinn can be interpreted in an entirely different manner.

This was first shewn by J. D. Thomson (1917) in a striking paper illustrated with a very fine plate, and bearing the very modest title 'Notes on Malaria.' It is not uncommon in infections with *P. vivax* for two young trophozoites to enter into the same red blood corpuscle. Now a trophozoite is destined to develop either into a schizont or into a gametocyte. Hence, as these two parasites mature, we may encounter within the same erythrocyte such combinations as (a) schizont plus schizont; (b) gametocyte plus gametocyte; or (c) gametocyte plus schizont. It was the combination of macrogametocyte plus schizont within the same erythrocyte that was mistaken by Schaudinn for parthenogenesis; and if such forms be carefully examined the thin line of cleavage between macrogametocyte and schizont will be seen. Such forms are illustrated in Figs. 29, 30, 31 of Plate I, also in text Fig. 10. Since the publication of Thomson's paper, almost every worker of experience has accepted his interpretation. The parthenogenesis hypothesis with regard to malarial relapses is now as dead as mutton, although it is still enshrined in many text-books.

To what, then, are relapses due? There have been all sorts of suggestions, such as the production of special and resistant forms; the suggestion that some of the parasites may retreat into areas in the blood stream where they are not exposed to the destructive influences of the body, or that they may have an intra-cellular phase within endothelial cells; and several other suggestions. To the writer, all such suggestions appear to be entirely unnecessary; since relapses can be very simply

explained by a persistence of the schizogony cycle as a low grade afebrile infestation.

A little reasoning will make this clear. The parasite of benign tertian malaria is the one especially associated with relapses in malaria ; and it is to be noted that it tends to produce fewer, and not more numerous, gametocytes in proportion to the total number of parasites present. Now there must be a certain number of parasites present in a patient's blood stream before fever can occur ; in other words, there is a febrile threshold in malaria. After a person has been bitten by an infected mosquito, the injected sporozoites become trophozoites and then schizonts. Every 48 hours a fresh cycle of schizogony occurs, and the number of parasites is rapidly multiplying. Finally they become so numerous that their numbers rise above the febrile threshold ; fever occurs and the patient has his first rigor.

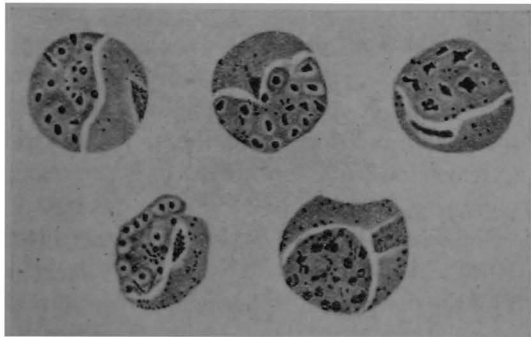


FIG. 10.—The combination of gametocyte plus schizont within the same erythrocyte in infections with *Plasmodium vivax*. (After J. D. Thomson, 1917.)

The febrile threshold will be influenced by such factors as the patient's powers of resistance, general state of health, condition of his blood, and so on, but the rough estimates given by Ross and Thomson (1910) may be accepted ; that in the case of *P. vivax* infections from 200 to 500 parasites must be present per c. mm. of blood to produce fever ; and in the case of *P. falciparum* infections, from 600 to 1,500 per c. mm. In the case of *P. vivax* we may take the lower reading of 200 parasites per c. mm. as the threshold value ; then, since the total volume of blood in a healthy adult is about 5 litres, or 5,000,000 c. mm., in order to produce fever  $200 \times 5,000,000$  or 1,000,000,000 (one thousand million) parasites must be present in the patient's blood stream.

Suppose that a single sporozoite, injected by the mosquito, becomes a schizont with, let us say, 20 merozoites ; and that the multiplication cycle goes on unchecked

by any outside influence, then the total number of parasites present in the patients' blood stream will be as follows :—

On the 2nd day after infection .. .. .	20
On the 4th day .. .. .	400
On the 6th day .. .. .	8,000
and so on, until it is	
On the 12th day .. .. .	64,000,000

This dose is not sufficient to produce fever ; but at the next schizogony cycle, on the 14th day, the total number of parasites in the body will be 1,280,000,000 ; this is above the febrile threshold, and accordingly the patient goes down with fever.

Suppose, however, that the multiplication still proceeds unchecked. By the 20th day the total number of parasites should have reached 102,400,000,000,000 ; this would correspond to 20,480,000 parasites per c. mm. of blood. Such an infection is utterly impossible, and the patient should be dead.

But he is not. The tendency, even in untreated cases of *P. vivax* infections, is for spontaneous recovery to gradually come about, to be followed by a more or less prolonged afebrile period, and then by a relapse. It is obvious, therefore, that there must occur a tremendous and continuous destruction of malarial parasites in the infected person.

This destruction of parasites is probably brought about in several different ways. When the mature schizont rosette ruptures, its hæmozoin pigment is liberated and is phagocytosed by the macrophages throughout the blood stream ; i.e., by the large mononuclear leucocytes and by the endothelial cells of the blood capillaries. The liberated merozoites—or possibly the remains of the destroyed red corpuscle—act as does an intravenous injection of a foreign protein, and the patient has a rigor which is the result of protein-shock. The liberated merozoites make for the surface of fresh red corpuscles. According to Yorke and Macfie (1924) death of a certain proportion of the merozoites—whether due to quinine treatment, or to the natural powers of the patient—results in these dead merozoites acting as an antigen ; this antigen stimulates the formation of the immune body on the part of the patient, and the immune body—if present in sufficient amount—destroys the remaining parasites, thus clearing the patient of infection. Interesting as such a hypothesis is, it remains at present a hypothesis only, and the evidence which these authors bring forward in its favour is all indirect.

McLay (1922) working with cultures of *P. falciparum* noted a tendency for red corpuscles infected with this parasite to adhere to the large mononuclear cells in preference to any other type of cell, whilst Wenyon (1926, Plate XIV) figures actual ingestion of malarial parasites by endothelial cells in the spleen ; D. Thomson (1914) has a similar coloured illustration ; and to some extent it is probable that

actual phagocytosis of malarial parasites by macrophages and endothelial cells is responsible for the destruction of malarial parasites. A study of spleen puncture findings in chronic and relapsing cases of malaria by Knowles, Acton and Das Gupta (1923); however, gave different findings. It would seem as if such parasites as fail to get a grip on the red corpuscles are swept off their surface into the blood stream, and are caught up in the intercellular meshwork of the spleen (and probably of other internal viscera). Here they simply go to pieces; the chromatin breaks up by karyorrhexis or swells up and dissolves by karyolysis; the cytoplasm becomes progressively vacuolated and disintegrates; finally there is left nothing except little sheets of residual parasitic cytoplasm, filled with pigment; lying between the cells of the splenic tissue, where the pigment is finally phagocytosed by the endothelial cells. In chronic and relapsing malaria the spleen appears to function as the grave rather than as the birthplace of malarial parasites.

In one way or another—probably in a variety of ways—the patient's natural powers of resistance reduce the infection. The production of gametocytes will be a still further factor in reducing the total infection, since the gametocytes are non-multiplying forms in man. In other words gametocyte production will act as a brake on the multiplicative cycle. Let us suppose that, at each 48-hour schizogony cycle, 50 per cent of the parasites are destroyed or are replaced by non-multiplying gametocyte-forms. Then, taking the above mentioned figure of 1,280,000,000 parasites as present in the body when the patient has his first rigor on the 14th day after infection by the mosquito, let us suppose that half of these parasites are destroyed; the remaining 640,000,000 parasites will produce 12,800,000,000 parasites within the next 48 hours, and the fever will continue. A 50 per cent destruction rate, in other words, will not be sufficient to check the fever. But suppose the destruction rate to be, let us say, 97 per cent. Then of the 1,280,000,000 parasites present at the first rigor, only 3 per cent, or 48,400,000 will survive to undergo schizogony; their progeny will number 968,000,000 at the end of the next 48 hours. This figure is under the febrile threshold of 1,000,000,000 defined above; in other words the infection will now not be heavy enough to cause fever.'

If the powers of the body to destroy parasites are such, therefore, that the infection is scotched but not exterminated, we may picture a state of affairs in which schizogony persists for very long periods, but at a very low level of total number of parasites in the patient's blood stream. Parasites will not be found in thin films of the peripheral blood—except very occasionally—but they *are* found in thick blood films taken from such patients during the afebrile period between relapses. If, now, for any cause—such as chill or other factor which lowers resistance, the patient's powers of destroying parasites are reduced, the schizogony cycle flares up, the total number of parasites rises above the febrile threshold, and the patient has a relapse.

Such—in the writer's opinion, at least—is the true explanation of malarial relapses. It is the explanation of them which has been put forward by Bignami, Sir Ronald Ross, James, and others. Whitmore (1918) studying *P. præcox* infection of birds, shewed that although parasites could only rarely be found in thin blood films up to the 4th to 5th month after infection, yet the birds' blood remained infective to clean birds by direct inoculation for a very much longer period—in one instance up to 29 months after infection; and further, that as long as parasites were found at all microscopically, the schizogony forms seen were true to type.

A patient once infected with *P. vivax* may remain infected for many years. The writer, when a child of four, travelled from Travancore to England in 1887, heavily infected with malaria, and repeatedly suffered from attacks of malaria in England up to the age of 16. (Blood examinations were not made, but the attacks were typical of ague, and were in each instance controlled by quinine administration.) Coming out to India in 1908, he remained free from malaria, until he contracted an infection with *P. vivax* at Shillong in 1917; this caused relapse after relapse between the years 1917 and 1920. The last attack occurred within a few hours of reaching Simla in the autumn of 1920, and was undoubtedly brought on by the effects of cold. It was countered by taking 90 grains of quinine in solution within 24 hours, followed by leave to England in 1921, and no further relapses have since occurred. This history is of interest in shewing that any 'immunity' to malaria which may be acquired in childhood may not be of a very persistent character. S. P. James (1922) gives the history of a patient who arrived in England from Palestine in February 1917, heavily infected with *P. vivax*; he had a typical relapse with parasites present in blood films in January 1920. He remarks, however, 'beyond three years, the occurrence of a true relapse is a rare event which always merits careful study.'

#### *Animal Inoculations in Malaria.*

The malarial parasites of man are very strictly specific to man. Very numerous attempts have been made by different workers to infect hosts other than man experimentally, but only in one instance with success. Bass (1922) points out that horses, mules, dogs, foxes, monkeys, rabbits, mice, guinea-pigs, hedgehogs, bats, wolves, cats, pigeons, doves, magpies, screech-owls, turtles, frogs and lizards have all been inoculated with human blood containing malarial parasites, without infections resulting, and records further negative experiments by himself. Mesnil and Roubaud, (1920) after a preliminary series of failures, succeeded in producing a mild infection with *P. vivax* in a chimpanzee by intravenous inoculation of infected human blood; the infection appeared on the 10th day, and lasted for a similar period; a second chimpanzee inoculated in a similar manner did not become infected. Reichenow (1917, 1920) discovered malarial parasites resembling those of human type in anthropoid apes in West Africa, and produced some evidence that it was only in those apes that were kept in association with man that the infections occurred.

Blacklock and Adler (1922, 1924), who studied the parasites of chimpanzees, failed to infect human beings by inoculating them with blood from an infected ape, and also failed to infect a young chimpanzee with *P. falciparum*. Direct inoculation of infected blood from man to man, however, readily produces infection, and this method of inducing malaria is now a standard method of treatment of general paralysis of the insane. Wenyon (1926, p. 955) records a very interesting instance where a salvarsan apparatus became infected with malarial blood from the first of a series of eleven patients to whom the drug was administered from the same apparatus in series. The ten succeeding patients [became] infected, and one of them died of malaria.

#### *The Nature of the Malarial Rigor.*

The views at one time entertained that 'toxins' are produced by the malarial parasites are to-day practically abandoned. It is true that the anæmia of malaria is more profound than can be accounted for by the number of red corpuscles destroyed by the parasites; and many workers have suggested that the malarial parasites produce a hæmolysin. Abrami and Senevet (1919), however, have shewn that the malarial rigor is in all probability of the nature of an anaphylactic or 'hæmoclastic' shock. They give the term 'hæmoclasia' to the phenomena resulting from inoculation of foreign proteids into the blood stream, these phenomena being chiefly lowered arterial tension, leucopænia, an alteration in the differential leucocyte count, a diminution in the number of red cells and changes in the coagulability of the blood. They have shewn that the paroxysms in benign tertian malaria are preceded by a hæmoclastic crisis, and regard the chill, the vomiting, the headache, backache and other symptoms of the cold stage of the malarial paroxysm as manifestations of this hæmoclastic shock. In all probability either the liberated showers of merozoites—or possibly the products of the destroyed red blood corpuscles—act as does a foreign proteid injected into the blood stream.

#### *Congenital Malaria.*

As has been noted above, Mühlens, on cutting sections of infected anopheline mosquitoes, found sporozoites in every tissue of the mosquito except the ovaries; and there is no evidence that malarial infection can be transmitted from the mosquito to its progeny. On the other hand, there are now numerous cases in the literature where malaria has been transmitted from an infected mother to her child. One of the most striking instances is that recently recorded by Buckingham (1925). A Chinese mother who was in labour was suffering from malaria. The presentation was a transverse one, and the hand of the fœtus prolapsed. It was cleansed, and pricked and a blood film made from the child's blood, before version was performed and the child extracted. Films from the child's blood, taken before birth, shewed



malarial parasites. The instances in the literature are now too numerous to overlook, and it must be admitted that congenital malaria does sometimes occur. Thus Heiser records the case of an infant seven days old which shewed the gametocytes of *P. falciparum* in its blood ; as crescents are practically never found before the 8th day of the infection, this child must almost certainly have been infected *in utero*.

On the other hand congenital malaria is very exceptional, and probably only occurs when accidental tears of the placenta allow the passage of parasites from the maternal to the foetal circulation. The true state of affairs in the malaria of pregnancy has been set forth in a very interesting paper, illustrated with a very fine colour plate, by Blacklock and Gordon (1925). These workers discovered that in Sierra Leone about 36 per cent of native women are infected with malaria, all infections found being due to *P. falciparum*. Parasites were very scanty in peripheral blood films, and these women do not constitute a danger to the community as far as the spread of malaria is concerned. In pregnant women, however, intense infection occurs in the placenta, the blood vessels of which are filled with sporulating parasites and its endothelial cells loaded with pigment. Pathological effects on the mother may be lacking, and some of the children born from such mothers appear to be healthy. On the other hand the enormous destruction of red blood corpuscles by the parasites in the placental circulation may deprive the foetus of nourishment, or toxins absorbed from the intensely infected placenta may poison the foetus, and severe destruction of the erythrocytes may occur in the umbilical cord in spite of the absence of congenital malaria. The result is that the foetus dies *in utero* and is expelled.

In medical practice in the tropics the importance of childbirth in waking up a latent malarial infection in the mother is very great, whilst surgical operations may also cause a recrudescence of a latent malarial infection. It is equally bad to mistake puerperal fever due to sepsis for malaria, and to mistake malaria after childbirth for puerperal sepsis. Microscopical examination of the patient's blood will usually at once shew which infection is present.

#### *Cultivation of the Malarial Parasites.*

Bass and Johns (1912) were the first to succeed in cultivating the malarial parasites of man. A modification of their method which the writer has found to be very uniformly successful was introduced by J. G. Thomson and D. Thomson (1913) ; details are given on p. 85. Amongst others Ziemann (1913, 1914), Rocha-Lima and Werner (1913), and McLay (1922) have succeeded in cultivating malarial parasites. At the Calcutta School of Tropical Medicine culture is resorted to as a rule only when it is likely that the patient has malaria, but where parasites cannot be detected in thin or thick films. In such cases the cultural test is a very valuable

one. It is not often required however. The schizogony cycle of *P. falciparum* can be readily studied in cultures. It is difficult to keep strains going by sub-culture, however, and the hope which was once entertained that the successful cultivation of malarial parasites *in vitro* would afford a means of indefinitely maintaining these parasites *in vitro* outside the body has not been realised.

McLay notes that it is difficult, if not impossible, to obtain successful cultures from patients who have taken quinine. Indeed, the taking of a little quinine by the patient is one of the greatest difficulties encountered in the laboratory diagnosis of malaria in the tropics. The ordinary intelligent European or Indian patient in the tropics who gets a chill and thinks that he is going to develop malaria usually takes a dose of 5 grains or so of quinine before sending for his medical attendant. This dose is sufficient to render search of thin films for parasites almost hopeless; and in such cases thick films should be taken.

Sinton (1922) has invented a most ingenious modification of Wright's capillary blood tube in which the whole process of culture of the malarial parasites can be carried out from the finger blood. He advocates incubation at from 35°C. to 38°C (*vide* p. 86).

#### *Blackwater Fever.*

We may perhaps at this point briefly consider the question of the ætiology of blackwater fever. Despite the researches of almost innumerable workers, the whole question of the ætiology of blackwater fever is still obscure. In India, blackwater fever occurs especially in the Dooars, Assam and Bengal, whilst it has recently proved a veritable curse in connection with the doubling of the main line of the Bengal-Nagpur Railway in the Singhbhum District of Bihar, where the new railway construction runs through an intensely malarious zone. Its chief home, however, is in tropical Africa; it was prevalent during the construction of the Panama Canal, but has since disappeared from the Canal Zone with the introduction of anti-malarial measures.

The actual condition present in the disease is a tremendous destruction of red blood corpuscles and hæmoglobinæmia, with consequent hæmoglobinuria, urobilinuria and albuminuria. It occurs usually—but not invariably—only in those who have resided for a long time in the tropics. Jaundice sets in early and is intense, together with a very marked destruction of red corpuscles—the number of which may be reduced by as much as 2,000,000 per c. mm. in two days. The reduction of hæmoglobin runs parallel with that of the red corpuscles. The urine shews spectroscopically the absorption bands of methæmoglobin, or more rarely of oxyhæmoglobin. The blood coagulability, alkalinity, and its pH are reduced. Schlesinger's test for urobilinogen in the urine is positive; and occasionally hæmatoïdin crystals may be found in the urinary deposit.

A good review of the whole subject, together with a good bibliography is that given by Warrington Yorke (1922). In the main one may say that there are four different views prevalent as to its causation. These are as follows :—

(1) That it is due to a specific parasite, as yet undiscovered. The symptoms of blackwater fever in man are very strikingly similar to those of acute piroplasmosis in animals. If blackwater fever is an instance of piroplasmosis, however, it is strange that the innumerable workers who have studied the disease have never been able to demonstrate the piroplasma. Leishman (1912) found certain cell-inclusions in the cytoplasm of endothelial cells found free in the blood stream in cases of blackwater fever ; he came to the conclusion that they might possibly

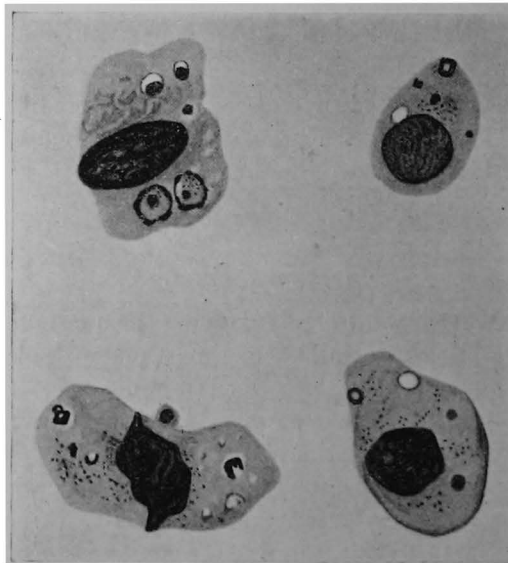


FIG. 11.—Cell inclusions in the cytoplasm of endothelial cells in blackwater fever. (After Leishman, 1912.)

be of parasitic origin (Fig. 11). On the other hand the endothelial cells and large mononuclear leucocytes may shew marked karyorrhesis and even karyolysis in blackwater fever, and it is not likely that the forms seen by Leishman are parasites at all ; they are probably nuclear remnants due to karyorrhesis of endothelial cells poisoned by the toxins of the disease. Blanchard and Lefrou (1922) state that by triple centrifugalization of the blood in three cases of blackwater fever they isolated a spirochæte of leptospira form, and that it was inoculable into guinea-pigs. J. G. Thomson (1924) in Rhodesia and Low and Duncan (1923) in London were entirely unable to confirm these findings in cases of blackwater fever, and it seems probable that the ' spirochætes ' seen were leptospira-like artefacts.

It is quite possible that blackwater fever may be due to a specific micro-organism, but, if so, that organism has yet to be demonstrated.

(2) A second view is that the disease is nothing more or less than hyper-virulent malignant tertian malaria. Stephens (1913) has examined the parasitic findings recorded in the literature on blackwater fever. Malarial parasites were found in blood films from patients in 73 per cent of cases examined the day before the onset of blackwater fever; in 47·5 per cent on the day of onset of the fever; and in 23 per cent on the day after onset of the fever. Occasionally infections with *P. vivax* and *P. malariae* have been encountered in such patients, but J. G. Thomson (1924) claims that in all instances the infection is with *P. falciparum*, and that when *P. vivax* or *P. malariae* is encountered, further search of films will shew a mixed infection with *P. falciparum* also. It is obvious that the parasites are destroyed in the blood stream in blackwater fever almost as rapidly as are the red corpuscles. Even when *P. vivax* or *P. malariae* is found, very aberrant and clearly degenerating parasite forms may be encountered.

A very fine memoir, with a complete bibliography, on the association of blackwater fever and *P. falciparum* infection is that by J. G. Thomson (1924), and it should be studied in the original by all interested in the blackwater fever problem. In Rhodesia, over the period 1913—1922 inclusive, graphs for (a) hospital admissions for malaria, and (b) for hospital admissions for blackwater fever shew a most striking similarity and the seasonal incidence of the two diseases absolutely coincides (Fig. 12). 'The whole evidence of my researches,' writes Thomson, 'incriminates repeated infections with *P. falciparum*. No definite incubation period can be detected in the onset of hæmoglobinuria, which indicates that the condition is not directly due to the action of the malarial parasite or any other organism, but is to be considered as a reaction of the body to a prolonged infection.'

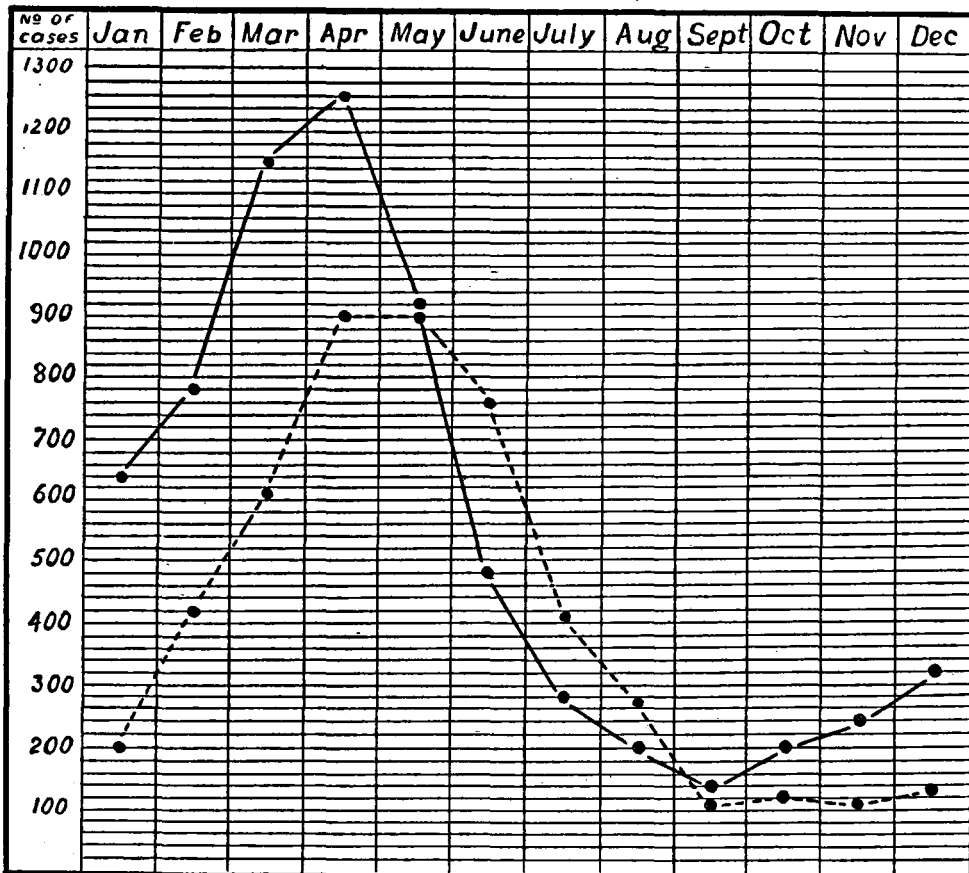
(3) Still a third view is that blackwater fever is quinine hæmoglobinuria, occurring in persons especially susceptible to quinine poisoning. Quinine normally circulates in the blood as quinine base, no matter in what form it be administered. But if the alkali reserve of the body be unduly lowered, quinine might possibly circulate as an acid salt in the blood and cause hæmolysis of the red corpuscles.

(4) The most usually held view is that blackwater fever is due to *P. falciparum* infection plus quinine poisoning in certain susceptible persons. Thus two typical cases were seen by the writer at Shillong, both of whom gave an identical history. One was the wife of a tea planter in Assam, the other a tea planter who had long been resident in Assam. Both were habituated, so to speak, to malaria. On commencing the long, cold motor drive from Gauhati to Shillong they felt chilly, took a dose of quinine, and arrived at Shillong—altitude 6,000 feet—suffering from blackwater fever. On the other hand a third—fatal—case seen by the writer in Shillong was a

Khasi prisoner in the jail, who had been taking a weekly prophylactic dose of quinine, but with no clear history of previous malaria.

### SOUTHERN RHODESIA .

*Hospital Admissions, Malaria and Blackwater, 1913-1922 (inclusive).*



*Malaria Curve ●—●. Blackwater Curve ●---●. Total Malaria Cases 6608.  
Note. Blackwater Curve is multiplied by 10. Total Blackwater Cases 492.  
(After J. G. Thomson. 1924.)*

FIG. 12.

Between these views it is at present impossible to judge. The argument that blackwater fever only occurs in intensely malarious districts, and that therefore

the disease must be a form of malaria, is a very weak one. Exactly the same argument was once used to support the view that kala-azar was a type of malaria, prior to the discovery of *Leishmania donovani*. At present the only possible attitude is to preserve an open mind with regard to the causation of the disease. A very large volume of biochemical investigation has been carried out on it, but scarcely perhaps sufficient from the possible parasitological side.

*Plasmodia of Hosts other than Man* (Fig. 13).

Excellent practice, and the possibility of very interesting researches, is obtainable by the study of the blood parasites of hosts other than man. In many such, birds and snakes especially, these are far more common in the peripheral blood than *Plasmodia* in the human subject, whilst nucleated red blood corpuscles are far more resistant to bad fixation and give the beginner better results than mammalian corpuscles.

The genus *Plasmodium* is a very widespread one, and includes parasites of many hosts other than man. Thus monkeys are frequently parasitised by plasmodia. Koch (1898) was the first to observe a monkey plasmodium in various species of *Cynocephalus*, *Cercopithecus*, and *Cercocebus* monkeys. As shown by Blanchard and Langeron (1913) the natural malarial infections of monkeys are apt to prove very fatal to these animals; thus they found an imported *Macacus cynomolgus* in Paris to be naturally infected, and from it infected two other monkeys of the same species. One died from an acute infection, the other acquiring a chronic one. Just as in man, so in monkeys, their natural malarial infections may be very acute, or may become chronic.

Of the different monkey species described *P. reichenowi* (Sluiter, Swellengrebel and Ihle, 1922) occurs in anthropoid apes—especially, as shown by Reichenow (1920) in anthropoid apes in close association with man. Apparently two and not one species are concerned, for forms resembling *P. vivax* and *P. falciparum* of man have been described from chimpanzees and gorillas. Blacklock and Adler (1922, 1924) have studied the malarial parasites of the chimpanzee in West Africa, and have published excellent descriptions of them. *P. kochi* (Laverán, 1899) is very common in monkeys of the genera *Cercopithecus*, *Cynocephalus* and *Cercocebus* in tropical Africa; in many ways it resembles *P. vivax* of man, but Gonder and Rodenwaldt (1910) record two unsuccessful attempts to transmit the infection from the monkey to man. Quinine had a definite action in controlling the infections. *P. inui* Halberstadter and Prowazek, 1907, occurs in *Macacus* monkeys and also resembles *P. vivax* of man, but small ring forms resembling those of *P. falciparum* also occur. *P. cynomolgi* Mayer, 1908, is probably identical with *P. inui*. *P. semnopithecii* Knowles, 1919, occurs in the hanuman ape of Assam, *Semnopithecus entellus*; the infection is associated with enlargement of the red corpuscles, and the parasite in many ways resembles *P. vivax* of man, but is deeply pigmented. *P. pitheci* Halberstadter and Prowazek, 1907, occurs in the orang-outang, and Dodd (1913) records an infection with it which proved fatal; this parasite is transmissible to the orang-outang, but not to the lower monkeys. *P. brasilianum* Gonder and Berenberg-Gossler, 1908, occurred in a monkey imported from the Amazon to Hamburg, and resembles *P. malariae* of man. *P. roubaudi* Leger and Bedier, 1921, was found in the gorilla in Senegal. With regard to transmission of these monkey plasmodia very little work has been done, though Mayer (1908) failed to obtain development of *P. inui* in *Culex pipiens*, but saw what he took to be small oöcysts in *Anopheles maculipennis*.

Bats and other small mammals frequently show plasmodial infections; and here a special difficulty arises, since deeply staining basophile red corpuscles and stippled corpuscles are a striking feature of the blood of bats, and such corpuscles may be mistaken for parasite forms. *P. murinum* (Dionisi, 1899) occurs in the bat genus *Vespertilio*, whilst *P. melaniptherum* (Dionisi, 1899) also occurs in bats, and both species rather resemble *P. malariae* of man. *P. brodeni* Rodhain, Bequert, Pons and Vandenbränden, 1913, was found in the Congo in the jumping rat, *Petrodromus tetradactylus*. A similar parasite was described by Rodhain (1915) from the flying fox in the same region. *P. pteropi* Breinl, 1912, rather resembles *P. vivax* and was found in the flying fox, *Pteropus gouldi*, by Breinl in West

EXPLANATION OF FIG. 13.

Plasmodia of Hosts other than Man. (From Wenyon, 1926, after various authors.)

- 1—4. *Plasmodium præcox* from the blood of a Bagdad sparrow.
- 5—7. Plasmodium of the chimpanzee, described by Reichenow (1917) as *P. falciparum* and named *P. reichenowi*.
- 8—10. Plasmodium of the chimpanzee, described by Reichenow (1917) as *P. malariae*.
- 11—12. *Plasmodium kochi* of *Cercopithecus* monkeys.
- 13—14. *Plasmodium pitheci* of the orang-outang.
- 15—16. *Plasmodium bubalis* of the Indian buffalo.
- 17—18. *Plasmodium vassali* of the squirrel.
- 19—21. *Plasmodium murinum* of bats.
- 22—26. *Plasmodium agamæ* of a Sudan lizard.
- 27—29. *Plasmodium mabuia* of the necklace lizard.

(Plate reproduced in black and white from Wenyon's coloured plate.)

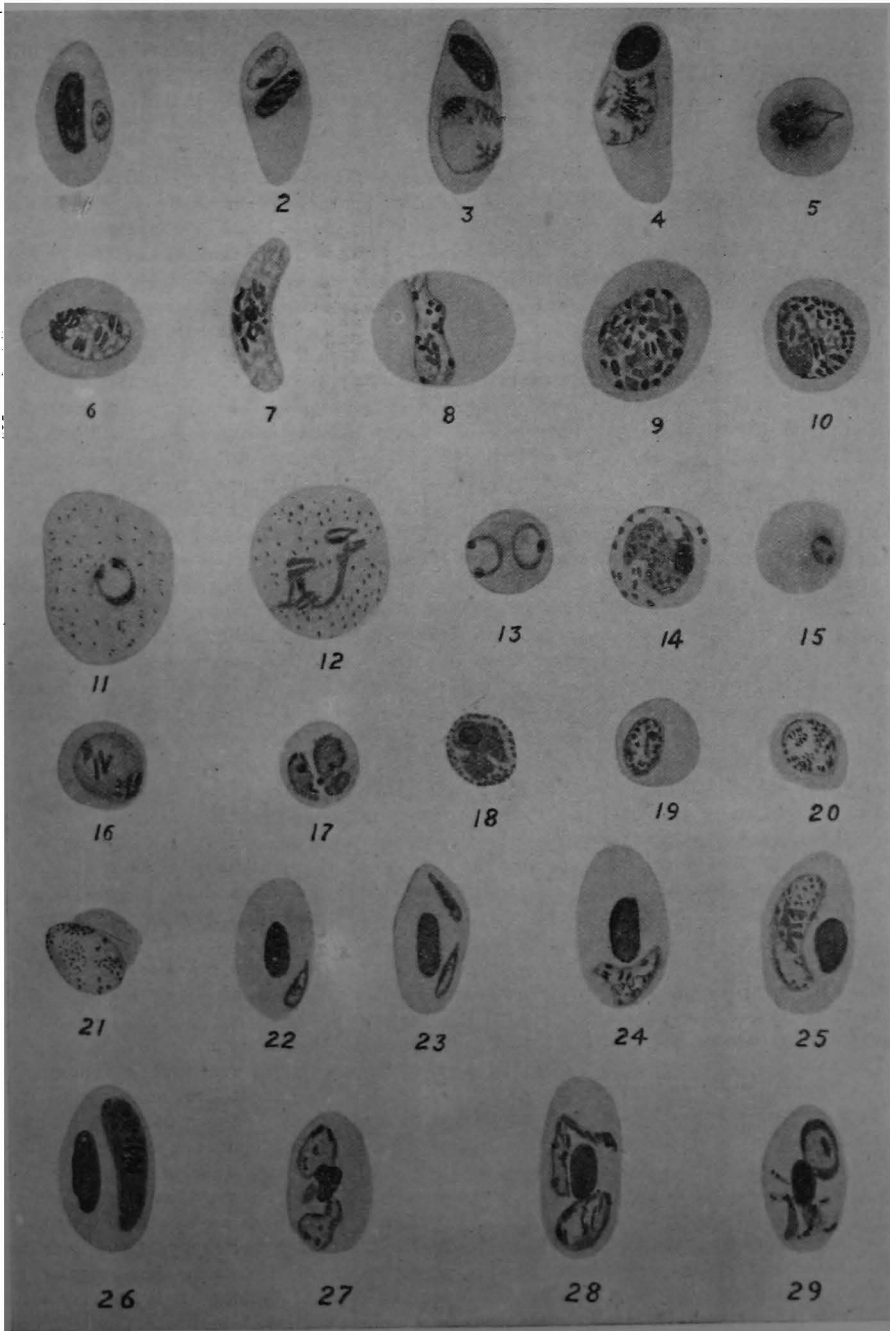


FIG. 13.



Australia and by Mackie (1914) in India. *P. vassali* (Laveran, 1905) is a plasmodium of squirrels; it was found by Vassal in squirrels of the genus *Sciurus*, and resembles *P. malariae*; a similar and possibly identical plasmodium was described by Donovan (1920) in the Indian squirrel, *Ratufa indica*.

Of the plasmodia of large mammals, *P. cephalophi* Bruce, Harvey, Hamerton and Lady Bruce (1913) was discovered in the duiker in Nyasaland by the Royal Society's Sleeping Sickness Commission. It showed resemblances to *P. malariae*, but the infected red corpuscle is markedly enlarged and pale. Sheather (1919) has given an account of an infection in the Indian buffalo and named the parasite *P. bubalis*. This parasite resembles *P. malariae*, and the same parasite has again been seen in Indian buffaloes by Edwards (1925). *P. caprae* (de Mello and Paes, 1923) occurs in goats in Angola and was further studied by Paes (1924). Wenyon (1926, p. 976) remarks that it is a little doubtful whether this form is a plasmodium or a piroplasma. *P. canis* Castellani and Chalmers, 1910, is described as a plasmodium of dogs in Colombo, and morphologically identical with *P. vivax* of man; but other observers have failed to re-discover this parasite, and Wenyon (1926, p. 976) notes that although he examined blood films from 500 pariah dogs in Colombo he failed to find *P. canis* in any of them.

The plasmodia of birds have been very extensively studied by many workers, and over many years. The common 'proteosoma'—or *Plasmodium praecox* (Grassi and Feletti, 1890) as is its true name—is a parasite which has been used by worker after worker over many years in the study of malarial problems. Thus Ross first discovered its sporogony cycle in *Culex fatigans*, before he and the Italian workers went on to apply the discovery to the further discovery of the sporogony cycle of the human malarial parasites in *Anopheles* mosquitoes. This parasite is a very favourite one for laboratory study. It has a world-wide distribution and occurs especially in small birds, such as the sparrow, finch, and lark, but also in larger birds, such as the pigeon, crow, owl, partridge, duck, hen, and guinea-fowl. It is curious how uncommon the infection is in Calcutta city itself; the writer has repeatedly tried to find the infection in Calcutta sparrows, crows, and pigeons, but has never succeeded. On the other hand sparrows in the *mofussil* are probably frequently infected; in reading Sir Ronald Ross' *Memoirs* it is interesting to note that he first succeeded in obtaining oöcysts by feeding mosquitoes on a mixed bag of pigeons, crows, sparrows and larks without, apparently first examining their bloods. On the other hand, *Hæmoproteus* infections are very common in birds in Calcutta.

The youngest forms of *P. praecox* are very tiny structures lying towards the pole of the infected nucleated red blood corpuscle, shewing a red-staining chromatin dot and a trace of blue-staining cytoplasm. A vacuole soon forms, however, and at this stage the young trophozoite resembles the ring forms of *P. falciparum*. Pigment next appears, and is very intense, and deeply brown-black in colour; *P. praecox* is much more deeply pigmented than even *P. malariae*. As the parasite grows in the pole of the cell it displaces the nucleus of the red corpuscle so that the nucleus lies askew across the cell; a very characteristic feature of proteosoma infections, whereas in halteridium infections the parasites (gametocytes) develop along the lateral aspect of the nucleus of the red corpuscle. It is not uncommon in proteosoma infections to find a developing schizont in each pole of the same red corpuscle, with the nucleus lying askew between them.

The mature schizont rosettes are deeply pigmented and two different types are described; small schizonts with a diameter of about  $4\ \mu$  to  $5\ \mu$  with about 6 merozoites, and large schizonts occupying half the cell, with from 16 to 24 merozoites. The gametocytes are slightly elongate, ovoid bodies about the size of the largest schizonts. The pigment in them is irregularly distributed, and the female has a more deeply staining and more compact nucleus than that of the male. The infections in naturally infected birds are usually light ones, but very heavy infections can be produced by passaging from bird to bird. Experimental infections are apt to be very heavy in canaries, and are frequently fatal in these birds with marked hypertrophy of the liver and spleen. In experimentally infected birds the schizogony cycle appears to be somewhat irregular, but the infection reaches its height in from 8 to 10 days and thereafter declines. Relapses may occur, but are rare; Ben-Harel (1923) attributes them to a sudden revival of multiplication which has been proceeding at a low level throughout the afebrile period (*vide p.* 46). Laveran and Lucet

(1905) attributed to *P. præcox* a fatal epidemic which occurred amongst partridges imported to France from Hungary.

Transmission is by *Culex* mosquitoes, but the brothers Sargent (1907) and Neumann (1908) have demonstrated that the complete sporogony cycle can also occur in *Aedes argenteus* (*Stegomyia fasciata*), but that only 11.4 per cent of these mosquitoes when fed became infected. The optimum temperature for the sporogony cycle in *Culex pipiens* was found by Et. Sargent (1919) to be from 20°C. to 30°C., and the time for the cycle to develop depends very markedly on the temperature; if the fed mosquitoes are kept at from 17.5°C. to 24°C., sporozoites may not appear in the salivary glands until two months after feeding.

That the proteosomata of different species of birds all belong to the same species, *P. præcox*, was shewn by Ross (1898), Koch (1899, 1899a), Euge (1901), and Wasielewski (1908), who shewed that strains from one species of bird are readily inoculable into other species, and that mosquitoes fed on proteosoma of one species can infect other species of birds.

The plasmodia of lizards are still another group, and have been especially studied by Wenyon. Thus *Plasmodium agama* (Wenyon, 1909) occurs in the nucleated red blood corpuscles of the Sudan lizard, *Agama colonorum*. The fully formed schizonts are from 4  $\mu$  to 7  $\mu$  in diameter with up to six merozoites; the gametocytes are elongated pigmented bodies, about 14  $\mu$  in length by 4  $\mu$  in breadth. *Plasmodium mabuia* (Wenyon, 1909) is a parasite of another Sudan lizard, *Mabuia quinqueteniata*. The mature schizonts are about 5  $\mu$  in diameter, with up to six merozoites, and the gametocytes about 8.5  $\mu$  by 5.5  $\mu$ . *Plasmodium tropiduri* (Aragão and Neiva, 1909) occurs in the Brazilian lizard, *Tropidurus torquatus*, and closely resembles *P. præcox*. *Plasmodium diploglossi* (Aragão and Neiva, 1909) occurs in the snake lizard, *Diploglossus fasciatus*, of Rio de Janeiro. As the parasite grows in the nucleated red cell, it comes to completely encircle the nucleus, and as many as forty merozoites may be produced. The infected red cell becomes pale and enlarged. *Plasmodium minasense* (Carini and Rudolph, 1912) occurs in the Brazilian lizard *Mabuia agilis*, and has been found by Wenyon (1915) in the iguana of Trinidad. The schizonts are from 4  $\mu$  to 5  $\mu$  in diameter and produce four merozoites which are arranged either in a cruciform pattern or in a fan; the gametocytes are spherical or ovoid, or sometimes elongated, in which case they lie around the nucleus.

## CHAPTER III.

### PRACTICAL LABORATORY WORK AND DIAGNOSIS IN MALARIA.

#### *On the Selection and Use of a Microscope.*

THE student will be well advised to purchase his own microscope. No medical man can possibly carry on the practice of medicine in the tropics without the constant use of the microscope, since the diagnosis of quite the majority of tropical diseases depends upon the use of the microscope. The medical man who attempts to practise medicine in the tropics without using a microscope is like the captain of a ship who puts to sea without a compass, and a microscope is more essential to the medical man in the tropics than a stethoscope.

Of the many suitable models on the market, amongst those by English makers Beck's London Model 22 B can be recommended for its careful construction and durability in use. Other excellent English models are Swift's 'B. S. G. Standard,' Watson's 'H. model, Edinburgh Student's' microscope, and Baker's 'D. P. H.' microscope. Of continental models excellent types are Zeiss' 'Stand A. S. A. 16' and their 'C. C. E.' model, Leitz's 'Stand G,' and Reichart's 'Laboratory model B II (3)'; also the Winkel 'Stand I f M/29.' Among American models the Spencer Lens Co.'s 'Medical Model 44 H' is an admirable microscope, also their 'No. 15 medical model,' and Bausch and Lomb's 'F. F. S.' model. For dissection and adult mosquito identification work either a 'Greenhough' model binocular or an ordinary low power monocular lens dissecting microscope is required in addition.

In using the microscope the student must accustom himself to keeping both eyes open. At first outside images may be a little confusing, but a little patience and effort soon teaches one to disregard them. If difficulty is experienced in keeping both eyes open, a shade can be purchased to fit the draw-tube, or a substitute cut out of thick cardboard to shade the eye which is not at the microscope. Most recent microscope models are made to take a binocular eyepiece, and the use of such a binocular eyepiece is especially recommended. With binocular vision one gets a sense of depth and contour impossible with monocular vision, and structures such as protozoal cysts shew up much better. A binocular eyepiece for a monocular draw-tube can be purchased, but in the writer's experience the use of such a heavy

binocular eyepiece tends to wear the fine adjustment, and a microscope with a built-in binocular tube, such as Zeiss', is much better. A very admirable model is Leitz's 'A. A. B. M.' which has both monocular and binocular fittings.

