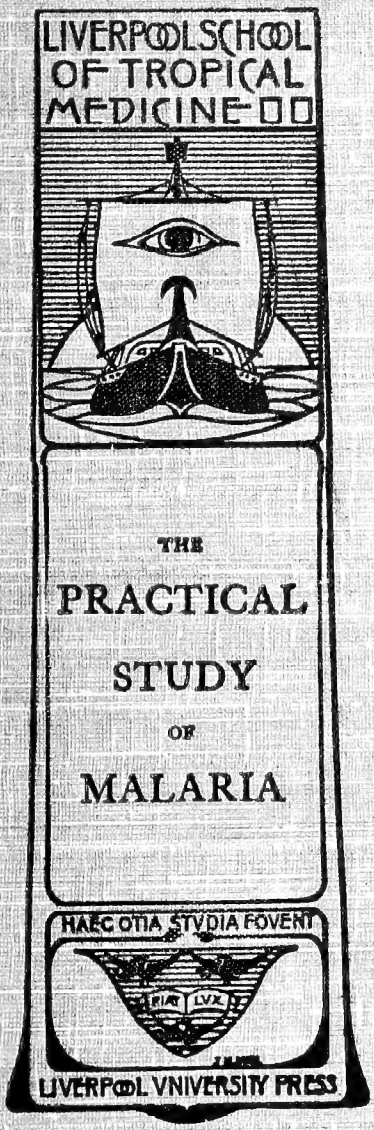
LIVERPOOL SCHOOL
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THE
PRACTICAL
STUDY
OF
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PLATE I

Fig. 1.—Simple tertian parasite. Fresh preparation.

1 and 2. Young forms, pigmented, actively amoeboid.

3. Large form with 'boiling' pigment. Corpuscle much enlarged.

Fig. 2.—Simple tertian parasite. Romanowsky stain.

1 and 2. Young and medium size forms, shewing stippling of the red cell (Schüffner's dots).

3. Female gamete, with much stippling of red cell.

4. Segmenting stage.

Fig. 3.—Malignant tertian parasite. Fresh preparation.

1. Young parasites. Horse-shoe forms.

2. Mikrogametocyte (male crescent).

3. Makrogamete (female crescent).

4. Oval stage of crescent.

5. Spherical stage of crescent.

Fig. 4.—Malignant tertian parasite. Romanowsky stain.

1 and 2. 'Ring' forms.

3. Large egg-shape form.

4. Large form shewing *coarse* stippling of red cell.

5. Segmenting form. Very rare in peripheral blood.

Fig. 5.—Quartan parasite. Fresh preparation.

1. Medium size.

2. Large size.

3. Presegmenting form.

4. Segmenting, daisy, or marguerite form.

Fig. 6.—Quartan parasite. Romanowsky stain.

1 and 2. Young forms.

3. Large form.

4. Segmenting form.

5. Mikrogametocyte (♂).

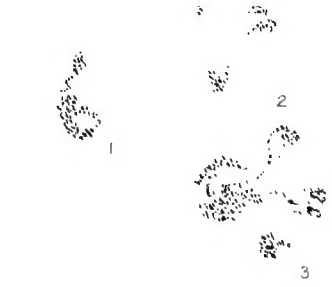

FIG. 1.

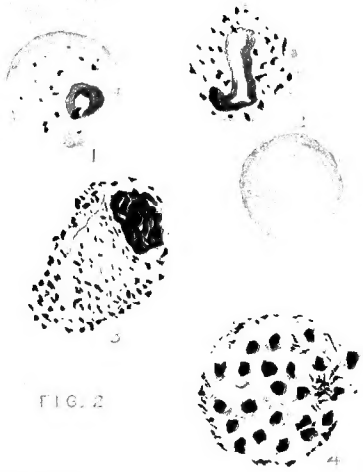

FIG. 2.

BENIGN TERTIAN

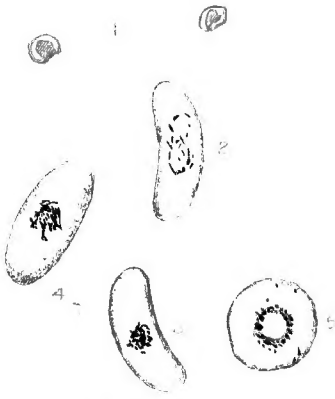

FIG. 3.

FIG. 4.

MALIGNANT TERTIAN

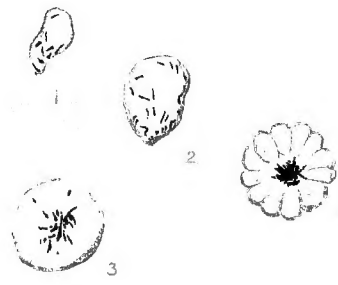

FIG. 5.

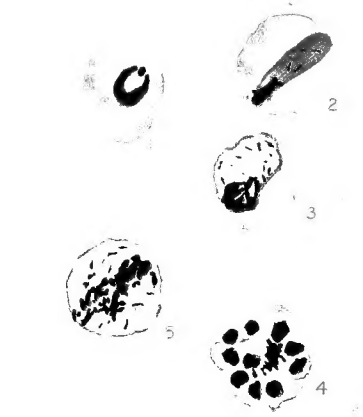

FIG. 6.