The microscope must have an adjustable substage condenser with rack and pinion movement and an iris diaphragm. A mechanical stage is absolutely essential for work in malaria, and no decent work can be done without it. The writer would recommend the purchase of not only one but of two oil immersion lenses—the usual  $\frac{1}{2}$ th inch and a 'fluorite'  $\frac{1}{4}$ th inch oil immersion lenses. Also of one ocular of very low power, e.g., a No. 1, and of one high power ocular, e.g., a No. 12 or 15. With Nos. 1, 2, 4 and 12 or 15 oculars, and  $\frac{3}{4}$ rd,  $\frac{1}{4}$ th,  $\frac{1}{2}$ th inch oil immersion and  $\frac{1}{2}$ th inch oil immersion objectives, one can get an enormous range of magnifications. Thus in searching a blood film for malarial parasites one can cover ground very rapidly by using the  $\frac{1}{2}$ th inch oil immersion objective with a No. 1 eyepiece; if a parasite be found, the  $\frac{1}{2}$ th inch oil immersion objective may be turned on, or a higher eyepiece substituted. On the other hand, for seeing fine detail the  $\frac{1}{2}$ th inch oil immersion lens is essential.

Even in the tropics the writer far prefers artificial light to daylight for general work. One sees far and away more detail by artificial light than by daylight. Most microscope manufacturers turn out excellent small and cheap paraffin lamps for use with the microscope. A brilliant illumination is not necessary. The writer usually uses a Zeiss binocular microscope with Reichart's 'Star' microscope model of electric lamp.

Further, it is of the utmost importance that the student shall use the microscope intelligently. Modern microscopes of to-day are such refined instruments that the best results are only to be obtained by using them with a full understanding of their principles of optics and construction. A small handbook on microscopical methods will be found very useful; such as Shillington Scales' *Practical Microscopy* (London: Baillière, Tindall and Cox. Third Edition, 1926. Price 5s.).

The oil immersion objectives require to be cared for. After use, at the end of the day's work, the oil should be removed by carefully wiping them with a soft silk handkerchief or with special lens paper. It is permissible in doing so to moisten the handkerchief with a little xylol, but it is not permissible to dip the front of the lens into xylol—as one sometimes sees a student do—as this will loosen the front lens of the objective and ruin the lens. In using the oil immersion lens, the writer's custom is to lower the lens until it just touches the oil, watching the operation from one side as one does so; then to focus downwards carefully, first with the coarse and then with the fine adjustment. If the objective touches the slide, it should on no account be forced downwards as this will break the slide and may ruin the objective. Forcing the fine adjustment when it has come to the end of its movement is another fault which the student should not commit.

The dry  $\frac{1}{8}$ th inch objective is a lens which is very useful for histological work but for protozoological work the  $\frac{1}{4}$ th inch oil immersion objective is far better. It gives one almost the same working distance, much more definition, and more critical illumination.

The microscope must always be used with its draw-tube drawn out to the correct tube length. For Leitz, Zeiss and Winkel microscopes this is 160 mm. ; i.e., the tube should be drawn out to the 16 mark ; for most British models, such as Beck's, it is 170 mm.

The commonest mistakes made by students who are unaccustomed to the use of a microscope are as follows \* :—

(1) The improper use of the iris diaphragm. It is almost better for the student at first not to use this part of his microscope, because light can be as easily cut down by racking down the condenser, although from an optical point of view this method is slightly inferior. In the East, where everything mechanical is done in the opposite way to the West, students in order to open the diaphragm pull the lever towards them, with the result that the iris diaphragm becomes closed. They then try to force it still further, with the result that the vanes get notched and distorted and the diaphragm becomes jammed.

(2) Improper use of the fine adjustment. In every microscope there are two lines and an arrow on the side near the fine adjustment. This gives the working distance of the fine adjustment, and instead of continually using the fine adjustment for focussing any considerable distance, the larger distances should be traversed by using the coarse adjustment. The student should get accustomed to almost getting a sharp focus with the coarse adjustment, and then using the fine adjustment for finally rendering the image clear and sharp. When the thread of the fine adjustment comes to the end of its limit, a student will often then try to force it still further and consequently damage its mechanism.

(3) Some students use traction on the mirror and pull the microscope about by it, instead of using the handle on the body of the microscope, which is built for that purpose, with the result that the mirror is torn away from its holder.

(4) If a triple nosepiece is on the microscope, it is important to mount the objectives in a fixed order and to stick to that order. Screw in the  $\frac{2}{3}$ rd inch,  $\frac{1}{4}$ th inch and  $\frac{1}{2}$ th inch oil immersion lenses into place in counter-clockwise order and keep them in that order. One then knows automatically that the lens after the  $\frac{2}{3}$ rd inch is the  $\frac{1}{4}$ th inch and then comes the  $\frac{1}{2}$ th inch oil immersion lens. One has known students by not adhering to this rule mistake the  $\frac{1}{2}$ th inch oil immersion lens for the  $\frac{1}{4}$ th inch, try to use it as a dry lens, fail to get a focus, and finally jam it against the slide. They may then try to force it on further, with the result that the slide breaks, and the front lens of the oil immersion lens

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\* The following paragraphs are from notes very kindly supplied by Lieut.-Col. H. W. Acton, I.M.S.

may be dislocated. It is very bad microscopy to ever break a slide with the oil immersion lens.

(5) For unstained objects the microscope should be set in the vertical position, with the draw-tube drawn out to its correct length, the substage condenser racked well down, and the concave mirror in use. For stained specimens such as blood films the microscope should be set tilted to a comfortable working angle, the condenser should be racked right up as far as it will go, the iris diaphragm be fully opened, and the plane mirror used. The student should get into the habit of automatically seeing to these points according to the character of the work in hand. In sitting down to examine stained blood films, he should automatically see that the draw-tube is out to its proper length, the condenser racked up, the iris open, and the plane surface of the mirror in position.

In a previous publication the writer mentioned the case of a veterinary officer whom he found using or trying to use the  $\frac{1}{4}$ th inch dry objective with immersion oil. Since writing that he has seen two qualified medical men—post-graduate students—make the same mistake; and has received a letter from a veterinary surgeon stating that he has seen a medical man try to use cedar-wood oil by removing the eyepiece and pouring the oil down into the tube of the microscope!

#### *Slides and Cover Slips.*

No decent work can be done in malaria unless slides and cover slips are most scrupulously clean and polished. Slides and cover slips not kept in alcohol in the tropics are very liable to become frosted, and such 'frosted' glass cannot be used. This especially applies to Japanese slides and cover slips.

When received from the maker, slides should be placed in a saturated solution of carbonate of soda, brought to the boil, then well washed in running water and wiped with a soft linen rag; they are then transferred to 50 per cent sulphuric acid for a few minutes, again washed in running water, and the excess of water drained off. They are then placed in glass-stoppered bottles containing rectified spirit. Before use they are taken out of the spirit, dried, cleaned and polished. For this purpose the writer uses old much-washed linen handkerchiefs, though perhaps a silk handkerchief is better. If the glass surface has been properly freed from grease, a drop of water will spread out evenly over the entire surface. If there is any grease on the slide it can be quickly got rid of by passing the slide three times through the flame of a Bunsen burner. To remove the last trace of grease Coles recommends rubbing the slide with whiting—a mixture of pulv. cræta preparata and water, and then polishing.

Cover slips received in the dry state from the makers in the tropics are very liable to be 'frosted.' Cover slips in the tropics should on no account be kept in the dry state. Those sold packed in oil of cloves are best, and for ordinary work No. 0 or No. 1 thicknesses are best. On receipt of the cover slips they should be

placed in 1 per cent lysol solution for an hour to remove the oil of cloves ; then transferred to a saturated solution of carbonate of soda in which they are brought to the boil, washed in running water, placed for 6 to 8 hours in 50 per cent sulphuric acid, again very thoroughly washed in water and stored in spirit in wide-mouthed glass-stoppered bottles.

#### *To Clean Old Slides.*

There are several methods by which used glass slides can be cleaned.

1. A fluid very commonly employed consists of 1 part each of potassium bichromate and commercial sulphuric acid with 10 parts of water. Slides are left to soak in this indefinitely ; they are then taken out, washed very thoroughly in running water, and transferred to spirit. The solution is very corrosive however.
2. An alternative is to leave the slides overnight in a mixture of 2 parts of glacial acetic acid to 100 parts of rectified spirit, and next day to wash them well and transfer to spirit.
3. Archibald recommends boiling dirty slides for one minute in a solution consisting of 6 parts per cent of sodium hydroxide and 2 per cent calcium carbonate. Then rinse in cold water and transfer to alcohol.
4. The Indian laboratory attendant is very fond of cleaning used slides by rubbing them under a running tap with *saja mutti* (crude carbonate of soda) full of gritty particles. This scratches the surface and ruins the slide. It should not be permitted.
5. A cheap and efficient method is to boil the slides in a solution of soft soap in water and then to thoroughly cleanse in running water.

#### MATERIALS.

##### *Preparation of Blood Films.*

If a knowledge of how to use his microscope is essential to the medical man in the tropics, a knowledge of how to properly prepare and stain a blood film is equally essential. The majority of blood films received in the laboratory, however, are badly prepared, and one can only conclude that the majority of medical men have not been taught this essential lesson.

Proceed as follows :—

1. Clean three or four slides. They must be spotlessly clean and polished. If any grease is present, pass through the flame of a Bunsen burner. Do not use old scratched slides. For cleaning an old much-washed linen handkerchief or a silk cloth is best. Lay the slides down flat on a clean piece of paper.

2. Select a slide with a smooth even end, as tested by passing the edge of the end of the slide across the palmar surface of the finger. This is the spreader. One corner of it may be cut off, as shewn in Fig. 14.

3. Prick the patient's finger. An ordinary hypodermic needle makes a good pricker, and must, of course, be sterilised by flaming before use. There is no necessity to swab the patient's finger with alcohol, ether, etc., beforehand; as long as the needle is sterile that is all that matters. The best place to prick is not on the palmar aspect of the finger, but on the dorsum just below the root of the nail.

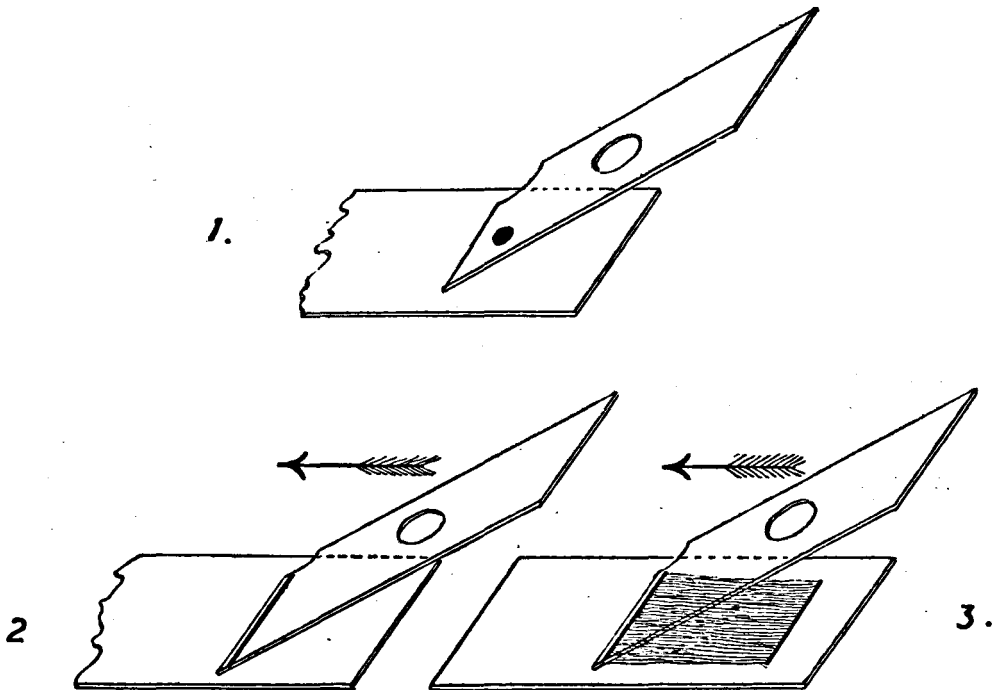


FIG. 14.—To illustrate the method of preparing a thin blood film. The blood should *follow* the spreader, and not be pushed before it.

4. Picking up one of the clean slides, invert it, and towards one end *just* bring it into contact with the oozing blood. Too large a drop must not be taken. Reinvert it on the table, and with the spreader held at an angle of  $45^{\circ}$ , touch the drop, so that a thin film of blood runs between the edge of the spreader and slide. Next draw the film; the blood must *follow* the spreader and not be pushed before it, (as the latter procedure distorts the cells). Then apply a little tincture of iodine to the patient's finger.



5. Allow the film to dry in air. It is best, in order to preserve the film from flies, dust, etc., to cover it whilst it is drying with a Petri dish. Drying is usually only a matter of a minute or so, but in wet weather may take longer. During the rains, hæmolysis often occurs before drying and the film is useless. In field work, under such conditions the only way is to hold the film *as soon as spread* over a spirit lamp until dry.

#### *Leishman's Stain for Blood Films.*

Leishman's stain is both a combined fixative and stain. The preparation and use of the stain, however, require the closest attention to details if good results are to be obtained. The best stain is either Burroughs Wellcome and Co.'s 'Soloid' Leishman's stain, which is very suitable for laboratories with only occasional films to examine; or Grüber's or Merck's powder for larger laboratories. (There seems to be a little difference in the keeping properties of 'Soloid' Leishman's stain. Fresh 'Soloids' give an excellent stain, but old 'Soloids' may give a very inferior stain.) The very purest methyl alcohol, free from any trace of acetone, must be used; preferably Merck's.

To prepare a small bottle of stain from 'Soloid' Leishman's stain. Take a 30 c.c. drop bottle; clean it out very thoroughly; dry it; and then rinse it out with a little pure methyl alcohol. Into it drop two of the 0.015 gm. 'Soloids'; add 20 c.c. of methyl alcohol; turn the stopper so that no drops can come out; and shake until every particle of the stain has gone into solution.

To prepare Leishman's stain in bulk. Take a glass (not porcelain) pestle and mortar. Clean them absolutely and rinse the mortar out with a little methyl alcohol. Weigh out 0.15 gm. of Leishman's stain and put it into the mortar. Measure in a cylinder 100 c.c. of pure methyl alcohol. Add a little of the alcohol to the stain in the mortar and grind. Add more of it and grind. Drain off the stain into a stoppered bottle. Continue adding the alcohol in portions and grinding until *every* particle of the stain has gone into solution. *This* is the most essential step of the whole procedure.

Next *incubate* the stain. Place the bottle of stain overnight in the 37° incubator. This causes the stain to ripen and greatly improves results, whilst it also ensures complete solution of every particle of stain. Do not leave the stain in the incubator, however, for more than 24 hours.

Every step in this process must be carried out with meticulous care. In many laboratories in India Leishman's stain is prepared from bad materials, or not properly prepared, with bad results. If properly prepared, the stain will keep for two weeks or longer in the plains.

*To Use Leishman's Stain.*

1. Lay the blood film, film surface upwards, on a staining rack. (A convenient way to make such a rack is to fix two pieces of glass tubing parallel with one another across a basin with plasticine. This answers very well where large numbers of films have to be stained, and avoids mess.) See that the rack is dead level.

2. From the drop bottle, drop Leishman's stain on to the slide until its whole surface is covered with the stain. The methyl alcohol in the stain fixes the film. This takes half a minute only, and the readiest way of estimating that time is to count twenty slowly mentally.

3. After the lapse of half a minute drop on to the slide double the corresponding number of drops of pure distilled water; (the water *must* be distilled water). By tilting the end of the slide, allow the stain and water to mix thoroughly. Stain for five minutes; (many authors recommend ten minutes or longer, but in the writer's experience, five minutes is usually ample with a well prepared sample of stain). If the stain has been properly prepared, a golden scum will rise to the surface of the fluid, and unless this happens the result will probably be bad.

4. Flush off the stain from the slide with distilled water. (The Romanowsky stains should never be drained off a slide, owing to the fear of deposit of the stain occurring. The stain should be flushed off the slide by immersing it in distilled water.) Place it in a Petri dish containing fresh distilled water and gently rock the dish. The film turns at first greenish-blue, then pink. This takes about one minute. When the film is turning pink, remove the slide.

5. It is always better not to blot slides, as all sorts of foreign bodies may get into slides from the use of dirty filter paper. The best plan is to lean the slide end-on against a vertical wall with its film surface downwards and to let it dry of itself.

6. Examine with the oil immersion lens. For rapid work it is good practice to search the slide with a  $\frac{1}{4}$ th inch 'fluorite' immersion lens and a No. 2 or 4 ocular; if a suspicious object be found, a higher ocular can be used to determine whether it is a parasite or not. An alternative, recommended by Wenyon, is to cover the whole surface of the film with a layer of cedar-wood oil, and to examine it with the  $\frac{1}{4}$ th inch dry objective. If an object believed to be a malarial parasite is encountered, the  $\frac{1}{2}$ th inch oil immersion objective can then be substituted. One may, however, miss small rings of *P. falciparum* when using the  $\frac{1}{4}$ th inch or  $\frac{1}{2}$ th inch objectives.

*Certain Difficulties with Leishman's Stain.*

1. *Deposit on the Slide.*—This is especially apt to occur if the film has been stained for too long, or if there has been too long a delay in adding the distilled water to the stain. To remove it, first remove the cedar-wood oil from the slide

with xylol ; then let the film dry completely. Flush the slide for an instant or two only with rectified spirit, and immediately immerse in distilled water to remove the spirit. This will usually clear the slide of deposit, but the flushing with spirit must be very rapid, in order to avoid decolourising the film. Or stain a fresh film from the case:

The use of a rocker upon which to rest the slide whilst staining will avoid deposit. There are several patterns of such rockers on the market, or a home made one can easily be made.

2. *Staining too blue.*—After removing the oil as before, immerse for a few seconds in 1 in 5,000 acetic acid in water and then wash thoroughly in distilled water.

3. Deeper staining can often be obtained if 1 in 2,000 watery potassium carbonate solution be used instead of the distilled water to dilute the stain with.

4. The water used to *dilute* the stain with *must* be distilled water. But in Calcutta and Bombay, ordinary tap water may be used for washing and differentiating the stain. In small *mofussil* stations however, the water supply is usually not suitable for this purpose and distilled water (or collected rain water) should be used.

#### *Giemsa's Stain for Blood Films.*

Giemsa's stain is probably preferable for general use to Leishman's, as it has the great advantage that the slide may be stained for a prolonged period without fear that deposit will form on it ; and it can be especially recommended to laboratory workers who experience trouble with Leishman's stain. The stain may be prepared as follows :—In a glass mortar with a glass pestle grind 3 grammes of azur-II-eosin and 0·8 gramme of azur-II into thorough solution in 250 c.c. of the purest anhydrous glycerine. Add 250 c.c. of the purest acetone-free methyl alcohol, and mix thoroughly. Allow to stand overnight and next day filter the stain through filter paper. It is then ready for use. The reagents used must be absolutely reliable and Grüber's or Merck's are the best. The methyl alcohol must be chemically pure and free from even the slightest trace of acetone, and the glycerine also chemically pure. Prepared Giemsa's stain however, is stocked by most of the large chemical firms in India, whilst the writer has for years used the ready prepared stain as issued by the Central Research Institute, Kasauli, and has always found it to give excellent results, much better than any 'shop' sample tried.

#### **In using the stain :—**

1. **First fix the film.** This may be done by covering it for 3 to 5 minutes with pure methyl alcohol, or by dipping it for 10 minutes into ordinary absolute ethyl alcohol. Then wash thoroughly.

2. Dilute the Giemsa's stain 1 part with 14 parts of distilled water. This is most readily done by measuring out 10 or 15 c.c. of distilled water and dropping into it the corresponding number of drops from a drop bottle of the stain.

3. Place the slide in a Petri dish, and flood with the stain. Several authors recommend that the slide should be stained in the inverted position resting upside down on two glass rods, but this is hardly necessary. Stain for half an hour or longer. The longer the staining, the better the result, and if desired the film may be left to stain for an hour or two. Or the stain may be made even more dilute and the film stained overnight, the Petri dish being covered to prevent evaporation.

4. Remove the slide from the staining bath; flush it with distilled water. Then soak it in a bath of fresh distilled water until it commences to turn pink. At this stage, remove the slide and place it to dry by leaning it against a vertical surface. Do not blot.

Deeper staining with Giemsa's stain may be accomplished by using a 1 in 1,000 solution of potassium carbonate to dilute the stain with instead of distilled water.

#### *The Panoptic Method of Staining.*

This gives very pretty pictures. Proceed as follows:—

1. Lay the slide on a staining rack and cover it for half a minute with undiluted Leishman's stain.

2. Dilute the Leishman's stain with double the number of drops of distilled water and allow to stain for 5 to 10 minutes. Wash the film with distilled water, and then

3. Lay the slide in a Petri dish and flood with diluted Giemsa's stain, one drop to each c.c. of water. Stain for one to twenty-four hours, covering the Petri dish to prevent evaporation.

4. Wash the film with distilled water and transfer it to a bath of 1 : 1,000 acetic acid. This commences to remove the blue colour in the film. At the instant when the film is turning pink, remove, wash rapidly with distilled water, and slant the slides against a vertical surface to dry.

An alternative and more rapid method is as follows:—

1. Cover the film with undiluted Leishman's stain for half a minute.

2. Dilute the stain with double the volume of diluted Giemsa's stain, one drop to each c.c., and mix thoroughly. Stain for 10 minutes.

3. Differentiate in distilled water as with Leishman's stain.

#### *The Original Romanowsky Stain.*

Both Leishman's and Giemsa's stain are somewhat expensive, and in large laboratories where many slides have to be stained every day, the original

Romanowsky method is more economical, and if properly used gives good results. It is specially recommended by Colonel Christophers.

Two stock solutions are needed.

*Solution A.* Medicinal (not pure) methylene blue 1 gramme.  
 Pure sodium carbonate .. 0.5 gramme.  
 Distilled water .. .. 100 c.c.

Dissolve and place the bottle containing the stain either in the 37°C. incubator or in full sunlight for two or three days. The solution acquires a deep purple colour which will be seen at the edges of the liquid, and it is then ready for use but not before.

*Solution B.* Eosin, extra B. A. water-soluble .. 1 gramme.  
 Distilled water .. .. 1,000 c.c.

For staining

1. Fix the film either by five minutes' exposure to methyl alcohol or for ten minutes in ordinary absolute alcohol. Wash and transfer to a Petri dish.
2. Dilute solution A : 1 part with 19 parts of distilled water. Also dilute solution B : 1 part with 19 parts of distilled water. Mix equal parts (about 4 c.c.) of diluted solution A with diluted solution B, and pour the mixed stain into the Petri dish to cover the slide. Do not let the mixed stains stand a moment before pouring on to the slides.
3. Stain for half an hour or longer. On tilting the Petri dish a red stain should be seen at the sides ; this indicates that the staining is proceeding properly.
4. Flush off the excess of stain with distilled water and differentiate in a bath of distilled water as with Leishman's and Giemsa's methods. When the film commences to turn pink, remove from the bath and allow it to dry by slanting it against a vertical surface.

#### *The Romanowsky Stains.*

Leishman's and Giemsa's and all other modifications of the original Romanowsky stain depend for their staining properties on certain loose chemical combinations. Medicinal (not pure) methylene blue contains a number of oxidation products, the most important of which is methylene-azur. When watery or alcoholic solutions of medicinal methylene blue and of eosin are mixed together a series of loosely combined chemical bodies are formed such as methylene blue-eosinate and methylene-azur eosinate, etc. These different compounds possess different affinities for different cell structures, and thus differential staining results. The red blood corpuscles stain a transparent pink or orange colour ; the nuclei of leucocytes, shades of violet ; eosinophile granules in the coarsely granular leucocytes, red ; neutrophile granules in the polymorphonuclear leucocytes, yellow to lilac ; mast

cell granules deep violet; blood platelets, purplish; the cytoplasm of malarial and other blood-inhabiting protozoal parasites a bright 'Cambridge' blue; and their chromatin a bright ruby red.

In all the Romanowsky methods it is very important that the distilled water used for diluting the stain shall be free from carbon dioxide, as fresh as possible, and of neutral reaction. To test the distilled water take 5 c.c. of it in a test tube and add to it 5 drops of a 0.04 per cent solution of bromo-cresol purple. If the water is acid a pale yellow solution will be formed, if it is alkaline it will be purple. The reaction of the water should be such that it gives a purple colour, which is turned to yellow by the slightest trace of acid. If the water is acid, take 100 c.c. of it, add 100 drops of a 0.04 per cent solution of bromo-cresol purple, and it will turn yellow; now add cautiously drop by drop a 1 in 1,000 solution of sodium carbonate till a purple colour appears. Excess of soda solution must not be used. The coloured water may now be used for diluting the stain without ill-effects.

'The best results with the Romanowsky stains are obtained with newly-prepared films. Blood films in the tropics, if not stained within a day or two of making, begin to deteriorate. With increasing age the staining becomes more and more defective, and although methods have been devised for rejuvenating old films, none of them gives the results which can be obtained by staining them directly after they are made. The defective staining of old films can be remedied to some extent by extracting the blue by treating the stained film with a 1 per cent solution of acid sodium phosphate' (Wenyon, 1926, p. 1319).

As a rule the stained films are examined as soon as they are dry. Films stained by the Romanowsky methods fade fairly rapidly with storage. In order to make permanent preparations, several authors recommend taking the stained film up through graded alcohols to xylol and mounting in 'neutral' Canada balsam. The writer has never had any success with this method, since however rapidly one passes through the alcohols a great deal of the stain is dissolved out, whilst he has never yet seen a truly neutral xylol solution of Canada balsam. A much better method is to let the stained film dry in the 37°C. incubator, and then cover it with either 'Euparal'—a mounting medium prepared by Flatters and Garnett, Ltd., 309, Oxford Road, Manchester; or with Gurr's Neutral Mounting Medium—prepared by G. T. Gurr, 136, New King Road, Fulham, London, S. W. 6—and then place on it a perfectly clean long cover slip 2 inches  $\times$  1 inch. Such preparations will keep without fading for at least six months, possibly for a year.

It is essential that the methyl alcohol used in all the Romanowsky methods shall be absolutely pure and free from any trace of acetone.

Old and faded stained blood films are very difficult to re-stain. The writer has tried many different methods, but none with any marked success. The following method is given by Daniels. Treat the slide for a few minutes before staining with a mixture of absolute alcohol 1 oz. and 3 to 5 drops of glacial acetic acid. Then

wash in distilled water and stain with Leishman's or Giemsa's stain in the usual manner. Old blood films take on a deep blue staining of the red blood corpuscles instead of the normal orange-pink. To some extent this can be got rid of by flooding the slide with a 1 per cent solution of acid sodium phosphate after staining, but the results are never too good.

#### *The Use of Thick Films.*

The use of thick films for the detection of malarial parasites is to be very strongly advocated. James states that if only one thin blood film be examined from patients with malaria, parasites will be detected in only 40 per cent of cases. By the use of thick films, however, they are but rarely missed, and the writer's own procedure is to take three thin films and one thick one from every suspected case of malaria. The thin films are stained and examined whilst the thick film is drying, and if no parasites are detected in the thin films, the thick film is then proceeded with. The thick film method is especially applicable to those all too frequent cases in which the patient has taken a small dose of quinine before the blood is examined. Throughout India classes in first aid for laymen are held in almost every large town or cantonment, at which they are taught how to splint fractures. It would be far more to the point if they were taught how to take a thin and a thick blood film and send them to a laboratory. What happens when the European or the educated Indian gets fever is usually that he takes a small dose of about 5 grains of quinine and then sends for the doctor. This dose is sufficient to make it impossible to find parasites in thin blood films, but wholly insufficient to control the fever. It is especially in such cases that the thick film method is useful, and the laboratory worker should thoroughly master the technique.

There are several different thick film methods. The following is advocated by Knowles and Das Gupta (1924).

1. The slide used for the thick film must be perfectly clean and polished and free from every trace of grease. The patient's finger is pricked and the glass slide—held upside down—is brought into contact with the issuing blood so that four drops are placed at the corners of a small square about half of an inch in diameter, as shewn in Fig. 15 after James. The four drops of blood should not be too large nor too small. With a round needle next pool the four drops so as to spread the blood into an even thick film covering the half inch square. Puddling should be avoided, and the film must not be made too thick (Fig. 15).

2. This film takes two hours to dry at room temperature, or one hour in the 37°C. incubator. If the film be taken in the laboratory, cover it with a Petri dish whilst it is drying in order to keep flies and dust away from it. If at a patient's house, leave the film covered with a saucer, and give instructions that

it is to be sent to the laboratory two hours or more later. If possible, it is best to take the thick film one day and stain it the next in order to make certain that it has dried.

3. Lay the film on a staining rack and gently flood the slide with the following mixture :—

Glacial acetic acid ; 2·5 per cent solution in distilled water . . 4 parts.

Tartaric acid, crystalline ; 2 per cent solution in distilled water . . 1 part.

This mixture keeps indefinitely, and it is better to keep the two solutions mixed, as fungi are apt to grow in the tartaric acid solution. It should be kept in a stoppered bottle.

This solution de-hæmoglobinises the film, and the process should be watched. An ordinary thick film quickly de-hæmoglobinises, but films with thicker patches in them may require a little longer. The de-hæmoglobinised film should shew a grey-white colour.

4. As soon as de-hæmoglobinisation is complete, drain off the fluid by gently tilting the slide. Next flood the slide with methyl alcohol, and allow it to remain on for five minutes. The film is now de-hæmoglobinised and fixed.

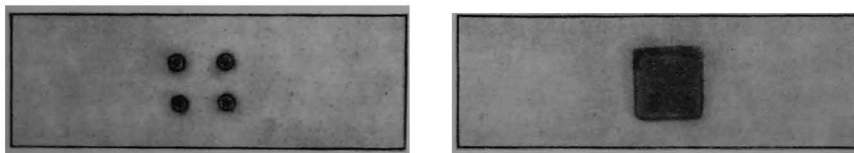


FIG. 15.—Method of preparing a thick blood film. (After James, 1920.)

5. Drain off the methyl alcohol and wash the film *very thoroughly* in distilled water. Every trace of acid must be removed from it.

6. Stain the film with Giemsa's stain, one drop to each c.c. of water, for 15 minutes or longer. Differentiate in the usual way with distilled water. Do not blot the film, but let it dry by slanting it against a vertical surface.

If careful attention be paid to details in this technique, the results are excellent. Further, in cases of kala-azar it gives some 67 per cent of positive findings of parasites in the peripheral blood, and is very helpful in differentiating between kala-azar and malaria. Leishmania parasites are not plasmolysed by this method, as they are with some other thick film methods ; they stain well and shew up clearly.

The film should be examined when dry with the  $\frac{1}{2}$  inch oil immersion objective and a No. 6 or so ocular. The red blood corpuscles are de-hæmoglobinised and the parasites shew up well. Occasionally it may be a little difficult to be certain which species of malarial parasites is present, but a further examination of the slide will usually settle the diagnosis.



For survey work in the field Sinton (1925) advocates the following and most ingenious method.

1. A small drop of blood is placed about half an inch from the end of a clean glass slide, and a second drop about one inch from the same end. The second drop is now spread into a thin film towards the opposite end of the slide in the usual manner, and the first drop is made into a thick smear of about  $\frac{1}{2}$  inch in diameter. The thick film should not be made too thick. The slide is next covered to protect it from dust and flies, and the film allowed to dry.

2. In order to label, write the patient's name and date on the thin film with a soft pencil or with the point of a pin or needle.

3. Immerse the part of the slide containing the thin film in a staining jar of methyl alcohol to such a depth that the alcohol does not come within three-quarters of an inch of the thick drop, because any alcohol on this drop would fix the red cells and prevent their de-hæmoglobinisation.

4. Withdraw the slide when fixed and place it upright, so that all the alcohol drains off without coming into contact with the thick film. Next allow the slide to dry.

5. Gently flood the slide with diluted Giemsa's stain and allow it to act from 10 minutes to 2 hours, as preferred.

6. When stained gently flush the stain off with distilled water and differentiate with distilled water. Do not blot the film but allow it to dry by slanting it against a vertical surface.

If a line is drawn across the slide with a grease pencil between the thick and thin films Leishman's stain can be used instead of Giemsa's. The undiluted Leishman's stain is allowed to act on the thin film for half a minute; it is then diluted with water, and the diluted stain drawn across the grease pencil line, where it is allowed to act for the usual length of time.

When the slide is dry both thin and thick films from the patient are ready for examination on the same slide. The value of this method in survey work is shewn in the following table from Sinton.

	<i>P. vivax.</i>		<i>P. falciparum.</i>	
	Thin films.	Thick films.	Thin films.	Thick films.
Parasites in 100 fields .. ..	1	4	1	16
Parasites found in 2 minutes ..	1	7.7	1	22
Time required to find parasites ..	6.6	1	23	1

For many years the writer relied upon prolonged examination of thin films in difficult cases of malaria where parasites were scanty ; but he has now abandoned this procedure in favour of the thick film method. In the preparation of thick films, however, it is of the utmost importance that the technique shall be irreproachable, since if this is not the case all sorts of difficulties may be encountered. The distilled water—if not perfectly fresh—may become contaminated with free-living *Bodo* or even ciliate protozoa from the atmosphere, and these may get into the film when staining it and be mistaken for malarial parasites. If the thick film be not covered whilst it is drying, protozoa from the atmosphere or yeasts may drop into it, be stained, and be mistaken on examination for parasites. Flies if they get at either a thin or a thick blood film will eat a large portion of it, deposit faeces on the film which may contain herpetomonads, and these also be mistaken for malarial parasites.

#### *Normal Cells of the Blood.*

Before commencing to examine blood films for malarial parasites it is absolutely essential for the student to be able to identify with certainty all the normal and abnormal cells which he may come across in blood films, in order that he may not mistake them for parasites. Hence a short note may be given on these cells, commencing with the cells normally encountered in blood films (Plate IV).

1. *The Red Blood Corpuscles.*—The colour of these after staining with the Romanowsky stains varies with the degree of differentiation in distilled water. In slides which have only been washed for a few seconds they are a bluish-green, but in properly differentiated films they are orange to pink. They are round or almost round biconcave discs having an average diameter of 7·5  $\mu$ .

2. *The Blood Platelets.*—These vary in size, and are usually from 1  $\mu$  to 3  $\mu$  in diameter, but may be up to one-third of the diameter of a red blood corpuscle. In the unshed blood the platelets have a definite capsule of their own, and this is particularly well seen in the corresponding thrombocytes of bird's blood. Very occasionally if a human blood film be taken and instantly stained, almost before it has time to dry, the capsule will be seen. As a rule, however, the platelets commence to disintegrate immediately the blood is shed, and they usually show an irregular, somewhat crenated outline. They are nearly always found in clusters of from five to fifty and stain a purplish-blue, the central part staining more deeply than the periphery. They frequently show several small filamentous projections from their margin, and a much drawn out platelet may simulate a flagellate protozoon. An isolated platelet lying on top of a red blood corpuscle is often mistaken for a malarial parasite, though it looks nothing like a parasite in reality. In such cases a pale halo area will be seen around the platelet.

3. *The Coarsely Granular Eosinophile Leucocyte.*—This cell is a most unmistakable one. It is about 12  $\mu$  in diameter, and the nucleus is bilobed, or very

occasionally trilobed. Frequently the nucleus consists of two more or less spherical masses of chromatin connected together by a thin strand of chromatin. The cytoplasm of the cell is stuffed full of large, deeply brick-red staining granules. It is this cell whose numbers are so greatly increased in ankylostomiasis and other helminthic infections, and in asthma and filariasis. The granules are probably of pre-enzyme nature, and this leucocyte is probably capable of digesting proteids and converting them into amino-acids, etc.

4. *The Polymorphonuclear Leucocyte.*—This cell is about  $10\ \mu$  in diameter and has an irregularly shaped and lobed nucleus, usually trilobed. The cytoplasm stains a very faint blue and is thickly dusted with fine, small, faintly pink staining neutrophile granules. This cell is increased in numbers in all conditions of sepsis and acute inflammatory states.

5. *The Mast Cell.*—This is a coarsely granular basophile cell. It is about the size of a polymorphonuclear leucocyte, and has a bilobed, sometimes trilobed, nucleus. Its cytoplasm contains numerous large granules which stain a deep blue to black colour. So deeply do the basophile granules stain that they often obscure the nucleus.

6. *The Finely Granular Basophile Leucocyte.*—This cell is very rare. It has a frayed out, bilobed or trilobed nucleus, and its cytoplasm is dusted with very small, fine granules which take a blue stain.

7. *The Small Lymphocyte.*—This cell is about the same diameter as the red corpuscle. It has a rounded or spherical nucleus which stains most intensely purple and occupies almost the entire cell, leaving around it only a narrow rim of rather deeply blue staining cytoplasm. In infants these cells may constitute 50 per cent of the total leucocytes, and this cell is also present in the blood in enormous numbers in lymphatic leukæmia.

8. *The Large Lymphocyte.*—A cell which in general resembles No. 7, but is larger. It has a round or sometimes oval nucleus which stains very deeply, and a considerable volume of rather pale blue-staining cytoplasm which frequently shows a few purple-staining granules. This cell is especially well seen in blood films from patients with typhoid fever.

9. *The Large Hyaline Mononuclear Leucocyte.*—This cell is of special importance in malaria, as it is greatly increased in numbers in that disease; also it phagocytoses the hæmozoin pigment of the parasites. It is a cell up to  $14\ \mu$  in diameter, about one and a half or twice the size of the small lymphocyte, with a nucleus which is large, oval, excentric in position in the cell, and much looser and more open in texture than the nucleus of the lymphocyte. The nucleus stains much less intensely than does the nucleus of the lymphocyte, and it is indented, notched, or partly bilobed. The volume of cytoplasm is considerable and stains a clear, pale blue colour. The cytoplasm often contains a few scanty azure pink-staining granules.

PLATE V.



PLATE V.

Normal and Abnormal Blood Cells, etc.

(Partly original and partly from Schleip's *Hæmatological Atlas*, 1920; Panton's *Clinical Pathology*, 1913; and Byam and Archibald's *Practice of Medicine in the Tropics*, 1921.)

- |           |     |   |
|-----------|-----|---|
| Fig.      | 1.  | Normal red blood corpuscle.   |
| "         | 2.  | Blood platelets.  |
| "         | 3.  | Coarsely granular eosinophile leucocyte.                                    |
| "         | 4.  | The same, ruptured.   |
| "         | 5.  | Polymorphonuclear leucocyte.  |
| "         | 6.  | The same, ruptured.   |
| "         | 7.  | Coarsely granular basophile leucocyte, or mast cell                         |
| "         | 8.  | Finely granular basophile leucocyte.  |
| "         | 9.  | Small lymphocyte.   |
| "         | 10. | Large lymphocyte.   |
| "         | 11. | Large hyaline mononuclear leucocyte.  |
| "         | 12. | The same, ruptured.   |
| "         | 13. | Large hyaline mononuclear leucocyte, containing malarial hæmazonin pigment. |
| "         | 14. | The same.   |
| "         | 15. | Transitional mononuclear leucocyte.   |
| "         | 16. | Poikilocytosis.   |
| "         | 17. | Microcytes.   |
| "         | 18. | Macrocyte.  |
| "         | 19. | Achromia.   |
| "         | 20. | Polychromatophilia.   |
| "         | 21. | Punctate basophilia.  |
| "         | 22. | Diffuse basophilia.   |
| "         | 23. | Denilune red blood corpuscles.  |
| "         | 24. | Howell-Jolly body.  |
| "         | 25. | Chromatin dust in red blood corpuscle                                       |
| "         | 26. | Cabot's rings.  |
| "         | 27. | Normoblast.   |
| "         | 28. | Megaloblast.  |
| "         | 29. | Myeloblast.   |
| "         | 30. | Neutrophile myelocyte.  |
| "         | 31. | Eosinophile myelocyte.  |
| "         | 32. | Basophile myelocyte.  |
| "         | 33. | Endothelial cell.   |
| "         | 34. | Türck cell.   |
| "         | 35. | Lymphoblast.  |
| "         | 36. | Rieder's cell.  |
| Figs. 37— | 38. | Platelets lying on red corpuscles.  |
| Fig.      | 39. | X-bodies of Horrocks and Howell (1908).                                     |
| "         | 40. | Distorted platelets.  |
| "         | 41. | Deposit of stain on a red corpuscle.  |
| "         | 42. | Vacuolated red corpuscle.   |
| "         | 43. | Yeasts on the slide.  |
| "         | 44. | A protozoon on the slide from an extraneous source.                         |
| "         | 45. | Pessary bodies, from destroyed red corpuscles.                              |

When this cell is ruptured as it often is in spreading blood films, its cytoplasm is lost, and the loose open nucleus is ruptured and frays out into a basket-like network staining rather faintly a dirty red colour. A similar disruption may occur in old polymorphonuclear leucocytes, leading to a similar but smaller 'basket-cell' appearance.

10. *The Transitional Mononuclear Leucocyte*.—This resembles No. 9, but the nucleus is more indented and not unlike that of the polymorphonuclear leucocyte. The nucleus is frequently horse-shoe shaped and takes a washed out violet shade of less intensity than that of the large hyaline mononuclear. This cell is also frequently ruptured in spreading blood films, giving rise to a 'basket-cell' appearance. For all practical purposes the transitional leucocyte may be included with the large hyaline mononuclear leucocyte.

#### *Abnormal Blood Cells.*

Turning first to abnormal red blood corpuscles, the red corpuscle may vary in shape, in size, and in its staining reactions. It may be distorted in shape—*poikilocytosis*; or be unduly small—from  $1\ \mu$  to  $6\ \mu$ , when it is termed a *microcyte*; or unduly large,  $10\ \mu$  to  $18\ \mu$ , when it is termed a *macrocyte*. Where marked variation in size of the red corpuscles is present, the condition is termed *anisocytosis*.

With regard to its staining reactions, we may have *achromia*, characterised by pallor of the central portion of the stained red cell, and in the fresh blood by a vacuolation in the centre of the cell. One has known students to mistake such vacuoles for malarial parasites. Immature red cells frequently show *polychromatophilia*, the stained cells taking on a brownish to a dirty-blue tint. *Granular basophile stippling* may be present in the form of blue-staining fine dots on the pink background of the stained cell, and such basophile stippling is found in many severe anæmias, in the leukæmias, in malarial cachexia, and above all in lead poisoning. This punctate basophilia may co-exist with polychromatophilia in the same cell. *Diffuse basophilia* is also commonly met with, the red corpuscles staining a diffuse uniform bluish tinge; in this condition most of the red corpuscles stain the normal pink but a certain proportion of them take on the basophile tinge. Blood films from bats and from white rats show marked basophilia.

A cell which is very characteristic of benign tertian malarial infections is the *demicrescent red cell*. This cell is the shadow-outline, as it were, of an enormously distended, pale and œdematous red corpuscle, and is in shape like the young crescent moon, the outer convex margin being smooth and even, but the inner concave border

The red blood corpuscle having been derived from a nucleated parent cell, remains of the nuclear apparatus may occasionally be encountered within it. These may take the form of small irregular fragments of chromatin, or *Howell's bodies*. Howell's bodies are spherical small masses of chromatin in the cytoplasm of varying size, taking on the violet stain of the chromatin. There are rarely more than two in the cell and the condition is often noted in blood which also shews punctate basophilia. *Chromatin dust* may also be present in a red corpuscle in the form of single or double tiny red granules, usually seen at the periphery of the cell and in size smaller than Howell's bodies. *Cabot's rings* are loops, and figures of eight, or irregular lines—apparently in the membrane of the red corpuscle—which take a red stain. They are especially well seen in Giemsa-stained films from cases of severe anæmia. One has known them to be mistaken for relapsing fever spirochætes.

*Normoblasts*, or nucleated red corpuscles, may be found in blood films from cases of anæmia of any type. They are of the same diameter as the non-nucleated red corpuscle and have a large round, intensely staining nucleus, which stains an intense violet—almost black—tinge. The nucleus nearly fills the cell, and looks as if it was embedded in it. The cytoplasm of the normoblast stains pink, or, not infrequently, shews polychromatophilic staining. The parent red cell or *megalo-blast* may also appear in blood films in cases of severe anæmia. It is a cell 10  $\mu$  or more in diameter, with a nucleus which is irregular in shape, and not infrequently of trefoil shape. The nucleus is poorer in chromatin than that of the normoblast, stains less intensely, and has a less distinct outline. The nucleus frequently stains more deeply in some parts than in others. The cytoplasm of the cell takes a polychromatophilic stain.

Turning to the abnormal leucocytes which may invade the peripheral blood stream, *myeloblasts*, which are the parent cells of the myelocytes, may occasionally be seen, and are characteristic of the blood in spleno-medullary leukæmia. They are cells rather like the large lymphocyte with a round nucleus which occupies the greater part of the cell, and stains intensely. The nucleus frequently shews three or four nucleoli within it. The cytoplasm is non-granular and intensely basophile. The azure-staining granules so common in the large lymphocyte are not seen in this cell.

The *neutrophile myelocyte*, which is the parent cell of the polymorphonuclear leucocyte, is seen in enormous numbers in blood films from cases of spleno-medullary leukæmia, and occasionally in malarial cachexia. The cell is about 15  $\mu$  to 20  $\mu$  in diameter, and the nucleus is rounded or may be indented or horse-shoe shaped. The outline of the nucleus shews up very indistinctly and the nucleus, which is usually central in position, stains feebly. The cytoplasm contains tiny neutrophile granules staining a faint pink. At times myelocytes may be encountered which shew both neutrophile and basophile granules in the same cell.

The *eosinophile myelocyte* is the parent cell of the coarsely granular eosinophile leucocyte. It is also well seen in spleno-medullary leukæmia. It may be as large as a normal coarsely granular eosinophile leucocyte, but is frequently rather smaller, and may not exceed the diameter of a red blood corpuscle. The nucleus is central in position and rounded and the cytoplasm stuffed with large deeply brick-red staining granules.

The *basophile myelocytes* are of two types, and are probably derived from the connective tissues and not from the bone marrow. They are cells which have a relatively small round nucleus shewing up indistinctly and staining feebly. The cytoplasm is stuffed with either coarse deeply blue-black staining granules, in the coarsely granular type ; or with fine small blue-staining granules in the finely granular type.

*Endothelial cells* or portions of them are very occasionally found in blood films, especially in the late stages of kala-azar. They are derived from the endothelial lining of the blood capillaries and appear as flattened cells with a flattened nucleus shewing a well marked nuclear network.

The *irritation cell of Türk* is a cell which is derived from the spleen and which is frequently encountered in cases of malarial cachexia with enlargement of the spleen. It is a cell with a faintly staining, excentrically placed nucleus, usually rounded in shape. The cytoplasm stains an intense opaque blue, and is not infrequently vacuolated. Türk's cells seem to vary a good deal in size, but as a rule are about the size of large lymphocytes.

*Lymphoblasts* may occasionally be found in blood films, and are especially characteristic of the blood in lymphoid leukæmia. As a rule they are larger than the normal large lymphocytes and shew poorer staining of both nucleus and cytoplasm. In the forms with a single nucleus a spherical chromatin-staining dot is often seen in the cytoplasm of the cell and would appear to be a centrosome. Forms are also encountered in which the nucleus consists of two separate lobes which touch one another at one point—the *Rieder's cell*.

Experience alone will teach the student how to recognise malarial parasites. A safe working rule is not to identify any suspicious form seen as a malarial parasite unless (1) it focusses with the margin of the red blood corpuscle ; (2) it has a definite form and shape and outline ; (3) it shews red-staining chromatin and blue-staining cytoplasm. Further, anything doubtful is almost certainly *not* a malarial parasite. Vacuoles into the outline of which the red of the stain has deposited ; platelets lying on the top of a red blood corpuscle ; and granules derived from broken down leucocytes lying in the same situation are possibly the three findings most commonly mistaken by beginners for malarial parasites. One has also known distorted red corpuscles to be mistaken for malarial crescents.



*The Leucocyte Count in Malaria.*

At one time great importance was attached to the value of the total and differential leucocyte count in the diagnosis of malaria, but with the increasingly common use of thick films in the diagnosis of difficult cases the leucocyte count has fallen into the background. Methods for carrying out the total and differential count will be found in every text-book on clinical methods, so need not here be described.

In carrying out the total count the blood should be diluted 1 in 20 with one per cent acetic acid ; (the half per cent solution recommended in many text-books is a little too weak). If the ruled lines on the hæmocytometer are faint they can be rendered distinct by very lightly rubbing its surface with a soft pencil and then dusting off the excess of graphite with a silk handkerchief. This practice is not recommended, however, as it may obscure details. The acetic acid solution should always be freshly filtered before use, in order to free it of any suspended matter. At least three counts, each of the total number of leucocytes present in the total square ruled area of the hæmocytometer should be made, and the average taken. An Ehrlich square ocular is very useful in doing such counts, as the eyepiece can be so adjusted as to exactly correspond with the size of the squares.

In general one tends to get leucopænia in association with malaria. Within a few hours of the onset of an attack the total leucocyte count has fallen to between 3,000 and 5,000 per cm., and the ratio of white corpuscles to red corpuscles changes during the apyrexial interval from the normal of about 1 to 500 to perhaps 1 to 900. It is important to remember, however, that sometimes in the course of the disease there may be an increase in the total number of leucocytes. For example, a true leucocytosis is by no means infrequent during the very early stage of an ordinary acute attack (especially in malignant tertian infections) and for a longer period in pernicious cases while the fever is rising. D. Thomson (1911*a*, 1912) has studied very thoroughly the leucocyte changes which occur in cured malarial patients ; he finds that there is a daily leucocytosis at about midday or at the period of the day when the patient was previously having rigors. The leucopænia of malaria is not as severe or prolonged as that of kala-azar.

The total leucocyte count is of some value in the prognosis of malaria. The reduction takes place essentially in the number of the polymorphonuclear leucocytes, and should any acute inflammatory state set in during the malaria such as pneumonia or acute sepsis, the patient's resistance to it will be reduced in proportion to the degree of leucopænia present.

In carrying out the differential leucocyte count, one should never count less than a total of 250 cells, and 500 is better. The simplest way of recording results is to use squared paper with an enclosed space of 250 or 500 squares marked on it, so that one knows mechanically when one has come to the end of 250 or 500. Or if

squared paper be not available, a rapid method of record is as follows. Mentally keep a tally of the polymorphonuclears in fifties, and record every other type of leucocyte encountered, using the symbols M = mast cell, P = polymorphonuclear, E = eosinophile, L = lymphocyte and small mononuclear, and H = large hyaline or transitional mononuclear. Thus an actual count read as follows:—

P 50 + 50 + 50 + 31

L.L.H.L.L.L.E.H.H.H.L.H.H.L.H.L.L.H.L.E.E.L.L.L.L.H.H.L.L.L.H.L.L.H.  
H.H.H.H.H.L.L.L.L.L.H.L.H.E.H.H.L.E.L.L.E.H.H.L.H.L.L.L.H.H.H.M.L.L.H.H.

in a chronic case of benign tertian infection. Now add up; thus the above count gives M 1 : E 6 : L 33 : H 29 : P 181. In order to get the percentage from 250 cells multiply by 0.4; thus the above count gives.

	Per cent.
Mast cells .. .. .	0.4
C. G. Eosinophiles .. .. .	2.4
Polymorphonuclears .. .. .	72.4
Lymphocytes and small mononuclears .. .. .	13.2
Large hyaline mononuclears and transitionals .. .. .	11.6

D. Thomson (1911*a*) has shewn that the percentage of mononuclear leucocytes present hour by hour during an attack of malaria gives a curve which is exactly the inverse of the temperature chart; during the rigor and at the height of the fever the percentage is at its lowest, during the afebrile period it rises again. Further, this fluctuation in the mononuclear count may persist for weeks and perhaps even months after the patient has been cured by quinine.

As a rule during the febrile phase the percentage of large mononuclears is from 10 to 15. Stephens and Christophers (1904) state that a figure of over 15 per cent (in the absence of kala-azar) is diagnostic of malaria, whilst if 20 per cent be encountered (in the absence of kala-azar) further search of the films will usually shew malarial parasites. Knowles (1920) contrasts the leucocyte findings in kala-azar and malaria as follows:—

	Kala-azar.	Malaria.
Total leucocyte count .. .. .	Usually under 3,000.	Between 3,000 and 5,000.
Leucopenia .. .. .	Constant and progressive.	Fluctuating.
Percentage of large mononuclears in the differential count .. .. .	20 per cent or more.	10 per cent to 16 per cent, not usually over 20 per cent.
Percentage of polymorphonuclears .. .. .	Usually less than 50 per cent.	Never less than 50 per cent

D. Thomson (1911), on a most careful examination of the whole question of the leucocytes in malaria, comes to the following conclusions :—

(1) Fever plus leucocytosis plus an increased percentage of polymorphonuclears is not malaria. It may be sepsis.

(2) Fever with leucopænia and a large hyaline mononuclear increase—e.g., 12 per cent to 15 per cent—(in the absence of kala-azar) is strong confirmatory evidence of malaria.

(3) A persistently high large mononuclear percentage with from time to time a leucocytosis should arouse the suspicion of malaria.

(4) Leucocytosis *per se* does not necessarily exclude malaria.

#### *Pigment-Bearing Leucocytes.*

The finding of large mononuclear leucocytes containing ingested hæmozoin pigment is proof that the patient is infected with malaria. Indeed, it usually happens that when grains, rods or blocks of hæmozoin pigment have been found within such a cell, further search of the film results in the discovery of a parasite. But the observer must be certain that it is true malarial hæmozoin pigment, and not something else. Dust may simulate it, but dust on focussing is seen to be obviously upon and not within the cytoplasm of the leucocyte, whilst it will also be present outside the cell as well. It is not at all uncommon to find tiny grains of chromatin in the cytoplasm of the large hyaline mononuclears and these must not be mistaken for hæmozoin. As a rule when there is pigment present in a large mononuclear, there is a good deal of it, the presence of only a few grains or of a single granular clump being exceptional. The pigment is either yellow-brown or brown-black and, as a rule, is very characteristic (Plate IV, figs.13, 14).

#### *Spleen Puncture in Malaria.*

Spleen puncture may be resorted to in malaria under two different conditions for purposes of diagnosis, and is a perfectly safe procedure, for the hard fibrotic spleen in malaria is less likely to bleed than the rather softer spleen of kala-azar.

(a) In cases of suspected malignant tertian malaria, it sometimes happens that parasites cannot be found in the peripheral blood. Under such circumstances if the patient has an enlarged spleen, if the spleen be punctured and films of the spleen juice be prepared and stained, large numbers of red corpuscles containing parasites at every stage of schizogony will be found, together with macrophages and endothelial cells loaded with hæmozoin pigment, and free hæmozoin pigment lying between the cells.

(b) In cases of chronic and relapsing malaria, where the diagnosis cannot be confirmed by other means ; e.g., by examining thick films, or by cultural methods. These are especially associated with the benign tertian parasite. In films from such

cases one finds (*a*) malarial pigment, both free and phagocytosed; (*b*) parasites in process of destruction lying in the intercellular spaces (*vide* p. 49).

In carrying out spleen puncture, the procedure should be as follows. The patient should be made to lie flat on his back in a comfortable position with his hands behind his head. For the puncture a Roux's 2 c.c. or 5 c.c. syringe is far preferable to any other pattern of syringe. (Roux's syringes may be obtained from the Central Research Institute, Kasauli; the Haffkine Institute, Bombay; or from Messrs. Collin Frères, Paris). The Roux syringe gives good strong forcible aspiration, whilst all its parts are replaceable and can be renewed. The syringe should be fitted with a rather fine and sharp hypodermic needle. The syringe must be sterile and dry, since the presence of water in it will hæmolyse the parasites. An assistant fixes the enlarged spleen upwards against the costal margin and diaphragm. The skin over the most prominent part of the spleen is next sterilised. Dr. Napier advocates two movements in doing the puncture: (*a*) to pierce the skin, and then wait a second or two, and then (*b*) to enter the spleen; but the writer has always performed the puncture in one movement straight into the spleen. The sensation of resistance when the needle enters the spleen is unmistakable.

Once one knows that the needle has entered the spleen, make strong steady aspiration with the plunger of the syringe, and then sharply withdraw the needle and syringe from the spleen. It does not matter whether blood enters the body of the syringe or not, since there is always sufficient spleen pulp in the bore of the needle for the preparation of two or more good films.

The patient should be kept lying down for two hours after spleen puncture, and his pulse examined before he is allowed to depart. If desired, an abdominal pad and binder may be applied.

#### *The Cultural Diagnosis of Malaria.*

Culture is one of the best methods of discovering malarial parasites in difficult and obscure cases of malaria; but, as with thick films, so here, it demands the most meticulous care and accuracy in technique. The best method is the modification by J. G. and D. Thomson (1913, 1913*a*) of Bass' original technique. It is as follows:—

Ten c.c. of blood is withdrawn into a sterile syringe from the patient's vein, and carefully ejected into a sterile test tube containing 0.1 c.c. of a 50 per cent sterile dextrose solution in distilled water. (The dextrose solution is sterilised by steaming daily for 20 minutes for three days.) Care must be taken in transferring the blood to the test tube that no air bubbles form. The blood is next defibrinated by gently stirring it with a sterile wire or glass rod, which is then withdrawn with the adherent clot. The defibrinated dextrose-containing blood is next transferred to a series of small sterilised test tubes not less than 1.25 centimetres in width and not less than 12.5 centimetres in depth. A quantity of blood giving a depth of 2.5 to

5 centimetres is run into each tube, so that when it settles there will be a column of clear plasma 1.25 to 2.5 centimetres deep above the blood cells when they have settled to the bottom of the tube. The tubes are incubated at a temperature of 40°C.; (Sinton, however, recommends a temperature of from 35° to 38°C.). The parasites live and develop in the red blood corpuscles at the top of the column of deposited cells just below the clear plasma in a layer of cells of from 0.05 to 0.1 centimetre thick. All parasites below this layer die off in from 2 to 20 hours.

In order to examine the culture carefully aspirate off a tiny drop of the uppermost layer, spread into a film, fix and stain, and examine.

In order to obtain a sub-culture, the procedure must be different. Wenyon (1926, p. 1306) gives the following method. 'The defibrinated glucose-blood must be centrifuged at a speed sufficient to cause the leucocytes to occupy the upper layers of the deposit, so that red cells free from leucocytes can be obtained. The supernatant serum (plasma) is then transferred to culture tubes which are better if they have flat bottoms. The column of serum in each should be from 1.25 to 2.5 centimetres in depth. A pipette is passed into the middle of the deposit in the centrifuge tube and red cells and parasites are drawn off. These are transferred to the bottom of the culture-tube. By this technique the presence of leucocytes, which devour the merozoites as soon as they escape from the red blood corpuscles, is avoided. The young parasites will enter red cells and again grow into schizonts. It has only been possible to conduct the parasites through three generations, after which they cease to grow and die.'

Row (1917) has given a method for the cultivation of malarial parasites from the finger blood. The blood is drawn from the sterilised surface of the finger into a small tube, in which it is defibrinated. The small quantity of defibrinated blood is then transferred by means of a pipette to a small, flat-bottomed tube containing serum to which the requisite quantity of dextrose has been added. The small tube is placed inside a larger tube—such as the ordinary bacteriological potato-tube—provided with a constriction on which it rests. Into the bottom of the potato-tube a quantity of pyrogallic acid is placed before the small tube is inserted. After the small culture tube has been inserted, 2 or 3 c.c. of a 10 per cent solution of caustic soda is run into the bottom of the large tube, to mix with the pyrogallic acid, and a rubber cork is inserted into the open end of the tube (1922, 1922*a*) which must be corked tightly. The pyrogallic acid and soda solution absorb the oxygen so that the culture takes place in a relatively anaerobic environment.

Sinton (1922, 1922*a*) carries out culture of malarial parasites from the finger blood in a specially constructed glass tube about 20 cms. in length (Fig. 16). It is made as follows. Take glass tubing having a bore of 0.4 to 0.5 cm. Draw one end out as in making an ordinary capillary pipette (A), place a narrow metal ring over the thin drawn out portion whilst it is still molten, and by pressing upwards produce a flat surface (B), from the centre of which the thin drawn out tube arises

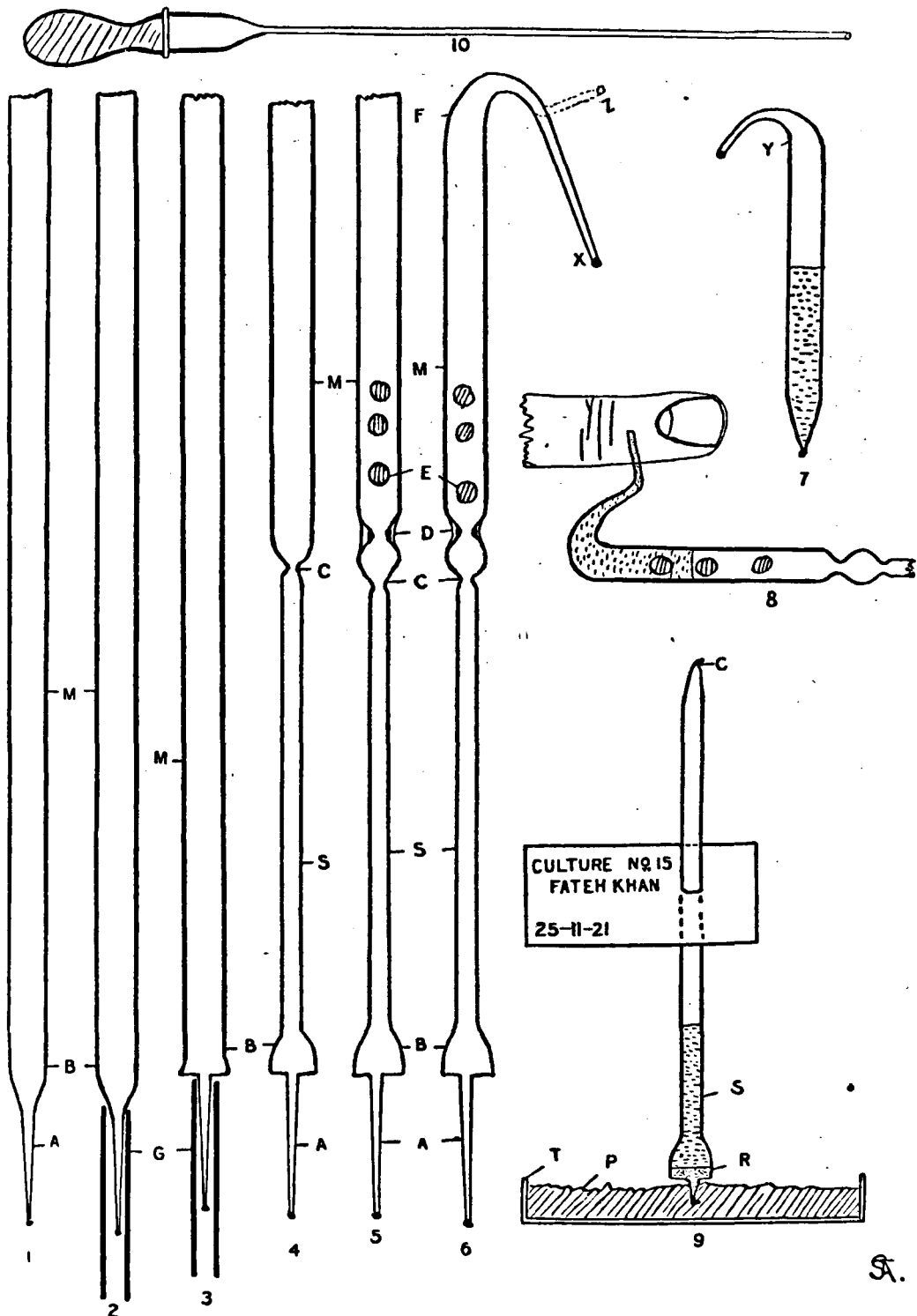


FIG. 16.—Sinton's method of cultivating malarial parasites from the finger blood. (After Sinton, 1922.)

(A). The tube is now heated a short distance above the flattened surface, and drawn out till it forms a tube about 0.2 cm. wide (S). A bulbous part with a flat bottom (B) is thus left between (A) and (S). At the upper portion of (S) a slight constriction is made (C), whilst about 0.4 to 0.5 cm. higher up the tube is again heated, and an indentation made on each side by pressing inwards on the softened glass with the point of an iron nail (D). Three glass beads (E) are then dropped into the upper portion of the tube (M), and come to rest above the indentation (D). The upper end of the tube is then bent, drawn out and bent, as in a Wright's capsule (X). Both ends of the whole tube are then sealed and the apparatus sterilised.

When culture is to be made, the tube is opened at both ends. The upper capillary end is heated in the flame of a spirit lamp and turned to a right angle (Z) to the plane of the rest of the tube, so that the whole will lie on a table with the open upper end pointing upwards. A Wright's capsule containing ascitic or hydrocele fluid to which 1.5 c.c. to 2 c.c. of 50 per cent sterile dextrose solution has been added per 100 c.c. is taken and opened (7). The open upper end of the tube is inserted into the capsule of ascitic or hydrocele fluid and the chamber (M) allowed to fill one-third to one-half full of the fluid by capillary attraction. The patient's finger is next carefully sterilised, and deeply pricked. With the arm hanging down to promote the flow of blood from 5 to 10 large drops of blood are taken into the chamber (M) by capillary attraction (8). The chamber (S) and bulb (B) are now heated to expel the air from them, and the capillary end at (A) sealed off. As the heated air in this chamber cools, the mixed ascitic fluid and blood is drawn into the chamber (M) containing the beads. The capillary end (Z) is now sealed. The tube is now shaken backwards and forwards so that the blood is defibrinated by the beads. When defibrination is complete, take the whole apparatus in the hand, with the chamber (B) furthest away and swing it round and round two or three times at the end of the extended arm. This drives the defibrinated blood mixture into chambers (S) and (B). A few minutes are allowed for the blood to settle, and the tube is then heated, drawn out at constriction (C), and sealed off. The culture tube thus obtained is set vertically in plasticine and incubated at 35° to 38°C. (9). The red corpuscles settle down on the flat shelf in (B), and the parasites grow here. In order to examine the culture, this layer is aspirated off by a capillary pipette.

Sinton was successful in 50 per cent of his earlier attempts at culture by this method, but as technique improved he obtained successful cultures from all his last 12 cases of *P. falciparum* infection.

## CHAPTER IV.

### NOTES ON THE TREATMENT OF MALARIA.

It is a little difficult at this period of time to discover the reasons which led to the universal use of quinine salts in the treatment of malaria, in preference to the use of the other alkaloids contained in cinchona bark. According to Sir Leonard Rogers (1910), the following periods may be traced in the history of the treatment of malaria in India :—

(a) 1657 to 1804. The use of cinchona bark by ships' surgeons who visited India,—notably Bogue (1657), James Lind (1765), John Clark (1768), and William Hunter (1804).

(b) 1804 to 1847. A period during which—largely owing to the influence of James Johnson (1804)—the cinchona treatment of fevers was entirely abandoned, and a course of treatment by violent purging, mercury administration in excessive doses, and copious venesection was resorted to. As much as 800 to 900 grains of calomel were given during a single attack of fever; the Presidency General Hospital, Calcutta, used 13,337 grains of this drug in a month, and necrosis of the jaws from mercurial poisoning was of frequent occurrence.

(c) During this interval, quinine alkaloid was discovered in 1820 by Pelletier and Caventon, but the so-called 'quinine' of those days was rather the sulphate of all the crystallisable alkaloids of cinchona than pure quinine sulphate. In 1828 James Annesley not only used this 'quinine' sulphate in the treatment of malaria but also advocated the use of mosquito nets as a preventative. Corbyn in 1834 was also one of the early pioneers of quinine therapy, whilst Edward Hare resorted to it in malaria. The publication in 1847 of Hare's pamphlet *On the Treatment of Malaria and Dysentery* led to an official investigation of quinine therapy by the Calcutta Medical Board, lasting for one year, and the routine use of 'quinine' sulphate in malaria became the established order of the day.

It will thus be seen that the use of 'quinine' sulphate came to replace that of cinchona bark almost accidentally. The bark treatment disappeared during the period 1804 to 1847; and when re-introduced in 1847, it was in the form of administration of 'quinine' sulphate. In 1866 the Madras Cinchona Commission was appointed, to test the relative merits in the treatment of malaria of the different alkaloids of cinchona—several of which had by then been isolated. The plan



adopted was to distribute the different alkaloids to different civil surgeons and collate their reports. No control microscopic observations were possible, as the malarial parasites were not discovered until 1881 to 1890, and the report rested on a purely clinical basis. Even so, it is difficult to understand how the Commission came to advocate quinine in preference to quinidine, for their results with the latter drug were distinctly better than those with quinine. Apparently they considered that when once pure quinine sulphate should become available, the ideal cure for malaria would be found.

Passing over the intervening years, during which quinine was regarded as the one and only specific for malaria, we come next to the work of MacGilchrist (1914 to 1916). His observations were made on jail prisoners, and the therapeutic activity of each alkaloid was tested by measuring the time that elapsed between the administration of the first dose of the alkaloid and the disappearance of asexual forms of the parasites from the peripheral blood. He placed the alkaloids in the following order of anti-malarial efficacy :—

1. Hydroquinine hydrochloride.
2. Cinchonine sulphate.
3. Quinine sulphate.
4. Quinidine sulphate.
5. Optochin hydrochloride.
6. Cinchonidine sulphate.
7. Quinoidine.

Further, with regard to the five chief natural cinchona alkaloids—quinine, quinidine, cinchonine, cinchonidine and quinoidine—the results *in vitro* upon free-living infusoria corresponded with the results *in vivo* upon cases of malaria.

Acton (1920) found that the dextro-rotatory cinchona alkaloids (with the exception of cinchonine) are more toxic to *Paramœcium caudatum* than are their lævo-rotatory isomerides. The order of toxicity was as follows :—

1. Ethyl-hydro-cupreidine.
2. Optochin ; (ethyl-hydro-cupreine).
3. Quinidine.
4. Cinchonidine.
5. Quinine.
6. Cinchonine.

The corresponding hydro-alkaloids were slightly less toxic to *Paramœcium* than were the natural alkaloids. Further, the degree of alkalinity of the environment is of very great importance. The minimum lethal concentration—i.e., the lowest concentration necessary to kill every individual in a standard dose of *Paramœcium* culture—varies with the hydrogen-ion concentration. At a pH of 7 it was 1 in 10,000 for quinine and 1 in 20,000 for quinidine ; whereas at a pH of 8

it was 1 in 70,000 for quinine and 1 in 100,000 for quinidine. Both experimentally when acting *in vitro* upon *Paramecium*, and clinically *in vivo* when administered to cases of benign tertian malaria, quinidine proved itself a far more potent alkaloid than did quinine.

Of these alkaloids, cinchonine and quinoidine are liable to cause vomiting, whilst optochin (ethyl-hydro-cupreine) tends to cause optic atrophy. Cinchonidine is the weakest of the natural alkaloids of cinchona bark, and S. P. James (1922) sums up the situation by saying that the decision as to which alkaloid it is best to employ rests between hydroquinine, quinine and quinidine. The same author also quotes Morgenroth, Giemsa and Werner, and Baermann as reporting that hydroquinine is superior to quinine in anti-malarial efficacy.

A most important point in the treatment of malaria is that it shall be continuous. In malaria there is some evidence of acidosis in the blood, according to Sinton (1924); and the pH of the blood is probably reduced. To kill off all parasites by a single dose of quinine or other alkaloid, therefore, it would theoretically be necessary to reach a concentration of the alkaloid in the blood stream of something like 1 in 8,000, and this is impossible. After the ingestion of a single dose of 20 grains of quinine sulphate Acton found the maximal concentration of quinine in the blood to be only 1 in 150,000; and after a single dose of 10 grains to be only 1 in 250,000. Again, in whatever form quinine be administered, it circulates in the blood as quinine base; and is present in the plasma, adsorbed on to the surface of the erythrocytes, but not within them. Hence such parasites as have become intra-cellular escape its action. For all of these reasons, and as the clinical experience of many decades has shewn, to be efficacious the cinchona treatment of malaria must be continuous, and a concentration of some 1 in 150,000 or 1 in 250,000 in the blood must be maintained over a period of at least three weeks.

In the clinical application of these findings to treatment, we may accordingly lay down the following principles:—

(1) The administration of the alkaloid must be continuous over a period of one week according to Sinton (1923), or three weeks according to Acton and Knowles (1924).

(2) Its administration should be so timed that it reaches the blood stream at the moment when the latter is at its most alkaline tide. Experimenting with fed and starved cats, Acton and Chopra have found that the portal blood stream reaches its most alkaline tide some  $2\frac{1}{2}$  hours after a meal.

(3) The alkaloid should always be administered together with alkalis. It is not possible experimentally to appreciably increase the pH of the blood stream, since the oxygen-carbon-dioxide interchange in the lungs, together with the defensive hepatic metabolism, tend to keep this at a very constant level. On the other hand alkalis tend to increase the absorbing power of the intestinal mucosa, and the chief absorption of the cinchona alkaloids takes place in the small intestine,

(4) It is very far from certain that quinine is the best alkaloid of cinchona to use. Both quinidine and cinchonidine are more efficacious with regard to anti-malarial power.

*Sinton's Method.*

Probably the best standard treatment for malaria to-day is that advocated by Sinton (1923). The total number of patients treated in connection with his paper was 336, and they received from 110 to 210 grains of quinine with alkalies within one week, and were under observation for eight subsequent weeks. Of these cases only 2 shewed malarial parasites in their blood at the end of treatment, as compared with 20 in the control series treated with ordinary quinine mixture. The chief drawback to Sinton's method is that it involves the administration of six doses of medicine a day, and strict adherence to a set time table. Sinton's treatment is as follows :—

1. The diagnosis of malaria having been confirmed by finding malarial parasites in blood films taken from the patient, he is first given an aperient, 3 grains of calomel, followed by 1 oz. of magnesium sulphate dissolved in an equal amount of warm water. This preliminary purgative is always advisable, or even necessary, in the treatment of malaria. If there is nausea or vomiting present, the calomel can be given in small, divided, and repeated doses, with sodium bicarbonate, or adrenalin given in 5 to 15-minim doses in water to check the vomiting.

2. The two mixtures used are as follows :—

(a) *Alkaline Mixture\**.

Sodium bicarbonate	..	..	60 grains
Sodium citrate	..	..	40 grains
Water to	..	..	1 oz.

(b) *Quinine Mixture.*

Quinine sulphate	..	..	10 grains
Citric acid	..	..	30 grains
Magnesium sulphate	..	..	60 grains
Water to	..	..	1 oz.

3. Treatment is commenced the morning after the case has been diagnosed to be malaria. One dose—i.e., 1 oz.—of the alkaline mixture is given at 7-30 a.m., repeated at 9-30 a.m., and again at 11-30 a.m. Fifteen to thirty minutes after the last dose of alkaline mixture one dose—i.e., 1 oz.—of the quinine mixture is given. At 6 p.m. a further dose of the alkaline mixture is given, followed fifteen to thirty minutes later by a second dose of quinine mixture.

For the next four days one dose of alkaline mixture is given thrice daily—at 7-30 a.m., 11-30 a.m., and 6 p.m., followed on each occasion by 1 oz. of the quinine mixture fifteen to thirty minutes after each dose of alkaline mixture.

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\* This mixture is not a solution, and the bottle must be well shaken before the administration of each dose.

For the remaining two days of the week one dose of the alkaline mixture is given each morning and afternoon, followed fifteen to thirty minutes later by one dose of the quinine mixture.

In seven days the patient thus receives a total of 180 grains of quinine in solution. In the case of women or where there is much gastric disturbance each dose of the alkaline mixture may be replaced by a mixture containing 2 drachms of sodium citrate in 2 ozs. of water, taken slowly. In cases with much vomiting it may be necessary to give a dose of tincture of opium before treatment. In severe cases a dose of alkaline mixture followed by 1 oz. of quinine mixture fifteen to thirty minutes later may be given at once without waiting for the effect of the purgative, but in these cases the alkaline treatment should be continued in the intervals between the doses of quinine until the urine becomes alkaline.

In severe cases of malignant tertian malaria, the amount of sodium bicarbonate in the alkaline mixture may be increased to 90 grains, dissolved in 2 ozs. of water.

With this treatment Sinton claims that only 28 per cent of cases of benign tertian malaria relapsed, as compared with a relapse incidence of 40 per cent in those treated with stock quinine mixture; whilst in cases of malignant tertian malaria the relapse rate with Sinton's method was 15 per cent as against 79 per cent for those on stock acid quinine mixture.

In cases of chronic and relapsing malaria, Sinton advocates giving repeated courses, each of one week's duration and according to the above time table, with intervals between them, rather than a long continued course of quinine.

#### *Quinine plus Alkalies in one prescription.*

Whilst Sinton's method is probably to-day the best standard treatment for malaria, it is hardly suitable for out-patients. In the out-patient department of the Calcutta School of Tropical Medicine, the following routine prescription is in use:—

℞			
Quininae sulphatis	..	..	.. 10 grains
Pulv. acidi citrici	..	..	.. 20 grains
Magnesii sulphatis	..	..	.. 10 grains
Spiriti anisi	..	..	.. 10 m.
Syrupi simplicis	}	āā	.. ½ oz.
Aquam			

Of this mixture 1 oz.—containing 10 grains of quinine in solution—is given three times a day two and a half hours after food on an absolutely empty stomach for one week. The dose is then reduced to 1 oz. taken two and a half hours after food twice a day, and continued for a further two weeks.

The rationale of this prescription may now be considered.

(1) Quininae sulphatis, 10 grains. 'From the numerous experiments conducted during recent years, it would appear that quinine in doses of less than 20 grains daily (for an adult) has no curative effect in malaria' (Sinton, 1923).

(2) Pulv. acidi citrici, 20 grains. This is to secure solution of the quinine. It is converted into carbonates in the gut, and thus may help to slightly raise the pH of the portal blood stream in which quinine acts, and to assist in its absorption from the gut.

(3) Magnesii sulphatis, 10 grains. Opinion is unanimous that aperients should always be exhibited in the treatment of malaria. If the continued repetition of this dose of magnesium sulphate leads to diarrhoea or gastric disturbance, however, it can be omitted from the prescription.

(4) Spirit anisi, 10 m. It is almost impossible to disguise the taste of quinine, but this flavouring seems to be the best available.

(5) Syrupi simplicis,  $\frac{1}{2}$  oz. This is added on the advocacy of Bishop (1924). Sinton and Hughes (1924) have shewn that in malaria there is a deficiency in the glycogen of the liver, and the administration of glucose is thus indicated.

Owing to the impossibility of following up Indian patients after their discharge from hospital, it has not been possible to collect figures as to the efficacy or otherwise of this standard line of treatment. It is much to be desired that medical officers in charge of troops or of jails who have a controlled population under them and can trace the after-histories of their patients should try this or some similar line of treatment, and publish their results.

#### *Cinchona Febrifuge plus Alkalies.*

Quinine is an expensive drug, its present retail price on the market being Rs. 19 per lb.\* Cinchona febrifuge is much cheaper, its present market price being Rs. 12 per lb. Further, as shewn by Acton and Knowles (1924) it is as efficacious in the treatment of malaria as quinine. According to MacGilchrist (1915), and Fletcher (1923) the composition of Indian and of Java cinchona febrifuge is respectively as follows:—

	Indian febrifuge.	Java febrifuge.
Quinine .. .. .	7.4	11.5 per cent.
Quinidine .. .. .	22.83	5.0 "
Cinchonine .. .. .	18.58	26.3 "
Cinchonidine .. .. .	5.84	20.0 "
Uncrystallisable alkaloids, ash, etc. ..	43.35	37.2 "

\* The prices quoted have been given by Messrs. Howards and Sons' representative, and are lower than the retail prices in the bazaar.

Now it has been shewn by Acton (1921) that both quinidine and cinchonidine are more toxic to *Paramæcium caudatum*—and presumably to malarial parasites—than quinine. Hence in using cinchona febrifuge it is better to prescribe the Indian febrifuge rather than the Java febrifuge, since its content of quinidine is so high; also its content of cinchonine—the chief factor in producing vomiting after the administration of cinchona febrifuge—is lower than that in the Java febrifuge.

For out-patient work, where cinchona febrifuge is prescribed in place of quinine, it may be given as follows:—

℞			
Cinchona febrifuge (Indian)	.. ..	10 grains	
Pulv. acidi citrici	.. ..	20 grains	
Magnesii sulphatis	.. ..	20 grains	
Spiriti anisi	.. ..	10 m.	
Syrupi simplicis	} āā ..	..	½ oz.
Aquam			

Dose: 1 oz. t.d.s. two and a half hours after food for one week\*; then 1 oz. b.d. two and a half hours after food for a further two weeks.

As a rule this prescription is well tolerated, *if given at the right time, two and a half hours after food and on an empty stomach*. Occasionally, however, it may cause nausea and even vomiting. This is very liable to occur when mass routine treatment of a working population is being attempted. Treatment can only be given after the day's work is over, when the average Indian is either cooking, or consuming, or has just consumed a meal. It is impossible to return to a household so engaged, and equally difficult to wait until say 8 p.m., when all have finished eating sufficiently long, as the majority are then asleep. In railway work, where men are going on and off duty at all hours, the difficulties are intensified. In such cases, it is probably better to give cinchona febrifuge pills, which necessarily dissolve more slowly. The Chief Medical Officer, Eastern Bengal Railway, recommends as a standard rule in quinine salts administration the exhibition of gr. ii of sodium bicarbonate for each grain of quinine salts prescribed. A dose of m. 15 of 1:1000 adrenalin in water, or ½ m. of tincture of iodine in water may be given before the cinchona febrifuge in order to check vomiting, or, if necessary, a dose of tincture of opium.

Fletcher (1923) sums up his experience with cinchona febrifuge as follows:—

(1) Many preparations of different alkaloidal strength are being sold under the name of cinchona febrifuge. Its constitution should be legally defined.

(2) The sample which we employed in the treatment of forty-two cases of malaria proved therapeutically as efficient as quinine, when given in doses of ten grains twice a day.

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\* This may be found to be too much for delicate or weakly individuals, and the dose may have to be given b.d. instead of t.d.s.

(3) In doses of ten grains twice a day it is not more toxic than quinine sulphate.

(4) Quartan gametocytes are more resistant than trophozoites to the action of cinchona alkaloids.

(5) Quartan malaria is not so prone to relapse within three weeks after treatment as benign tertian.'

#### *Quinidine Sulphate.*

Quinidine sulphate has been advocated by Acton (1920) in the treatment of malaria as being more parasitocidal than quinine. Unfortunately its price is high, Rs. 32 per lb. Also it may have a toxic action on the heart. On the other hand it is a drug which is of special value in chronic and relapsing benign tertian malaria. In such cases, before prescribing it, the practitioner should make sure that the heart is sound. Quinidine should be given in doses of not more than 5 grains t.d.s., together with citric acid, etc., as in the above prescriptions for quinine and cinchona febrifuge.

#### *Rules in the Treatment of Malarial Cases.*

Quinine being a very expensive drug, it is frequently adulterated, or the prescription not made up to the strength prescribed. The practitioner should make certain :—

(1) That the quinine prescription is up to full strength.

(2) That the patient swallows and retains every dose prescribed, and that he omits not a single dose of the standard course given.

(3) That he is absorbing the quinine into his system.

To make certain on points (1) and (3) certain elementary laboratory tests can be applied.

#### 1. *Is the quinine prescription up to full strength?*

Colonel Proctor, I.M.S., informs the writer that, on examining the stock quinine mixture in four dispensaries under his charge he found that in three the stock mixture only contained 5 grains to the ounce instead of 10, whilst in the fourth the mixture only contained 3 grains to the ounce. This is typical of what goes on all over India.

To apply a simple clinical test on this point the following method is recommended by Sinton (1925a).

1. Prepare Tanret's modification of Mayer's reagent as follows. Dissolve 1.35 grammes of mercuric perchloride in 75 c.c. of water and 5 grammes of potassium iodide in 20 c.c. of water in a 100 c.c. measuring flask. Pour the mercuric solution into the iodide solution, gently shaking the flask to agitate the liquid, and then fill up with distilled water to the 100 c.c. mark.

2. Heat a portion of the quinine solution to exclude the presence of albumin. If albumin is present it must be removed before testing the solution. If albumin is present acidify with acetic acid, bring to the boil, and filter the solution before test.

3. Take 4 c.c. of the quinine solution—freed from albumin—place it in a flask, and dilute with distilled water to 250 c.c. In one of the special spare tubes provided with each set of Brown's opacity tubes for vaccine preparation \* place one volume of the quinine solution. Add to it one volume of Tanret's reagent, and shake well. The solution will become more or less opaque.

4. Match the opacity with Brown's tubes. This is done by taking the tube under test in turn with each of the standard opacity tubes, and 'laying the tubes side by side in a good light, upon some clearly printed book. The lighting must be equal in the two cases. The opacity of the two suspensions can then be readily compared by rolling the tubes from side to side, and raising them slightly from the surface of the print.'

5. Brown's tubes are numbered from 1 to 10, and Sinton states that these numbers may be taken to represent the number of grains per ounce in the quinine solution under test. A quinine solution containing 10 grains of quinine to the ounce should shew an opacity of 10.

6. As a control to the test a fresh solution of 10 grains of quinine to the ounce should be made up and tested at the same time.

7. If the solution is weaker than 10 grains to the ounce, solutions of different strengths can be prepared from the control solution by diluting it, and the tests repeated until a match is obtained.

### 2. *Retention of the dose by the patient.*

If the medical practitioner is to be successful in the treatment of his malarial patients, it is of the utmost importance that he must see that every dose of quinine prescribed is taken and retained by the patient, and not leave the administration to compounders or assistants. Any one of many causes may lead to the omission of one or more doses. The patient may vomit the dose; he may absent himself from the ward at the time a dose is due; and, as quinine is an unpleasant drug to take, he cannot be trusted to carry out the full treatment by himself. MacGilchrist (1915) treated 149 cases of malaria in the Presidency Jail, Alipore, personally seeing to it that every patient took every dose prescribed; there was not a single case of relapse in his series. D. Thomson (1917) at the Liverpool School of Tropical Medicine between 1910 and 1913 treated 200 cases of malaria,

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\* Brown's opacity tubes are available from the Director, the Central Research Institute, Kasauli; or they may be prepared from the instructions given by Brown (*Indian Journal of Medical Research*, 1919, Vol. VII, No. 1, p. 343).



examining some 30 films from each patient during the course of treatment; in only one instance were parasites discovered after the first few days of treatment, and in this case there was some doubt as to whether the patient had taken the quinine prescribed for him. Fletcher (1923) investigated 8 cases of malarial relapse amongst convalescent soldiers at Southampton, and 44 'quinine resistant' cases at Kuala Lumpur. In every single instance investigation shewed that the 'quinine resistance' was due to the quinine prescribed not having been swallowed—or, in a few instances, to the quinine having been adulterated. Glennie, Municipal Health Officer, Singapore, found that 50 per cent of the quinine on sale at some of the dispensaries in that city was adulterated. Colonel Megaw records that he 'once found that the whole supply of quinine mixture of a large hospital was only about one-third of the prescribed strength. There was ample evidence in this case that the drug was being stolen and sold to a shop in the bazar.' 'In conclusion,' writes Fletcher, 'we cannot deny the possibility of a case of malaria being absolutely resistant to quinine, though we have never encountered such a phenomenon ourselves. . . . but these exceptions are so few and so seldom seen by those who take scrupulous care with the details of the treatment that for practical purposes they can be ignored by the practising physician. The mistake is all too common of imagining that the fact of having written a prescription amounts to evidence that the quinine has been properly compounded and that the prescribed amount of the drug has been swallowed by the patient. This is certainly a case in which there is many a slip between the cup and the lip.'

### 3. *Is the patient absorbing the quinine into his system?*

This can easily be ascertained for quinine, cinchona febrifuge, and quinidine by a simple daily test applied to the urine. Proceed as follows:—

1. Prepare Mayer's reagent, which consists of 13·55 grammes of mercuric chloride and 49·8 grammes of potassium iodide in one litre of water.

2. Examine the patient's urine for albumin. If albumin is present remove it by adding acetic acid, bringing to the boil, and filtering.

3. To a few c.c. of the clear, albumin-free, urine in a test tube add three or four drops of Mayer's reagent. If any of the cinchona alkaloids are present in the urine, cloudiness or opacity will follow.

Should the urine be clouded with phosphates, etc., as is not infrequently the case in malaria, it should be filtered clear before the test is applied. Mayer's reagent precipitates the great majority of alkaloids, including strychnine, so if the patient is on nux vomica a positive result will be obtained with this also.

Fletcher records some very interesting results on applying this daily test to the urine of patients who were supposed to be on full quinine treatment. Of 233 such patients 66, or 28 per cent, failed to shew quinine in the urine. In several more the reaction was so slight as to make it improbable that the dose previous to

examination had been taken. Patients who were supposed to be swallowing up to 120 grains of quinine daily failed to shew any quinine in the urine. A man who was stated to have taken 120 grains a day for three consecutive days failed to shew any quinine in his urine. Fletcher concludes that 'at least 25 per cent of the quinine prescribed in hospitals is not swallowed by the patients..... There are many reasons why a very large proportion of the quinine prescribed is never taken by the patient. No doubt some is stolen by dishonest hospital attendants and others; but one of the principal causes is the patient's disinclination to swallow it. The prescription of multiple mixtures for malaria patients plays an important part, because it makes it far more difficult to administer quinine regularly. For instance, if a man has bronchitis while he is undergoing treatment for malaria he is given a cough mixture; a few days later, perhaps, he is ordered an iron tonic because he looks anæmic, and so it happens that the quinine is forgotten among the multitude of remedies. The convalescent malaria patient is seldom bed-ridden; he often wanders about the hospital compound and frequently misses his dose of quinine because he is not in the ward at the hour when the medicines are served out. We strongly recommend that rosters of all patients on quinine treatment should be kept in malaria wards, and that each dose should be entered opposite the patient's name when it is given. The administration of quinine should be under the direct supervision of some responsible person and controlled by the daily examination of the urine.'