TAN

THE PRACTICAL STUDY OF MALARIA

AND OTHER BLOOD PARASITES

BY

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PREFACE TO THE FIRST EDITION

In the authors' experience many medical men in the tropics are only deterred from undertaking researches in tropical diseases by the impossibility of obtaining the necessary knowledge of methods apart from personal instruction in some laboratory. Numerous works on technique exist, they are, however, more adapted for work in a laboratory than for the conditions under which the average practitioner in the tropics must be prepared to conduct his researches. As a result of an experience of several years, during our work on the Royal Society's Commission on Malaria, of the difficulties that Indian and Colonial medical officers experience in making the first start in what must often be work of the greatest interest to themselves and the utmost value to science, we have deemed it wise to give instead of full and elaborate technique, as usually given, only that which we have found the best, the simplest, and the most generally useful. In reality, the necessary methods required to undertake research of the highest value in Malaria are very simple, yet most of these cannot be found in books, and they are with considerable difficulty learnt except by the personal direction of those who are familiar with the small details which go to make success.

In the present handbook we propose to give the essentially practical methods, by which those not familiar with laboratory methods may, under

their own microscopes, follow all the most recent work on Malaria, and eventually be in a position themselves to add new facts to our knowledge of this important disease.

For instance, with very little apparatus it is possible to undertake many most important researches, *e.g.*, to work out the rationale of infection in any station or cantonment; the form of the parasite present; the percentage of adults and children infected; the species of *Anopheline*; where each species is found and where it breeds; the percentage of each species carrying sporozoits and zygotes.

In fact nearly the whole technique of Malaria can be conducted with a microscope, a few slides and coverglasses, a needle, a stain, some tubes, pins, and cardboard. (*Vide* Appendix).

While our original intention was to write a practical guide to Malarial Study solely, yet the opportunities for research on other blood parasites are so numerous in the tropics, that we have thought it to be of practical value to add short supplementary chapters on other Haematozoa and on the Trypanosomidae, etc.

November, 1903

PREFACE TO SECOND EDITION

Our aim in writing this book was primarily to assist the beginner who had little or no experience of the study of malaria or kindred diseases of man and animals. While keeping this view steadily in mind, we by no means intended that its scope should be restricted solely to this, but endeavoured to make the book a convenient and complete reference book for those investigating blood parasites. Progress in the study of blood parasites is extremely rapid, and we have to add to this new edition, besides many new *Anophelines* and mosquito genera and details of mosquito life, many new haemogregarines, trypanosomata, etc., and an account of SCHAUDINN's remarkable investigations on 'Halteridium.' Two new additional chapters have also been added, one on the LEISHMAN-DONOVAN bodies; a second on spirillar fever. The chapter on biting flies and fleas has been much expanded, and in response to requests we have added four new coloured plates.

November, 1904

CONTENTS

The Practical Study of Malaria

Chapter I

TO PREPARE BLOOD FILMS

FOR ordinary work we have no hesitation in saying that it is better to use almost entirely dry films. The advantages of using these are many :—

1. They are much less trouble to make, especially under the adverse conditions one has to contend with in the tropics.

2. They need not be looked at at once, but can be put aside until one has the leisure to examine them.

3. Large numbers, fifty or one hundred, may be taken at once, as will be found constantly necessary, and examined weeks or months later, a thing quite impossible with 'wet' films.

4. They give information as to the leucocytes if this is needed, and may be examined over and over again when some new point of view demands a re-examination.

5. By the use of exceedingly simple methods the routine of blood examinations may be reduced to the greatest simplicity.

For studying movement, delicacies of structure, for watching the process of exflagellation,

phagocytosis, fertilization, and other phenomena of the living parasite, it is necessary, however, to be able to make good wet films, and the points of importance in the preparation of these will be described.

The Preparation of Dry Films

The simplest and by far the best way of making films is by the use of no other apparatus than—

1. A straight surgical needle about two inches in length.
2. Clean glass slides.

To Clean Slides

Slides should be dipped in water and rubbed dry and clean with a soft cloth, *e.g.*, a clean handkerchief. To ensure the best results it is well to heat the slides in the flame of a spirit lamp, or smokeless paraffin lamp, and allow them to cool on a sheet of clean paper. For ordinary purposes this is quite unnecessary. But if a perfectly clean slide is required, then heat it 'red hot' over a flame. In this way grease is completely removed.

Before proceeding to take specimens of blood, the prepared slides may be placed in a small pocket slide box or wrapped in a sheet of clean dry white paper. A packet of half-a-dozen prepared slides wrapped in a sheet of note-paper, afterwards transfixed with the needle is a most convenient form of carrying the necessaries for taking specimens of blood. The needle should be an ordinary triangular-pointed, straight surgical needle. (It is best to nip off the eye with pincers).

To Clean the Patient's Finger

If the finger of one's subject is obviously dirty, and especially if damp with sweat, the finger should be roughly wiped with a cloth. If considered necessary, precautions may be taken to avoid all skin contaminations by the routine of water, alcohol, and ether, but in ordinary examinations for malarial parasites this is quite unnecessary.

To Prick the Finger

The last phalanx of the finger (the third finger of the right will be found most convenient and the skin usually soft) is taken between the finger and thumb of the left hand of the operator and *gently* pressed to force the blood towards the pulp. A slight prick with the triangular pointed needle will in most cases cause a fair-sized drop of blood to exude.