#### *The Treatment of Special Cases.*

For algid, cerebral and comatose cases of malaria, or where there is vomiting and intolerance to the cinchona alkaloids by the mouth, quinine should be administered intravenously. As far as one can ascertain, the present, and almost universal practice—in Bengal at least—is to give quinine or cinchonine intramuscularly; in fact, many practitioners appear to treat *all* their cases of malaria with intramuscular injections of quinine or cinchonine.

One wishes that the advocates of intramuscular quinine—and, above all, those who are to-day teaching this pernicious mode of administration to medical students—would but pause to try such injections experimentally in animals before they advocate the procedure for man. Whether quinine or cinchonine or other cinchona alkaloid be used, after intramuscular injection into a rabbit, the invariable sequel is local necrosis at the site of injection. If quinine or cinchonine base be used, there is necrosis and necrosis only. If—as is usual—an acid salt be used, in addition to this necrosis, is super-added acute hæmorrhagic inflammation and exudation. The plate reproduced below shews the conditions present 24 hours after an injection of two minims of a 50 per cent solution of cinchonine dihydrochloride into the gluteal muscles of a rabbit, and is typical of the results which occur.

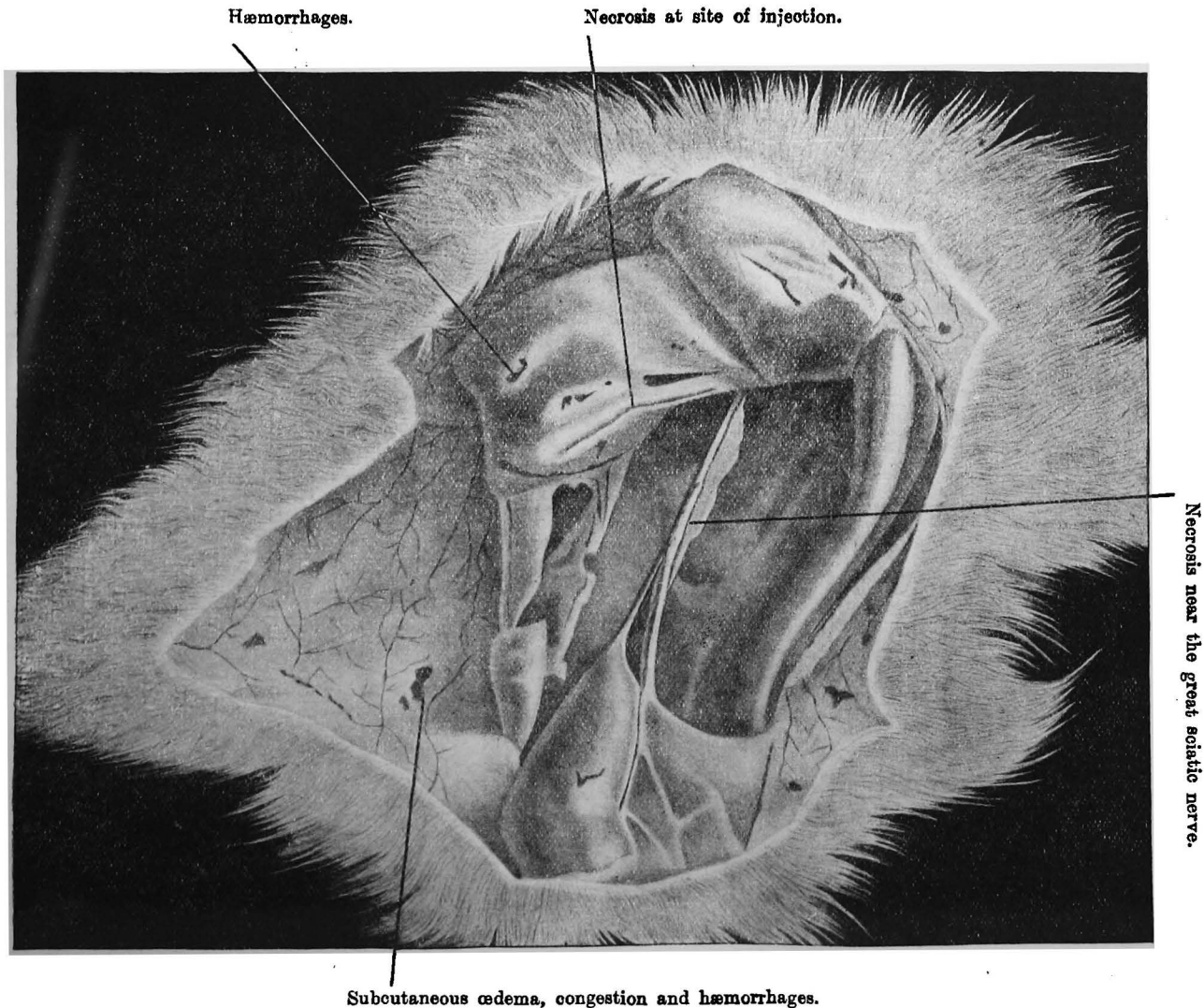


FIG. 17.—The conditions present in the gluteal muscles of a rabbit 24 hours after an intramuscular injection of two minims of a 50 per cent solution of cinchonine dihydrochloride.

In man, the same changes always follow. Usually the focal necrosis is limited and is very gradually absorbed and replaced by scar tissue, leaving a painful lump which persists for some weeks or even months. If a nerve, such as the sciatic, be accidentally struck, however, permanent paralysis may ensue; whilst if sepsis be super-added, the consequences are disastrous. The following cases may be quoted from Fletcher's *Notes on the Treatment of Malaria* (1923).

1. A young and healthy child crippled for life from paralysis of the sciatic nerve, which had been injured by an injection of quinine.
2. A man who, a year before had been earning good wages as a motor-car driver, but who had become a pauper, on account of ankylosis of the left knee and ankle—the result of an injection of quinine.
3. A man with a healing abscess in his right deltoid, the result of an intramuscular injection of quinine 47 days before; and also a healing abscess in his left deltoid from the same cause. This patient in the meantime, had nearly died of septicæmia.
4. A mechanic bed-ridden for several months with abscesses and discharging sinuses in the buttocks, the result of injections of quinine.
5. An engine driver who had been given injections into the deltoids for a mild attack of malaria; and who had been unable to work for several weeks in consequence. A large discharging sinus was present over the right deltoid.
6. A patient with three incisions leading into an enormous abscess in the left gluteal region, due to an intramuscular injection of quinine 55 days before. The gluteal muscles were largely destroyed and the use of the limb was permanently impaired.
7. A patient with three sinuses which had been discharging pus for several weeks, with the greater part of the affected muscles destroyed.

Also many other such cases. One of the worst of such instances which the writer has seen was the case of an officer in the Royal Engineers who sustained two abscesses in the *right forearm* as the result of intramuscular injections of quinine. To such a highly trained officer the use of his right forearm is invaluable, and the folly which had led his medical attendant to select such a site for the injections is almost incredible.

Finally, as Fletcher (1923) has shewn, 'after intramuscular injection, quinine is absorbed less rapidly than after oral administration, and the method does not maintain an effective concentration of quinine in the body for a longer period than does quinine orally. The view that quinine injected into a muscle forms a reservoir which keeps up the supply of quinine in the peripheral blood, has no basis in fact.'

Under these circumstances, for algid, comatose and cerebral malaria, or where the patient cannot tolerate cinchona febrifuge or quinidine sulphate by the mouth, quinine should be given intravenously. (Quinidine is too toxic and cinchona febrifuge unsuitable for intravenous use.) The preparation which the writer prefers is quinine acid hydrobromide.

In giving such injections, certain precautions must be observed. As McCarrison and Cornwall (1918) have shewn, intravenous injections of quinine cause a profound fall of blood pressure and affect the respiratory centre. Hence they must be given with the patient in the recumbent posture. Since, however,

these injections should be reserved for critical cases, this will follow almost automatically.

Secondly, the injection must be a 'clean' one into the lumen of the vein, since any quinine which escapes into the tissues will cause acute necrosis. Thirdly, the injection must be given *very slowly*. J. D. Thomson (1917) advocates using a very fine needle and taking at least 20 seconds over the injection of each c.c. of solution, whilst McCarrison and Cornwall (1918) advocate taking a blood pressure reading as a preliminary, and adding 0.3 c.c. of commercial adrenalin solution to the injection in cases where the blood pressure is below 100 mm. of mercury. Lastly, the solution injected should be freshly prepared, since moulds grow very readily in old quinine solutions.

The writer considers, however, that the dangers of intravenous injections of quinine have been greatly exaggerated. He has given several hundreds of these injections without seeing any of the dramatic sequelæ described by some writers; whilst he has also received some 30 such injections at different times in the treatment of a chronic benign tertian infection as a patient. The injections are followed by a transient feeling of dizziness, lasting for only a few seconds, whilst quinine can often be tasted in the mouth before the injection is completed. The dose which he usually uses is from  $7\frac{1}{2}$  to 10 grains—*not more*—of quinine acid hydrobromide freshly dissolved in from 15 to 20 c.c. of sterile saline. He considers the dose advocated by S. P. James (1922) of 15 grains of acid hydrochloride in 5 c.c. of saline as being rather too large, and rather too concentrated.

In the majority of such critical cases, one or two such intravenous doses of  $7\frac{1}{2}$  grains each, given during the first 24 hours, will speedily bring the patient into a condition of comfort, with a normal or sub-normal temperature. The intravenous injections should then be discontinued and the patient should commence the full 21-day oral course of quinine or cinchona febrifuge. Intravenous quinine, although the strongest weapon in the physician's armamentarium for the immediate treatment of a critical attack of malaria, is useless in the prevention of relapses, and fails to sterilise the patient of parasites.

#### *Intramuscular Quinine.*

As far as the writer is aware it is only in India that the intramuscular administration of quinine salts is being widely resorted to. All the leading authorities in tropical medicine in England are opposed to this practice. Colonel Clayton Lane (1925) remarks with regard to it 'It is possible to push into a patient with a syringe a lasting conviction that if it has come to a choice between a bitter taste and a bitterly painful experience, it is better to take quinine by the mouth after all.' Professor Swellengrebel 'very much applauds' this remark of Colonel Clayton Lane's. Sir Ronald Ross (1914), Fletcher (1923), Bassett-Smith (1923) and

Acton and Knowles (1924) all record severe necrosis and even death following such injections. McLay (1922) compared blood films taken from patients after the administration of similar doses of quinine (*a*) by the mouth, and (*b*) intramuscularly; he found that parasites disappeared more quickly after oral than after intramuscular administration.

In the writer's opinion, the only occasion upon which it is necessary to resort to intramuscular quinine is in young children or fat patients where the veins are difficult to find or to inject. It is true that one may give hundreds of intramuscular injections of quinine without a disaster, but the very next injection may lead to such. The necrosis after intramuscular quinine is often of slow onset and its results may not become apparent until weeks or months after the injection. One of the worst cases which the writer ever saw was an Anglo-Indian female patient admitted to the Carmichael Hospital for Tropical Diseases, Calcutta, suffering from typhoid fever. Some medical practitioner in Calcutta had treated her for this disease by intramuscular injections of quinine into the deltoid muscles without even examining her blood for malarial parasites. When admitted to hospital in one arm there was an extensive, sloughing and dirty ulcer at the bottom of which the humerus lay bare and partly necrosed. In the other arm a large abscess was forming in the deltoid muscle. The fatal issue in this case was largely brought about by the lowering of the patient's resistance by the severe sepsis and prolonged suppuration in the arms.

#### *Malaria in Pregnancy.*

Malaria occurring during pregnancy is no contra-indication to quinine treatment, as is so often supposed to be the case. As has been shewn on p. 52, the blockage of the placental circulation by sporulating masses of the parasite is in itself likely to lead to the death of the foetus *in utero* and to abortion; in fact the malaria itself is far more likely to lead to abortion than is quinine administration. In such patients, however, it is advisable to give the daily dosage in small divided doses rather than in two or three large ones; doses of 6 grains or so may be given in alkaline solution every four hours or until the patient is receiving a full 18 or 24 grains a day.

#### *Malaria in Children.*

For the treatment of malaria in children relatively large doses of quinine should be administered, considering the age of the patient. Quinine as a rule is very well tolerated by children; but for babies and young children euquinine, which is quinine-ethyl-carbonate and is tasteless, may be substituted. For a baby 1 year old  $1\frac{1}{2}$  grains of euquinine should be given every six hours; for children from 3 to 5 years of age 5 grains of euquinine every six hours are suitable; and for children of from 5 to 10 years of age 7 grains of euquinine or 5 of quinine should be

given every six hours. Most children 10 years of age can stand a 10-grain dose of quinine, but it is preferable not to exceed 5 grains at a single dose for children of this age; the best plan is to get the child well under quinine therapy by doses of 5 grains, given in alkaline solution every four hours for the first day, and thereafter to give 5 grains t.d.s. for the three weeks' standard course.

#### *Intolerance to Quinine.*

In a few individuals—but in far fewer than is usually supposed—there is intolerance to quinine, and the administration of a dose of 10 grains leads to such anaphylactic reactions as superficial or deep-seated giant urticaria, hæmoglobinuria, severe abdominal colic, and intense headache. This intolerance is no contra-indication to treatment with quinine, but it is necessary to de-sensitise the patient by small doses before proceeding to larger ones. A preliminary dose of  $\frac{1}{2}$  grain of quinine may first be tried every four hours, and thereafter the dose be cautiously increased daily. Such patients can almost always be worked up gradually to a dose of 5 grains t.d.s. and kept on this dose for the full three weeks of standard treatment.

#### *After-Treatment.*

After-treatment is almost as important in malaria as the primary treatment of the disease with quinine or cinchona febrifuge. At the end of his course of quinine or cinchona febrifuge the patient will be afebrile, but still—in all probability—anæmic and run down. In this condition of post-malarial debility arsenic is an invaluable drug. It is the writer's custom for all such patients to prescribe Burroughs, Wellcome and Co.'s 'Tabloid' Ferri Citratis Comp., one tabloid after each meal three times a day for one month. The formula of this 'tabloid' is as follows:—ferri et ammonii citratis, grs. 3; quininæ sulphatis, gr. 1; acidi arseniosi, gr. 1-60th. Or Fowler's solution can be given, or some such general tonic as the following:—

R̄

Liquor arsenici hydrochloridi	..	..	..	m. 3
Liquor ferri perchloridi	..	..	..	m. 15
Spiritus chloroformi	..	..	..	m. 5
Infusum quassie	..	..	..	ad oz. 1.

Dose: one ounce t.d.s. after meals.

It is highly improbable that the cinchona alkaloids ever succeed in exterminating all the malarial parasites in a patient's system, and the destruction of the remaining parasites which are not affected by the course of quinine treatment is effected by the patient's own natural powers of resistance. In building up this resistance arsenic and iron are the two sheet-anchors of treatment.

It may be mentioned here that occasionally one comes across cases of chronic and relapsing benign tertian malaria where the patient has relapse after relapse despite repeated courses of quinine treatment—prescribed at least, if not swallowed. In some of these cases an intravenous injection of novarsenobillon given before the course of quinine treatment is prescribed seems to have a stimulating effect in aiding the patient to finally overcome his malarial infection.

#### *The Treatment of Blackwater Fever.*

The treatment of blackwater fever hardly comes within the scope of this small book, but a few notes on the subject may be added—culled chiefly from Dr. Manson-Bahr's admirable eighth edition of *Manson's Tropical Diseases*. The blackwater fever patient should be put to bed at once, and not even allowed to sit up in bed until all risk of sudden heart failure has passed. When the blackwater has commenced and the urine tends to be suppressed, caffeine citrate 2 grains twice in the twenty-four hours should be given as a bland diuretic. Plenty of bland fluids should be given and an exclusive milk diet prescribed until albumin has cleared from the urine. Hot fomentations should be applied to the loins, or cupping by Fenwick's glasses. High rectal lavages with hot water have a marked diuretic effect.

If, owing to persistent vomiting, fluid cannot be retained by the stomach, enemata of warm normal saline should be repeatedly administered, 6 to 8 ozs. every half hour or hour. Sodium bicarbonate in 5-grain doses dissolved in water should be given at frequent intervals by the mouth. Sterile saline may be slowly introduced into the subcutaneous tissues of the flank or elsewhere by means of a hollow needle attached by a rubber tube to some improvised reservoir placed one or two feet above the level of the patient. In cases with convulsions or coma, 5 per cent glucose solution given intravenously to the extent of 2 or 3 pints, is a useful measure. Marked restlessness may require morphia hypodermically by way of treatment, but the drug must be used with the utmost caution for these patients, and not more than  $\frac{1}{8}$ th of a grain or so given.

Oxygen inhalations are valuable if available, and blood transfusion may be indicated. James and Christophers (1922) sum up the treatment of blackwater fever in the following terms. 'The most important points are :—

1. Absolute rest in bed.
2. The most skilled nursing available.
3. Immediate cessation of all quinine treatment.
4. The free administration of alkaline drinks, barley water, and saline enemata or injections.
5. A continuous watchful endeavour to allay troublesome and dangerous symptoms.'



A remedy which may be of considerable value in blackwater fever is 'Hæmostyl, Roussel.' This is an activated horse serum for the treatment of hæmorrhagic states and anæmias of all grades. In preparing it young healthy horses are first selected, and a first copious bleeding resorted to. After this the animal reacts and its blood is drawn off at the moment when blood examination shews that the hæmopoietic reaction is most intense. The serum from this blood is sterilised by heating to 55°C. on five occasions, and this serum constitutes 'Hæmostyl.' It is put up in ampoules for subcutaneous administration or for rectal injection, as a syrup for oral administration, and also as desiccated tablets. The writer has not yet had an opportunity of trying Hæmostyl in blackwater fever, but has found it of great value in two patients with symptoms of pernicious anæmia.

#### *Prophylactic Quinine.*

Such contrary opinions are entertained as to the value of quinine prophylaxis against malaria that it is almost impossible to come to any conclusion at present as to its value. In all probability quinine has no destructive action on the malarial sporozoites injected into man by the infected mosquito. These enter the red blood corpuscles and become trophozoites. Prophylactic quinine in reality acts as very early treatment of a malarial infection rather than as a preventative of infection. Where other measures are not available—e.g., persons on survey work in heavily infested malarial country, or in camp—it is advisable to take 10 grains of quinine every evening in solution in the hope of warding off actual malarial fever, rather than of preventing becoming infected.

The 'good old custom' among tea planters in Assam of handing round a bottle of 5-grain tablets of quinine every evening at dinner with the sherry and the soup has nothing to recommend it, for it is very doubtful whether such a prophylactic measure has any value at all.

#### *Quinine and Cinchona Febrifuge Tablets.*

In general quinine or cinchona febrifuge should always be prescribed *in solution*, since only in this way can the medical practitioner make certain that the quinine will be absorbed. The tablets prepared by such well-known British firms as Burroughs, Wellcome and Co., Parke Davis and Co., and Howards are reliable and soluble, but quinine tablets and pills on the Indian market may consist of anything, even perhaps of concrete. Swellengrebel (1925) records the results of examination of tablets of quinine manufactured in Italy by the State factory. The coating of the tablet proved to be insoluble in water, and after 12 hours' immersion in water it floated off the surface of the tablet in an undissolved state. 'It has been shewn,' writes Clayton Lane (1924), 'that properly made tablets of quinine hydrochloride produce at least as much urinary excretion of quinine base as does the same quantity

in solution. There has been a tendency to blame sugar coating as preventing absorption, but sugar cannot but rapidly dissolve in any aqueous fluid. The fault really lies in the tablet's menstruum which, as Blanchard (1922) has shewn, may resist solubility for months and defy the attack of a hammer. It is as useless to expect absorption by solution of the quinine which lies in the centre of an impervious lump of concrete as it is unreasonable to condemn the popular sugar-coated rapidly disintegrating tablets of proved efficacy, and yet the quality of the menstruum is rarely considered in the reports of these cases; a state of things which perhaps prompts the generally recognised advantage of solution over quinine in tablet form.'

## CHAPTER V.

### ON THE CARRYING-OUT OF A MALARIAL SURVEY.

BEFORE any anti-malaria measures can be undertaken, it is necessary to carry out a malaria survey of the area under consideration. This may be a large town, a village, a tea or rubber plantation, a railway station, a mining camp, or a tract of country through which a railway or canal is to be constructed, or which it is proposed to open up for agricultural or industrial purposes. Experience of trying to live in the area may have already only too clearly proved that the location is malarious, or the directors of the enterprise may desire to know in advance the health difficulties likely to be encountered, and to formulate schemes to meet them.

Moreover, financial considerations rule anti-malaria works as everything else. Save in war time, such works are very seldom carried out without first counting the cost, and the latter cannot be arrived at without a proper survey as a basis. The Health Officer almost invariably has to justify his proposals before a coldly calculating and non-scientifically minded Finance Department or Board of Directors, and, if his proposals involve engineering works, the engineers will want exact definitions of what is required before estimating for their part of the scheme.

The area to be surveyed may vary from the mass of tanks, ponds, puddles, streams and canals that make up the environs of such a city as Calcutta to an almost trackless forest on a mountain side, where movement may be extremely difficult. How then to set about it under the varying conditions likely to be encountered ?

In every case certain questions have to be answered.

- I. What kind of area are we dealing with ? Its topography and meteorology.
- II. How much malaria exists in the area ?
- III. How is it distributed over the year ?
- IV. Does the species of parasite vary during the year ?
- V. What are the Anophelines of the area, and where do they breed ?
- VI. Have the various species seasonal distributions ?

- VII. Which of them are responsible for the malaria of the area ?  
 VIII. Under the conditions we are investigating, is the malaria becoming, or is it likely to become worse or better if nothing is done ?

When the answers to these questions are known, and not until then, is it possible to consider the problem effectively, and to formulate proposals for dealing with the situation envisaged. This chapter will be devoted to the consideration of the eight lines of investigation mentioned above, the writing up of the results of which in a proper report is usually known as a Malarial Survey.

### I. *Topography. Meteorology.*

The report should commence with a general description of the area surveyed. The configuration of the country, flat or hilly, forested or cultivated, its river system, and the like, should be the subject of a brief paragraph or two. Try and make your readers see in their mind what you have seen with your eyes, but remember that you are probably writing for busy men who are not specially interested in your subject, and try to be brief and concise. Likewise, the habits of the people, their chief occupations, and whether these afford them a steady or a precarious livelihood, should also be touched on, for if it is shewn, for instance, that fluctuations in the price of their staple money crop vitally affect their nutrition, and in certain years lower their vitality, fluctuations in the malaria curve are in part explicable, and indicate the necessity of undertaking a consideration of 'prices,' as was first done by Christophers (1908).

The meteorology of the tract under examination requires consideration, for by the study of such records much is often explicable, though the effects of climatic factors differ in different parts of the world. What, for instance, is explicable in the Punjab may totally fail to elucidate malaria fluctuations in Madras. And here, at once, the investigator will encounter difficulties in the shape of the paucity of recording stations in the tropics. In wide areas of the plains, the records of a station one hundred miles away may, with caution, be utilized as representing the meteorology of the area under survey, but among hills this is not the case. Rainfall, for instance, is often extremely local in hilly country.

If the survey is to be an extended one, it might be worth while starting a series of rainfall, maximum and minimum, and wet and dry bulb observations, but such is very seldom the case, and usually one is fortunate if a few years' rainfall figures are obtainable. Such are to be prized when found, for comparison with the epidemiological statistics considered in the next section. More often one has to be content with stating something of the following nature: 'the rainfall is about fifty inches annually, nearly all of it during the monsoon. Light January rains are sometimes experienced.' None the less, it is worth making the effort of gathering what information one can under this head.

II. *Amount of Malaria.*

This is obtainable by the study of four separate considerations :—

- A. Mortality Registration.
- B. Hospital Records.
- C. Splenic Index.
- D. Parasite Index.

Each of which requires a separate section.

A. *Mortality Registration.*—In civilized countries, where a qualified practitioner's certificate is required as a pre-requisite to burial, these records have a high value, but nowhere in the tropics is this happy state of affairs found. Vital statistics are nearly always recorded by a village headman or other totally unskilled minor official, and at first glance might therefore be thought to be entirely useless. As Christophers (1908), Gill (1922) and Cragg (1922), among others, have shewn, though utterly valueless directly, these figures, if properly treated have a considerable comparative value.

The Indian village watchman's register contains the following causes of death : 'Plague,' 'Cholera,' 'Smallpox,' 'Diarrhoea and Dysentery,' 'Respiratory Diseases,' 'Fever,' 'Injuries,' 'All other causes.' It is therefore obvious that the sixth will compete with the eighth as a 'column-filler'! The diseases most commonly confounded with malaria under 'fever' are typhus, relapsing fever, and enteric, and in the 1920 report of the Sanitary Commissioner of the United Provinces the opinion is given that the chowkidars' malaria figures are five times greater than they should be! Likewise, when the actual disease causing a mortality-rise is known, it is observed that the numbers of deaths entered under various other headings also tend to rise. An epidemic of malaria is invariably associated with a great relative increase in mortality entered under the head of 'diarrhoea and dysentery.'

Faced with this state of affairs, it may well be wondered how it can be claimed that such 'statistics' have even a comparative value, but Christophers (*loc. cit.*) has shewn that in general the percentage of error is constant. Further, in India at least, the various major epidemic diseases have their maxima at various times of the year. Plague, for instance, is generally a disease of the cold weather and early spring. In its septicæmic form, especially, it is liable to be entered as 'fever.' Relapsing fever is a disease which reaches its height in the late spring, whereas malaria attains its maximum usually in the autumn, and is distinguished by a rise in infantile mortality which is not shewn by any other epidemic disease. Bearing these considerations in mind it will be understood that comparisons can be instituted between the records of a series of years which possess a definite value.

In the actual tropics, however, seasonal incidence of the various diseases is less marked, whilst malaria shews maxima in various months according, among other

little-understood factors, to the causative species of Anopheline, and facts elucidated for Upper India must be accepted with caution in the actual tropics themselves. Kala-azar in an area will, of course, render vital statistics accumulated under the eight official heads quite valueless. The whole subject of Indian vital statistics is too complicated for detailed treatment here, and must be consulted in original in the papers quoted and in others, but a comprehensive article on the subject has yet to be written. Enough has been said to point out the pitfalls with which the subject is surrounded, and to indicate the extreme care with which local statistics must be accepted and handled.

After the malaria incidence in amount and time has been ascertained by other means, however, vital statistics again have their uses. There are few diseases except malaria and kala-azar that slowly kill off a population over a number of years, and malaria, as stated above, is peculiarly a disease causing infantile mortality. If in a hyper-malarious place it can be shewn that in certain months of the year the total deaths, and particularly the infantile deaths, exceed the births (and total deaths and births have been found by comparison with census figures to be quite accurately recorded), then obviously a most serious state of affairs exists. When the local population cannot hold their own against a disease, there is small hope for the future of the district unless anti-malaria measures are undertaken, and less chance still for any new-comers to it to thrive. An area of increasing malariousness has probably been encountered, and the cause of this must be sought. Portions of Bengal, the south side of Vizagapatam Harbour, and Manaar Island between India and Ceylon are examples of such places; which, fortunately, are comparatively few.

B. *Hospital Records*.—In most localities in India, at least, something of this sort is usually obtainable. But again, such must be accepted with a certain amount of caution. Assistant and sub assistant surgeons in charge of remote posts are not usually distinguished for the extent of their knowledge, (India has still to throw up a Jenner or a Robineau-Desvoidy from the depths of the *mofussil*), and their diagnoses are not seldom at fault. Whilst free, of course, from the gross errors that distinguish the vital statistics, the para-typhoids in particular are often entered and, worse, treated, as malaria. Only too seldom is a microscope, or knowledge of its use available, and even if such were the case, what chance or incentive has a heavily worked man in charge of an out-station dispensary to devote the necessary time to accurate microscopical diagnosis? But, here again, the percentage of errors is probably constant, and for comparative purposes over a series of years, may be safely used. In this way the records of an out-patient department often have great value.

Of course there are numerous areas in the less developed tracts of the tropics where neither, and especially the latter, of the foregoing figures are available, and like the writer in exploring the Vizagapatam Agency Tracts in

the last year, the observer must trust to himself alone for his facts, and in any case *without exception*, must collect such for himself to obtain any accurate idea of the actual and not merely the comparative malariousness of the area under survey.

C. *Splenic Index*.—From the very early days of malaria investigation, the examination of a community for splenic enlargement has been the criterion of the malariousness of a locality. In this work it was soon found that children between the ages of two and ten formed the only safe guide (Stephens and Christophers, 1908), for among the actual inhabitants of a hyper-malarious tract, the adults shew an immunity characterized by the reduction of the splenic enlargement caused by their infantile malaria morbidity. The infantile spleen rate, *per cent*, then, is the basic measure of the endemic malariousness of a locality, the adult spleen rate of the actual amount of malaria that is occurring at the moment of examination. The former alone is useful for the purpose under discussion. Moreover, it is easier to obtain than the adult rate, which in an undisciplined community such as is commonly encountered, has to be confined to the male population only.

The amount of splenic enlargement may be measured with the child standing up or lying down, and as Watson (1915) has shewn, considerable differences in the results obtained are given by the two methods, the recumbent position yielding the higher values, as by its means very small degrees of enlargement are more easily palpated when the organ descends during inspiration. In the Southern United States, where the average amount of enlargement is small, the recumbent seems to be the position most usually adopted, but in the tropics, with the gross degrees of enlargement commonly encountered, the standing position is nearly always used. In any case, it is necessary to adopt one or the other, and stick to it. Occasionally, a doubtful result in the erect position may need checking by laying the child on its back with the knees flexed.

A few hints on examining children for spleen may be useful. If opportunity permits, it is best to send a chaprassi to the village about to be examined some hours in advance, with a message to the headman to collect the children at some convenient central spot at a certain hour, and to remember in this connection that the villager never has aught but the vaguest ideas of time. It is seldom indeed that such a message is disregarded. If it is, or if the village is visited unexpectedly, such as must occur in working up a district by a road with motor transport for instance, then less time is wasted by perambulating the village than by waiting whilst an attempt is made to collect the children.

Many books mention that small change is useful in attracting children. Indiscriminate scattering of largesse in this fashion I have never found necessary. Save in very jungly tracts, where a supply must be carried—a sticky performance—there is nearly always a sweet-meat seller to be found, and eight annas worth of his wares

will suffice for the child population of the average small village. Begin by offering a sweet or two to a few of the bolder or better dressed children, who are obviously those of village notables, and then give each child a sweet at the completion of its examination, not before. Commence by examining older boys, who are less likely to be frightened, and try and avoid struggling to palpate a screaming child before a mob of as yet unexamined children. Motion the parents of such to keep them aside until the rest have been dealt with. Then make a fresh attempt, preceded if necessary by a sweet to instil confidence and attract interest. (A word here to European investigators: leave the actual palpation work to your Indian or native medical assistant if there are general symptoms of fright. Remember that you are in any case something strange and rather frightsome in the average remote village, and though a distinct 'draw' in yourself, your closer approach may often start a child screaming.) At the end of an examination, it will invariably be the case that the sick of the village will be brought before you. Often the nearest dispensary is miles away, and there may never have been a medical man in the village before. A few simple remedies should therefore be carried on spleen-index expeditions, for it makes for general good-will if a little free doctoring is done before the party leaves the village, and in any case smooths the way wonderfully if blood films are wanted. Many most interesting cases in advanced stages are encountered under such circumstances, and the writer has seen in villages remote from medical aid enough clinical material to keep a whole research staff happy!

Reverting now to the actual spleens palpated, in addition to the percentage shewing enlargement, the spleens examined are usually classified in finger-breadths of enlargement below the costal margin. In some of the earlier work in India, five classes of enlargement were used, but it is better to make seven, in which the 'four-finger-breadths' degree is a spleen reaching to the level of the umbilicus, and the 'seven fingers' category spleens reaching right down to the pubis. The results of each examination should be called out by the observer and noted down by another of the party, the children being numbered consecutively. It is seldom necessary to record their names, unless the village is to be visited again for a subsequent examination, when it may be required to trace individual children.

Obviously, the above method of examination affords entrance to fallacies. The distance from the costal arch to the pubis is very different in an infant of two years and a child of ten, whilst the breadth of the observer's finger remains constant. Likewise, in various individuals, the position of the costal arch varies considerably. Christophers and Khazan Chand (1924) have devised a method for overcoming the first difficulty, by applying to the actual measurement from the costal margin to the apex of the enlarged spleen a correction based on the nipple-umbilicus length of the child, though sitting-height can also be used as a basis for the correction. Table II



Standard chart for Indian child (60 cms. sitting height).

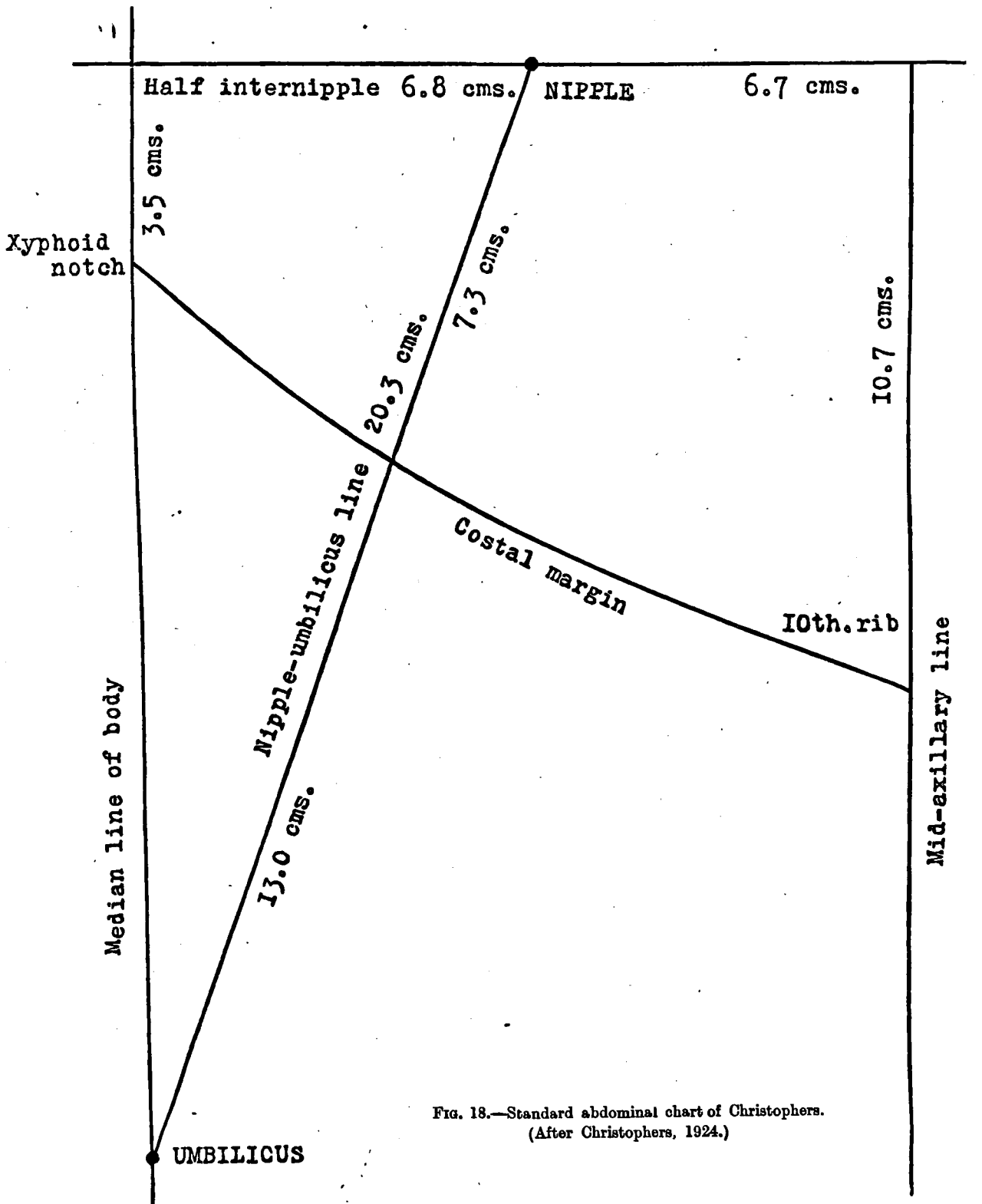


FIG. 18.—Standard abdominal chart of Christophers.  
(After Christophers, 1924.)

cms.

TABLE II.

CORRECTION TABLE FOR SPLEEN MEASUREMENTS.

Shewing correction for measurements of spleen of other abdominal measurements by the sitting-height, nipple-umbilicus line or age (recession 0·8).

S. H. N. U. Age.		Measurement in centimetres.																			
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
40	2	3	4	5	7	8	10	11	12	14	15	16	18	19	20	22	23	25	26	27	
41		3	4	5	7	8	9	11	12	13	15	16	17	19	20	21	23	24	25	27	
42		3	4	5	7	8	9	11	12	13	14	16	17	18	20	21	22	24	25	26	
43	16	3	4	5	6	8	9	10	12	13	14	16	17	18	19	21	22	23	25	26	
44		3	4	5	6	8	9	10	11	13	14	15	17	18	19	20	22	23	24	25	
45		3	4	5	6	7	9	10	11	12	14	15	16	17	19	20	21	22	24	25	
46	17	3	4	5	6	7	8	10	11	12	14	15	16	17	18	20	21	22	23	25	
47		2	4	5	6	7	8	10	11	12	13	15	16	17	18	19	21	22	23	24	
48		2	4	5	6	7	8	10	11	12	13	14	15	17	18	19	20	21	23	24	
49		2	4	5	6	7	8	9	11	12	13	14	15	16	18	19	20	21	22	23	
50	18	4	2	3	5	6	7	8	9	10	12	13	14	15	16	17	18	20	21	22	
51		2	3	5	6	7	8	9	10	11	12	14	15	16	17	18	19	20	22	23	
52		2	3	5	6	7	8	9	10	11	12	13	15	16	17	18	19	20	21	22	
53	19	5	2	3	4	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21	
54		2	3	4	5	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	
55		2	3	4	5	6	7	9	10	11	12	13	14	15	16	17	18	20	21	22	
56	20	6	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19	20	
57		2	3	4	5	6	7	8	9	10	11	12	14	15	16	17	18	19	20	21	
58		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	
59	21	7	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
60		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
61		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
62	22	8	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
63		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	19	
64		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	19	
65	23	9	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
66		2	3	4	5	6	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
67		2	3	4	5	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
68	24	10	2	3	4	5	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
69		2	3	4	4	5	6	7	8	9	10	11	12	13	14	14	15	16	17	18	
70		2	3	4	4	5	6	7	8	9	10	11	12	13	14	14	15	16	17	18	
71	25	12	2	3	3	4	5	6	7	8	9	10	11	12	13	14	14	15	16	17	
72		2	3	3	4	5	6	7	8	9	10	10	11	12	13	14	15	16	17	17	
73		2	3	3	4	5	6	7	8	9	9	10	11	12	13	14	14	15	16	17	
74	26	14	2	3	3	4	5	6	7	8	8	9	10	11	12	13	14	15	16	17	
75		2	2	3	4	5	6	7	7	8	9	10	11	12	12	13	14	15	16	17	
76	17-20	16	2	2	3	4	5	6	7	7	8	9	10	11	12	12	13	14	15	16	
77		2	2	3	4	5	6	7	7	8	9	10	11	11	12	13	14	15	15	16	
78	27	2	2	3	4	5	6	6	7	8	9	10	10	11	12	13	14	15	15	16	
79		2	2	3	4	5	6	6	7	8	9	10	10	11	12	13	14	14	15	16	
80		2	2	3	4	5	6	6	7	8	9	9	10	11	12	13	13	14	15	16	

Note.—Measurements of 1 cm. are unchanged.

Note.—Readings for correction by nipple-umbilical line or age should be taken along the line opposite the figures in the columns referring to these.

S. H.—Sitting-height.

N. U. = Nipple-umbilical line.

The observed measurements are those given in the top line of figures; the corrected values are in columns below these.

is a reproduction of their table. For a discussion of the reasons and formula underlying it, the original paper should be consulted. This correction is applicable to the correction of any other abdominal measurement. All that it is necessary to do in the field is to mark with a bit of grease pencil on the skin of the child the position of the apex of the enlarged portion of the spleen, and with a short, (18-inch) length of flexible tape to measure the two distances, costal arch-apex, and nipple-umbilicus.

From the foregoing measurements, Christophers (1924) has described the construction of an 'abdominal chart' (Fig. 18). His directions are as follows :—

'Draw a line representing the median line of the body. At right angles to this draw another line crossing it. Mark on the second line the position of the left nipple by measuring off along the line a distance equal to-half the inter-nipple line. Continue the inter-nipple line outwards the measured distance to the mid-axillary line. Make a line from the nipple of the length of the nipple-umbilicus line to intersect the median line of the body at the umbilicus. Measure off the distance from the umbilicus to the mid-axillary line and draw a line to represent the latter. Mark on the median line of the body the distance of the xiphoid notch below the inter-nipple line ; and on the nipple-umbilicus line the distance of the costal margin from the nipple ; also on the mid-axillary line the position of the lower edge of the 10th rib. The costal margin will be along a curved line, convex downwards, joining these last-mentioned three points.'

In order to compare the results of various observers, variously recorded, Christophers (*loc. cit.*) has also prepared a 'standard abdominal chart,' (*vide* Fig. 18) which is that of a child of 60 cm. sitting height. On such can be studied (after correction by Table II for size of child), measurements and locations, etc., of the spleen. By its means any anatomical reference to the position of the spleen can be easily translated into measurements. For instance, 'a spleen reaching half-way between costal-margin and umbilicus would on the standard chart be one of 6 to 7 cm., and if the child on which the observation was made was aged 12, this spleen must have actually measured 7 to 8 cm., as shewn in the correction table reproduced above. This would be a small four-finger-breadth spleen on the usual Indian observer's notation, or one reaching to a level of about 4 cm. below the 10th rib in the mid-axillary line.' Observe how, when the child grows larger, the simple finger-breadth method breaks down. As stated earlier, in the average (i.e., 6 years old) child in the 2 to 10 years class the 'four-finger spleen' reaches to the level of the umbilicus.

Christophers (*loc. cit.*) has distinguished three classes of enlarged spleens :

- (a) those in which the position of the costal margin is about normal ;
- (b) those with the costal margin 'raised' ('distant' spleens) ;
- (c) those with the costal margin depressed ('near' spleens) ;

and shews how, due to the effect of such differences in the position of the margin, spleens of the same measurement may be of different degrees of enlargement and vice versa, (*vide* Fig. 19).