To Make the Film

When the drop of blood reaches the size of the head of a small pin, a slide is taken in the right hand and lowered on to the drop (taking care not to 'dab' it on the skin). If the drop is too large, wipe it away and squeeze a small fresh one. The drop should be transferred to the slide about one-third inch from the far end. The slide is then changed to the left hand, the finger and thumb grasping the end nearest to the drop. The right hand again takes the needle, and holding it by the pointed end lays the cylindrical shaft transversely to the slide and across the drop of

blood. After waiting about a second, that is until the drop spreads to the extent of about one-third inch between the slide and the needle, the needle is evenly and not too quickly carried to the right and so along the whole length of the slide. The right amount of pressure is very

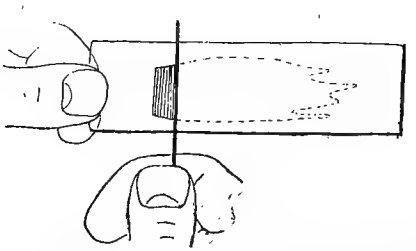


Fig. 1. *Authors' method of making blood films*

quickly learnt, and the making of a useful and good film is far easier in this way than in any other known to us (Fig. 1). Immediately the film is made it should be waved to and fro until it is seen to be quite dry. The quicker the film dries the more perfectly preserved will be the red cells of the blood.

The Characters of a Good Film

1. As a film is needed to detect even the most minute forms of the parasite within the red cell, it is necessary that the red cells be well spread out and not distorted or lying over one another. To ensure this the film must be uniform and as thin as possible.

2. Films should be made so that, if desired, the leucocytes may be differentially counted. A

little practice will enable one to make films with the upper and lower edges more or less parallel with the edges of the slide, and terminating in a pointed manner about half an inch from the right hand end of the slide. Practically the whole of the drop of blood is then upon the slide, and the edges to which the leucocytes tend to find their way are in a suitable position for examination (see differential counting of leucocytes, p. 41).

3. In the case of very anaemic bloods, *e.g.*, those of 'malarial cachexia,' difficulty will arise from the film being too thin. The needle in this case must be carried very loosely and rapidly along the slide and a thicker film thus made. When blood with difficulty adheres to the slide, good evidence of extreme anaemia is obtained.

The Preparation of Wet Films

A wet film is not so easy to make as a dry film, and requires cleanliness and rapidity of manipulation. Wet films are therefore difficult to make in dusty countries, where a single particle of grit will mar the process.

Before proceeding to make films, several glass slides and coverglasses should be carefully cleaned and polished with a dry pocket handkerchief, and wrapped in clean smooth paper, to ensure the absence of dust or grit. In making a wet film the result may be marred by—

1. Too small or too large a drop of blood.
2. Too slow manipulation allowing the drop of blood to partially clot.
3. An uneven coverglass or a coverglass with a minute bubble or speck in its substance.
4. Dust of any kind.

To Make a Wet Film

The same procedure is gone through as in the case of a dry film. It is well to polish the slide and coverglass immediately before use with a clean handkerchief. When the exuding drop of blood reaches the size of a *small* pin's head, a coverglass is picked up rapidly with forceps, or *the edges* are grasped between finger and thumb, and allowed to touch the drop without 'dabbing' the skin, and again rapidly placed drop downwards upon a slide. A gentle tap or two with a needle or forceps may aid in the film formation, but the pressure must not be great or the corpuscles will be found laked and invisible.

The requirements of a suitable wet film are:— There should be a central transparent area shewing no sign of a granular appearance, and even looking quite free from blood. If this appearance is present, the film is probably a good one. If the centre of the film appears reddish or granular, it is useless to examine it (for young parasites), as the corpuscles will be massed together and the parasite not seen. Under the microscope a good film should shew clear, even, circular discs, lying side by side and not overlapping each other. It may be necessary to make several before a sufficiently good one is obtained.

To Label Films

Films should always be labelled as soon as possible, otherwise uncertainty and annoyance are sure to arise. The use of paper labels is not at all necessary in routine work.

1. The most convenient method is that of writing on the end or back of the slide with ordinary ink. This should be quite dry before placing in alcohol. There is then no fear of its coming off.

2. An excellent and extremely simple method of labelling has been described by Dr. POWELL, (Bombay), viz:—After making a dry film, as described above, the name, date, and other necessary information, are scratched on the film with the head or point of the needle. The films used being extensive, the writing in no way injures them. The first half inch or so of the film is frequently rather thick, and much information as to name, date, temperature, etc., may safely be written on it.

TO STORE FILMS

Slide boxes may be used, holding the slide vertically. These should be well cleaned out if made of wood, otherwise fine sawdust accumulates on the slide. A size which will go in the pocket, and holds about twenty-five slides, will be found a great convenience for daily work. Larger boxes to hold one hundred or so are best for use at home; half-a-dozen of these may be enclosed in a stronger outside case. In a square foot of space something like 1,500 slides can be stored in this way.

If no box is at hand, films may be wrapped in clean white paper; a fold of paper being placed between each slide. For transmitting half-a-dozen films or so this is quite the most convenient way, the whole of course being packed in a box or tin with wool.

Both fixed and unfixed films rapidly develop moulds in damp and hot climates. Moulds appear as branching threads under the microscope. A certain amount of mould does not much interfere with the utility of films if they are intended only for detecting the presence of parasites, counting leucocytes, etc.

Unstained films kept for six months or more stain as a rule badly, and are not of much use. Stained films (as we shall see, without putting on Canada balsam and a coverglass) will shew excellent results after many years. Films that have been unfixed at the time of making, and that may have subsequently become damp, will have their red cells destroyed, but the parasites will still stain.

The method of wrapping one's slides up in paper (after duly labelling) is in practice the most convenient one, especially if travelling, when not unfrequently boxes of slides suffer much damage.

To Fix Films

Until a film has been 'fixed' it is soluble in water, and will be immediately washed off if placed in water or any *watery* solution of a stain. When fixed it is insoluble, and may be treated in almost any way without destruction. Except when using Leishman's combined fixing and staining solution, the film must always be 'fixed.'

On returning home or to the laboratory, place the films in a glass-stoppered cylindrical jar, about four inches high and one-and-a-half inch diameter, containing absolute alcohol. When a number of slides are taken, they are placed in

known order, and a blank one added *at the last*, to avoid the possible mistake of reversing the series. A few minutes is all that is necessary if one is in a hurry. Otherwise they may be left in from one-half to twenty-four hours as may be convenient. They are then taken out, allowed to dry, and placed in series in a slide-box, and labelled as desired.

There is the possibility that absolute alcohol may not at times be obtainable. Other fixing methods are :—

1. Methylated spirits. (This is in fact quite adequate for the fixing of blood films in the absence of absolute alcohol).

2. By the use of LEISHMAN'S stain. (The stain itself contains the fixative).

3. By heat. Heating to 100° – 110° for five minutes on a copper plate gives very beautiful leucocyte preparations with EHRLICH'S stain. This method requires care, and a copper plate for heating the slides. If greatly pushed, one may often satisfactorily fix by passing the slides several times through the flame of a spirit lamp.

4. For other methods, *vide* Appendix.

To Stain Films

A large choice of methods is usually given for the demonstration of the malarial parasite in blood. There is one stain, however, so much more strikingly effective and generally satisfactory than other stains that, for routine use, no alternative method need be considered. This stain is ROMANOWSKY'S chromatin stain ; the modifications of LEISHMAN will also be found simple and certain.

In some circumstances the one will be found more convenient; in others, the other. Both methods are therefore given. The making up of the stain, although apparently rather complicated, is not in reality so, and the staining of blood films is one of the simplest, most rapid, and certain of methods. It is useless to attempt to prepare the stain unless suitable methylene blue and eosin are obtained. These stains are very inexpensive, and the only care necessary is to order the exact stain from a good firm (C. BAKER, 244 High Holborn, London).

By the use of Method 2, the staining of blood films is so simplified that a bottle of stain and a supply of water is all that is necessary for the process. Process No. 1, however, is in some ways very convenient and rather more certain, and was largely used by us.

Method 1. The following materials are necessary for the making of the stain, viz :—

'Medicinal' methylene blue.
Eosin extra (B.A. or A.G.) or simply pure eosin for blood staining.
Sodium *carbonate*, pure.

Two stock solutions are made :—

Solution A.	Methylene blue .	1·0 part.
	Sodium carbonate .	0·5 parts.
	Water . . .	100 parts.

The solution is then placed in a hot incubator or by the kitchen fire, or in the tropical sun for two or three days. By this time a deep-purple colour will be noticed at the edges of the liquid. The colour depends upon the formation of a new red body which, combined with eosin, forms the active staining principle of ROMANOWSKY. Until

the purple colour is developed the solution is quite useless.

Solution B. Eosin . . 1 part.
Water . . 1,000 parts.

For staining, these stock solutions are diluted one in twenty, respectively, with water, *i.e.*, five parts of the stock are made up to one hundred parts with water.

To Stain.—Mix equal parts (say one c.c.) of each solution and pour on the slides.

Leave the stain on the slides any time from ten minutes to half-an-hour or longer. Wash off the excess of stain with water, and allow them to drain or dry them with blotting paper, but do not dry them by heating over a flame. The red corpuscles may have a bluish tinge. This can be got rid of if desired by washing in water or very rapidly in equal quantities of spirit and water.

Placed under the microscope, while still wet, the blood platelets should appear as ruby red granular masses, if they are bluish the film should be replaced in the staining solution.

Slides may be decolourized to any required extent by soaking in water, in fact, if left long enough the stain is entirely washed out. Such a specimen can, however, easily be stained a second time.

The exact position and relations of pigment are best seen in specimens lightly stained, as deep ROMANOWSKY staining may completely obscure pigment.

Method 2. LEISHMAN'S stain :—

LEISHMAN'S stain consists of the product of inter-action of the eosin and the methylene blue of the first method.

Make the following solution, viz. :—

LEISHMAN'S stain . .	0·15 grammes.
Methyl alcohol . . .	100·0 c.c.

Place sufficient solution on the slide to cover the film, and allow it to stand for about half-a-minute. Add about twice as much water. Mix by moving the slide to and fro, or stir gently with a glass rod. Allow it to stain five minutes or longer.

The stain is also sold in the form of 'soloids' (by BURROUGHS, WELLCOME & Co.), each 'soloid' = 0·015 grammes. If it is impossible to procure methyl alcohol (pure), dissolve the 'soloid' in methylated spirit (ten c.c.) and proceed as above. The results got with methylated spirit are perfectly satisfactory for diagnostic purposes. There is no preliminary fixing.

TULLOCH recommends making a saturated solution of the stain in twenty-five c c. of methylated spirit, to which two drops of a ten per cent. solution of potassium bicarbonate have been added.