In spite of these differences, however, when results are plotted on one chart, the apex in all three series of positions is situated in approximately the same line, since differences are not due to any lateral displacement of the axis of the spleen,

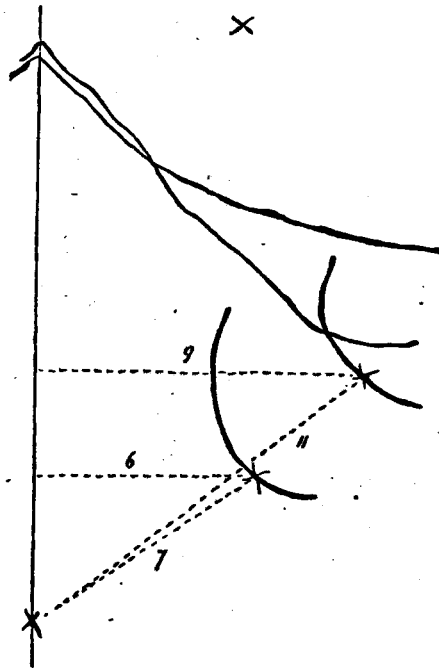


FIG. 19.—'Distant' and 'near' spleens; shewing spleens of the same degree of enlargement at different distances from the umbilicus, owing to variations in the position of the costal arch. (After Christophers, 1924.)

but to the fact that 'distant' spleens are further back, and 'near' spleens further forward in the same line representing the advance of the apex of the spleen from the rib margin to the umbilicus. All that is necessary, therefore, is to take two more measurements, (i) from the apex of the spleen to the umbilicus, and (ii) from the apex to the mid-line of the body, measured at right angles to

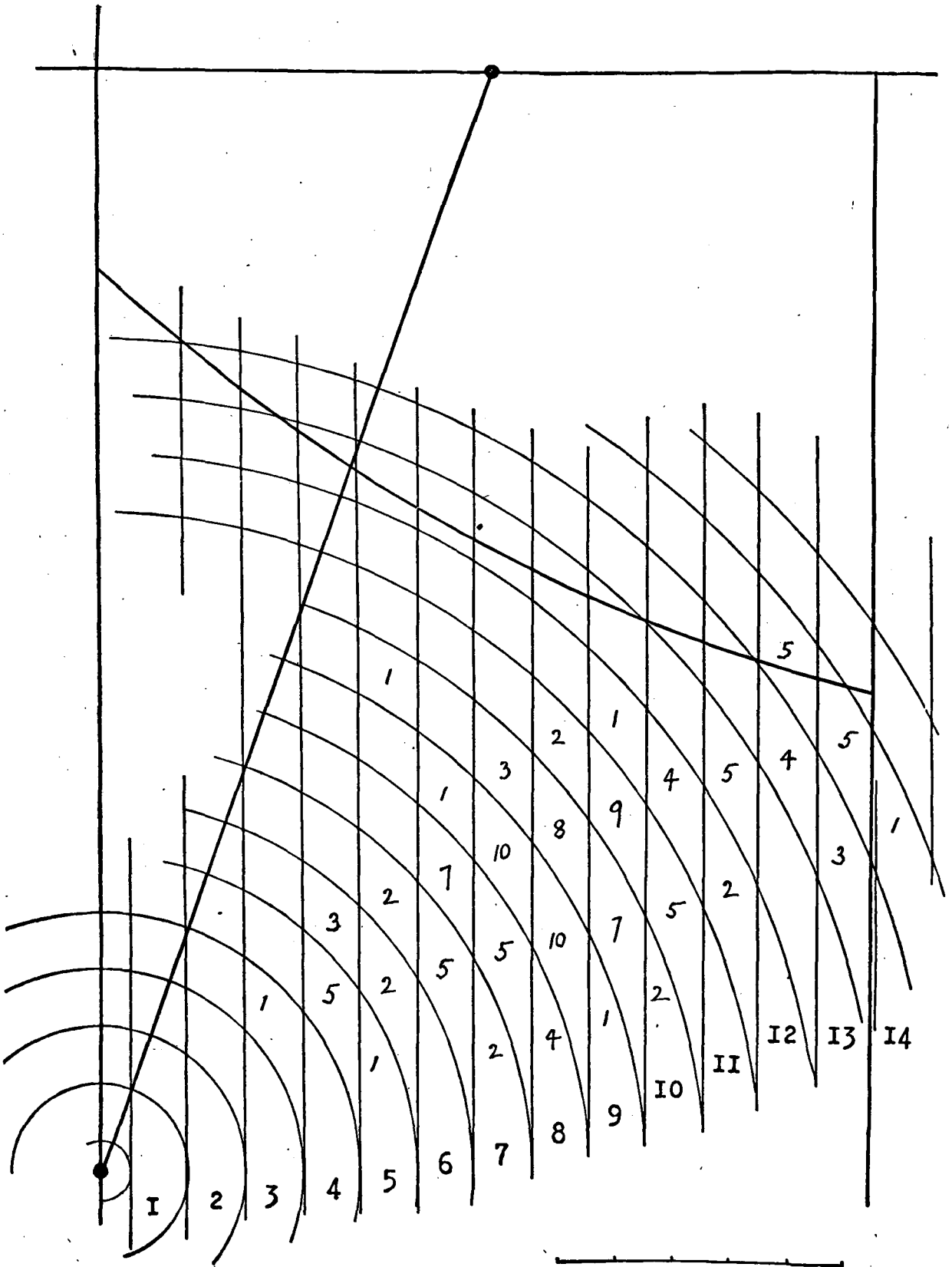


FIG. 20.—Abdominal chart, with results of a spleen census entered thereon. (After Christophers, 1924.)

TABLE III.

*Apex to umbilicus measurement.*

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
14															1	1	
13														3	5	8	
12													4	5		9	
11												2	5			7	
10											2	5	4			11	
9									1	7	9	1				18	
8								4	10	8	2					24	
7								2	5	10	3					20	
6								5	7	1						13	
5						1	2	2			1					6	
4						5	3									8	
3					1											1	
2																	
1																	
0																	
<b>Total</b>					1	6	5	9	16	22	21	16	7	5	7	11	126
																	<b>Mean apex to umbilicus.....9.9 cms.</b>
																	<b>Mean apex to median line.....8.3 ,,</b>

TABLE III.—The same information as in Fig. 20 in tabular form. (After Christophers, 1924.)

this line, and the second difficulty, that of the varying position of the costal arch, can be overcome.

With these measurements, only one position of the apex in the abdomen is possible, unless the apex lies beneath the level of the umbilicus, as in a spleen of very large size, in which case an appropriate symbol (a stroke under the recorded measurement) is to be made to indicate that the measurement was made below the level of the umbilicus. In case of measurements to the right of the middle line of

the body a minus sign is to be given. Moreover, as the apex-costal arch measurement has also been made, the position of the latter is also fixed, and practically complete information is on hand regarding the proportions of the abdomen and the position of the spleen.

Since every double measurement indicates a definite point on the abdomen, the position of the apices of the spleens in any series examined can be marked on the standard abdominal chart, i.e., after reducing the actual measured values to those of a child of 60 cm. sitting-height by means of Table II, using the nipple-umbilicus line as an indicator. The chart may be ruled with two sets of lines, one set being circles giving distances of 1, 2, 3, etc., cm. from the umbilicus, the other being straight lines drawn parallel to the median line of the body at distances of  $\frac{1}{2}$ ,  $1\frac{1}{2}$ ,  $2\frac{1}{2}$ , etc., cm., so that the spaces correspond with measurements of 1, 2, 3, etc., cm. The lines form a series of diamond-shaped spaces each equivalent to a spleen measurement on the double notation. Into each space, therefore, can be entered the number of spleens shewing this particular measurement. The results of such a procedure carried out by Christophers is shewn in Fig. 20, and the same information entered in tabular form in Table III. To fill in such a chart, place the point of the pencil over the number in the line of figures at the bottom of the chart and follow the *curved* space until in the straight column over the figure that corresponds to the apex-median line measurement.

This method of charting gives the frequency distribution of the position of the apex for the community. In the series illustrated in Fig. 20 the mean for the series lies between spaces 10:9 and 10:8, the first figure referring to the apex-mid line, and the second to the apex-umbilicus values, and corresponds with an average enlargement of about three finger-breadths in the usual notation.

Unless time is of no importance, however, refinements of this nature in spleen-rate work seem impracticable. The writer has only seen the method used once since Lieutenant-Colonel Christophers described it, and in studying the report in question came to the conclusion that the author thereof had devoted so much time to spleen measurements that the practically much more important question of malaria causation had had to be somewhat perfunctorily dealt with! None the less, the value of Christophers' method for comparative purposes is so great that it is necessary to describe it. The writer has no personal experience of its use, however.

When the spleen census has been obtained by the usual rough field method, classified into eight classes, from 'nil' to 'seven finger-breadths,' there are still comparative methods open to us, which can be worked up and utilized at leisure. The 'average spleen' may be obtained. This is a 'weighted average' as explained

by Christophers (1915), and may be arrived at for our purposes as in the following example :—

Amount of enlargement.	Number of children.	Splenic volume.	Value.
Nil .. .. .	89	V <sub>0</sub>	0
1 finger-breadth .. .. .	38	V <sub>1</sub>	38
2 " " .. .. .	36	V <sub>2</sub>	72
3 " " .. .. .	26	V <sub>3</sub>	78
4 " " .. .. .	10	V <sub>4</sub>	40
5 " " .. .. .	9	V <sub>5</sub>	45
6 " " .. .. .	1	V <sub>6</sub>	6
7 " " .. .. .	1	V <sub>7</sub>	7
<b>TOTAL</b> .. .. .	<b>210</b>	<b>..</b>	<b>286</b>

then  $\mu$ , the average spleen, =  $\frac{286}{210} = 1.36$  finger-breadths.

Comparison of figures yielding equal percentage rates, but taken in localities where the number of spleens enlarged slightly or largely differs considerably, will at once shew how greatly the total mass of enlargement caused by the malaria of a community may vary, even though the actual percentage with enlarged spleens may be the same. Thus a further idea of the malariousness of the area under examination is obtainable.

But this is not all that can be obtained from the spleen figures. Clinically, it is impossible to differentiate a relapse from a re-infection, but McKendrick has devised a mathematical method of solution, whereby it is possible at least to arrive at the ratio of one to the other in any community, which is invaluable for calculating what is happening through the agency of mosquitoes at the moment. Though individual cases are still impossible of differentiation, still, knowing the ratio, this can be applied to hospital statistics, when available. For instance, admissions may shew a tendency to decrease, whilst various signs may shew that mosquito



breeding is on the decline. If, in addition, it can be shewn that the ratio of relapse to re-infection is high, then everything tends to prove that the peak of the epidemic we may be investigating is past.

The formula, for the comprehension of the proof of which very high mathematical acquirements are required, and for the proof of which the original paper must be consulted, is:—

$$\frac{l}{k} = \frac{m-\mu}{1-m-\epsilon^{-m}}$$

where

$$m = \text{nat. log. } \frac{N}{V_0}$$

$N$  = total number of children examined.

$V_0$  = number of children with nil splenic enlargement.

$\mu$  = the average enlarged spleen, as explained above.

$l$  = relapse.

$k$  = re-infection.

The natural logarithms and the exponential can be obtained from any book of tables of hyperbolic functions, such as those of Becker and van Orstrand (1921). As previously mentioned, the result is of course only applicable to the period of examination, and not to the year for instance, for obvious reasons. A series of figures, worked out for the same place over a year, would undoubtedly yield results of the highest importance, if correlated with the mosquito breeding findings.

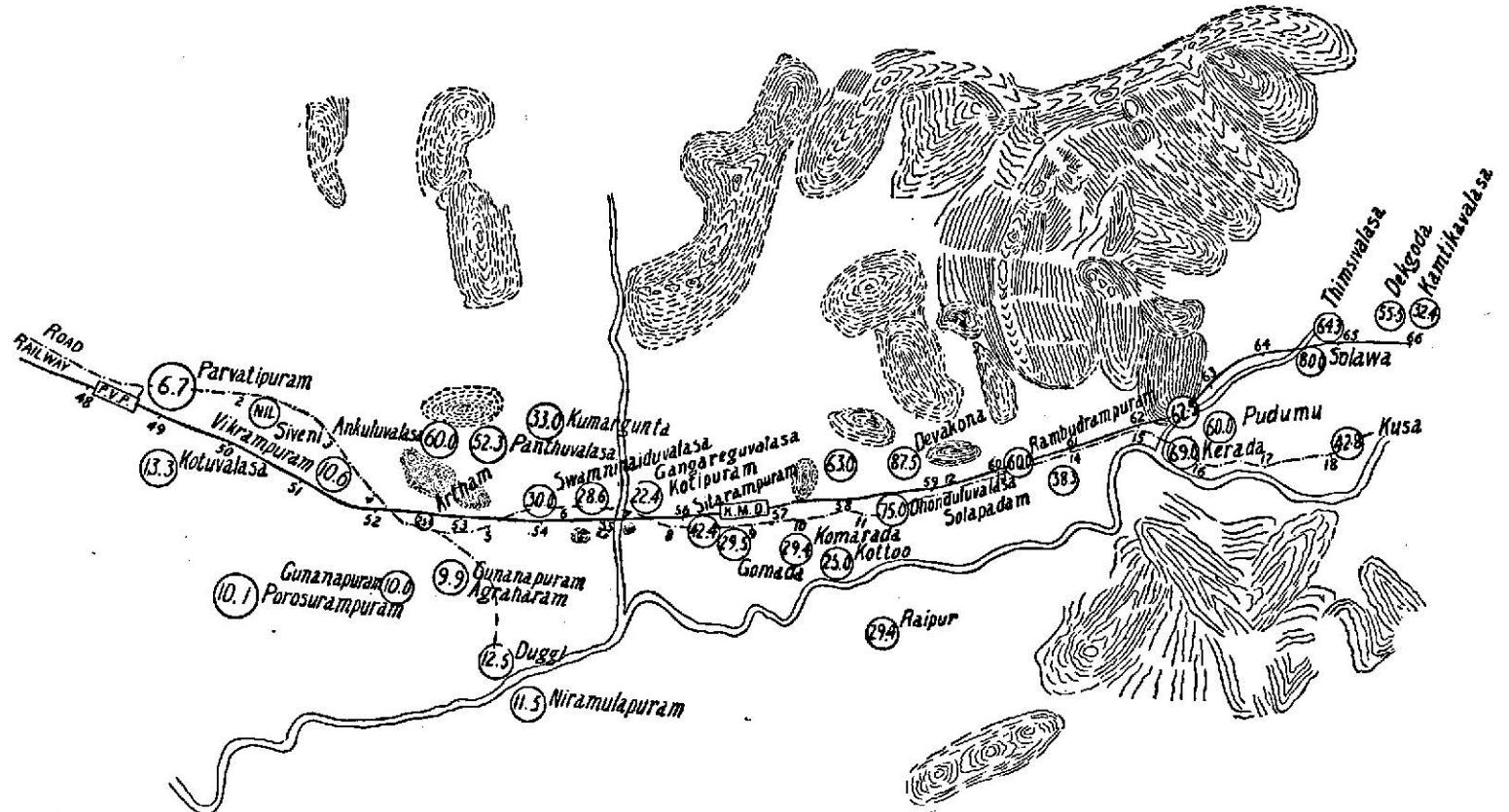
*Classification of Malariousness.*—In the numerous provincial malaria surveys carried out in India during the first decade of the century, the following rough classification, based on the crude child spleen rate, was adopted, and is to some extent still in use:—

Spleen rate greater than 50 per cent	..	HYPER-ENDEMIC.
Spleen rate 25 per cent to 50 per cent	..	HIGHLY ENDEMIC.
Spleen rate 10 per cent to 25 per cent	..	MODERATELY ENDEMIC.
Spleen rate less than 10 per cent	..	HEALTHY.

*Spleen Index Map.*—In a survey of an extended area, such as a valley, a spleen index map is extremely useful. By its means increases in the rate towards the hills, for instance, are well brought out. A 1 inch to the mile scale is usually sufficient, unless the incidence in the various quarters of a large town is being shewn. Various methods are in use:—

- (i) Various forms of shading or colouring.
- (ii) The placing beside or within the site of the village or town-quarter, of a circle, divided into one hundred divisions, with the angle between the two radii the number of divisions between which equals the spleen index, filled in black.

PLATE VI.



Spleen rates in villages on a section of the Raipur-Vizianagram Railway, Vizagapatam District.

(iii) The writing in figures within the circle representing the village or town of the actual spleen rate.

The writer prefers the first or last method. It is impossible to read accurately the rate from small circles such as are generally used in method (ii). Even if colour washes for the various limits of incidence are used, it is usually necessary to supplement this by adding figures. Unless, therefore, it is desired to be especially graphic, method (iii) is the best. Part of such a map is reproduced in Plate VI.

*Effect of Kala-azar.*—This is the only other common disease which causes splenic enlargement, and in known kala-azar areas and in those where the existence of the disease is suspect or possible it is necessary to be on one's guard against attributing to malaria the results of leishmaniasis. At first sight it might be thought that in kala-azar areas the spleen rate is too likely to be a compound effect to have any meaning, but Dodds-Price and Strickland (1925) consider that this is not the case. Their conclusion is 'that when the clinical signs of kala-azar are well marked such cases must not be used in a malarimetric spleen index.' For malarimetry other cases only should be taken for a spleen count, and the index read in conjunction with a parasite index of all members of the community. (For parasite index, *vide* next section.)

The clinical signs of kala-azar are well known and it is easy to differentiate a typical case. An emaciated child, with the well-known 'distress' facies, with a hard spleen of at least three finger-breadths, should be tested by the formol-gel or aldehyde reaction (Napier, 1922), before accepting its splenomegaly as due to malaria. A differential point worth remembering is that in acute kala-azar the spleen will be enlarged to several fingers' breadth below the costal arch in a fortnight's time after the initial outburst of high fever. Such rapid enlargement is not seen in malaria.

D. *Parasite Index.*—This is a simple percentage figure for the number of persons actually shewing parasites in their peripheral blood at the time of examination, and, again, the child and adult figures should be kept separate, as the spleen rate falls with age more rapidly than does the parasite rate, which shews how many of the adults of a community may be 'healthy carriers' (James, 1920), especially in hyper-malarious localities.

But here, for the first time, we get to know what species of parasite we are dealing with, a most important point vitally affecting our ultimate recommendations on the treatment side. Also we get an idea of what chance the local Anophelines stand of acquiring infection, if we keep a separate count of gametocytes, noting the number counted per hundred leucocytes of all kinds seen. For instance, if malignant tertian is dominant, we may fear pandemics with fatalities; if quartan is prevalent, we may look for many intractable relapses which will try our medication arrangements to the utmost. Many gametocytes

will indicate that the local Anophelines are probably intensely infected at the moment.

The parasite rate should always be obtained from the study of thick as well as thin films, as set out in Chapter III. When a large number of persons are being examined, the two are best made and dealt with on one slide, as described by Sinton (1925), (*vide* p. 76).

A further refinement is due to Christophers (1924*a*) whereby a measured quantity, such as 10 c. mm. of blood is spread out over a measured surface of the slide, and the actual number of parasites counted. One can only wish that the requirements of practical work permitted generally of such methods of procedure.

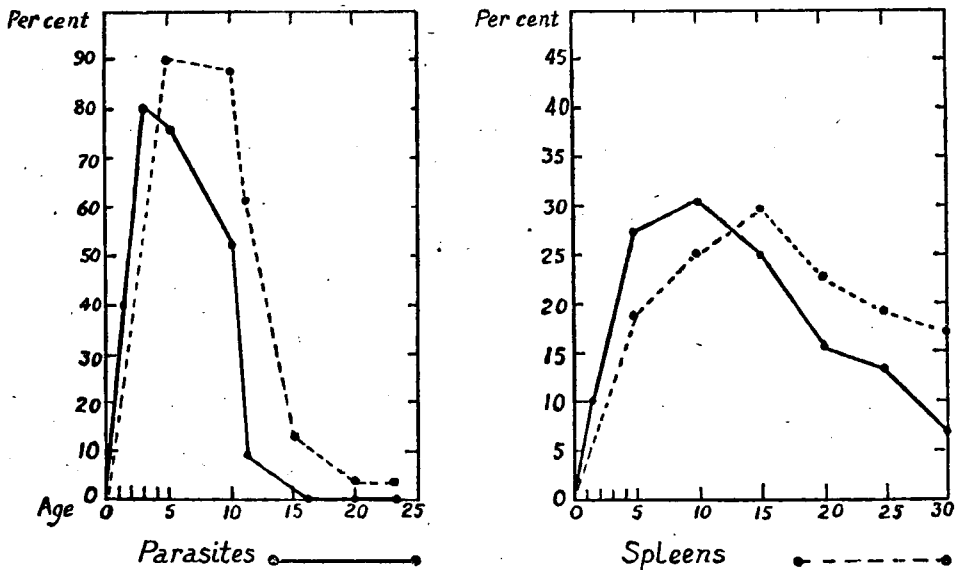


FIG. 21.—Shewing differences between spleen and parasite rates according to age (After James, 1920.) (Hyper-endemic area on the left; area of lesser intensity on the right.)

A convenient method of carrying slides for taking numerous films is to place them, ready cleaned, in a cabinet box with slots in which they stand closely approximated on their long edges. The slots may be numbered, so that any interesting case can have its number easily recorded.

On whatever work one is engaged, it is always well to have a few cleaned slides, each wrapped separately in tissue paper, in one's pocket. A fever case may be encountered by the roadside, or in a hut one passes, and in the writer's experience a blood film is more easily granted by a fever case than by an apparently well person. To the ignorant villager the taking of the blood seems to be accepted as part of the 'cure' which they hope for from you. It is hardly necessary to point out that parasites are more easily found during a paroxysm than at other times.

We are now in a position to answer question II. We know the child spleen-rate, which gives us a measure of the *endemicity*, the average amount of enlargement, which gives us a measure of the *intensity*, we have some idea at least of the proportions in which the three parasites are present, and can adapt our proposals to meet the varying indications we can draw from our findings. Knowing the amount of malaria existing at the moment, we can take the vital or hospital statistics of the moment as unity, and use past records for a comparison on this basis.

### III. *How is Malaria distributed over the Year?*

Unless we have been able to extend our studies over a twelve-month, we are driven back on hospital and registration statistics for this information, but, after making our own studies of the amount of malaria obtaining at the time of our visit, as mentioned at the close of the last section, we are in a better position to use them. Knowing the amount of malaria existing at the moment, and the relapse-re-infection ratio, we can compare current hospital records with the same over a year, and in the corresponding month of previous years; we can guess at the amount of reliance to be placed on registration figures, can use them comparatively. In months of active spread of infection, we can concentrate our efforts on the mosquito, in months where most of the cases are relapses, on the eradication of the parasite.

### IV. *Does the Dominant Species of Parasite vary over the Year?*

This question can only be answered if the observer is prepared to remain in or re-visit the locality at frequent intervals, unless he can arrange for a periodical supply of blood films to be sent to his headquarters. With enough assistance, this is possible, and if carried out, yields most interesting results of great value. For instance, if the only malignant tertian forms found were crescents, and all active infections shewed only benign tertian schizogony in progress, we could judge that the latter was causing the active malaria of the moment, and that malignant tertian had previously been largely present, but was now dying down.

The writer has seen the most intensely interesting series of observations of this nature kept at a hospital for several years, and it is an extreme pity that their author, after his indefatigable labours in thus examining all his malaria cases, has not published his results. Imitation is possible to anyone in charge of a hospital who will give the necessary time and trouble. The results in question shew how at that particular station benign tertian dominated the spring cases, and malignant tertian those of the autumn, whilst quartan infections could always be traced to changes in population involving transfers from a certain other station where evidently this form of parasite is common, though it was never contracted locally.

The information as to parasite distribution is useful in arranging for medication. Whilst it is preferable to attack malignant tertian with quinine, benign

tertian is better dealt with by other cinchona alkaloids, and hospital standard mixtures can be arranged accordingly. The months in which preparation should be ready for promptly dealing with urgent cerebral symptoms, and the like, also become known.

V. *What are the Anophelines of the Area? Where do they breed?*

Though tabulated as question V, it is best to commence work with the study of this point, as it is more time-consuming than any of the others. It may take longer to search many blood films, but this can be done at leisure in the base laboratory, whereas the study of the Anopheline fauna ties the investigator to the actual spot. Moreover—and it is no small point when it comes to spleen and blood investigations—having seen the party moving about for some days the local inhabitants will have become interested in what they are doing, familiar with their appearance, and more likely not to resent closer contact with them later. Never let your underlings rudely drive off persons shewing an interest in your strange performances!

How then to set about the study of the Anopheline fauna? It is seldom indeed that a map affording the necessary details of water location is available. One is fortunate if a large scale map shewing the chief topographical features of the locality is obtainable at all, and if it is, such as a plan of a plantation or railway station, it is always too crowded with detail irrelevant to our purpose to form a direct medium on which can be inscribed one's mosquito findings. If several copies are available, one may be taken into the field and scribbled on as required, but if not, then make during your peregrinations a very rough sketch map, shewing the principal points that will be identifiable on the proper map. Then prepare a tracing on draftsman's linen cloth of the actual surveyed map, but shewing only the salient features of the vicinity, such as hills, streams, bridges, etc., and add thereto from the notes on your field copy or from your rough sketch the approximate location of the ditches, ponds, etc., that you have examined. These will not, of course, be accurately located, but when the engineer comes later to carry out your recommendations, he will have little difficulty in identifying the spots mentioned in your report:—400 feet to 1 inch is the minimum scale of any use, twice this is preferable. In absolutely unmapped country a large scale sketch, roughly oriented, is the best that can be done, but for the report a map of some kind there must be.

Making notes for the above map as we go along, and starting from a fixed point to which we shall ultimately return and know that we have completely encircled the area to be surveyed, examine one by one every piece of water, however small, that is encountered. *On the thorough hunting of each and every body of water for larvæ, and on the omission of none, depends absolutely the ultimate success of any anti-malaria scheme based on mosquito control.*

The writer takes the following equipment with him into the field :—

1. Box containing 24 4" × 1" flat bottomed specimen tubes, the corks of which are serially numbered. The tubes fit through holes in a partition three-quarters the height of the box, the bottom and lid of which are lined with sheet rubber. The box has a flexible handle attached to the box and *not to the lid*, which slips aside for opening, but obviates an upset if the catches are pulled back when pushing through thick bush.
2. Two 6" pipettes,  $\frac{1}{2}$ " bore with aperture not less than  $\frac{1}{8}$ ", with rubber teats large enough to enable half the volume of the pipette to be sucked up. These lie on the partition in the box between the rows of tubes.
3. An enamelled soup ladle, the inside of the bowl white, and of 4" to 5" diameter, with handle about 2 feet long. It is best to use a pattern with the end of the handle hooked, so that it can be hung on a belt or pocket whilst writing notes, saving much stooping.
4. A loose-leaf pattern note book, the individual leaves about 6" × 4".
5. Pocket lens. Curved forceps, flat pointed (entomological pattern). One or two tubes of spirit.
6. Hydrogen-ion reagents and the colour chart from Clark (1922), which can be purchased separately. The set fitting into a box with carrying handle.

A fieldman and chaprassi carry the above, and after a little training soon learn their respective duties.

Arrived at a piece of water, one proceeds to search it systematically for larvæ by dipping with the spoon. If such are found they are transferred by means of the pipette to a numbered tube, and if pH work is being done, another tube, filled absolutely full of water, without larvæ or vegetation, from the same spot is taken. The spot is given a serial number in the note book, one page of which is devoted to each spot examined. The actual notes made at the spot should be in the following fashion :—(*vide* Fig. 22, p. 128).

Even if no larvæ are found, a sheet should be filled up.

The location of the spot, by number corresponding with the note book number, should be entered on the field sketch. Always try and obtain at least ten larvæ from each spot, and for preference take the larger ones, unless it is suspected that the smaller ones represent a different species. Pupæ should, of course, be taken also.

It is impossible to give a good word description of the procedure of hunting water for larvæ. It is an art that can only be learned by experience in the field. An unskilled worker will miss larvæ where a skilled performer will find them. In any case a negative result should not be entered without a prolonged search, at least until long experience has given familiarity with certain signs which generally indicate absence of larvæ.

Ustamratasa, 23.xi.26.

19

Ravine leading down in S.W. direction  
from rocky hill with temple on top.  
About 5 ft. deep, stream running fairly  
fast over sandy bed. Pools with easy  
current in places. A green Alga (sample  
in tube 22) in places.  
Larvae only plentiful where grass margins  
touch water.

Tubes 2, 23.

pH 7.2 (7.8)

FIG. 22.—Page of field note book giving collection notes and locality particulars for insertion of breeding places on sketch plan when made from field sketches.



Larvæ of Anophelines, unlike those of many Culicines, are almost invariably thigmotropic, not only actively, but also passively, as Hacker (1925) has shewn. They are therefore seldom found floating at large in the water, but nearly always in contact with the margins, or with floating vegetation or débris. Place the inclined bowl of the spoon against the margin, with its lip just below water, and pull or push it along fairly quickly whilst water runs into it, or place it quietly under a mass of water plants or floating débris from the side, and lift it through the mass, are both methods provocative of good results. Where a film of water, such as a seepage, lies on the surface, too shallow to dip into, scraping a depression with the sharp edge of the spoon (which should therefore be of stoutish metal), either accumulates enough water to dip into, or shews up larvæ carried in with the inflowing water against a muddy background, whence they can often be directly pipetted off. Puddles and hoof-prints in soft ground often reveal their contents when disturbed and rendered turbid. A difficult place to hunt effectively is the shallow sandy margin of a swiftly running stream without vegetation. Larvæ of especially dangerous species favour such habitats, and it may be necessary to go on to one's knees and carefully scrutinize the water. Scraping with a tilted spoon-edge, or making a depression just outside the edge of the stream so that marginal water for some inches upstream flows into it, can be tried. Examine carefully natural small bays in the sand, for here larvæ are especially likely to be found. In a really swift stream, places out of the full current, or where grass or other vegetation trails in the water, are favoured spots likely to yield larvæ when long stretches of the bank are sterile.

Wells require special apparatus, owing to its being impossible to reach the water with the spoon. The writer uses a muslin net tightly stretched across a 12" brass ring, from which arise three chains at 120° apart, running together at a swivel about 18" above the net. At the upper end of the swivel is a ring to which the lowering string is attached. A second string, attached to one side of the brass ring, enables it to be lowered into the water edge-on, so as to cause the minimum of disturbance. The net is worked about by the lowering string, at a depth of about 2 inches and directed under floating débris and around the sides. It is pulled up at intervals and washed out in a white enamelled basin, any larvæ found being pipetted off.\*

For tree holes that are too narrow in aperture, or too deep, for the spoon to be used, the complicated but effective apparatus of Blacklock and Carter (1920) is required. For details of its construction, the original paper should be consulted.

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\* The writer is indebted to Major C. H. H. Harold, R.A.M.C., for the following description of an alternative well-hunting apparatus:—A round tin, about 5" diameter and 18" long, has the top and bottom removed, and in the middle of its length is fitted with a circular flap valve, so hinged by solder stops that it will only move upwards. It is attached to the lowering string by a 3-point suspension and rapidly dropped on to the water surface. The inrushing water from above closes the valve and holds any larvæ that may have been within the perimeter of the tin above the latter. It is then drawn up and emptied into a dish. The inside of the tin and the top of the valve may be white painted to facilitate seeing larvæ enclosed.

In spite of the foregoing notes, the writer realizes that nothing but experience and practice makes an efficient mosquito-hunter!

If pH work is being done, from time to time, say at hourly intervals, sit down and determine the pH of the water samples accumulated, entering them on their proper sheets in the note book. By this method tubes can be emptied of water and used for larvæ if required, but a good morning's work seldom results in filling the whole 24 tubes of a box with larvæ. In view of the laboratory work which must be undertaken in the afternoon, it is inadvisable to accumulate too many samples in one morning.

Though hydrogen-ion determinations are perhaps not part of a routine survey, they are at times very useful, in revealing that under no circumstances could a species that the water looked as if it might contain have been present, thus confirming a negative result (Senior-White, 1926), but such work belongs more to research than to the routine of a malaria survey.

A good fieldman is an enormous advantage. Under a hot sun, knee deep in mud, it is tiring and temper-trying to stand with a full spoon in one hand and a pipette full of larvæ in the other, and not find an uncorked tube next in number held convenient. A fieldman proved trustworthy and with a previous experience of a locality can repeat a survey of bodies of water of which he remembers the location, though through the inability of men of this class to read or make a map, their usefulness in this respect is limited.

On return to the field laboratory it is necessary to determine the day's catch, and for this purpose the following is the writer's equipment:—

1. Several dozen white bowls, about 3" diameter and 3" height, flat bottomed. These are purchasable very cheaply from Messrs. Smith, Stanistreet & Co., Calcutta, who call them 'common country gallipots.'
2. Squares of window glass, 4" × 4," to form covers for the bowls.
3. Compound microscope with lenses up to  $\frac{1}{2}$ "th. The mechanical stage must be easily detachable for larval determination work.
4. Compound binocular dissecting microscope giving magnifications up to × 50.
5. Plain slides, on each of which has been fixed by a drop of Canada balsam a fine entomological pin, at about 1" from one end.
6. Cover slips and mounted needles.
7. Chloroform in a drop bottle.
8. A 1" cube of plasticine.
9. Fine entomological pins and a small specimen box.

To this is added the usual reagents, dishes, etc., for staining blood films, though as a rule the writer prefers to bring blood slides back to examine in the comfort of a properly equipped base laboratory, though it is always necessary to be prepared to do blood work in the field laboratory. Blood films for storage until the return to the base must be fixed but not stained as soon after taking as possible. If working in a tent, or in Provinces such as Madras with unfurnished rest houses, camp tables in sufficient number must be added to an already large equipment. The writer has a long, narrow, folding table on detachable trestles especially for breeding bowls that is very convenient.

Arrived at the field laboratory, the first thing is to turn out the morning's catch from tubes to bowls. Under each bowl is placed the sheet from the note book which has reference thereto, and the glass covers of the bowls are numbered with a grease pencil to correspond.

Next, it is necessary to identify the larvæ caught. Though we are endeavouring to obtain a check on our identifications by breeding out the adults, larval identification, so far as this is possible, is essential, on account of the heavy mortality which is almost unavoidable with Anopheline larvæ in captivity.

The writer's procedure is as follows. Each bowl with its notes' sheet is brought in turn to the microscope table. A second bowl of clean tap or other water is placed contiguous. One by one the larvæ in the original bowl are pipetted to a drop of water on a slide with pin affixed. The size of the drop of water should be neither too great nor too small. Otherwise, in the first case, the cover slip, which is now dropped over the larva so that it also rests on the pin, floats about, and the larva, which should be immobilized without being crushed by the slip, wriggles free; in the second case, the water collects round the larva, and the meniscus obscures the view of the hair appendages, and more has to be run under the cover slip. The larva is now identified (*vide* Appendix III), after which the slip is raised by a mounted needle and the drop of water with the larva shaken or flushed into the bowl of clean water. The identification is entered on the note slip. If more than one specimen of a species is found, as is usually the case, a series of strokes after the name are added as each specimen is identified, and a total made at the end.

When the bowl has been worked through, the now larva-filled clean water bowl and the note slip are returned to the breeding bowl table, and the cover replaced.

It is necessary to feed the larvæ in tap water, and for this purpose nothing is better than compressed yeast, powdered, as originally described by Boyd (1926). Very little powder is required, as much as will go on the point of a knife is ample. Yeast water should be changed every two or three days, or it becomes so polluted with bacteria, etc., that the larvæ are killed. By this means nearly every species of Anopheline can be bred up, from the egg if necessary. Change at once if the

water develops the least sign of a sour smell. The yeast tablets are hygroscopic, and should be kept in well fitting tins. Enough powder for a month's work can easily be carried in a 2-oz. wide-mouthed bottle, well corked.

Feeding and water changing should be carried out by the fieldman, who during examination also keeps the microscopist supplied with bowls. Do not trust a chaprassi, for instance, to do the latter. He may mix up bowls and note slips!

Do not overcrowd your bowls with larvæ. Mortality is always higher in crowded bowls.

Adults which have emerged from pupæ are held in the bowl by the glass cover and are easily seen. Stupify by slipping a piece of blotting paper on which a little chloroform has been dropped under the cover, and when the mosquito has fallen on to the water, pick off with forceps, pin by inserting the point between the legs of the insect, laid on its back, and drive the head of the pin into the lump of plasticine. Do not replace the cover on the bowl for a few minutes, until the chloroform fumes have evaporated, or you may kill still-feeding larvæ, and unemerged pupæ. Leave it off, as a routine, until the result of adult identification can be entered on the note slip. Place the adult determinations in juxtaposition to the larval. Adult determinations are best made under the binocular microscope, but with experience, the common Anophelines of India are all determinable with a hand lens, when the binocular need not be taken about unless required for other purposes. In such circumstances a doubtful specimen can be preserved for subsequent high power examination in the base laboratory by cutting the head off the mounting pin, and storing, with label bearing reference number to the note slip, in the insect store box.

This completes the actual mosquito field survey, though see also Section VII. Owing to having to wait for larvæ to breed up, it is a somewhat lengthy business; which is why it is suggested that the field catching of larvæ be the first work undertaken, leaving the days whilst they are feeding up free, save for each morning's determination of emergencies, for spleen work, etc. Another advantage is that one at once becomes familiar with the neighbourhood.

The writer ultimately transfers the whole of the notes and observations which have accumulated on a note slip to a printed card which is filed in a card-index cabinet, with guide card for the place surveyed. Thus the whole of the information on Anopheline distribution and breeding is available for reference purposes.

What has been discovered has now to be shewn on the plan of the locality. On it, each body of water is numbered in agreement with the note slip (which has now been destroyed), and the record card that has taken its place. Following the method first used in tsetse fly surveys, the writer has adopted a standard series of symbols, which are drawn on the plan as near as possible to the outline of the

breeding place. This has the advantage of economising space on the plan, and if the latter covers investigations made at two or more seasons of the year, the symbols can be repeated in various colours so as to shew the changes in fauna which take place. The names of species known to carry malaria the writer underlines in red in the reference key to the symbols in the corner of the plan for the convenience of

No. 19	Locality	Usktemvalac. 23. XI. 26.	Plan no. 14
No. 10	Description	Ravine no. 14 on plan. Sides about 5 ft. deep, stream with fair current, sandy bed, pools at intervals. Spirogyra in masses in the pools. Grass touching the water edge at intervals, and here only larvae plentiful.	
	Weather	Fine.	
	pH	7.2 (7.8) $\times 10^4$ 410	
	Mosquitos	<u>A. punctatus</u> 4. ♂ 38 ♀. <u>A. tritaeniorhynchus</u> 1. (Adult failed to emerge)	

Notes. Overhanging vegetation will require cutting back to allow of passage of ailing coolies. Outlet channel from many of the pools will want deepening to give straight through flow.

(Vide also cards 14-18 for seepage areas at head of this ravine.)

FIG. 23.—Record card for a breeding place to correlate with final survey plan and control instructions.

the non-professionals who may study it. The set of these symbols for India are given in Fig. 24, and it is suggested, as in African tsetse fly work, that they be generally adopted by all workers in India and Ceylon. A similar set could easily be devised for other countries.

STANDARD SIGNS FOR THE  
ANOPHELES  
OF  
INDIA, BURMA & CEYLON.

⊖	<i>Anopheles</i>	<i>aitkeni</i>	James
↑	"	<i>annandalei</i>	Prashad
↓	"	<i>barianensis</i>	James
⊗	"	<i>barbirostris</i>	Wulp
□	"	<i>culicifacies</i>	Giles
◇	"	<i>culiciformis</i>	Coghill
●	"	<i>fuliginosus</i>	Giles
■	"	<i>funestus</i>	Giles
⊞	"	<i>gigas</i>	Giles
○	"	<i>jamesi</i>	Theobald
▲	"	<i>jeyporiensis</i>	James
▽	"	<i>karwari</i>	James
⊕	"	<i>kochi</i>	Dönitz
⊖	"	<i>leucosphyrus</i>	Dönitz
⋈	"	<i>lindesaii</i>	Giles
⊗	"	<i>ludlowi</i>	Theobald
▼	"	<i>maculatus</i>	Theobald
⊖	"	<i>maculipalpis</i>	Giles
⊖	"	<i>pallidus</i>	Theobald
⊖	"	<i>pulcherrimus</i>	Theobald
⊗	"	<i>rhodesiensis</i>	Theobald
⊗	"	<i>rossi</i>	Giles
⊗	"	<i>sinensis</i>	Wiedemann
+	"	<i>stephensi</i>	Liston
⊕	"	<i>superpictus</i>	Grassi
⊗	"	<i>tessellatus</i>	Theobald
△	"	<i>theobaldi</i>	Giles
⊖	"	<i>turkhudi</i>	Liston
⋈	"	<i>umbrosus</i>	Theobald
⋈	"	<i>vagus</i>	Dönitz
⊖	"	<i>willmori</i>	James

FIG. 24.—Standard signs for the Anopheles of India, Burma and Ceylon.

At times the work of the malaria investigator carries him into places where all the apparatus detailed above cannot be transported. In the Vizagapatam Agency Tracts, for instance, roadless or almost so, damage to microscopes and chinaware would be immense if bumped over the boulder-strewn ravines which the maps of the Survey Department dignify as 'first class roads,' and the former at least would suffer should coolie transport lose its footing in dubious fords of unbridged rivers, whilst transport of any kind may be so scarce that full equipment cannot be carried. Moreover, the worker may be steadily on the march day by day working up a valley. In such cases, larval determinations alone have to be relied on. The larvæ, if no microscope is available, are first killed by placing them in water in a test tube and bringing the contents just to the boil over a travelling spirit lamp, which kills them fully stretched out, and then crowded into 1"  $\times$   $\frac{1}{2}$ " specimen tubes in water, and preserved by adding to this a few drops of a saturated solution of mercuric chloride. The tubes are labelled *inside* in pencil with reference to the note slips (which are then not removed from their book cover), and the contents identified on resuming touch with civilization. Larvæ thus preserved are found less contracted and crumpled, and with their diagnostic points more visible, than when placed in spirit or formalin, provided the interval between capture and examination is not too long—a month or so. Also, when equipment must be cut down to the uttermost, a few ounces of saturated mercuric chloride solution occupy less space than spirit in some quantity.

By such means, equipment enabling some idea to be obtained of the malariousness and malaria-causative species of a tract can be cut down to a spirit lamp, two or three test tubes, a hundred or so small specimen tubes and enough slides for blood films, with oddments such as hand lens, needles, forceps and pipette, the whole easily packing into a small box much less than a man load; but such methods should only be adopted when absolutely unavoidable. They do not compare in accuracy with the proper procedure and are really only applicable to preliminary flying surveys.

Occasionally, it may be worth while starting a spot-map of malaria cases, if such can be continued over a year. By this means comparison with the survey map may shew the breeding places mainly responsible for the trouble.

#### VI. *Have the various Species a Seasonal Distribution?*

In general this question can be answered in the affirmative. Quantitatively as well as qualitatively, the Anopheline output of a piece of water almost invariably varies throughout the year, save perhaps in the perennially moist tropics, such as Malaya. Very little is known about the reasons underlying this; for an exposition of what is known, and the lines on which further research should proceed, the reader is referred to Senior-White (1926) and Senior-White and Williamson (1927). If the factors controlling these variations were understood, it would be possible to

control malaria with certainty and cheapness. Research along these lines, however, is not touched on further here. However, it is extremely necessary that, in surveying, an idea at least of the seasonal specific changes should be obtained. A rice field, for instance, that we find freshly flooded with clear rain water in July and breeding *A. culicifacies*, will after ploughing, become populated only by *A. rossi*. Later in the season, this species will in all likelihood be replaced by *A. sinensis*, though *A. rossi* will return when the last few pools are drying up in January after reaping. As a source of malaria, then, we are only concerned with the rice field for a few days or weeks at most.

It will thus be seen that an inexperienced worker, making a single examination, may either condemn the rice field as dangerous, and elaborate expensive schemes for ameliorization, or pass it as harmless and have his protection scheme vitiated, according to the month in which his survey took place.

The ideal method, and the only one which should be adopted when a large scheme is envisaged, is monthly surveys over a whole year. This is not always practicable. If the survey is made about the height of the local fever season, the results of a single survey can form the basis for a scheme. It is true that the insufficient data thus obtained will probably result in a certain amount of unnecessary treatment, but the result will be sure, though more expensive than it need have been. If the survey is made in a month when active propagation of malaria is not taking place, a return visit must be made in a more suitable month. After long experience of similar country, a worker can often envisage what will be the mosquito fauna of the dangerous period of the year, and devise a scheme based on probability and not on actuality, but this is a very risky method of procedure, and one not to be adopted unless driven by urgent necessity. In any case, especially in hill country, no idea of the extent and location of seepage water can be gained from a dry season visit. With a water plan of the locality, as previously described, in existence, re-survey becomes a much more rapid matter than the original work, and in India an effort should always be made to re-visit a locality surveyed during any other time of the year between August and October. In many localities it is probable that active propagation of malaria is confined to less than four months of the year. An intensive anti-mosquito attack during those months is all that is required entomologically, whilst effort over the remaining months is to be confined to effective treatment of those infected. It is useless spending money killing off species which do not carry malaria, and in every anti-malarial scheme, on the mosquito side, only *species* control is to be aimed at.

#### VII. Which Species of *Anopheles* are responsible for carrying the Disease?

In the answer to this question lies the whole reason for the species control mentioned in the preceding paragraph. In Calcutta, for instance, it does not matter,



malarially speaking, in the slightest if the place pullulates with *A. vagus*, so long as the much fewer but better hidden breeding places of *A. stephensi* are known and controlled. A seepage spring may breed *A. karwari* to the top of its bent, and we are not concerned, but let the closely allied *A. maculatus* get in, and immediately we may expect an outbreak unless the place is dealt with.

Not only, therefore, do we encounter species which do, or do not, carry malaria, but, in two places with the same species comprising the anopheline fauna, in each a different species may be responsible for most of the malaria causation.

In the first place, therefore, we require to know what species of Anopheles are capable of carrying malaria, given suitable conditions. The Oriental fauna may be divided into the following categories:—

TABLE IV.