After some minutes the slide will be stained. The same red scum and precipitate are seen as in Method 1, and are of the same significance. The slide should, when stained, be washed in water and allowed to remain in this for a minute or so. This intensifies the ROMANOWSKY staining and removes the remains of the deposit. The red cells also by this process are changed from greenish to faint pink.

To obtain the most brilliant results with these stains is perfectly easy, and no one who has used them will, except for special reasons, use any others at present in use.

The advantages of the ROMANOWSKY stain (either method) as stated by LEISHMAN are :—

1. The great beauty and brilliancy of the staining.

2. The greater certainty of the detection of young forms of the parasites.

3. The ease of application and certainty of result.

4. The staining of the red cell in simple tertian (SCHÜFFNER's dots).

In malignant tertian also a peculiar staining of the red cell is sometimes seen, especially in overstained specimens not washed too much. It consists of coarse blotches or clefts, and the cell also as a whole stains a deeper tint than the surrounding cells (*Vide* Fig. 4).

By fixing the films in chloroform instead of alcohol the 'malignant stippling' is well brought out.

Chapter II

NORMAL BLOOD*

It is difficult, without considerable experience, to know exactly the interpretation to put upon many appearances seen in blood films under the microscope. The less carefully the film is prepared, the more numerous are artifects and various contaminations all broadly included under the designation 'dirt,' and, until one is used to recognize these, mistakes from this cause are extremely likely to happen. There is no way to get over this difficulty except by experience. After a time, however, artifects and 'dirt' can never be mistaken for a parasite.

We may point out some of the artificial appearances that may be encountered :—

1. If in any portion of a film the red cells shew as double outlined circles, or have a central spot, or indeed give any other appearance than

---

* In examining unstained specimens use :—
  1. Concave mirror.
  2. The Iris diaphragm partly closed.
  3. The condenser may be racked down or dispensed with.

For stained specimens use :—
  1. The condenser racked up.
  2. The flat mirror.
  3. The Iris diaphragm almost wide open.

Always keep an eye-piece in the tube of the microscope, if a lens is at the other end, to prevent dust getting in, and always wipe the oil off the oil-immersion lens with a little xylol before putting away. An oil-immersion lens generally keeps quite well in the tropics, especially if in use.

that of perfectly uniform disks, this portion of the film should be passed over. If the whole of the film is of this character, it should be discarded.

2. It is well to have had the parasite demonstrated to one both fresh and stained. After this, little or no difficulty will be experienced in recognizing the parasite when it is really present. Should there be any doubt, the object seen is probably *not a parasite*. A parasite stained properly by the ROMANOWSKY stain has always a *blue body* with a *bright* red dot or dots, and a more or less clear, unstained, whitish (vacuolic) area. Its *definite* outline, whether circular or elongated, should be quite clearly made out—then no possibility of mistake should arise.

3. Artificial bodies in the red cells are generally to be detected by their occurrence in nearly every corpuscle in one portion of the field, and not in another. When a really well-spread portion of the film is reached, they are no longer seen. A common artifect of this nature is a small granular mass stained reddish, apparently in the red cell. It is caused by the staining of vacuoles in the cell. The *body* has a granular appearance, but the *blue body*, red spot, unstained area, and clear-cut outline of the parasite are quite wanting.

4. In fresh specimens, crenations or vacuoles may simulate young ring parasites. It is, however, impossible to get a clearly defined edge to these by focussing. Crenations appear as black dots in one focus, and as bright dots in another. Vacuoles have not the peculiar solid look of young parasites, and cannot be clearly focussed.

5. Masses of bright yellowish-brown pigment derived from the skin are common in films made

without cleansing the finger. They have no relation, however, to any surrounding parasite. Micrococci, yeast, etc., are commonly found in blood films, especially in the tropics. Further, we have found from much experience that the commonest bodies to mistake for parasites are platelets and, strange to say, leucocytes. Platelets (stained)

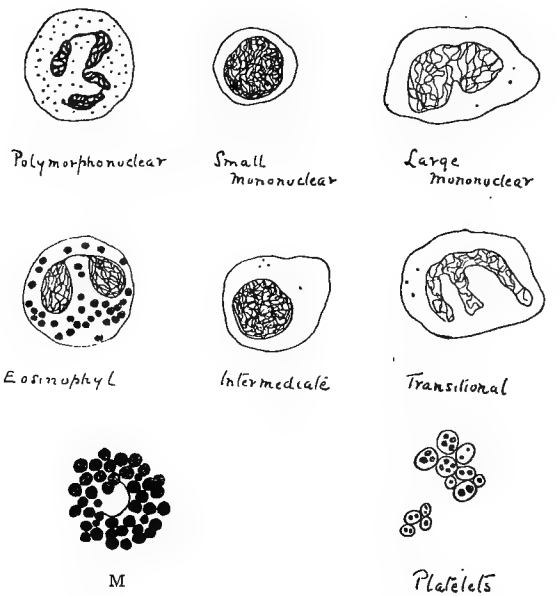


Fig. 2. Normal constituents of Blood:—
M = Mast Leucocyte

may show a great variety of shape. They may be sausage-shaped, oval, or elongated, or they may vary in size from about one-fifth of a red cell to masses three or four times, or even more, as great

as the red cell. Frequently, too, platelets are surrounded by what looks like a definite clear outline, but a closer examination will show that the resemblance to a parasite is only superficial. The mass is *granular* throughout, a parasite is not. The staining is uniformly reddish or blotchy, blue and red, it is not divided as in the parasite between a definite blue area and a definite red dot or dots.

Leucocytes, we have said, are also not uncommonly taken for the larger forms of parasites (*e.g.*, gametes), but only a beginner could possibly make such a mistake, as the leucocytes have a large densely staining mass of red (the nucleus) forming a considerable proportion of the whole cell mass, whereas in the gametes there is only a patch or so of red amidst the blue.

Dust on the eye piece is at once detected by rotating the eye piece when the body shifts its position.

The Normal Constituents of the Blood

Normal blood should be carefully studied in fresh and stained specimens.

1. *The Red Cells.*—With Romanowsky's stain these are only faintly stained reddish (Method 2), greenish or bluish in colour (Method 1). Their apparent size, *i.e.*, the area they occupy when flattened out, depends upon the thickness of the film; in well-made thin films they are large, and stain with beautiful uniformity. In fresh specimens (wet films) they ought to appear as perfect, uniformly straw-coloured discs; if crenated, it is impossible for the beginner to detect parasites.

2. *Leucocytes.*—The following types should be clearly made out in stained films :—

The Polymorphonuclear Leucocytes (Fig. 2).—These are very characteristic, and a reference to the diagram will make their recognition easy. It will be noted that they have a very irregular nucleus and fine granulations (stained red by ROMANOWSKY).

The Small Mononuclear Leucocytes (Fig 2).—These, the lymphocytes, are readily seen and can scarcely be mistaken for other forms. Their appearance varies somewhat with the thickness of the film. The thinner the film the larger they appear and the greater the area of protoplasm surrounding the nucleus. In typical forms the nucleus is dark staining and nearly spherical.

In wet films, a proportion of these cells shew a dark refractile spot (MANSON'S spot), which might be mistaken for a pigment granule, but, as we shall see later, they cannot possibly be confused with a typical pigmented leucocyte.

The large Mononuclear Leucocytes (Fig 2).—It is well to get thoroughly familiar with the appearance of this type of leucocyte, as the percentage of these is of great diagnostic importance in malaria.

Typical large mononuclear leucocytes are readily and clearly distinguishable. They are the leucocytes which may contain malarial pigment, and the recognition of a pigmented leucocyte gives a clear idea of their characters.

(i) They are in thin films of considerable size, half as much again to twice the size of the small.

(ii) The nucleus is large, oval, eccentric, not nearly so dense as in the case of the small—as is

shown by its less intense staining. They also present indentations giving a partly bi-lobed appearance.

(iii) The area of protoplasm surrounding the nucleus is considerable. It is clear, and contains at most a few scattered granules (ROMANOWSKY stain). The only difficulty will be found to arise in the case of a comparatively small number of 'intermediate' and 'transitional leucocytes.'

Intermediate Leucocytes (Fig. 2).—These are forms intermediate between the large and small mononuclear forms. They are usually classed along with the large forms, the characteristics of which they generally more nearly approach.

Transitional Leucocytes (Fig 2).—These are very characteristic, and when seen will be at once recognized. In shape, the nucleus approaches that of the polymorphonuclear forms, being trident-shaped or S-shaped. In consistence, however, it is obviously related to the nuclei of the large mononuclear cells. As a rule these cells are small in number, and from their close resemblance to the large mononuclears may be included with these.

Eosinophil Leucocytes (Fig 2).—The large granules with which these are packed suffice to distinguish them. The granules are stained pink or blue (peripherally) by ROMANOWSKY. The nucleus in the eosinophil cells is frequently characteristic, consisting of two spherical portions united by a thin strand of nuclear material; it is really of the polymorphonuclear type.

Mast Leucocytes (Fig. 2, M).—These, in a film stained by ROMANOWSKY, are cells crowded with granules stained deep blue or nearly black.

These cells occur as isolated specimens in normal blood. They form about 0·5 per cent. of all white cells.

3. *Platelets* (Fig. 2).—Bodies of various sizes up to one-third diameter of the red cell, nearly always lying in clumps of from six to fifty, and stained bright crimson. They often show a considerable amount of differential staining, but differ entirely in appearance from parasites, more especially in having no *blue* stained mass. An isolated platelet lying upon a red cell may simulate a parasite. For the difference between it and a parasite, *vide* above, platelets often occur in large numbers in cases of malaria and, perhaps, especially in blackwater fever.

4. *Blood Dust or Granules.*—Small granules, smaller than micrococci. In fresh films they exhibit active motion (? Brownian).

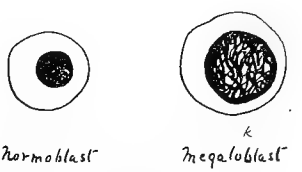


Fig. 2A

Among abnormal constituents of blood we may mention :—

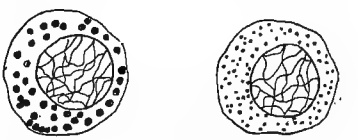
1. *Nucleated Red Cells.*—In conditions of loss or destruction of blood cells, *e.g.*, malaria, it is common to see nucleated forms of the red cell in the blood. They are characterized by a small globular nucleus with sometimes one or more little buds, staining almost black with ROMANOWSKY. If the

film be counterstained with eosin, the fact that the surrounding pale area is red cell will become evident.

Two forms may be seen :—

(a) Normoblasts, *i.e.*, nucleated red cells the size of a red cell (Fig. 2A).