Completely refractory.	Carrying experimentally but not yet found so naturally.*	Found infected in Nature.*	No information available.
<i>A. aitkeni</i> ..	<i>A. bariensis</i> .. (S)	<i>A. barbirostris</i> .. (Z)	<i>A. annandalei</i> .
	<i>A. karwari</i> .. (S)	<i>A. culicifacies</i> .. (S)	<i>A. culiciformis</i> .
	<i>A. kochi</i> .. (Z)	<i>A. fuliginosus</i> .. (S)	<i>A. gigas</i> .
	<i>A. maculipalpis</i> .. (Z)	<i>A. funestus</i> .. (S)	<i>A. jamesi</i> .
	<i>A. rossi</i> .. (S)	<i>A. ludlowi</i> .. (S)	<i>A. jeyporiensis</i> .
	<i>A. tessellatus</i> .. (Z)	<i>A. maculatus</i> .. (S)	<i>A. leucosphyrus</i> .
	<i>A. theobaldi</i> .. (Z)	<i>A. pallidus</i> .. (Z)	<i>A. lindesayi</i> .
		<i>A. pulcherrimus</i> .. (Z)	<i>A. rhodesiensis</i> .
		<i>A. sinensis</i> .. (Z)	<i>A. vagus</i> .
		<i>A. stephensi</i> .. (S)	
		<i>A. superpictus</i> .. (S)	
		<i>A. turkhudi</i> .. (S)	
		<i>A. umbrosus</i> .. (S)	
		<i>A. willmori</i> .. (S)	

\* Z = Zygotes found.

S = Sporozoites found.

This is the commonly accepted position, but following the recent work of James (1926), the whole of the results will obviously have to be re-done by better experimental methods. This is not work for a malaria surveyor, who may

be compared with a general practitioner, but for the research worker with full laboratory and hospital facilities at his hand. Pending this being done, the third column in the foregoing table must form our working basis, always remembering that when we encounter adults of species still in columns 2 or 4, we stand a chance of accumulating information which will transfer them out of it.

In the second place, we require to know what is the local carrier or carriers of the spot we are investigating. Because, for instance, search in houses yields a heavy catch of *A. fuliginosus*, a natural carrier, it is not safe to assume that by especially attacking this species we shall control the disease. Among the catch, if numerous enough, we may find a few *A. funestus*, and in all probability we are then looking at the real culprit, numerically insignificant though it appears to be.

To complete a malarial survey, therefore, it is necessary to make a catch of adult Anophelines, not only in the houses of the place we are investigating, but in other spots likely to be day-time resting places, such as cowsheds and among thick bush growth. This alone may yield very valuable information leading to the incrimination of the true culprit-species. *A. funestus* and *A. culicifacies* are both excellent natural carriers, but as Perry (1915) shewed, in the area he surveyed on that occasion the former was much the more common in human dwellings, and the latter in cowsheds. This would not be applicable to every locality, be it remembered, which shews the necessity of making a really separate investigation of each piece of work, and not accepting as applicable to one place conclusions formed from the study of another. Local conditions are all important.

How then to set about this piece of work ?

The writer usually takes with him an electric torch, a box of perfectly dry tubes, each lightly plugged with cotton-wool, an ordinary butterfly net with very fine mesh on a short handle, and a killing tube made by setting potassium cyanide in plaster-of-Paris to a depth of about  $\frac{3}{4}$ " in a 3"  $\times$  1" specimen tube. Over the cyanide mixture is placed a layer of cotton-wool, which is changed from time to time as it becomes damp or dirty. In a village house the places to search for resting adults by day are the corners of the roof with the walls, the thatch of the roof, the underside of bamboo shelving on which household goods are stored, and similar quiet places. When a mosquito is spotted, cautiously approach a tube over it, and then disturb it by a sideways movement of the tube held against the resting surface. The insect will fly up, and when it is well up the tube, which should be a fairly long one, and not of a lesser diameter than 1", rapidly plug it with the cotton-wool, which has meanwhile been held as in bacteriological manipulations. Practice is necessary to achieve this little operation successfully, and nothing can take its place. Do not try and subsequently use the same tube for a second mosquito ; almost invariably the original capture will be lost.

After completing the 'quiet' search, tap the underside of the roof, the shelving, clothes piled in corners, etc., with the net, and catch insects which fly up.

Occasionally such captures can be transferred to tubes alive, but usually they have to be killed at once in the cyanide bottle owing to the difficulty of manipulating them in the net.

In better class houses, in addition to the likely spots described above, almirahs full of clothes are favourite day-time resting places. Usually, however, the insects are only found after disturbing the contents, and have to be caught in flight.

In cowsheds, the underside of the thatch is the most usual resting place. Among bushes, only active disturbance will generally reveal the insects by day.

The best time for search are the morning hours, when more of the insects are likely to be sluggish whilst digesting their overnight meal, and so less easily disturbed or rapid in flight.

In hunting houses, especially amongst an ignorant population, great difficulties are often encountered. The help of the headman or other official known to the people should be enlisted, so that he can explain with good chance of belief the object of your disturbance of their privacy.

When as many as possible of the tubes have been filled, the catch is taken to the laboratory, and for the first time is examinable at leisure.

Arrived there, examine each tube, with a hand lens if necessary, and discard the Culicine mosquitoes which will inevitably form part of the bag. Next, set aside in a shady place those tubes containing mosquitoes obviously engorged with blood, to complete its digestion. It is practically impossible to make a proper dissection of an insect whose stomach is full of semi-solid dark blood. But, examine tubes thus put aside at least once daily. Fatalities are common, and an insect dead any length of time is undissectable. Should an engorged insect be found dead, make an attempt to tease it apart somehow.

The remainder of the catch, and those put aside in due course, has now to be first identified, and then dissected. With experience, the former procedure is frequently practicable with a hand lens through the tube, whilst the insect is living, but if not, and in any case for the subsequent procedure, kill it by allowing a few drops of chloroform to fall on to the cotton-wool plug. Then pin it as in examining a bred-out specimen, but inserting the point of the pin as lightly as possible, and determine it specifically. In dealing with a badly rubbed specimen all the characters given in the identification key in Appendix II may not be apparent, and experience and judgment is required to arrive at a correct diagnosis.

If the insect is a male, it can now be destroyed. Males do not suck blood, and their presence in a house in any numbers is indicative of a very nearby breeding place, or of a steady influx of wind-driven insects, both points useful to know. However, the majority of the catch is sure to consist of females, and it is necessary to dissect them for parasites.

The procedure is as follows :—

1. With fine forceps pull off the wings and legs, trying to get the latter, especially, away by the roots, as this loosens all the tissues.

2. Lay each insect, thus treated, in a watch glass of 0·9 per cent saline (which is preferable to the 'normal' strength); but if you are dealing with more than one species, be careful, as the now completely denuded insect is indeterminable. A series of watch glasses should then be set up.

3. On a plain, perfectly clean slide place a small drop of saline. As much as can be transferred on the tip of the handle of a mounted needle is usually sufficient. Place the insect on its side in this with its head towards your left hand. Trouble with capillary attraction is experienced if the slide is not perfectly clean. In that case, work with as little saline as possible, or a very large quantity, but either way the subsequent procedure will be more troublesome.

4. Holding the insect by the shaft of the left-hand needle a little way behind the point pressed on the side of the thorax, with the right-hand needle make a nick in the integument of the abdomen between the last and the penultimate segment. Turn the insect over, and do the same on the other side.

5. Revert the insect to its original position (this is not essential), and work the right-hand needle between the two segments thus separated, not holding the needle vertically, but well inclined, so that its point only is in contact with the chitinous wall of the last segment, and then proceed to make a *steady* slow traction on the last segment, holding the thorax steady with the left-hand needle. The last segment will separate, and draw with it the stomach and hind intestine, with the Malphigian tubes arising from their junction. The stomach will separate from the œsophagus at its junction with the latter.

6. Draw the segment with attached organs well clear of the rest of the mosquito, cover with more saline if necessary, and make a cut with the right-hand needle at the point of insertion of the Malphigian tubes. The latter, with the hind gut and last segment, are now lifted from the slide and thrown away. The stomach has thus been isolated.

7. Now return to the mosquito. Make cuts as shewn in Fig. 25, and holding the thorax steady, put slow, steady traction on the neck against the back of the head. It is usually easier to turn the slide round so that the head faces the right hand, and so to use the right-hand needle. The head will separate, and should drag with it the œsophagus and two glistening masses of white tissue from above the base of the front legs in the thorax, which are the salivary glands. Without much practice (and it is very easy to get 'out of practice'), this is a far from easy operation, and if it fails, it is necessary to try and tease the glands out of the thorax. For this a dissecting microscope is almost essential. The writer prefers to use a binocular for all the foregoing operations, with the slide on a black and white porcelain tile, which greatly facilitates the various procedures.

8. When the glands have been freed, they are separated from the head if still attached thereto.

9. By now the saline is a mass of detached scales from the insect, and other debris, ruptured fat body, and the like. Take another clean slide, and on it put two very small drops of fresh saline, well separated. With a needle point transfer the glands and the stomach to the two drops, and place cover slips on them.

10. Examine the stomach for oöcysts with a  $\frac{1}{8}$ th inch, the glands for sporozoites with a  $\frac{1}{2}$ th inch oil immersion lens. A good light and the diaphragm nearly closed are essential, as we are looking at unstained objects.

To make permanent preparations of the parts is no part of a malarial survey. If such are required, the procedure will be found in James (1920).

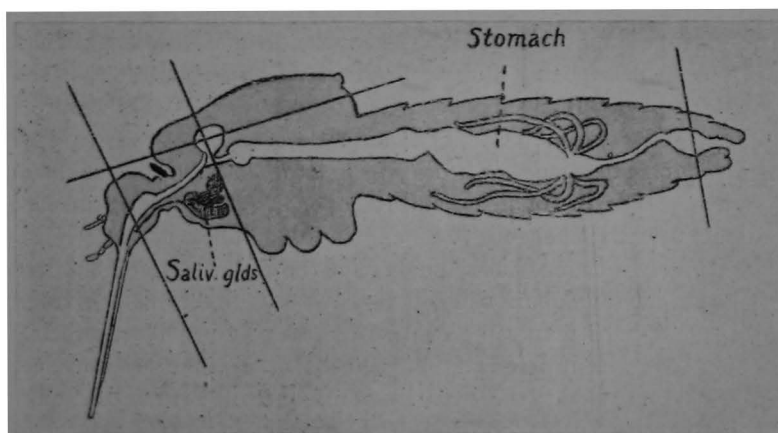


FIG. 25.—Dissection of a mosquito for oöcysts and sporozoites. (After James, 1920.)

The great secret of success in the foregoing procedure is perfectly clean slides and really sharp needles. Of the latter, one should be round, the other flattened and sharpened on both edges. It is best to possess a small oil stone for keeping needles in order, and check sharpening by examination under a low power of the binocular. The least trace of rust on the needles will cause the tissues to adhere to them in a most maddening way. Needles should be carried with corks to protect their tips.

The last piece of information required has now been obtained. We have found the carrier species, and from our breeding place survey know where it is coming from. Do not, however, hope to achieve results of any value from a few captures and dissections. Seldom, indeed, is more than 1 per cent of the actual carrier species found infected in Nature. The more insects are worked through, the more chance

there is of results being accurate. Do not neglect to examine species tabulated as non-carriers, totally or in Nature. On none of them has enough work been done to be dogmatic. Many interesting results, well worthy of publication, await the patient investigator.

VIII. *Is the Malaria becoming, or is it likely to become, worse or better if nothing is done?*

The following sets of circumstances have to be considered :—

(i) *A community in a locality unlikely to change in constitution or topography.*—In this case, be the malaria incidence what it will, no change is to be apprehended.

(ii) *A community gradually taking up agricultural pursuits, such as an aboriginal tribe being taught better methods by the Agricultural Service.*—In this case, following on the better utilization of water, conditions are likely to improve slowly, but if there is an influx of non-immunes attracted by cheap land, or a new irrigation scheme, the first few years will probably shew such a flare-up of the disease that the whole scheme may be ruined. Badly managed irrigation has upset the health of many places, as in some of the Punjab Canal Colonies.

(iii) *A highly or hyper-endemic locality in which commercial operations are to be initiated.*—Whether these are of an agricultural or engineering nature, so long as they involve local labour, little change is to be apprehended, unless it is foreseen that the proposed work involves changes in the nature of the water system of the country. Massing even semi-immune aboriginals on tunnel works that will involve exposing numerous seepage springs may cause enough breeding to upset even their 'fever-proof' economy! Again, if deforestation is likely to let in a dangerous species by exposing streams to sunlight, the situation is full of peril. If non-immunes are to be imported in quantity, such as the use of Chhatisghris for a new tea garden or railway construction, a flare-up is certain if enough breeding is present. Where the proposed works entail the creation of fresh breeding places, and especially if they are liable to let in a new and dangerous carrier, such as railway or road construction through heavy forest in Malaya, then an outbreak on a big scale is absolutely certain. In any work in highly or hyper-malarious country the management of the project, principal and subordinate, being necessarily imported, are certain to suffer in either case, and numerically small though their total will be, their continued health and efficiency is vital to success.

We now possess all the information necessary for the presentation of a detailed report.

## CHAPTER VI.

### THE DESIGN, CONSTRUCTION AND MAINTENANCE OF ANTI-MALARIA MEASURES.

In the previous chapter the carrying out of a malaria survey was described in detail. In the present chapter the action to be taken thereon is considered. It is presumed in the first place that the disease has been found to be of sufficient importance to warrant control measures.

*Evaluation of Loss.*—Now in the first place it is necessary to bear in mind that enormously expensive schemes are only justifiable when the disease is correspondingly costly to the interested party. It is, for instance, totally useless to produce a scheme costing lakhs for the protection of a malarious village. Save perhaps in war time, malaria control must be shewn to 'pay' directly in relation to what the disease is costing or is likely to cost. In the first place, therefore, it is necessary to evaluate the cost of malaria to the community concerned. In India this is usually impossible save in the case of places controlled by capitalist concerns. A plantation, for instance, will possess records of the number of days work lost by each coolie through illness in the estate 'check-roll.' Correlation of these records with those of the dispensary or hospital on the estate will give the number of days work lost by malarial attacks. It is seldom indeed that an estate is so well stocked with labour that any large proportion of the force can be off sick without loss of crop. Tea, if not plucked at the correct moment of maturity of the 'flush' gives leaf of a lowered value, and the subsequent producing power of the bushes is lessened until the next pruning season. Rubber, if not taken out of the tree on the proper day of the tapping round, is lost for ever. In addition, direct losses are involved through expenditure on quinine and other drugs, the issue of free 'sick-rice' to coolies unable to work, and the recruitment of excess labour to cover malarial wastage. Adequate accountancy knowledge is required to properly evaluate all these factors, and such is not part of the professional equipment of a medical man or entomologist, but an idea of how to proceed with such enquiries must be acquired if the malariologist is to be in a position to convince the powers-that-be that his scheme is not impossibly expensive.

In railway working, direct losses due to sick pay are easily obtainable from office records, but the much higher indirect losses from delayed trains, loss of traffic, men moving on sick relief, etc., are very difficult to arrive at. Figures cannot be obtained

on the spot, and the assistance of other branches of the administration is required. In investigating railway malaria, do not forget that the unhealthy station is responsible for more malaria than shews in the records of its own dispensary. Men infected there may shew the developed disease elsewhere, on the books of another dispensary, so find out the runs on which the train-men from your particular spot work, and from what other stations men work in and 'book-off' for the night.

Less than a year's records are of little value, and owing to the annual fluctuations of the disease the average of as many years as possible is desirable, but having obtained a figure, the capitalized value of the loss must be arrived at. This is indeed a matter for expert accountancy, on which the writer has yet to secure an *ex-cathedra* pronouncement. Till such is obtained, it is recommended to follow the practice hitherto adopted by him and base the calculation on the 'eight-years-purchase-of-profits' principle of valuation used in tropical agriculture, which he has hitherto had accepted, though probably only for lack of expert argument to counter it!

Supposing therefore that the annual (or much better the average annual) loss from malaria on a plantation or mine, or at a railway station, is  $n$  rupees, then its capitalized value is eight times this figure. In other words, any sum of less than this amount expended on malaria control, if it will totally eradicate the disease, is sound finance. If only a reduction in its incidence can be hoped for, then the sum justifiably expendible must be reduced proportionally with the percentage of reduction considered possible. In expending the capital sum finally shewn as justifiable, a portion must be set aside, the normal interest on which will yield enough to maintain the works for the future. This sum must include expendible stores such as oil, as well as wages.

*Temporary versus Permanent Measures.*—This is the next point needing consideration. The latter, in any case, are of course not justifiable where only temporary human occupation of a site is contemplated, such as a construction camp, but when permanently occupied sites are concerned, the two alternatives have to be balanced. Temporary measures, which are mainly open drains and oiling or other chemical treatment of breeding waters, are much cheaper in the beginning, but the work is never done; there is a recurring cost for chemicals, and the intensely fallible human factor is prominent. Permanent measures, by which are meant the definite occlusion of the breeding waters from the mosquito by sub-soil drains, etc., cost far more initially, but once completed require little watching, and that by skilled and therefore far more reliable labour, with very low upkeep charges. If funds will permit, which is seldom in this country, permanent works are infinitely preferable. Where they are installed the responsible malariologist can sleep soundly, and not lie awake wondering if the oiling coolie at some place five hundred miles away skimmed his work last week!



*Design of Anti-Mosquito Measures.*—As the result of the malaria survey, we possess a plan of the area with the Anopheline findings entered on it. In addition, we have our mental photographs of the actual terrain, if we make out our protection scheme whilst the survey is fresh in our minds. Otherwise it is best to go back and do it on the spot.

From Table IV we know what are the dangerous ones among the species which we have found. Our index cards and house capture records tell us their relative abundance, but generally not more than two species appear responsible for the local malaria out of more than a dozen which we may have found. These two may co-exist, or may have different seasons of abundance. For instance, the onset of the

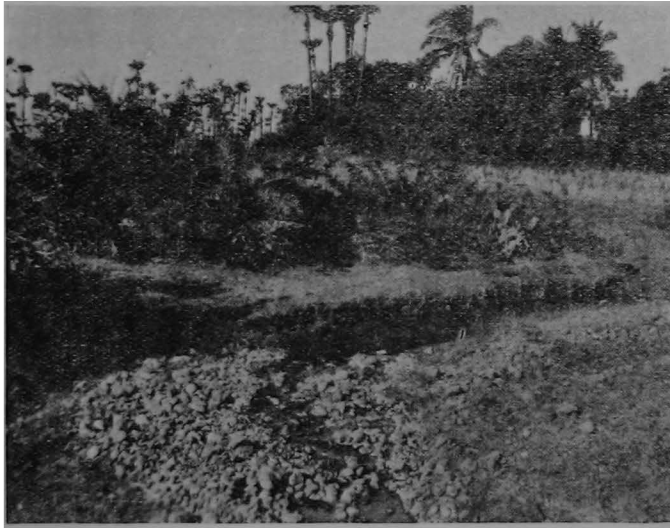


FIG. 26.—Grass-edged stream with *Spirogyra* near Waltair, Madras Presidency. *A. funestus* present in large numbers.

monsoon may create clear rain pools on grass full of *A. culicifacies*, whilst towards or just after its cessation, streams now running slowly down from high ground may be producing *A. funestus*. Dangerous species which appear sparsely in peculiar spots should be borne in mind, as they may greatly proliferate in a year of special conditions, but usually such places can be covered by a note on the working plan for the control staff about annual examination during particular months, with directions for temporary treatment if Anopheline larvæ (which is the most a sanitary inspector can be expected to recognize) are found therein abundantly. Short of residence by the malariologist on the spot under control it is never possible to be

certain that all breeding is being dealt with, or that oil is not being wasted on killing harmless species, or on already sterile water. A certain amount of hit and miss work is the best that can be expected in the existing state of our knowledge. If there is not enough 'hit,' the weekly malaria return will very soon show it! In any case, no control scheme should be instituted which does not contemplate and estimate for periodical return visits by a malariologist. Nature is never static, and we have to anticipate the possibility of 'breeding place deviation' (Swellengrebel, 1919), by which is meant the occupation of other and less suitable water by species driven by control measures from their normal habitats.

A quiet study of the survey plan thus enables us to decide on the culprit localities from which the malaria arises. How is one to deal with them? Every place requires consideration, and the ap- ledge which can be by actual exper- ously impossible methods for all typical ones com- must suffice. No can of course be many of the types shade off one into

#### *Grassy-edged*

The great thing is swift a flow as pockets or back- words, the stream possible, be 'cana- The sides should about 45° right



FIG. 27.—Canalized stream, Mauritius.

tom. To prevent bottom scouring, a layer of ballast stone is excellent where funds permit. Embayments in the banks too deep to bring into line during earth work should have a line of large stones (not necessarily masonry), fixed into the soil on the proper line, and the embayment behind filled in with earth from 'capes' cut off near by, right up to the general ground level behind the stream. If floods are anticipated before the earth can consolidate or vegetation bind it, then the whole should be roughly stone pitched after filling, but in general anti-malaria engineering works should be carried out in dry weather in the first half of the year. Working in water never affords as satisfactory a job. Another simple method of dealing with embayments in shallow water courses and which is very suitable for repairing small flood erosions in drains is an XXX arrangement

tion on its own plication of know- only be acquired ence. It is obvi- to detail suitable localities—a few monly met with hard and fast line drawn between described, which the other.

*Streams.*—(Fig. 26). to get as even and possible, without waters. In other should, as far as lized' (Fig. 27). be evenly sloped at down to the bot-

of sticks thrust into the earth in line with the true edge, stones and earth being rammed in behind to fill the socket. Meandering areas in a stream, which frequently have swampy margins, are best dealt with by a straight-through new channel, with stone and earth banks where it crosses the windings of the former bed.

A stream thus treated, so long as its flow is in fair volume, and the margins bare of all trailing grass and creepers, is practically self-sterilizing, unless we are dealing with *A. maculatus*, specially adapted for life in swift rocky streams and able

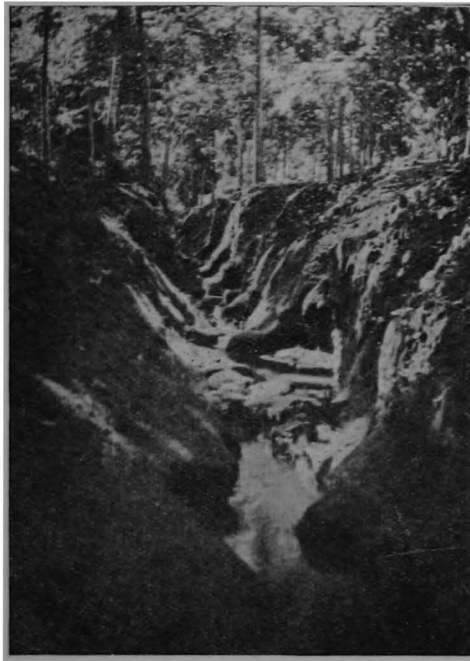


FIG. 28.—Estate Ravine, Matale District, Ceylon. *A. maculatus* and *A. funestus*.

to find sustenance in clear running water, but as in any case the stream may slow down after rain has ceased and become dangerous, it is wiser in all cases to supplement canalization by oiling.

Sub-soil drainage can of course be used in lieu of training and oiling. Its installation forms the subject of a separate section (Appendix IV).

Oiling alone will not act efficiently. In an untrained stream overhanging vegetation will catch the oil and leave clear areas between it and the bank where larvæ will be able to develop.

Irrigation channels fall into this class of stream. They should be especially well trained and maintained, as oiling them is apt to lead to trouble with rice field owners.

*Streams in Ravines* (Fig. 28). Here we usually find the stream in a deep bed, full of bush and creepers, leaping from pool to pool by rapids or actual water-falls, and generally (*A. maculatus* and *A. willmori* are again exceptions) larvæ are only found in the pools. The first thing\* to do is to clear off the overgrowth of bush and get a proper look at the whole ravine, so as to decide on what measures to adopt. If the fall is very great and the bed mainly in earth, it is usually useless to take levels and make an even slope right down, filling up the pools to the general level

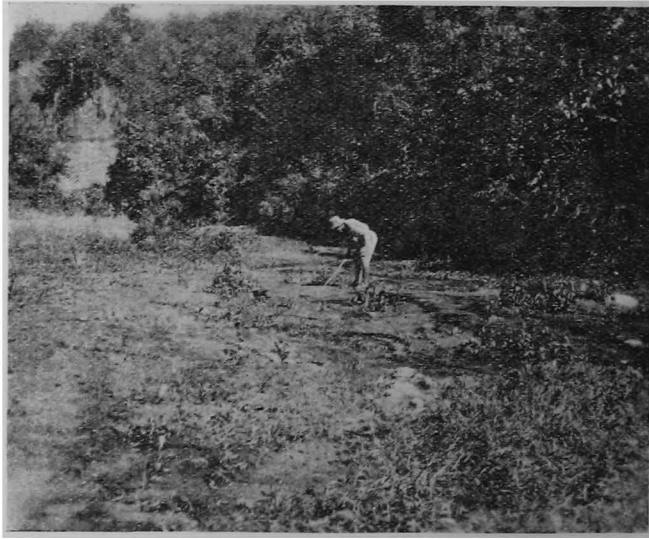


FIG. 29.—Seepage at foot of cliff in valley-like ravine, Vizagapatam Agency Tracts, Madras Presidency. *A. funestus*.

with stones. Certainly a beautiful looking job results, but a few days of really heavy rain will show further pot holes in the course of formation in the new bed, and the grading process cannot be repeated indefinitely. If money permits and stone is plentiful near by pitching the whole bed can be considered, so that the

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\* In *maculatus*-country the utmost caution must be exercised, however, in letting sunlight on to running water. As has been stated previously, in really heavy shade *maculatus* does not exist, and clearing lets it in. But a stream should not be passed as sterile from *maculatus* owing to shade, and the conservation of the latter given as a control measure, without repeated and thorough examination throughout its course through the area to be controlled. Even then a steady watch must be kept. Silt accumulations may kill shade trees or a big tree may fall, and let in sunlight and *maculatus*.

re-grading stands up to floods. If not possible, then sub-soil drainage must be considered.

A stream cascading over a rocky bed may require a considerable amount of blasting, and of concrete filling of embayments, before anything can be made of it. Of course in temporary protection works less engineering and more oil will probably prove the cheapest, but here the probabilities must be balanced according to length of occupation of the site.

*Hill-foot Seepages* (Fig. 29). The method *par excellence* for these is a system of hill-foot contour drains to catch seepage at the point where it arises (Fig. 30). Any attempt to drain the swampy area below by a central and herring-bone lateral drain is usually disappointing, but this depends on the nature of the ground. It is tempting to try the latter, because the contour drain wants taking deeper, and

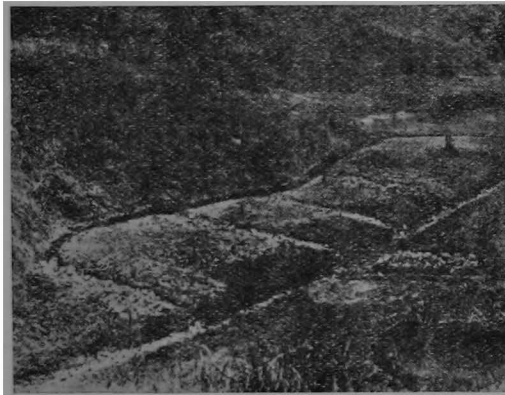


FIG. 30.—Hill-foot contour drains, Kuala Lumpur, F. M. S. *A. maculatus*.  
(From a lantern slide kindly lent by the Wellcome Bureau of Scientific Research.)

encounters boulders, etc., in its course, but unless the herring-bone drains are put in at the time of maximum discharge, new seepages have a maddening way of breaking out, and every one of them has to be led to the drainage system by a new channel. Supervision becomes unending, also coolie work, and the oil expenditure rises steadily. We have seen cases where there was almost more drain than ground as a result of this measure.

Hill-foot contour drains also may be open and oiled or sub-soil piped, according to the funds available and the necessities of the case.

In many places seepage is also found under tank bunds and beneath raised irrigation canals. It may be apparent to the eye, or it may arise in rice fields below the bund. Investigation of thus placed rice fields will show surprising results. Among the usual innocuous rice field Anopheline fauna of scanty *A. pallidus*,

*sinensis*, etc., an area will be found containing *maculipalpis* or other inhabitant of seepage waters. Unless it is possible with Government intervention and compensation to discontinue rice growing on such areas, the only possible solution is Paris green.

Seepages can also be dealt with by filling, but the writer's only experience of this is not encouraging. Though he buried the seepage area under a layer of coarse ashes many inches deep, and imagined that the seepage water would run under this to the nearest drain, yet it broke through the following year in several places and he had hastily to cut and oil drains in the ashes to lead it off. As these areas were not marked for oil treatment in the control plan, only timely personal



FIG. 31.—Running swamps, Railway yard, Singbhum District, Bihar and Orissa.  
*A. culicifacies*.

inspection saved the scheme from a break-down, which is another instance proving how necessary it is that fairly frequent inspections of a completed scheme in operation should be made.

Exceptionally, as when seepages appear on the banks of a ravine stream in a gorge or narrow valley, they can be dealt with by damming the stream to submerge the springs. An ornamental lake in Kuala Lumpur town fulfils this useful function. In one such seepage locality known to us (*vide* Fig. 29) no other effective way would appear possible, as the seepage arises at the foot of vertical earth cliffs, only separated from the stream by a few feet of sloping ground, on which neither contour drains nor even herring-boning is possible. Damming valleys is, however,

emphatically a subject for expert civil engineering advice, as certain kinds of fissured rock cannot retain water dammed above it. Such a locality might well defy all known methods of control, save wasteful soaking of every yard of the area with oil.

*Running Swamps* (Fig. 31).—Such frequently occur in the course of a stream in fairly level country. They are often dangerous sources of *A. funestus* especially. The first measure in dealing with them is to remove the Cyperaceous and other vegetation which covers them. Oiling is then at least possible, but definite treatment consists of taking levels up and down stream and driving a channel through the swampy area low enough to drain it. This of course involves lowering the level of the stream bed below the swamp, which may not always be easy.



FIG. 32.—Borrow pits on the Raipur-Vizianagram Railway, freshly excavated. *A. vagus* only.

*Borrow Pits* (Figs. 32 and 39). The menace of these has probably been frequently exaggerated in the past. The majority of them are not dangerous, our experience agreeing with that of Coggeshall (1926) in America, that they have little potentiality for ill until several years old, and then seldom to an extreme degree. When freshly dug out the contained rain water becomes too muddy for most Anophelines other than the *rossi*-group, but when vegetation edged and filled, they may produce *culicifacies*. In such a case, clean cutting the edges and treatment with cresol will meet the situation if draining is impossible, and unfortunately engineering considerations often over-rule this even when a fall is obtainable, for a connected series of borrow pits along the foot of an embankment may during heavy rains become a river

and scour the bank foot. However, the rules on the subject are not absolute, and an engineer in sympathy with the malariologist may see his way to helping him. In any case, with Sir Malcolm Watson we would stress the necessity of engineers remembering that 'work which without excuse leaves a trail of malaria behind it is bad engineering.' Borrow pits may be excusable, badly placed culverts emphatically are not.

The badly placed culvert is the *bête noire* of the malariologist, and he encounters it everywhere. Railway culverts certainly, and probably road culverts also, are constructed to 'type plans,' in the drawing up of which malariologists were never consulted. Almost invariably the sills are too high, and the effective drainage of land on their upper side is thereby often rendered difficult or impossible.

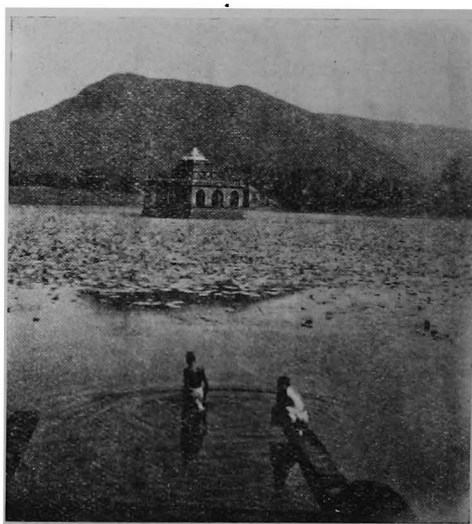


FIG. 33.—Tank at Simhachellam Temple, Vizagapatam District, Madras Presidency.  
*A. fuliginosus* in large numbers among marginal vegetation.

Borrow pits in which seepage springs arise are definitely dangerous in the first degree. Moreover, as it may be only a few in a long series of such pits that possess seepage water additions, letting in usually *A. funestus*, the occurrence of dangerous species in borrow pits may be overlooked unless very careful survey is made, as no eye-indications exist that here and there in the rain-filled series, one is spring fed in addition.

Finally, in connection with borrow pits, if such contain larvivorous fish such as those of the genera *Haplochilus*, *Danio*, *Barbus*, *Rasbora* and *Lepidocephalichthys*, after clean cutting the edges, pause before adding cresol (which is also fatal to the fish), to see if the latter will keep the larval breeding under control. But, unless the



edges are 'maintained,' i.e., kept weeded or stone pitched, a fresh growth of plants will soon again shelter larvæ from their enemies.

*Tanks* (Fig. 33). These are seldom dangerous. However, in localities (if any) where *A. fuliginosus* is really of importance as a carrier, then proper maintenance, (as above for borrow pits) of the edges is the only true remedy. The writer has experimentally controlled the breeding of this species in a typical small village tank by removing the marginal algal growth, mainly *Spirogyra* twining over *Utricularia* in that particular instance, with rakes and adding copper sulphate by dragging bags of the crystals through the water at the end of poles, but as this substance is dangerous to life in quite moderate concentrations, and every tank in India is used for

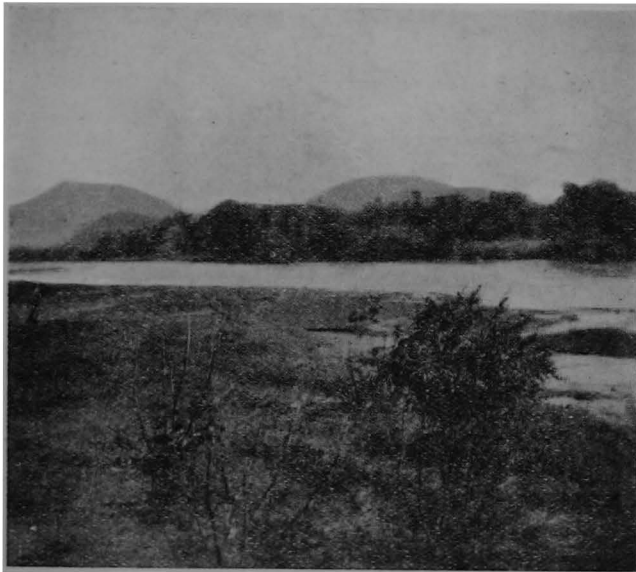


FIG. 34.—The Nagavali River, Vizagapatam Agency Tracts, Madras Presidency.  
*A. culicifacies* in shallow marginal embayments off main stream.

drinking water, its use without careful supervision is decidedly unwise. At the concentration of one in a million which is said to inhibit algal growth it is, of course, quite harmless to man and animals.

Dhobying in a tank seems to be invariably fatal to larvæ. The pH attained may be much less than that produced by photosynthesis that does not inhibit breeding, so another chemical factor is at work. Tanks regularly used for dhobying, also streams for some distance below a dhoby ghat are invariably sterile. This would not excuse omission to make a proper survey examination, however.

*Large Streams and Rivers* (Fig. 34). Only exceptionally dangerous. Malaria caused by such is sometimes characterized by outbreaks at unusual times of year

in the dry season. What really happens is that the stream is reduced to a series of pools and *A. culicifacies* is the culprit species. Cresol or Paris green is the best remedy, preferable to oil for reasons which will be considered later, but here again consideration must be given to the fact that such relict pools may form the only water supply of the countryside at that time of year.

An occurrence to be guarded against during the monsoon is pools left after the subsidence of extra high floods. It may be a week or more before such are either swept out by a further freshet, or dry up. In this time a generation of *culicifacies*

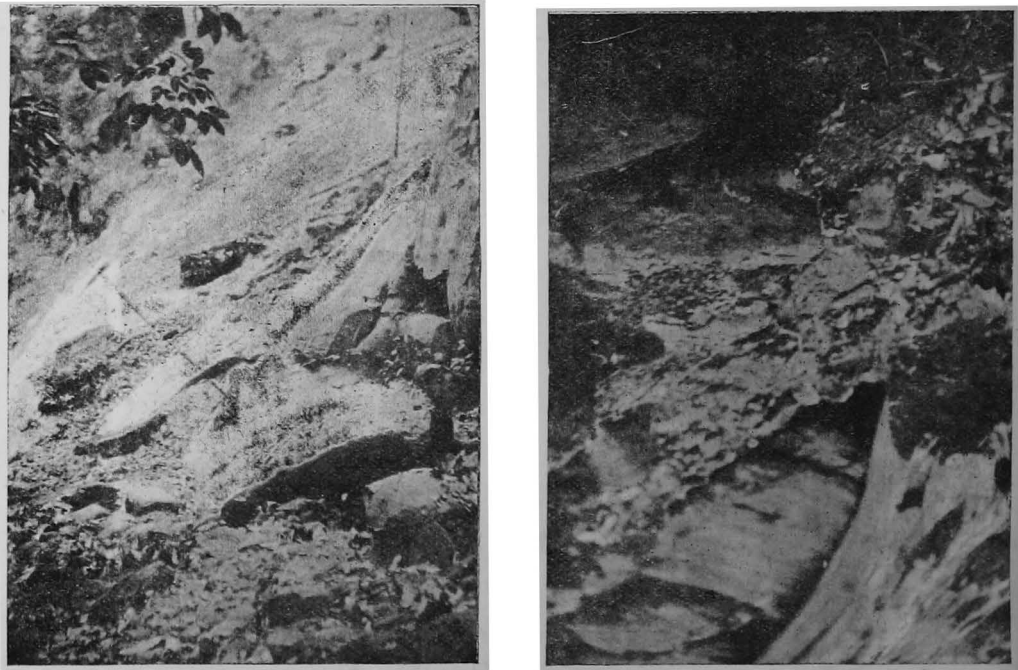


FIG. 35.—Springs arising among rocks, Rubber Estate, Matale District, Ceylon. *A. maculatus*.  
Rocks with water film at foot.

can get through. Cresol is the remedy. There is plenty of other drinking water about at such times. This phenomenon connects with dry season pools via post-monsoon pools in temporary rivers on the decrease in hilly country especially. Often the local inhabitants sterilize such pools by dhobying in them. If not, clear by cresol or Paris green.

*Holes in Black Cotton Soil.* In dry weather this, as is well known, cracks deeply and extensively. When the monsoon is well established these cracks become full of clear rain water, though the intermediate grass-covered areas are dry.

Whilst *A. rossi* is the principal species present in these holes, *A. culicifacies* is quite common. None the less, the majority of such collections of water are sterile. Several holes are found free of all larvæ, in one not two feet away they may be abundant. The controlling factor is not known. Cattle dung pollution reveals itself by letting in *Culex*. Paris green sown down-wind is a possibility, but the writer has no experience of a locality where such holes are the chief cause of malaria. Such a case would present an unusually difficult problem.

*Springs among Rocks* (Figs. 35 and 40). These are a not uncommon source of *A. maculatus* and *A. karwari* in montane regions. *A. karwari* is the commoner species, and if repeated examinations indicate that it is never joined by *maculatus*,



FIG. 36.—Rice fields, Assam. An example of correct cultivation, in itself an anti-mosquito measure. The irrigation channel in the corner is a typical breeding place of *A. funestus*. (Photograph kindly lent by Dr. G. Strickland.)

the spot can be neglected. If *maculatus*, however, is present, such may be a prolific source of malaria. The actual springs are usually of insignificant extent in spite of their potentialities for ill, an instance of the necessity in survey work of overlooking no body of water, however small. It is little use cutting rock channels to lead the little spring away. *A. maculatus* is equally at home in these. Oil swabs, to be described later, at the source are the remedy.

Seepage into track side drains in rock cuttings on railways has its closest natural affinity here, and responds to the same treatment. In earth cuttings where the importance of the station or town warrants it, subsoil drainage may be justified. The latter in rock has also been utilized, but where money was far from 'tight'!

*Rice Fields* (Fig. 36). Very exceptionally dangerous, but may be so when the water is in continuous motion through the fields, as in hilly tracts where water is abundant, or under the circumstances described for seepage water. This is bad agriculture, and the best remedy is education by the Agricultural Department. Meanwhile, oil in the irrigation channel, though it may damage a little paddy at the inlets, will often dissolve enough poisonous constituents to effect control. Paris green is a possibility. Drying off of the fields throughout a tract on one day each week has been legally imposed in Java (Darling, 1926) and is efficacious without

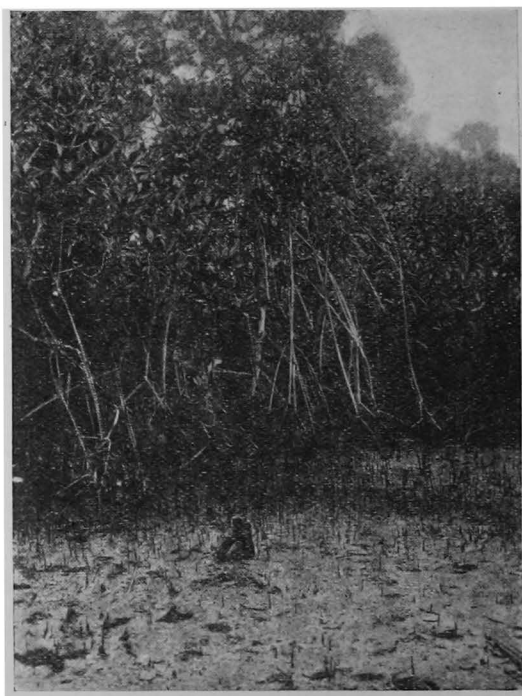


FIG. 37.—Mangrove zone, Trincomali, Ceylon. The area in the foreground is subjected to daily tidal influences.

damaging the growth of the paddy. Uncultivated plots in terraced fields that are allowed to become flooded are especially dangerous (Senior-White, 1926), (Darling, 1926), and such should be mercilessly oiled, failing the necessary Ordinances to compel cultivation or keeping dry. The presence of *A. funestus* in rice fields has been shown by one of the authors (Senior-White, 1926), to depend on a certain degree of oxygenation of the water, and an interesting and extremely useful piece of research is open to any worker in trying to obviate this concentration value being reached.

*Standing Swamps.*—In the author's experience, these are practically never dangerous, though this is not the case in many other parts of the world. The hostile population of predaceous insects and fish is too great to allow much Anopheline breeding. As Sella (1921) has shown, vertical vegetation in swamps is inimical to Anophelines, whilst horizontal is favourable. This European conclusion seems to hold good for Asia also. No method of dealing with these when undrainable seems to have been devised, other than the American one of dusting with Paris green from an aeroplane, a solution not likely to be practicable in Asia for many years to come. Than this, more hope could be placed in pressure of population compelling their cultivation under rice! Quite small swamps, of course, it may be possible to fill up.

*Mangrove Zones* (Fig. 37). The virulent malaria of these areas is due to *A. ludlowi*, and in working on the seaboard the utmost care must be paid to differentiating between larvæ and adults of this species and of the *rossi*-group, lest this deadly species be overlooked. Reference to Appendix III shows that the larvæ are inseparable from *rossi*, whilst the adults are very similar, especially when rubbed or dried. Again, *rossi* will breed in salt water (though apparently *vagus* will not), so the two species may be found together.

As sunlight is necessary for the development of the larvæ, the dense virgin mangrove forest is healthy so long as it is daily traversed by the tides, but when trees are cut down or when bunding operations interfere with tidal movements, trouble begins. Relict pools are formed, gradually diluted by rainfall to a salinity where breeding commences, whilst interference with tidal movements is inimical to the health of the mangrove, which means letting in sunlight.

If work has to be done in the mangrove zone, the only real remedy for the malaria which is bound to follow is drainage. The larvæ cannot stand a current, and whilst they flourish not only in relict pools but in ill-kept drains with marginal vegetation or floating sticks and débris, a clean edged drain with a flow is sterile.

Drainage at sea level is very difficult unless there is a big tidal range. Malaria workers in Malaya have been able to take advantage of the sixteen foot tides of their coast to instal automatic sluice gates in the bunds which have to be erected to keep the sea out of the cleared land behind, which open as the tide falls giving outlet to drains taken well below mean sea level, thus drying off the land and giving a good current in the drains themselves, but in places where there is little tide, as along most of the coast of the Indian Peninsula, this procedure would be necessarily much less effective. Where it fails, the only remedy would seem to be raising the land around a settlement above highest tide level for a distance of over half a mile, (*A. ludlowi* is a species of strong flight powers), so that there can be no breeding grounds within range; but this, of course, would be impossibly expensive unless it could be carried out in connection with some other work yielding the necessary material, such as dredging. It is, of course, only pools left by spring tides, that do not get flushed

again for a month, that become sufficiently diluted with rain to become breeding places, but the gross extent of such pools round a settlement would be so great that any idea of oiling the lot is hardly to be considered. It is thus seen that the problem of *ludlowi* malaria is a difficult and expensive one, involving considerable engineering works. Fortunately there are not many parts of India where it will have to be faced. In the Andamans it has been run away from, by moving the settlements inland. But this is not always practicable.

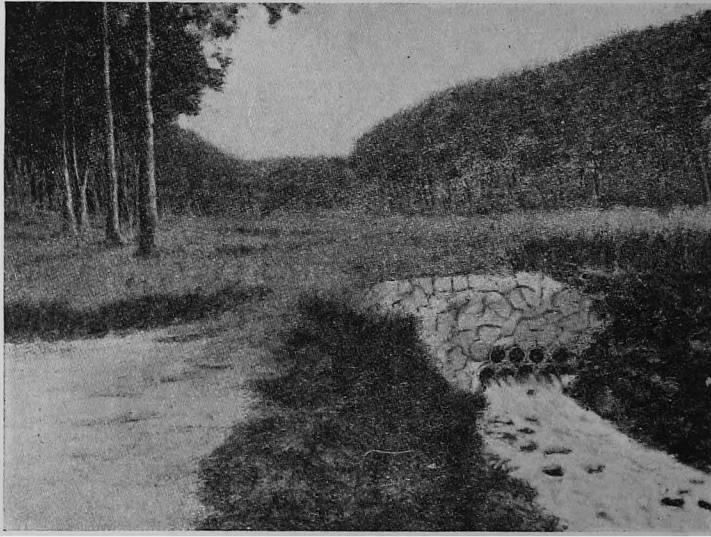


FIG. 38.—Sub-soil drain discharging at end of protected area into open drain. Rubber estate, F. M. S. *A. maculatus*. (After Watson, 1921.)

In the foregoing brief account of the general procedure of anti-mosquito works, the following methods have been referred to :—

Surface Drains.	Cresol.
Sub-soil Drains.	Paris Green.
Oil.	Copper Sulphate.

Notes on these are given below.

*Surface Drains.*—These should be as narrow as possible in reference to the volume they have to carry. The cross section of a drain 12" × 12" is the same as one 18" × 8", but the latter has 50 per cent more surface area to use oil on unnecessarily. Vertical sides look very nice when new, but they are very difficult to maintain, so slope the sides to at least 60°, even less steeply in friable soils. Grass growing at the bottom of drains should not be cleared out by scraping with a

kodarli-hoe, its use gradually lowers the level and may interfere with the outlet flow or cause pools when flow is ceasing. In the soft mud of the drain bottom hand-pulling of the roots is perfectly easy, but no coolie will do it unless made to. Repairs to flood erosions in the banks have already been referred to. In leading lateral drains into a main channel, do not bring in one from each side at the same spot, but alternate the entrances. Very sharp turns in direction should be avoided if possible. In flood, scouring of the bank takes place most at sharp angles. Do not put in contour drains at a greater slope than 1 in 30.

In places with a long dry season, the ground at the commencement of the rains will be clear of grass. With heavy run off, this tends to make channels on the

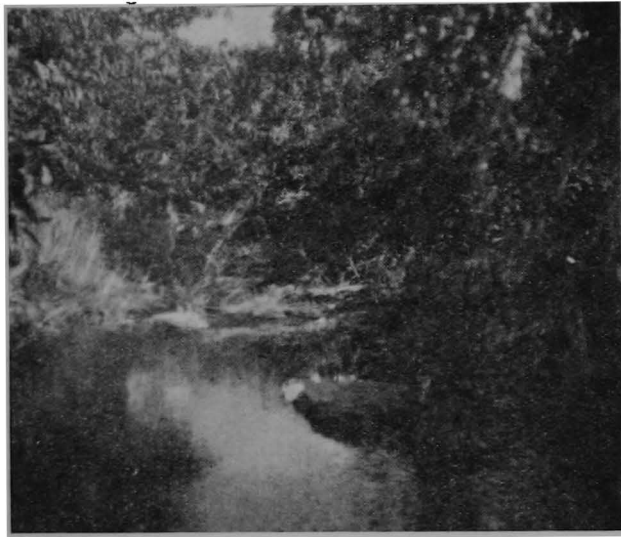


FIG. 39.—Stream through old borrow pits connected for engineering drainage purposes, main line station, Singbhum District, Bihar and Orissa. *A. funestus* in large numbers.

open ground towards the drains. In permanent work, the remedy is to lay a line of stone at the edges of the drains. This, of course, considerably increases the cost. Ballasting the bottom of the drains to prevent scour (and also weed growth), has already been mentioned. As the first year's experience of the working of a scheme often shows the necessity for minor changes in drain direction and depth, bottom ballasting and marginal paving may well be postponed until after the first season's working.

*Sub-soil Drains* (Fig. 38). These consist of rows of unglazed collarless pipes buried beneath the floor of the drain, which remains above to carry storm water. Only sufficient pipes are laid to carry the normal flow from springs and seepages.

Engineering formulæ for the pipe capacity must be used. Nothing but quite transient flood water is thus above ground, and no breeding places can possibly exist so long as the pipes function. Their failure is shown by the sustained appearance of surface water. A ravine properly piped should be surface-dry a few hours after the heaviest tropical downpour.

Laying pipes is emphatically a matter for an engineer with experience of such work. Many points have to be attended to for successful installation. The ground above the pipes should be at least three feet thick up to the floor of the ravine, lest heavy floods scour the pipes out. Silt has to be prevented from reaching the pipes and blocking them, therefore, though these may need burying under a heavy layer



FIG. 40.—Pools in rocks, Gunong Pulai Water Works, Singapore, F. M. S. *A. maculatus*.  
(From an illustration kindly lent by Dr. P. S. Hunter, Singapore.)

of stone of graduated size with the smallest on top, the actual floor of the ravine must be of silt itself in the topmost layer. In wet climates it may be hoped that the ravine will grow a grass floor, most efficacious against scouring, but in localities with long dry seasons the first monsoon floods will traverse soil that has been sun-pulverized for several months, and this must be reckoned on regarding depth of laying.

Unless the pipes will be actively functioning for several months each year we doubt if the cost of their installation would be justified. In regions where ravine streams only run in mosquito breeding volume for two months or so after the slackening of the monsoon, oiling is probably preferable.



Trees growing near pipe lines have to be rooted out. Roots naturally tend to grow towards moisture, and hence into the pipe junctions, blocking them, and necessitating lifting the blocked length and re-laying it after clearing.

The pipes end in a wall beyond which the ravine continues in the ordinary way. No method is known whereby a ravine stream running in the open can be made to enter pipes where it crosses an area to be protected. Pipes must be used from the source of the water downwards as far as is required.

In Appendix IV the official specifications for anti-malaria drainage of all kinds in the Federated Malay States are reproduced in full.

*Oil*.—The crude 'liquid fuel' of the petroleum companies is in every way preferable to the much more expensive kerosine oil. Its spreading power can be

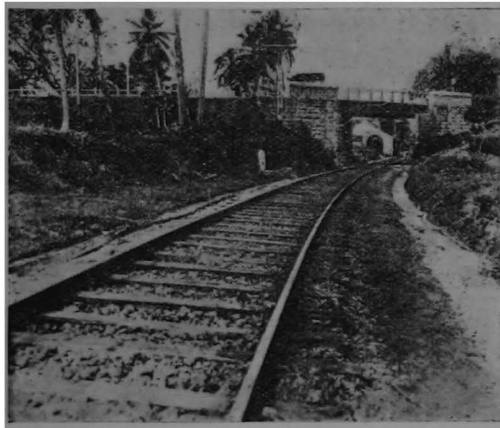


FIG. 41.—Railway trackside drain, Kurunegala Station, Ceylon. *A. maculatus* and *A. funestus*  
The drain is well cut, but can only be rendered safe by oiling.

(After Manson-Bahr, 1914. *Parasitology*, VII. From a lantern slide kindly lent by the Wellcome Bureau of Scientific Research.)

improved by the addition of from 1 per cent to 2½ per cent of a vegetable oil, castor, coconut or whatever is locally cheapest. Consignments of liquid fuel vary from time to time in the amount of thick black sediment they settle out on standing. This material is very useful for use in springs, etc., and it should therefore be carefully conserved when storage tanks are cleaned.

Oil may be applied to water in several ways :—

*Spraying* (Fig. 42) consists of forcing the oil under pressure through an atomizing nozzle from a special machine. The 'Four Oaks' Company's make are the best, and of these the 2½-gallon capacity 'Kent' sprayer best suits the carrying capacity of the Indian labourer. This pattern has a self-contained pump by which

pressure is brought up to a standard figure marked on the gauge prior to using the machine. Patterns with a pump which has to be worked continuously by the operator whilst the machine is in use involve carrying out two operations at once, which does not suit the coolie, who, in rough country, wants one hand free. As sent out by the makers these machines are fitted with rubber hose to the nozzle and rubber valves. Petroleum oils perish the rubber in a few days, and before purchase the vendors should be asked to fit the machine with leather valves and flexible metallic hose. The great fault of these machines is air leaks, and some mechanical knowledge is required to keep them in working order. In plantation, mining and railway work this is easy to arrange for. No great degree of skill or many tools are required, but an untrained coolie cannot re-pack joints and valves. Sanitary inspectors in charge of anti-malaria work must know how to do so. Metallic hose is



FIG. 42.—Oil Spraying.

not as flexible as the rubber pattern, and leaks at the base of the hose are especially troublesome and wasteful of oil. In charging the machine, the oil should be filtered in (a sieve is supplied to fit the machine), as it contains particles of dirt which clog the nozzle. The spraying coolies must in any case know how to unscrew the latter and clear it of grit in the field. No tools are required for this.

Nozzles are of many designs. A suitable one for anti-mosquito spraying should deliver a cone spray about 18" in diameter four feet in front of the nozzle. Patterns No. 1, 'Marvellous' nozzle and No. 2, I. T. A. attachment with 'Marvellous' nozzle of the 'Four Oaks' Company have been found most suitable by the writer. The second pattern mentioned has a double nozzle, one side of which can be directed directly downwards and the other straight ahead. The tendency is for

all nozzles to deliver too much oil per minute. This either forces the coolie to move at a run or wastes oil. Coolies will not move at a run for long, nor should they be expected to. A slower delivering nozzle is a desideratum.

*Oil Swabs.*—Where there is much machinery, there accumulates masses of used cotton waste which already contain oil. These should be worked by manipulation into elongated ‘sausages,’ and if they are not of themselves sufficiently oily, can be dipped into liquid fuel and wrung out prior to use. By their use running water is more effectively oiled with less expenditure of oil than by a machine, as the emission time is longer. In swift water they can be weighted down with a brick or stone. They are ideal for small rock springs. Bits of old sacking are fairly efficient substitutes for cotton waste. Neither these nor drip cans can take the place of the sprayer when a stream contains isolated lateral water in hoof marks, etc.

If the cotton waste is ‘on charge’ it is not hurt by its second use, and can be collected when the following week’s supply is put down and returned to store.

*Drip Cans.*—The efficient drip can that can be installed at a reasonable cost has yet to be designed. Their use usually only engenders a false sense of security. Moreover, being usually based on a kerosine tin as a container, they are very liable to be stolen by coolies.

*Brushing.*—Standing rain pools on grass can be quite effectively dealt with by means of the ordinary sweeper’s broom dipped some inches into a bucket of liquid fuel. The broom is brushed round the edge of each pool, where the larvæ congregate. The process is, of course, slower than spraying.

*Oil must be applied at seven-day intervals*, and any deviation in application periods will sooner or later degenerate into such slackness that a generation will get through. An oiling programme must lay down definite orders as to dates of application for every spot, and no excuse whatever be accepted for deviation therefrom. This is one of the reasons against entrusting oiling to an already overworked medical assistant who may have an urgent call just when oiling is due. The work is of sufficient importance to entrust to a special inspector. Oil need not be replaced if washed out by heavy rain unless this occurs within an hour of application. Oiling during heavy rain should not be attempted in drains or streams.

Successful oiling largely depends on adequate organisation. Supplies of oil must be ready to meet the spraying coolies at convenient spots on their daily rounds, having reference to the capacity of the machines and the areas to be sprayed. A system of records is necessary, to keep track of dates of application, quantity used, and the like, so that there is no chance of main depôts running short at a critical period of the year.

After each day’s use the machine should be washed out by pouring in a pint or so of kerosine oil and blowing it out by pressure. This clears the ball valve at the bottom of the container, and the nozzle discs.

Besides larval sterility, the ultimate criterion, at least two other tests of thorough oiling exist. (i) A properly sprayed drain should have the marginal vegetation burned brown by the oil for a foot on each side, and after a few applications bare earth margins should appear. (ii) Poisonous solutes from the oil alter the macroscopic algal vegetation in the water. *Spirogyra* will not stand oiling. In regularly oiled drains it is replaced by a bottom growth of a matted Cyanophyceous alga that is correlated with absence of mosquito life, as first pointed out by Watson (1921).

*Cresol*.—This is the basis of various disinfectants with trade names, such as 'Jeyes' Fluid,' etc. The cheapest variety locally obtainable will suffice for anti-mosquito work, providing a guaranteed analysis of the cresol-content is given, which should be about 15 per cent. It forms an excellent larvicide, though it kills all other life in the water at the same time. Fish killed by it are violently poisonous if eaten, so warnings must be given. Disregard of such by one oilman would have ended in a tragedy had not a stomach pump fortunately been at hand. Cresol should be stirred into water until a faint milky tinge is observed. In salt water this does not take place at as low a concentration as in fresh, which should be remembered to avoid waste in littoral areas, though the efficacy of application is not diminished. A dilution of about 1 in 40,000 is enough to kill larvæ in three hours. It should be well stirred into the water. It is only useful in ponds and standing water and where such are of any extent is preferable for the reason that a steady wind will blow an oil film clear to one side of the water. It is true that to a large extent Anopheline larvæ are likewise carried along and so into the oil, but the windward side is left clear for fresh generations. Whether it or oil should be preferred where there is no objection to its use is discoverable from the following formula by Marshall (1922).

$$\text{Paraffin : Cresol } 1 : \frac{1500 \times c \times d}{n}$$

Where  $c$  = ratio of cost of cresol to liquid fuel.

$d$  = depth of water in inches.

$n$  = dilution.

A cubic yard of water contains 168 gallons.

*Paris Green*.—This is an expensive compound, the best quality costing about Rs. 2 per lb. in Calcutta. It is liable to adulteration, indicated by the absence of a deep emerald colour to the powder. A guaranteed analysis should be called for. It is a copper-sodium-arsenite compound, but in quantities used for mosquito work is said to be harmless to everything else. Even Culicine larvæ, being mid-water or bottom feeders, do not ingest enough of the floating grains to suffer noticeably, but surface feeding Anophelines perish, though not so rapidly as with oil. It should not be used on a just discovered breeding place with mature larvæ and pupæ, therefore. The powder should be intimately mixed with one hundred times its weight of finely sifted perfectly dry road dust, and sown by hand down wind over the water at the rate of 170 grains of Paris green (not the dust mixture), per ten square feet

of water surface. One pound of the compound will therefore only cover 410 square feet.

In America dusting machines exist, but all the beautiful anti-mosquito machinery of that country, ditch digging machines, power sprayers, and the like, will have to wait long before Indian conditions are ready for them.

*Copper Sulphate.*—The use of this has been referred to in discussing dealings with tanks. Where sheets of algæ are sheltering larvæ it is very useful, but it can only be used under strict supervision owing to its poisonous nature. Its action is not direct on the larvæ themselves, but one of starvation by killing the vegetable microplankton on which they feed.

*Returns.*—These are absolutely necessary for keeping check on the working of a scheme. They should shew the number of cases occurring each month, and the total number of days off work due to malaria. Dependents should be recorded separately, so as not to muddle the 'lost days per employé' column.

In the case of railway staffs, the 'stationary' staff, that is those whose duties keep them within the protected area, should be recorded separately from the 'running' staff, who perforce stop at unprotected malarious spots. Such details will often indicate that much of the malaria of running staffs is contracted elsewhere than at their protected home station, their malaria incidence being far higher than that of the stationary staff.

A typical month's return should be in the following form:—

* STATION .....	MONTH..... 19.....
	RAINFALL.....
POPULATION.	
Stationary Staff.....	
Running Staff.....	
Dependents.....	
Total for month.....	
<hr/>	
Total cases malaria.....	Per cent of total employés attacked.....
Total days lost.....	Average days lost per employé.....
<hr/>	
Stationary staff cases.....	Running staff cases.....
Dependents cases.....	Per cent of running staff attacked.....
Per cent of total stationary population attacked.....	
Days lost by stationary staff.....	Days lost by running staff.....
<hr/>	
Average days lost per man of stationary staff.....	Average days lost per man of running staff.....

\* In railway working it usually happens that a hospital serves a length of line as well as the actual station, and figures from the whole area are entered in one case-book. Care must be taken to exclude 'line' cases. It is best to enter these in the daily case-book in different coloured ink.

The information should be in graph form in the malariologist's office, with pre-control years' figures, in a different colour, preceding or superimposed. Correlation of hospital returns with 'sick' and 'fit' certificates will afford details of past years' happenings, though its working out takes time. These figures are the malariologist's justification for the existence of his section of the medical department to which he is attached. Busy men can learn at a glance from a graph more than from anything but detailed study of columns of figures, and the head of an administration has too many calls on his time for the latter, so all records as far as possible should be in graph form.

Take a spleen rate of the children of all protected places at least annually, better, six monthly; at the end of the 'fever season' of the locality in any case. Under control the reduction will be most striking.

On construction work on railways, or where the population is very fluctuating in constitution and number, and widely scattered, so that the sick cannot all be seen daily by a medical man, the only figures useful for comparison are 'man-days,' got by totalling the daily labour return for the month and multiplying by the number of days therein, and working out the percentage attacked. In such circumstances the number of days off work per case cannot be ascertained. Even the number of attacks, of '*bokhar*' only, cannot be safely left to contractors to record, as they are often wilfully exaggerated, in the hope of enhanced rates of payment for work in feverish country, or minimized, for fear of interference by the medical staff. Unless a special system of itinerant recorders be substituted (we have never tried this method for financial reasons, but it might most usefully be combined with the duty of dispensing a few simple remedies of general application), the only remaining course is to make the medical staff record such information as is obtainable by questioning the coolies in each camp, and taking population figures from the contractor's agent resident therein. The data thus obtained are of course far from accurate, but they afford at least comparisons and serve to indicate any camp in which the rate is rising. A rising rate, however, is not always indicative of failure to control malaria. Construction coolies are the dregs of the population, underfed, ill-housed, often originally from malarious areas, and loaded on arrival at the scene of their work with parasites. Climatic vagaries alone will serve to precipitate numerous relapses amongst them, and half the 'fever' recorded may be catarrhal in nature and not malaria at all. Frequent inspections will alone reveal this, and such also serve to check the regular returns.

## APPENDIX I.

### NOTES ON THE BREEDING PLACES AND DISTRIBUTION OF THE INDIAN ANOPHELINES.

- A. aitkeni* .. Found in clear slowly running streams, usually in shade. Frequents the depths of the forest in such.
- A. annandalei* .. A tree-hole breeder.
- A. barianensis* .. A tree-hole breeder. Confined to the Himalayas.
- A. barbirostris* .. A species usually associated with large ponds and tanks. It is common, however, in slowly moving water against a temporary dam such as a flood débris block. Requires the presence of aquatic vegetation.
- A. culicifacies* .. In clear rain pools on grass, and slowly running streams and drains. Primarily a species of temporary water. Much commoner in areas with a long dry season than in the wet zones.
- A. culiciformis* .. A tree-hole breeder.
- A. fuliginosus* .. Pre-eminently a tank-breeding species. Common in late autumn.
- A. funestus* .. A running water species. Found at the margins of streams, in trickles running down from seepage springs, etc. The commonest species of rice field irrigation channels, and associated with most of the malaria of hilly tracts.
- A. gigas* .. A stream breeder at elevations of 5,000 feet and over.
- A. jamesi* .. Breeds in rice fields where the water is clear.
- A. jeyporiensis* .. Found in streams and rice field irrigation channels.
- A. karwari* .. In seepage springs, and trickles running down from them.
- A. kochi* .. In small grassy pools and drains. Rather tolerant of polluted water. A Malayan species only found in Assam and Burma.
- A. leucosphyrus* .. Breeds in streams in heavy forest. If found in cultivated land has probably been washed down from forest above.
- A. lindesayi* .. A stream breeder, in pools in mountain torrents. Not found much below 5,000 ft.
- A. ludlowi* .. A brackish water breeder. In pools among mangroves, etc., not covered save at very high tides. Does not breed in pure sea water. Only occurs in India in the Andamans and Ganges Delta. Always associated with very high malaria incidence.
- A. maculatus* .. In clear running hill streams, in full sunlight. Common where there is no marginal vegetation, but only bare earth or rock sides. Not found where there is really heavy shade over the water.
- A. maculipalpis* .. A seepage spring breeder.
- A. pallidus* .. Breeds in rice fields with some depth of water, and in large muddy ponds, often in company with the closely allied *fuliginosus*.
- A. pulcherrimus* .. In swamps and reedy water. Becomes progressively rarer East of the Indus, but occasionally found as far as the U. P.
- A. rhodesiensis* .. In pools in river beds and springs in rocks. Not found East of the Indus.
- A. rossi* .. In puddles, hoof marks, rice fields, etc., and seems to prefer muddy water to clear. Can tolerate salinity up to 50 per cent of sea water.

- A. sinensis* .. Breeds in pools, but not generally against bare earth margins. Often swarms in rice fields which are still flooded towards the end of the crop season.
- A. stephensi* .. Pre-eminently a well breeder. Has followed civilization into the centres of large cities in the track of water carriage sanitation. The larvæ have special adaptations for staying 'down' longer than most other species.
- A. superpictus* .. In pools in nullahs and river beds. Can tolerate a fair current. Does not come East of the Indus.
- A. tessellatus* .. In pools, usually with decaying leaves in swamp jungle or secondary growth.
- A. theobaldi* .. In running streams, ditches and drains, but seems to require vegetation in the water itself.
- A. turkhudi* .. In pools in dry river beds, etc. Has been found in a large grassy tank with *fuliginosus* (Sinton, 1917).  
The Palearctic *A. multicolor* has been recorded from Baluchistan and the North-West Frontier by Christophers (*Indian Journ. Med. Res.*, XII, 301). The adult is not certainly distinguishable from *A. turkhudi* save by examination of the male genitalia, a procedure not dealt with in this book and only suited to the specialist. The larvæ are likewise indistinguishable from *turkhudi*. The egg is very distinctive from that of the latter species however (*vide* Edwards, *Bull. Entom. Res.*, XII, 268, figs. 2 k and 2 l).
- A. umbrosus* .. In pools in heavy forest with peaty water and fallen leaves. Not in water with any appreciable current. Only found in Assam and the Andamans in our sub-region.
- A. vagus* .. Has the breeding habits of *rossi*, and takes its place almost completely in those parts of India with Malayan faunal affinities. But is not found in brackish water.
- A. willmori* .. In mountain streams, in clear pools. Purely a Himalayan species.

The reader is cautioned against accepting the foregoing notes in any but the most general sense. EXCEPTIONS ABOUND. The writer has found *A. rossi* in a seepage trickle, *A. funestus* in a tank and in borrow pits, *A. culicifacies* in a well! The underlying reasons, both for the common ecology, and for the exceptions, are not at all understood, but for what is known, *vide* Senior-White (1926). Other valuable papers on the subject are those of Hacker, Lamborn and Williamson in the Federated Malay States Malaria Bureau Reports. The subject has hardly been touched on in India.

None the less, certain of the foregoing ecological generalities tend to explain the mechanism of malaria causation in certain localities, and give hints for its eradication. For instance:—

In the plains tracts of India the monsoon causes clear rain pools on grass, with trickles of moving water. This lets in *culicifacies*, but if the land under water is ploughed for rice, the then muddy water becomes populated by *rossi* or *vagus*, according to the locality. Should it again stand at some depth and become clear, *A. sinensis* or *A. pallidus* may appear, but it is an outstanding fact that the older the water the less the mosquito fauna, owing to the number of predatory enemies that find their way into permanent or semi-permanent water.

The malaria of hillside rice fields is seldom in the rice itself, unless water continuously runs through the fields, which is bad agriculture. The irrigation channels are the source of *A. funestus* and the malaria.

A forest stream under really heavy jungle is inhabited by *A. aikeni*, and is harmless. Clearing the jungle lets in sunlight, and with it possibly *maculatus*, with dire results; but if the forest is on flat land clearing it and drying it off may have the best possible results, by ousting *A. umbrosus*.

In a valley, survey will often shew spleen rates rising as the hills are approached. Hill-foot seepage is frequently the explanation, the species concerned being *maculipalpis* and *funestus*.

Epidemic malaria is usually the work of *culicifacies* in temporary water. Hyper-endemic areas are characterized by seepage and stream breeders. Watson (1921) has shewn that in the perennially wet tropics *maculatus*-caused malaria has its peak in May, that due to *umbrosus* in October. This



means that for some as yet undiscovered reason these two species have their respective maxima in the areas they inhabit about a month previous. The question of seasonal maxima may be very important. *A. fuliginosus* is a natural carrier, but yet its maximum is not necessarily associated with the peak of the malaria curve in localities where it is common. The reason possibly is that it has its maximum in the cold weather, in Bengal and Bihar at least, and possibly temperatures are too low for effective transmission.

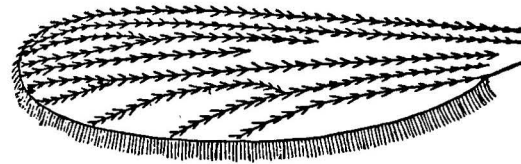
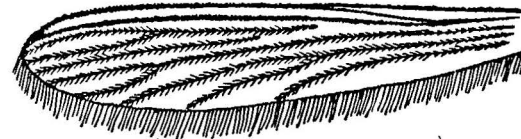
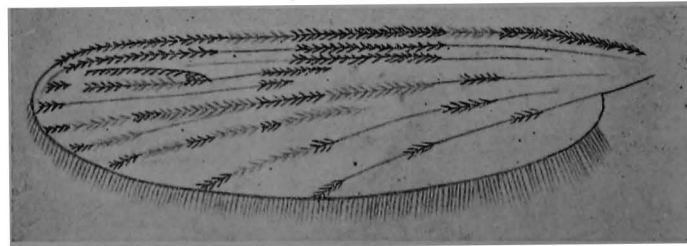
Numerous further examples could be quoted, but if these notes stimulate the reader to make observations on the 'why and wherefore' of Anopheline distribution, ecological and chorological, its most useful object will have been attained. The crying need to-day is for cheaper and more effective methods of control.

## APPENDIX II.

KEY TO THE ADULTS OF THE GENUS *Anopheles* IN THE INDIAN SUB-REGION.

Based on Christophers (1916), with acknowledgments to Strickland (1925).

- |   |                       |
|---|-----------------------|
| 1. Wings unspotted . . . . .  | 2.                    |
| Wings spotted . . . . .   | 4.                    |
|   |                       |
| 2. Distinct white knee spots at apices of femur and tibia<br>of hind legs . . . . .                 | <i>barianensis</i> .  |
| No definite knee spots . . . . .  | 3.                    |
|   |                       |
| 3. Anterior forked cell nearly double length of posterior<br>(fig. 1) . . . . .                     | <i>aitkeni</i> .      |
| Anterior forked cell relatively shorter (fig. 2) . . . . .  | <i>culiciformis</i> . |
|   |                       |
| 4. Costa with less than four dark spots involving both costa<br>and 1st longitudinal vein . . . . . | 5.                    |
| Costa with four dark spots involving both costa and 1st<br>longitudinal vein . . . . .              | 10.                   |
|   |                       |
| 5. Inner third of costa without any pale interruption . . . . .                                     | 6.                    |
| Inner third of costa with a pale interruption (fig. 3) . . . . .                                    | <i>gigas</i> .        |

FIG. 1. Wing of *A. aitkeni*.FIG. 2. Wing of *A. culiciformis*.FIG. 3. Wing of *A. gigas*.

6. A broad white band on hind femur . . . . . 7.  
 No broad white band on hind femur . . . . . 8.
7. White band on hind femur associated with a scale  
 tuft (fig. 4) . . . . . *annandalei*.  
 White band on hind femur without a scale tuft  
 (fig. 5) . . . . . *lindesayi*.

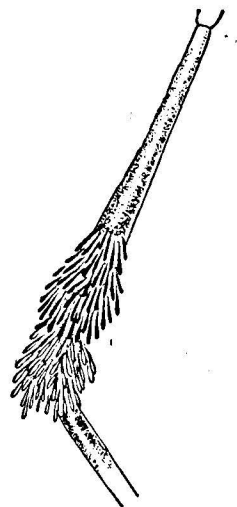


FIG. 4. Hind femur  
 of *A. annandalei*.

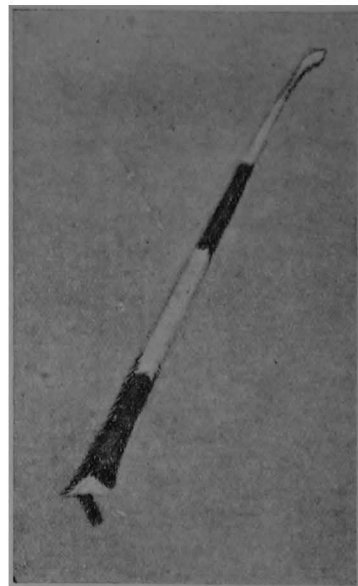


FIG. 5. Hind femur of  
*A. lindesayi*.

8. Palpi unbanded . . . . . 9.  
 Palpi banded. A broad golden spot at wing tip  
 (fig. 6) . . . . . *sinensis*.

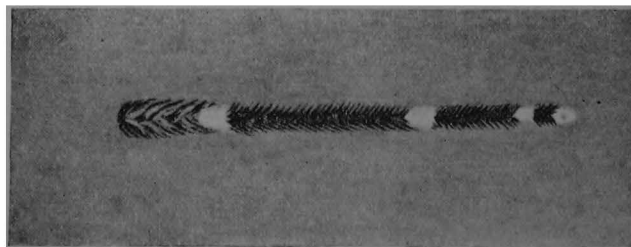
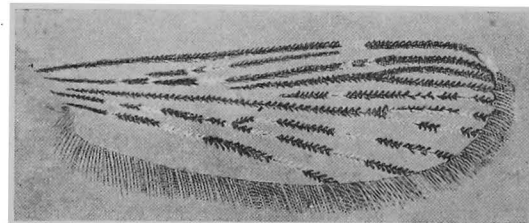
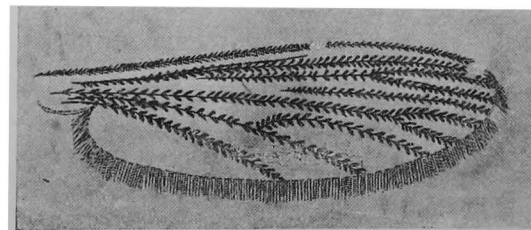


FIG. 6. Palp of *A. sinensis*.

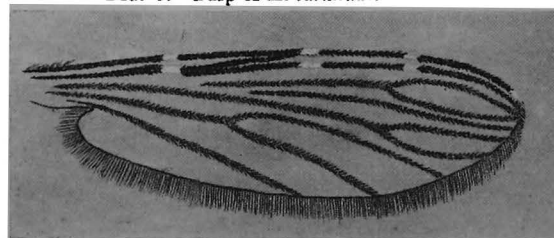
9. A pale spot on wing fringe opposite lower branch of 5th vein. Most veins with black and white scaling (fig. 7) . . . . . *barbirostris*.

FIG. 7. Wing of *A. barbirostris*.

- No pale spot on wing fringe opposite lower branch of 5th vein. Wing scales all dark (fig. 8) . . . . . *umbrosus*.

FIG. 8. Wing of *A. umbrosus*.

- |  |                       |
|--|-----------------------|
| 10. Tip of hind legs not snow white . . . . .                              | 11.                   |
| At least extreme tip of hind legs snow white . . . . .                     | 20.                   |
| 11. Femora and tibiae not speckled . . . . .                               | 12.                   |
| Femora and tibiae speckled . . . . .                                       | 19.                   |
| 12. Front tarsal joints not broadly banded . . . . .                       | 13.                   |
| Front tarsal joints broadly banded. Pleura with dark markings . . . . .    | 18.                   |
| 13. Tip of palpi black (fig. 9) . . . . .                                  | <i>turkhudi</i> .     |
| Tip of palpi pale . . . . .  | 14.                   |
| 14. Fringe spots not more than two . . . . .                               | 15.                   |
| Fringe spots at all veins but 6th . . . . .                                | 16.                   |
| 15. No pale spots on veins other than on costa and 1st (fig. 10) . . . . . | <i>rhodesiensis</i> . |

FIG. 9. Palp of *A. turkhudi*.FIG. 10. Wing of *A. rhodesiensis*.

Pale spots on cross veins and bifurcations (fig. 11) *culicifacies*.

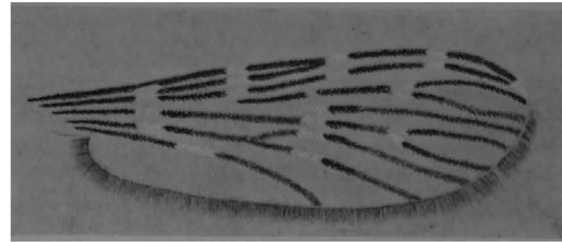


FIG. 11. Wing of *A. culicifacies*.

16. Inner quarter of costa entirely dark (fig. 12) . *funestus*\*.  
Inner quarter of costa with one or more small pale spots . 17.

\* [With fringe spot at 6th vein and outer half of proboscis golden. There may or may not be a very small pale spot in inner quarter of costa—var. *aconitus*.]

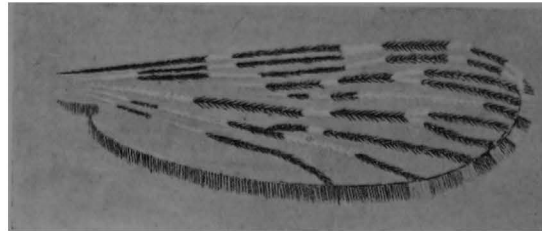


FIG. 12. Wing of *A. funestus*.

17. Hind tarsi narrowly but distinctly banded. Dark  
mosquito (fig. 13) . . . . . *jeyporiensis*.



FIG. 13. Hind tarsus of *A. jeyporiensis*.

Hind tarsi not distinctly banded. Pale mosquito  
(fig. 14) . . . . . *superpictus*.

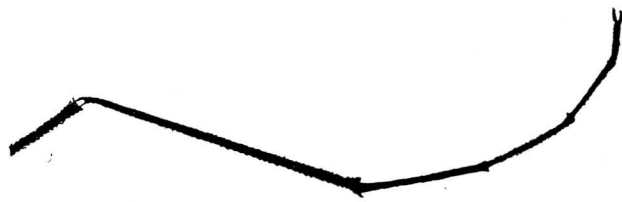


FIG. 14. Hind tarsus of *A. superpictus*.

18. Apical white band on palp equal to succeeding dark band  
(fig. 15) . . . . . *rossi*.

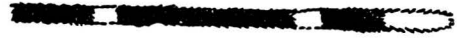


FIG. 15. Palp of *A. rossi*.

Apical white band on palp more than twice succeeding  
dark band (fig. 16) . . . . . *vagus*.  
[The males of these two species are indistinguishable.]

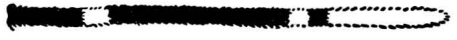


FIG. 16. Palp of *A. vagus*.

19. Not more than three spots on 6th vein. Palpi unspotted  
(fig. 17) . . . . . *ludlowi*.



FIG. 17. Wing of *A. ludlowi*.

More than three spots on 6th vein. Palpi spotted  
with white (fig. 18) . . . . . *stephensi*.

20. Hind legs with at least two tarsal segments wholly white . 21.  
Hind tarsi with only last segment or less wholly white . 26.

21. Abdomen with conspicuous lateral scale tufts  
(fig. 19) . . . . . *pulcherrimus*.  
No such lateral tufts on abdomen . . . . . 22.

22. Femora and tibiae not speckled . . . . . 23.  
Femora and tibiae speckled . . . . . 24.

23. 5th vein not extensively pale. Joint above pale  
area on hind tarsus invariably picked out with  
white (fig. 20) . . . . . *fuliginosus*.

5th vein extensively pale. Joint above pale area on  
hind tarsus never picked out with white (fig. 21) *pallidus*.

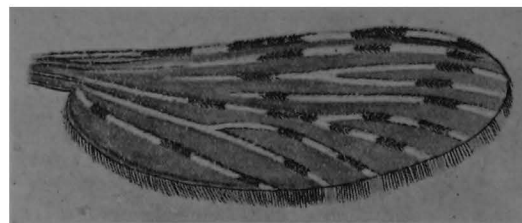


FIG. 18. Wing of *A. stephensi*.

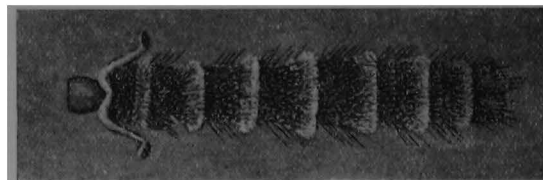


FIG. 19. Abdomen of *A. pulcherrimus*.

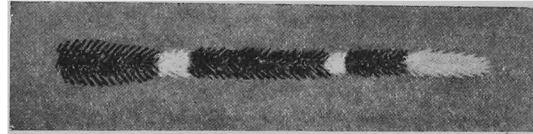


FIG. 20. Hind tarsus of *A. fuliginosus*.

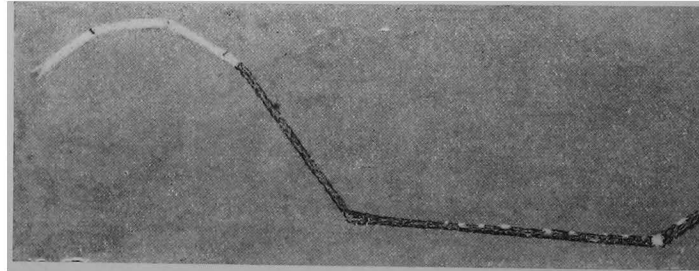


FIG. 21. Hind tarsus of *A. pallidus*.

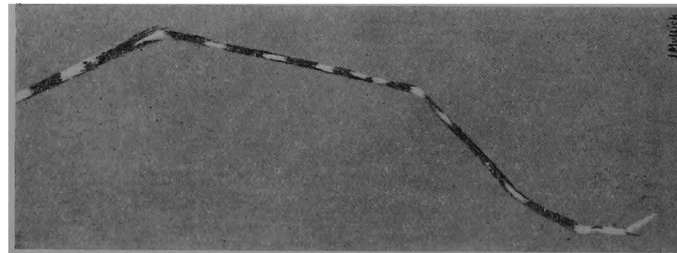
24. Palpi with one broad apical pale band.  
 The 2nd pale band narrow (fig. 22) . . . *jamesi*.  
 Palpi with two broad apical pale bands . . . . . 25.

FIG. 22. Palp of *A. jamesi*.

25. Last three hind tarsal segments uninterruptedly white. Middle area of palpi  
 white speckled (fig. 23) . . . . . *maculipalpis*.

FIG. 23. Hind tarsal of *A. maculipalpis*.

- Only last two hind tarsal segments  
 uninterruptedly white. Palpi not  
 speckled (fig. 24) . . . . . *theobaldi*.

FIG. 24. Hind tarsus of *A. theobaldi*.



26. Last tarsal segment wholly white . . . . . 27.  
 Last tarsal segment not wholly white, only extreme tip . . . . . 29.
27. Femora and tibiae not speckled (fig. 25) . . . . . *karwari*.  
 Femora and tibiae speckled . . . . . 28.

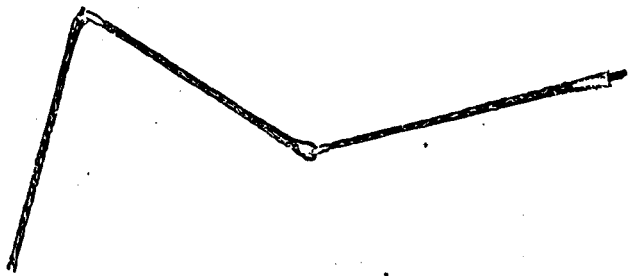


FIG. 25. Hind femur, tibia and 1st tarsus of *A. karwari*.

28. Scales on all abdominal segments dorsally (fig. 26) ; *willmori*.

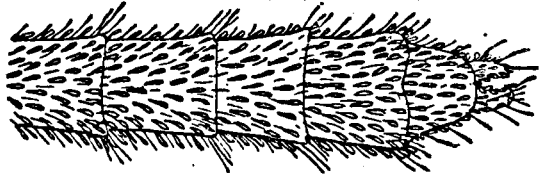


FIG. 26. Abdomen of *A. willmori*.

- Scales on dorsum of last two or three abdominal segments only (fig. 27) . . . . . *maculatus*.

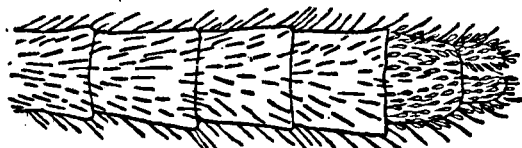


FIG. 27. Abdomen of *A. maculatus*.

29. Half last tarsal segment white. Three spots on 6th vein. Abdomen with ventral scale tufts (fig. 28) . . . . . *kochi*.  
 Only extreme tip of last tarsal segment white . . . . . 30.



FIG. 28. Abdomen of *A. kochi*.

30. Hind legs with broad white band at tibio-tarsal joint (fig. 29) . . . . . *leucosphyrus*.

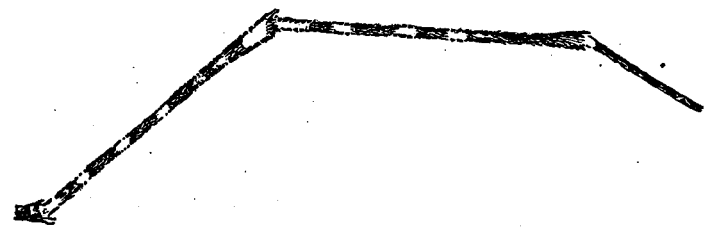


FIG. 29. Hind tibia and 1st tarsus of *A. leucosphyrus*.

No such band. Outer half of proboscis all pale (fig. 30) . . . . . *tessellatus*.



FIG. 30. Hind tibia and 1st tarsus of *A. tessellatus*.