(b) Megaloblasts, *i.e.*, nucleated red cells much larger than a red cell (Fig. 2A).



Myelocytes

Fig. 2B

Normoblasts are the form usually seen. Megaloblasts in excess are found in 'pernicious anaemia.'

2. *Deformed and Small Red Cells may be seen.* —These are known as poikilocytes and microcytes. They are common in severe anaemias, especially pernicious anaemia. It is quite exceptional to find deformed cells in blackwater fever. The red cells are generally quite normal in shape, though anaemic in varying degree.

3. *Abnormal Leucocytes.*—Under certain conditions, *e.g.*, malaria, but especially myelogenous leukaemia abnormal leucocyte forms are seen which normally are only found in the marrow, *i.e.*, myelocytes. These belong to the large mononuclear class, and may be of two kinds, either with large eosinophil granules as in the eosinophil cell, or fine neutrophil granules as in the polymorphonuclear leucocytes (Fig. 2B). If large

mononuclear forms are seen crowded with granules, films should be stained with EHRLICH'S triacid stain, in order to accurately determine the forms of leucocyte present.

4. Frequently in malaria films (stained) large open meshworks of nuclear matter are seen with little or no surrounding protoplasm. These are degenerated or dropsical, or, according to others, mechanically damaged leucocytes, and often occur in great numbers.

5. *Red Cells with Long Wavy Processes.*—These are seen especially in anaemic bloods after the fresh film has been under examination for some time. They occasionally break off and float about. Shorter and more granular processes emitted by the red cell are even commoner.

6. Further, we must point out an extraordinary appearance of the red cells in stained films, so far as we are aware not hitherto described. In anaemic (malarial) bloods, we find red cells, ten, thirty, or forty times the diameter of a normal cell, and these huge swollen structures shew at one side a crescentic area which is granular, and is the only remaining part of the red cell that can be recognized; the remainder is practically unstained. These gigantic structures may or may not be occupied by parasites.

Chapter III

THE DETECTION OF THE MALARIA PARASITE

Examining The Film

After staining and drying, the film is ready for examination. *No Canada-balsam or coverglass need be applied.* A drop of cedar-wood oil is placed upon the film and the oil immersion lowered into it.

After the examination is completed, if it be desired to keep the film, the cedar oil is dissolved off by dropping a little xylol over the film and allowing this to drain off and then to dry. After drying, the film can be put away and kept indefinitely. If not needed the slide is placed on one side with others and eventually cleaned.

To Clean Dirty Slides

1. Rub with turpentine (benzine or xylol) to remove any adherent oil.
2. Wash with soap and water.
3. Rinse in water.
4. Dry and rub well with a clean cloth.

The Detection of the Malaria Parasite

We propose to describe first the actual appearances which are likely to meet the eye, and later to give a systematic description and mode of distinguishing the various forms of parasite. A stained specimen (Romanowsky) should always be used for the purpose of making a diagnosis.

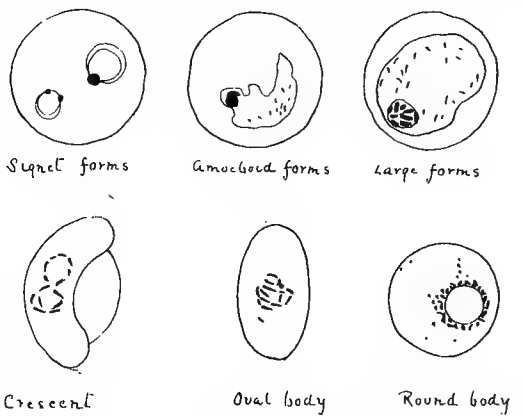


Fig. 3. Forms of the malaria parasite commonly met with in the blood:—The dark dots in the first line represent chromatin, the fine dots, pigment.

We may first note that it is not necessary, as is often thought, to examine the blood at any particular time, but it is very necessary that the patient should not have taken quinine previously. Even five grains of quinine may so diminish the number of parasites as to make detection a laborious task, and a negative result under these conditions is not conclusive.

In examining the slide it is a very convenient method to begin at the edge of the film and to work systematically towards the 'tail' end.

The following forms of parasites may be seen :—

(i) Small forms looking more or less like rings, or stained streaks lying across or apparently stuck to the side of the red cell.

N.B.—Parasites free in the plasma are practically never seen.

(ii) Larger stained bodies of various shapes and sizes more or less filling the cell.

(iii) Crescents or large round or oval bodies with a cluster of coarse pigment placed more or less centrally.

1. *Ring Forms* (Fig. 3).—These may be quite small, one-sixth of a red cell in diameter, or much larger, one-third in diameter.

Rings are parasites of very distinct outline and structure. The part of the parasite that will first be noticed in a ROMANOWSKY specimen will be the red nucleus (chromatin), a clearly stained bright red dot (or dots). This is generally situated on the margin of the blue ring, which is equally distinct in outline, though often only a faint blue. The blue ring encloses an unstained vacuolic area. These rings stand out so sharply that they appear to project from the corpuscles. The red dot generally forms the signet of the ring (signet forms), but also may occur in the centre of the vacuole. The red nucleus or dot is often also rod-shaped or angular. The rings may shew a very faint blue outline or a thicker portion on the side opposite to the nucleus.

Though generally called 'rings,' these parasites are really discs, or saucer-shaped bodies, adhering to the sides of the red cells.