*ANOPHELINE LARVA*  
*showing features used in identification*

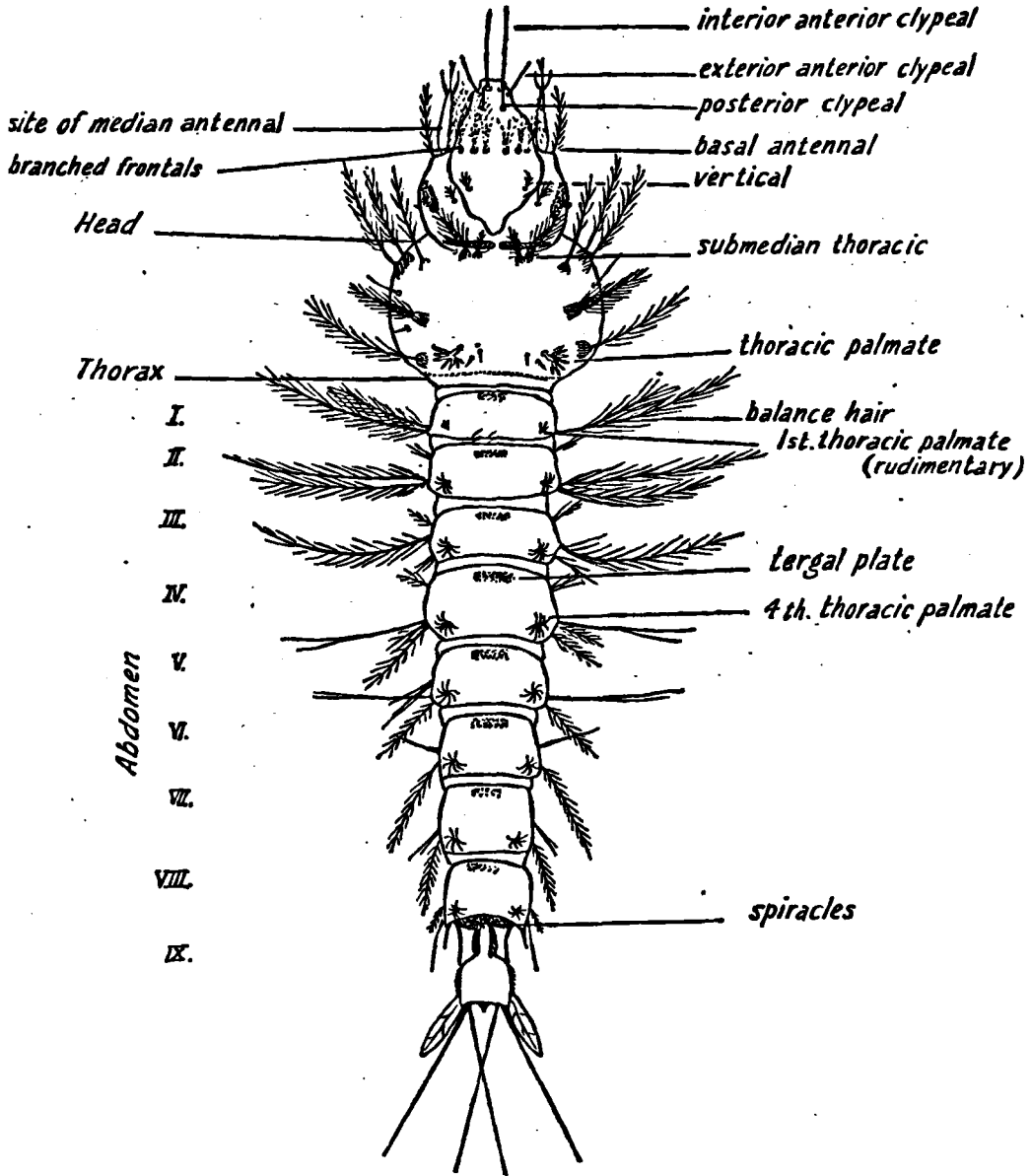


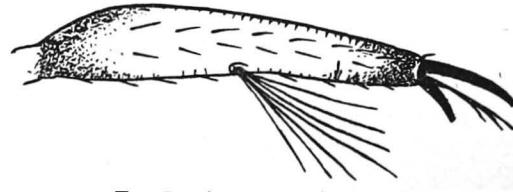
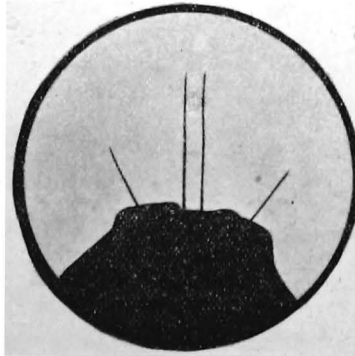
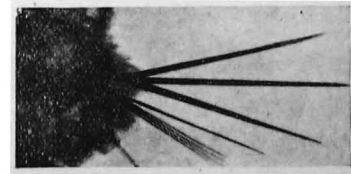
FIG. 43.—Anopheline Larva. Showing features used in identification.

## APPENDIX III.

KEY TO THE MATURE LARVÆ OF THE GENUS *Anopheles* IN THE INDIAN SUB-REGION.

1. Inner anterior clypeals more or less approximated . . . . . 2.  
 Inner anterior clypeals well separated. No branched median hair on antenna . . . . . 10.  
 2. Antenna with branched median hair. Clypeals normally strong (fig. 1) . . . . . 3.

Antenna without branched median hair. Clypeals very weak. No branched frontal hairs. A lateral tri-radiate spine on abdominal segments i-vii (figs. 2 and 3) . . . . *barianensis*.

FIG. 1. Antenna of *A. sinensis*.FIG. 2. Head of *A. barianensis*.FIG. 3. Abdominal spine of *A. barianensis*.

3. Thorax with well developed palmate hairs (fig. 4) . 4.  
 Thorax with palmate hairs weak or absent (fig. 4A) . 7.

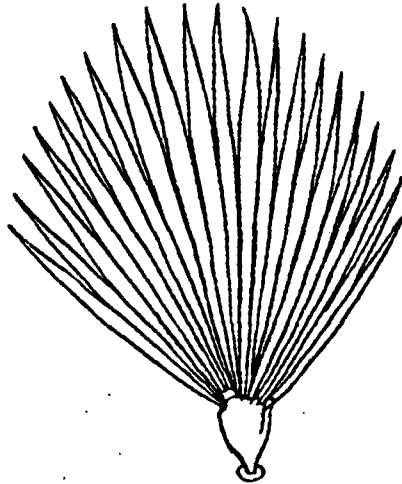


FIG. 4. Thoracic palmate of *A. sinensis*.

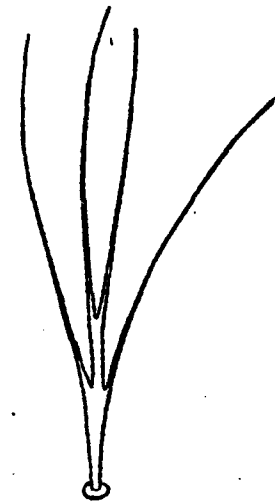


FIG. 4A. Thoracic palmate of *A. gigas*.

- 4. Outer anterior clypeal simple . . . . . 5.
- Outer anterior clypeal branched . . . . . 6.
  
- 5. Basal hair of antenna with nearly bare shaft and apical club. Balancer hair of 3rd abdominal segment simple (fig. 5) . . . . . *culiciformis*.
  
- Basal hair of antenna and balancer hair of segment iii normally formed. Inner anterior clypeals varying in form (figs. 6 and 7) . . . . . *aikeni*.

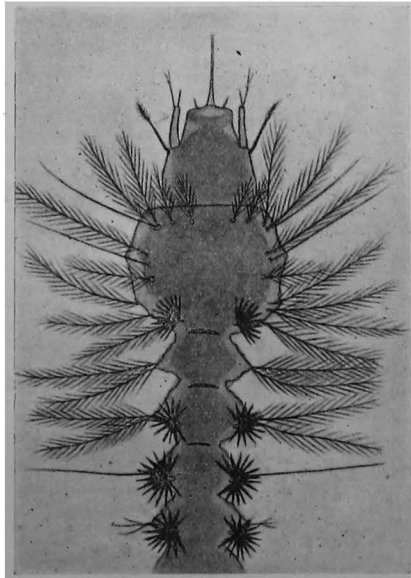


FIG. 5. Larva of *A. culiciformis*.

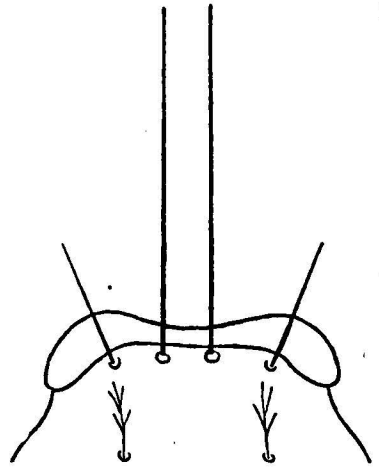


FIG. 6. Head of *A. aikeni* var. *insulæflorum*.

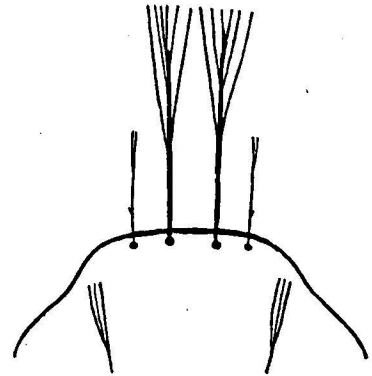


FIG. 7. Head of *A. aikeni*.

6. Innermost submedian thoracic hair  
 branched from base (fig. 8) . . . *barbirostris*.
- Innermost submedian thoracic hair  
 simple, sometimes branched apically  
 (fig. 9) . . . . . *sinensis*.
7. No abdominal palmate hairs . . . *umbrosus*.
- Abdominal palmate hairs on at least  
 last five segments . . . . . 8.

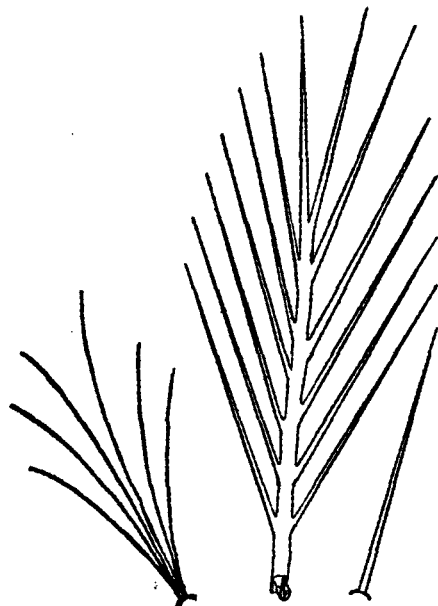


FIG. 8. Right side submedian thoracic hair of *A. barbirostris*.

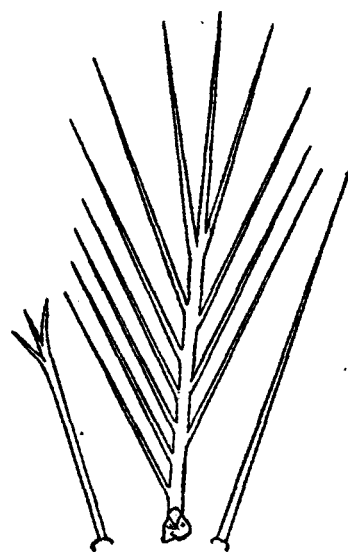
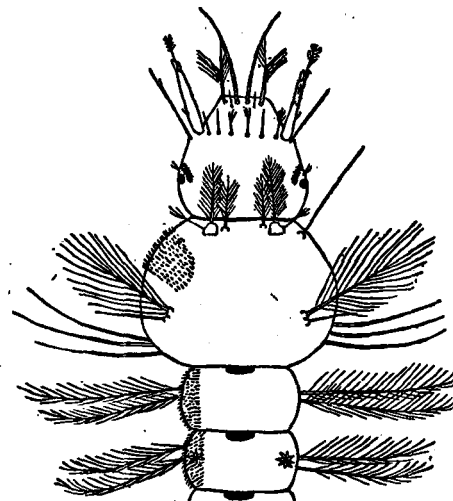
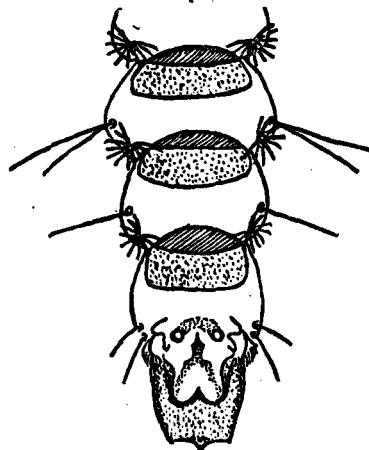
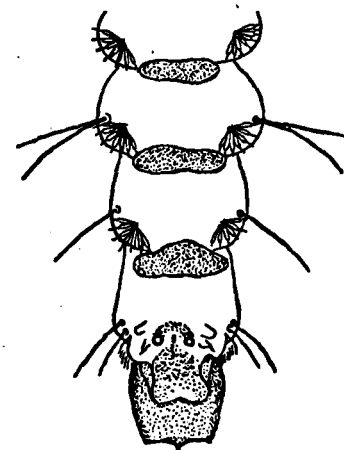


FIG. 9. Right side submedian thoracic hair of *A. sinensis*.

8. Thorax and abdomen completely covered with minute setae (fig. 10) . . . . . *annandalei*.  
 Thorax and abdomen with normal integument . . . . . 9.  
 9. Palmate hairs on abdominal segments ii-vii . . . . . *lindesalii*.  
 Palmate hairs on abdominal segments iii-vii . . . . . *gigas*.

FIG. 10. Larva of *A. annandalei*.

10. Abdominal tergal plates very large, occupying at least half of the dorsal surface (fig. 11) . . . . . *funestus*.  
 Abdominal tergal plates normally small (fig. 12) . . . . . 11.

FIG. 11. Tergal plates of *A. funestus*.FIG. 12. Tergal plates of *A. cuticifacies*.



11. Outer anterior clypeals simple, or with a few short branches. Posterior clypeals simple or faintly branched (fig. 13) . . . . . 12.  
 Outer anterior clypeals thickly plumose. Posterior clypeals branched, usually from base (fig. 14) . . . . . 25.

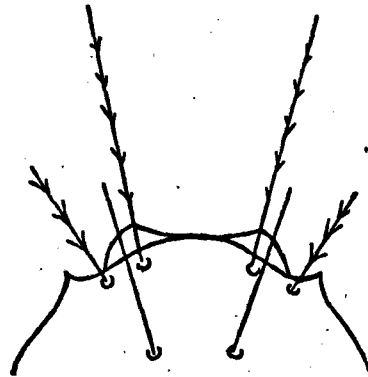


FIG. 13. Head of *A. karwari*.

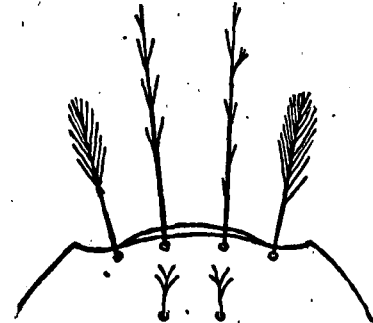


FIG. 14. Head of *A. jamesi*.

12. Inner and outer clypeals quite bare, or inner only very finely branched (fig. 15) . . . . . 13.  
 Inner and outer clypeals nearly always with short branches . . . . . 21.

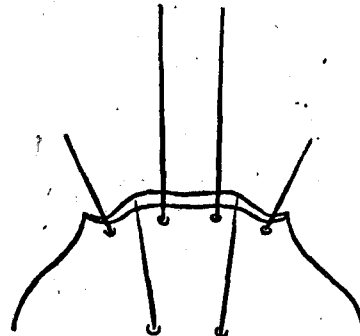


FIG. 15. Head of *A. culicifacies*.

13. Palmate hairs of thorax vestigial (fig. 16) . . . . . 15.  
 Palmate hairs of thorax well developed. Submedian thoracic hairs strong, innermost with conspicuous dark root (fig. 17) . . . . . 14 .

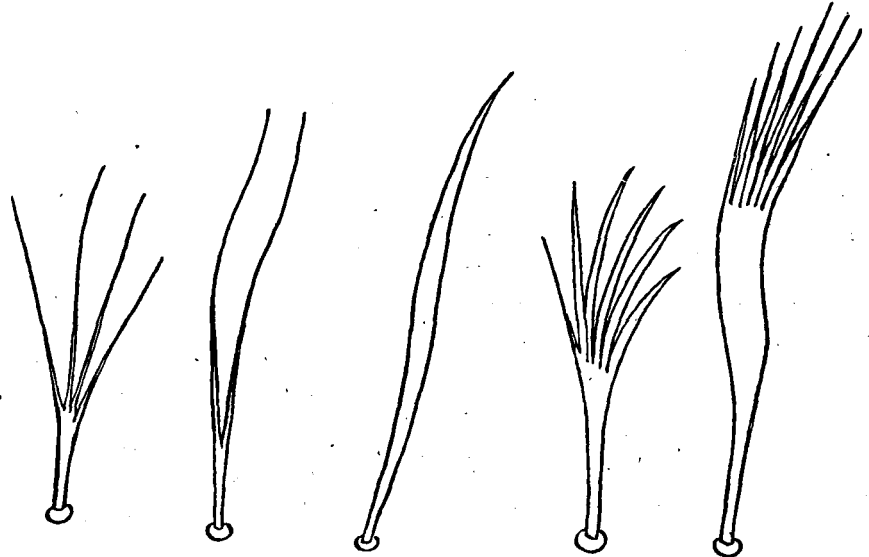


FIG. 16. Palmate of *A. rossi* (3 forms).

FIG. 17. Palmate of *A. culicifacies* (2 forms).

14. Posterior clypeals exterior to inner anterior (fig. 15) . . . . . *culicifacies*.  
 Posterior clypeals interior to inner anterior (fig. 15A) . . . . . *rhodesiensis*.

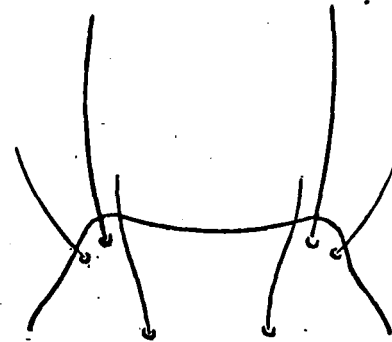


FIG. 15A. Head of *A. rhodesiensis*.

15. Posterior clypeals very long, projecting between the anterior pairs. Abdominal palmate hairs without long filaments (fig. 18) . . . . . *turkhudi*.
- Posterior clypeals much shorter, only slightly passing the front of the head in one species . . . . . 16.
16. All clypeals always simple. Leaflets of abdominal palmate hairs with long filaments (fig. 19) . . . . . 17.

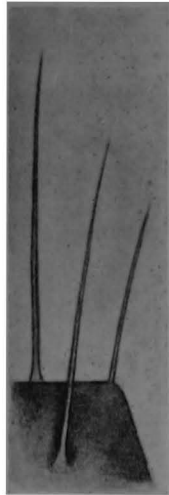


FIG. 18. Part of head of *A. turkhudi*.

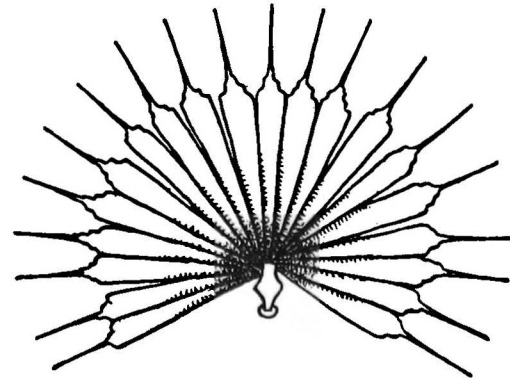


FIG. 19. Abdominal palmate of *A. culicifacies*.

- Inner anterior clypeals usually with short branches. Leaflets of abdominal palmate hairs blunt pointed (fig. 20) . . . . . 20.

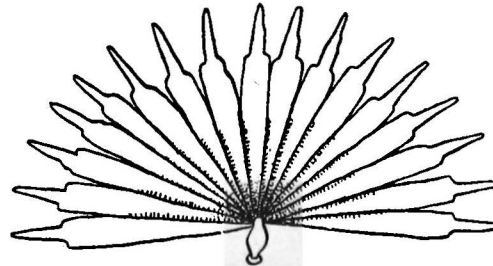
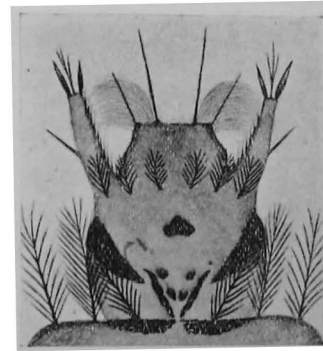
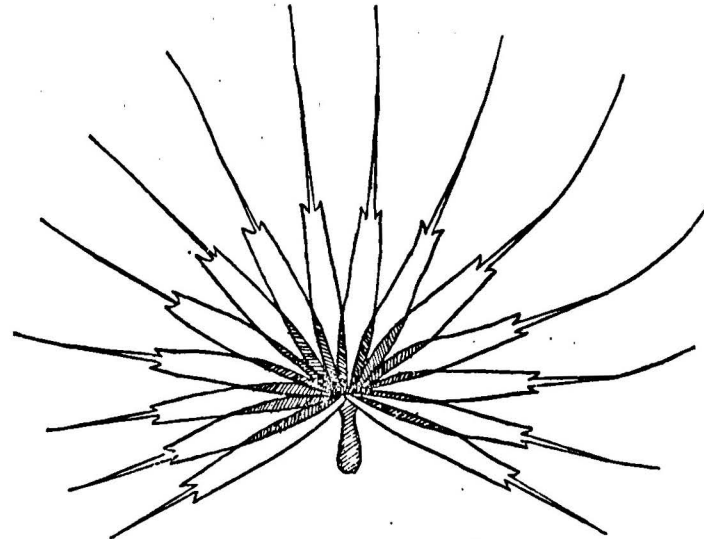


FIG. 20. Abdominal palmate of *A. tessellatus*.

FIG. 21. Head of *A. rossi*.

17. Head usually with characteristic V mark. Filaments of abdominal palmate hairs very long (figs. 21 and 22) . . . . . 18.  
 Head markings otherwise than as above . . . . . 19.

FIG. 22. Abdominal palmate of *A. vagus*.

18. Posterior clypeals interiorly placed to inner anterior and slightly projecting beyond head. Outer anterior pair very short (fig. 23) . . . . . *vagus*.

Posterior clypeals slightly exteriorly placed to inner anterior and not always reaching front margin of head. Outer clypeals not so short (fig. 24) . . . . . *rossi*,  
*ludlowi*.

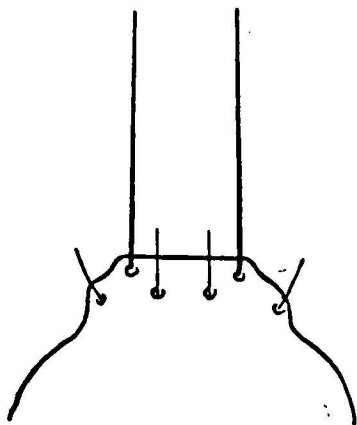


FIG. 23. Head of *A. vagus*.

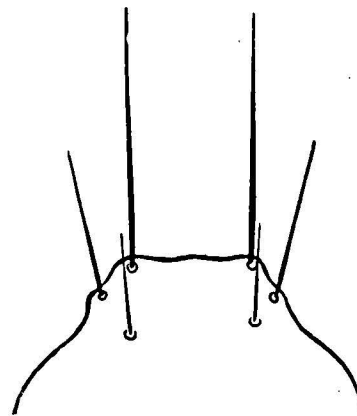


FIG. 24. Head of *A. rossi*.

19. Vertex of head with suffused dark patch. Filaments of abdominal palmates distinctly shorter than *rossi* group (fig. 25) . . . *stephensi*.

Head with three post frontal and one large occipital spot, the latter with four dots around it (fig. 26) *superpictus*.



FIG. 25. Head of *A. stephensi*.

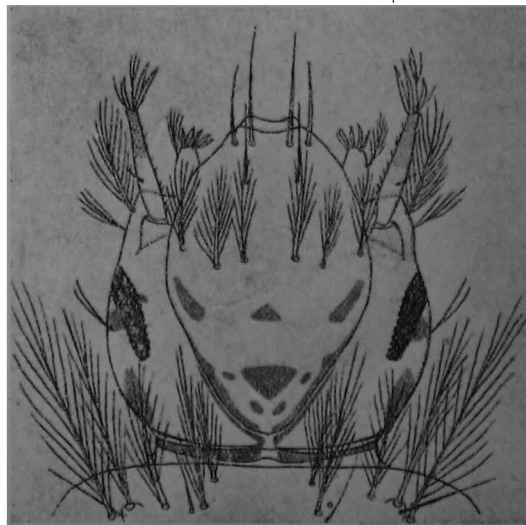


FIG. 26. Head of *A. superpictus*.

- 20. Innermost submedian thoracic hair short, simple or 2-3 branched only, root inconspicuous (fig. 27) . . . . . *tessellatus*.  
 Innermost submedian thoracic hair long, with numerous branches and conspicuous root . . . *leucosphyrus*.
- 21. Palmate hairs of thorax well developed . . . . . 22.  
 Palmate hairs of thorax weak or vestigial . . . . . 23.
- 22. Outer anterior clypeal finely branched. Palmate hair of abdominal segment i well developed . *jeyporiensis*.  
 Outer anterior clypeal quite bare. Palmate hairs on abdominal segments ii-vii only . . . . . *kochi*.
- 23. Palmate hairs on abdominal segments i-vii . . . . . *theobaldi*.  
 Palmate hairs on abdominal segments ii-vii . . . . . 24.

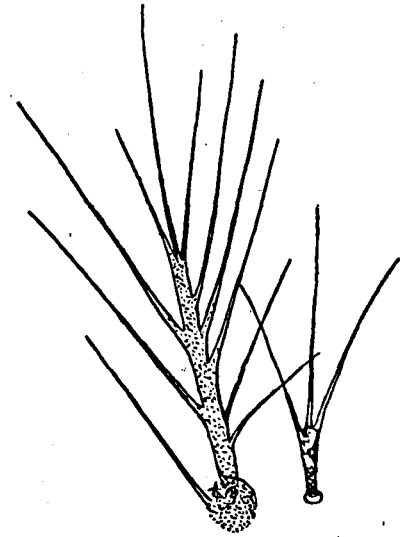


FIG. 27. Right side thoracic palmate of *A. tessellatus*.

24. Filaments of abdominal palmates very short and truncate (fig. 28) . . . . . *karwari*.

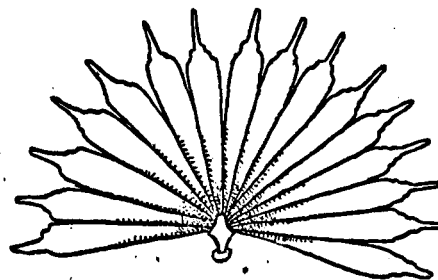


FIG. 28. Abdominal palmate of *A. karwari*.

Filaments of abdominal palmates more distinctly elongated (fig. 29) . . . . . *maculatus*.  
*willmori*.

25. Posterior clypeals absent . . . . . *maculipalpis*.  
 Posterior clypeals present . . . . . 26.
26. Inner anterior clypeals more or less branched . . . . . 27.  
 Inner anterior clypeals completely simple . . . . . *pulcherrimus*.
27. Inner anterior clypeals weakly branched . . . . . *pallidus*.  
 Inner anterior clypeals distinctly branched . . . . . 28.
28. Vertical hair simple or bifid. 1st abdominal palmate weak, only 7-8 rayed . . . . . *jamesi*.  
 Vertical hair with 5-6 branches. Palmate hair of 1st abdominal segment well developed, 10-12 rayed . . . . . *fuliginosus*.

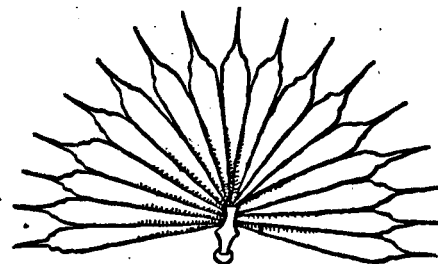


FIG. 29. Abdominal palmate of *A. maculatus*.

## APPENDIX IV.

### PUBLIC WORKS DEPARTMENT, F. M. S.

#### *Specification for construction of under drainage.*

##### *A. M. E. and Health Officer to be informed.*

1. The Engineer shall notify the Anti-Malaria Engineer and the nearest Local Health Officer before works in connection with land drainage are begun and shall co-operate with the Health Officer in preventing mosquito breeding as the work proceeds.

2. This specification is to be observed by all Engineers who will see that their subordinate officers in charge of work thoroughly understand and comply with it. Where alternative methods or materials are authorised by this specification the Engineer in writing shall specify in each case the methods or materials to be used. If cases occur when a departure from specification is deemed necessary, reference shall be made to the Director of Public Works for his decision.

##### *Clearing vegetation.*

3. No vegetation or trees shall be cut down or clearing be done without instructions in writing by the Engineer.

During the progress of work clearing shall be limited to the bare necessities of work in hand and final clearing shall not be undertaken until drains and trenches effectively dry out surface. Within ravine bottoms and in the vicinity of underdrains trees shall be felled and removed and tree stumps shall be grubbed out and removed.

##### *Works to begin from outlet.*

4. All drainage work including laying of pipe underdrains shall begin and continue from the outlet and upwards.

Temporary drainage trenches shall be opened up ahead of final work as may be necessary to dry out surface soil and facilitate cutting to final depth.

##### *Drain Trenches.*

5. Drain trenches shall be cut on lines and to levels and grades as shown on plans and aligned on site. The grades shall in every case be indicated by sight rails set up by the Engineer or his authorised representative and boning rods shall be used to determine depth. Trenches shall normally be about 5 feet deep and no underdrain shall be formed so that the pipe has less than 4 feet of cover over top of pipe. Trench lines shall be as straight as may be practicable and give the most direct discharge to drainage water.

6. Turf removed when trenching shall be stacked conveniently for use as required.

7. Excavated material shall be placed well away from trenches leaving not less than 2 feet clear to trench side and material shall be further removed should stability of trench be in danger.

8. Trenches which are to be filled in after underdrains are laid in them shall be cut of sufficient width only to permit of safe and easy excavation and pipe laying. Whenever trench faces will not stand firm supporting timbering of approved form shall be used.



9. Trench bottoms shall be accurately excavated to the precise depth as given by boning rods. Should the bottom be cut below grade, gravel or other approved material shall be well trodden in to bring bottom to grade.

10. When any trench bottom is soft and unsuited to receive pipes it shall be cut below grade and a layer of sand or other approved material shall be put in and consolidated to form pipe bed.

11. In unsuitable soils no trench shall be opened to full depth for a greater length than can be piped and completed the same day, unless the trench be securely timbered. The open end of pipe drain shall be suitably protected to prevent entry of silt.

12. No pipes shall be laid in any trench until the trench has been inspected and approved by the Engineer or his authorised representative.

#### *Pipes.*

13. Pipes to be used shall conform to specifications for pipes of the class required, and shall be carefully transported from place of delivery on nearest cart road to place where used. Imperfect pipes shall be sorted out and disposed of as may be ordered.

#### *Pipes without sockets.*

14. Pipes without sockets shall be well and firmly laid on trench bottoms with their ends abutting true and close one to another. Especial care shall be taken to ensure that the ends are in contact over the upper three-quarters of periphery. The pipes shall be held in place by clay packed between lower half of pipe and trench sides, the lower part of joints being left clear. The pipes shall not be trodden on nor displaced in any way.

15. All pipe lines shall be straight unless otherwise required by plans or ordered by the Engineer or his authorised representative.

#### *Curves and bends.*

16. Changes of direction in pipe lines 6 inches in diameter and upwards shall be formed of tangents and curves and the curves shall be as gradual as may be practicable. Reverse curves shall not be put in.

17. Socketted pipes, with special bends if necessary, shall be used for all changes of direction where the joints cannot be closely abutted as provided in Clause 14 unless other special methods of using pipes without sockets or inspection wells or silt traps are provided for.

#### *Junctions.*

18. T-junctions shall only be used in connecting 4-inch collector drains to a common outlet or in forming special connections on pipe lines as may be ordered.

Y-junctions shall be used to connect 2 smaller size pipes to a larger pipe. Side junctions shall be used to connect a small pipe to a larger pipe. The angle of intersection of a side junction shall be approximately 45 degrees.

#### *Special protection of joints for pipes without sockets.*

19. In fine, easily disintegrated soils, the pipe joints shall be covered with a wrapping of canvas or other approved material or pipe collars, as may be ordered.

#### *Inspection wells and silt traps.*

20. Inspection wells and silt traps shall be used at changes of direction or junctions as shewn on plans or as may be ordered.

#### *Relief wells and under inlets.*

21. Relief wells and under inlets shall be formed as shewn on plans or as may be ordered.

22. All inspection wells, silt traps, sumps, relief wells and under inlets shall conform to the drawings issued in respect of them and shall be subject to special instructions in each case.

*Socketted pipes.*

23. Socketted pipes shall be used where shewn on plans or where otherwise ordered.
24. Spigot and socket joints shall be closely butted and the full length of pipe shall be firmly bedded on trench bottom. The joints shall be filled with tarred twisted yarn or other approved material tightly packed in over a length equal to socket space and the outer portion of joint shall be well packed with clay of approved quality.
25. When shewn on plans or specially ordered cement mortar or other approved material shall be used for joining instead of clay.

*Inspection of pipe lines.*

26. Before any section of a pipe line is filled over it shall be inspected and approved by the Engineer or his authorised representative.

*Blinding pipe lines.*

27. Following inspection and approval, joints in pipe lines shall be blinded by clay packing at least 4 inches in thickness in case of pipes 8 inches in diameter and under, and of such greater thickness as may be ordered for larger pipes.

The clay blinding shall be well pressed down so that water can only enter pipes through the lower  $\frac{1}{4}$  of joint periphery. Where clay is not available subject to special instructions joints may be blinded by covering pipes with bertam leaves or other approved long leaf material laid without folding to cover upper  $\frac{3}{4}$  of periphery of pipes and be then packed over with approved material.

*Percolation screens.*

28. Gravel screens to allow of water entering over whole periphery of joints shall only be used as may be ordered.

Such screens shall be formed of approved graded material 12 inches thick or more of which the portion next pipe shall be coarser than the outer part. The outer 6 inches at least shall pass a  $\frac{1}{8}$ -inch mesh and be retained on a  $\frac{1}{4}$ -inch mesh.

The inner three inches shall pass a 1-inch mesh and be retained on a  $\frac{1}{8}$ -inch mesh. The trench filling over such gravel screens shall be formed of earth gravel or other material as may be ordered.

*Filling trenches.*

29. Back filling of trenches shall not be commenced until blinding as specified above has been inspected and approved by the Engineer or his representative. Back filling shall be done with such material and in such manner as may be ordered.

The material shall be loose without large lumps spread in layers about 3 inches and in no case exceeding 6 inches in thickness and be well trodden down before the next layer is filled in.

The material shall be compacted by well treading down and not by ramming and shall be broken up and damped if necessary but wet soil shall not be used. The filling shall be brought up to the level of surrounding ground which shall be cut and filled as necessary to form an even surface.

*Defective filling.*

30. Filling shewing defects shall be taken out down to pipe level and be properly replaced. Any sinkage in filling is evidence of defective work. Pipe lines under defective filling shall be examined and relayed if silted or displaced.

31. The surface of ravines shall be evened and graded so that no depressions or obstructions remain which are likely to prevent the free flow of water, cause channelling, or the formation of pools on the surface.

*Masonry drains.*

32. Masonry drains shall conform to the specification for drains of the class required by drawings or ordered to be used.

33. Masonry drains in underdrained areas shall be on lines and to levels as shewn on plans. The grades shall be indicated by sight rails set up by the Engineer or his authorised representative and boning rods shall be used to determine depth of drain invert.

34. All masonry drains shall be formed true to grade and straight or with tangents and easy curves or with simple curves as may be directed.

Any deviation from line ordered shall be made good.

35. Changes in grade of masonry drains are to be gradual so that water will flow without shock. Steps or abrupt changes shall not be used.

36. No masonry drain shall be placed at a lower depth than is necessary and the ground along masonry drains shall be protected from damp by suitably placed underdrains. Weep holes are to be avoided unless specially ordered.

37. The underdrains protecting sides of masonry drains from damp shall be at a lower level than inverts of masonry drains as shewn on plans. Where such underdrains discharge into masonry drains the shallow portion shall be formed with spigot and socket pipes with sealed joints.

*Weep holes.*

38. Weep holes where used shall be made at intervals shewn on plans or as ordered and shall be formed with a slope from back of drain to face of drain wall. The hole shall be covered at back of drain with a piece of tile or approved flat material to prevent earth back washing into drain.

*Concrete block drains.*

39. Concrete block drains shall be truly laid on a firm bottom with closely butting joints. No joint shall exceed  $\frac{1}{4}$  inch in width. In event of displacement the alignment and grade shall be corrected.

*Brick or concrete drains made in situ.*

40. Brick or concrete drains formed in place shall be built on a firm well drained bottom, true to grade and alignment. Precautions shall be taken to prevent disturbance until work is thoroughly set.

41. Back filling of masonry drain sides shall not be commenced until the Engineer or his authorised representative has inspected and approved the masonry work. Earth filling shall be compacted as specified for trench filling above, and brought to slopes as shewn on plans.

*Connections to masonry drains.*

42. Underdrains shall be connected to masonry drains with socketted pipes not less than 2 feet long taken into masonry wall at such height above invert as may be shewn on plans or otherwise ordered. The socketted pipes shall be well bedded in 1--2 portland cement mortar to masonry wall and the discharge opening in wall shall be neatly finished off with the mortar. Great care shall be taken that flow of water is not checked by irregular work and that mortar is not disturbed while setting.

43. The angle of intersection between masonry drains and underdrains connections shall not in the case of pipes 6 inches in diameter and upwards exceed 45 degrees. Special bends shall be used as may be necessary for this purpose but reverse curves shall not be used.

*Open earth drains.*

44. Open earth drains shall be straight and true to grade and line as laid out and determined, sight rails and boning rods being used as specified for other drains. The slopes shall be exactly formed to template as shewn on plans or otherwise ordered.

*Surplus spoil.*

45. Spoil from open earth drains shall be filled as may be ordered into unused channels, holes, or depressions if existing, or otherwise be evenly spread over surface of surrounding ground well away from drain sides. Spoil banks shall not be formed.

46. Connections of underdrains to open earth drains shall be by means of not less than two lengths of socketted pipes jointed with cement mortar as shown on plans.

47. Connections of masonry drains to open earth drains shall conform to plans issued in respect of each case.

*Earth filling.*

48. All filling of depressions, small holes and unused drainage channels shall be done as specified in Clause 29 for trench filling. The filling of large holes and general filling not affecting stability of underdrainage or ravine surfaces may be done as otherwise ordered. Turf, vegetation, and timber shall be removed before filling is done. Turf so removed shall be replaced after filling.

49. No filling shall be done in any hole or depression in which water is lying until such water be drained away, and any water lying below drainage level shall be pumped out until filling is raised above drainage level.

*Protection of surfaces.*

50. Surfaces subject to wash or damage by rain or drainage water shall be protected as shewn on the plans or as may be otherwise ordered, and the protection shall be effectively maintained.

51. Turf protecting surfaces shall be laid as directed and pegged down where ordered. The turf shall be sods of even thickness well trodden into place. Pegs shall not be less than nine inches long. In dry weather turf shall be watered after placing until it has well rooted.

52. Unless special instructions are issued to the contrary strips of turf or spot turfing shall be sunk level with the surrounding ground.

(Sd.) G. STRACHAN,

*Assistant Director of Public Works, F. M. S.,*

*for W. EYRE KENNY,*

*Director of Public Works, F. M. S.*

KUALA LUMPUR ;

*The 14th November, 1922. .*

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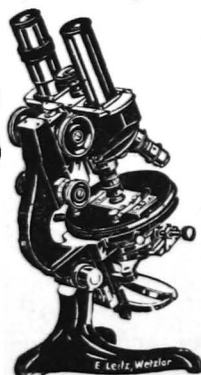
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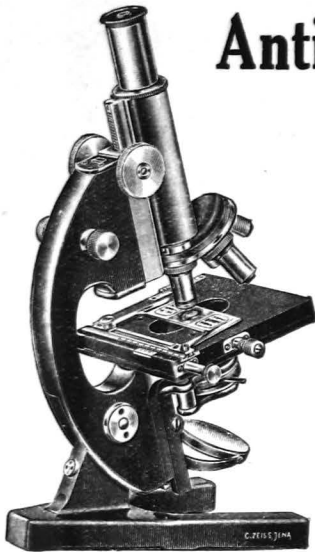
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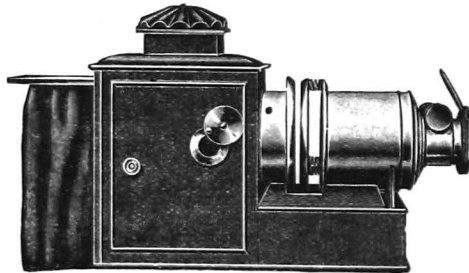
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*Chief Assistant in Malaria Surveys of Assam and Bengal from the School of  
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**With a Foreword**

By **SIR RONALD ROSS, K.C.B., K.C.M.G., F.R.S.,**

*Director-in-Chief, The Ross Institute, London.*

Nothing is more urgently required than a book on the larvæ of Anopheline Mosquitoes. The information can indeed be found more or less accurately stated in text-books and in many scientific journals, but workers are obliged to consult all such works one after the other in order to gather a complete knowledge of the subject. Drs. Strickland and Choudhuri's handbook will therefore greatly facilitate the work of malarialogists and medical men in the field. It is most necessary to distinguish the species of larvæ without waiting for them to hatch out, which is at present an unfortunate necessity for busy Health Officers or students of the subject. Dr. Strickland is one of our greatest authorities on the subject of mosquito-control and has paid special attention to larvæ during many years. His book is simply and efficiently arranged, and is sure to meet a want much felt amongst the many persons who are now taking up the important study of the Prevention of Malaria in the field.—SIR RONALD ROSS.

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# BENGAL IMMUNITY CO., Ltd.

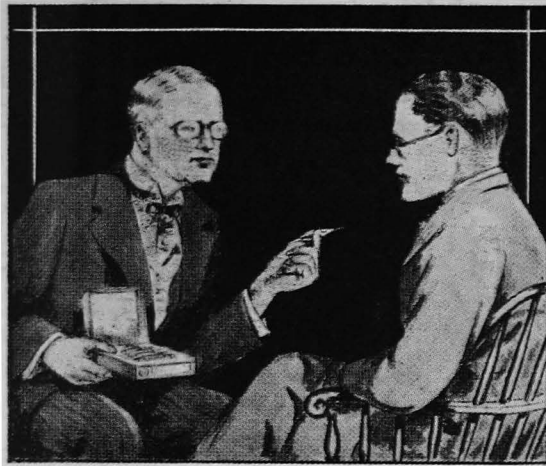
ESTABLISHED 1919

153, Dharamtolla Street, CALCUTTA.

The premier establishment for the manufacture of Sera, Vaccines, Injectables and organotherapeutic products.

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**Y**ES, DOCTOR, when you use any of the FRAISSE AMPOULES you *know* you are injecting controlled solutions of absolute uniformity and that definite results accrue instead of ineffectiveness and discouragement. Your patients respond in a remarkably brief time. Subjective symptoms are promptly ameliorated. And, remember, these standard, unequalled and *safe* ampoules are available at the leading druggists and physicians' supply houses throughout India.

**M**ORE than a quarter of a century ago, to be exact in 1900, THE FRAISSE LABORATORIES, Paris, France, first introduced to the medical profession at the Great Universal Exhibition, Paris,

### **Fraisse Ferruginous Ampoules**

Almost in the twinkling of an eye an excellent combination, such as iron, arsenic and strychnine, effective in action, but destructive to good digestion, passed out of the picture, *going way to the simple, effective, resultful and harmless Ferruginous Ampoules.*

Not only do the contents of these ampoules leave the gastro-intestinal tract in all its pristine activity, but *they do not affect the kidneys.* So far as physiological disturbance is concerned, the system knows it *not.* It receives nothing but physiological benefit.

Under the old regime, the physician could not tell the amount of iron his patient was receiving, on account of the possibility of inertness, and the inability of many a stomach to assimilate the drug. He labours under no misapprehension to-day. Every time he injects the contents of a Ferruginous Ampoule (Fraisse) he is positive that there enters the patient's system,

**Cacodylate of Iron gr. 1/6**  
**Cacodylate of Strychnine gr. 1/120**  
**Glycerophosphate of Sodium gr. 1½**

Potency, accuracy of dosage, ease of assimilation and administration and freedom from any type of after-effects are the outstanding characteristics of this excellent combination. No more will the doctor become discouraged by ineffectiveness of his medication. By the means of this unexcelled hematinic,

### **The Physician Has the Case in Hand at All Times**

He realizes that the patient's system will not be upset and that he can give each person as much treatment as the individual can properly take.

The popularity of the hypodermatic method of treatment amply attests its value. The increasing number of physicians who use Ferruginous Ampoules (Fraisse) and secure definite results from its administration, amply attests its therapeutic worth.

For the treatment of anemia or any other condition in which it is essential that there be an increase in the white blood corpuscles and in the hemoglobin index, physicians will find a remedial agent of superior worth in Ferruginous Ampoules (Fraisse).

### **Administration by Mouth :**

If preferred, Fraisse's Ferruginous may be given by the mouth, as it is supplied in solution form in 25-gramme bottles.

This method of administration is preferable with young children ; of course, with appropriately reduced dosage. It is strongly advised, however, that the hypodermatic method should be employed with adults whenever it is not especially contra-indicated.

*Sole Agents for Fraisse Laboratories :*

**J. CHARLES & ALBERT DAVID BROTHERS,**  
**12, Dalhousie Square, CALCUTTA.**