Besides these young rings, we have irregular forms of considerable variety, *e.g.*, a mere faint

bluish line stretching across the corpuscle, yet always shewing somewhere a red nucleus, or a mere streak along the margin of the red cell, with, however, a red nucleus in the blue protoplasm (accolé forms).

Finally, no small structure should be diagnosed as a parasite unless it is clearly made out that it has three distinct parts.

(i) A red nucleus.
(ii) Blue protoplasm or body.
(iii) A vacuolic area within the ring (in the irregular forms this cannot be distinguished).

No confusion can then possibly arise with a platelet or stained vacuole or dirt.

A nucleated red cell has not these characters. Nor, again, has a red cell shewing polychrome or basophil staining, *i.e.*, a purplish or bluish mottling all over. In fact, no other body has the definite, quite easily distinguished characteristics of a parasite.

2. *Large Intra-corpuscular Forms* (Fig. 3).—They appear as more or less extensive areas of blue protoplasm, with one or more distinct, red areas. Pigment may be seen scattered over the parasite. These large forms are generally simple tertian or quartan parasites.

3. *Crescent and Crescent-derived Bodies*—These are most definite bodies, and readily recognized by the coarse pigment granules centrally situated. The presence of this pigment should absolutely preclude the possibility of mistaking distorted red cells crescentic in shape, or a crescentic mass of platelets, for parasites. In neither of these is there a definite central pigment mass, nor should a foreign body be mistaken for a crescent.

Moreover, crescents again have quite definite outlines, and shew a red-stained central portion and blue extremities.

Fig. 3A. *Pigmented Large Mononuclear Leucocytes*

The same criteria apply to the spherical form of the crescent.

4. *Pigmented Leucocytes* (Fig. 3A).—Large leucocytes with a large nucleus. Pigment (melanin) may occur scattered about the periphery of the cell, or in little clumps, or even in very fine powdery grains. The pigment is brownish-black in colour. Skin pigment may be seen in epithelium scales or free in the plasma, but the definite position of the pigment in the protoplasm of the leucocyte characterizes melanin.

Appearances in a Fresh Specimen

1. *Rings.*—The very small forms of these are characteristic of malignant tertian infection. They measure about one-seventh the diameter of a red cell. A 'ring' is characterized by its rather opaque white look, its very definite contour, and by the fact that the central portion is of the same colour as the red cell, which is in fact seen through its substance. The ring has often a thickening at one point giving the 'signet ring' appearance. On watching such a ring from time to time

it is seen to alter its shape. The diagnosis of a ring from a vacuole is not difficult if the latter is carefully focussed, when it will be found impossible to get a sharp outline as in the parasite, the outline on the contrary 'opens out.' Crenated corpuscles are very frequently taken to be parasites. The crenations focus alternately as dark and bright points, and they have no clear ring outline.

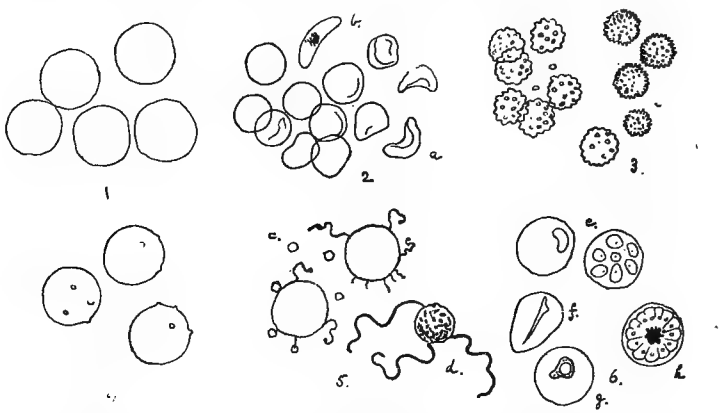


Fig. 3B. 1. *Thin portion of film.* 2. *Thicker portion:* (*a*) *a distorted corpuscle with no pigment;* (*b*) *a crescent with pigment.* 3. *Crenated corpuscles.* 4. *Commencing crenation.* 5. (*c*) *Red cells with processes;* (*d*) *a male gamete with flagella and pigment.* 6. (*e*) *vacuoles;* (*f*) *a crack;* (*g*) *a young parasite with very fine pigment;* (*h*) *a segmenting parasite with central pigment mass.*

Isolated platelets in the plasma or lying on a corpuscle appear as little granular masses, but again they have not the opaque white look of parasites nor the definite ring form, nor are they amoeboid. Here, as in the stained specimen, the definiteness of the body should prevent mistakes being made.

In young tropical or malignant tertian rings pigment is very rarely seen. In larger rings pigment may be seen, *e.g.*, in simple tertian rings.

2. *Large Forms.*—They may occupy the whole of the cell and may shew active amoeboid movement and pigment in active motion may also be seen. The pigment may be reddish-brown, very fine, or rather coarser black pigment. The differentiation of these forms will be considered later.

3. *Crescents and Spherical Bodies* (Fig. 3). The former are at once as in the stained specimen characterized by their shape. They are distinct, fat, plump-looking bodies, unmistakable when once seen. They always have, besides, a central clump of distinct pigment. Stretching across between each end of the crescent is seen the curved edge of the red cell. The spherical bodies also possess this definite, easily seen pigment mass.

In fresh specimens, further, the extremely beautiful and striking process of flagellation can be readily seen, provided a suitable case is used.

Flagellation

Select a case of (simple tertian or) malignant tertian infection, in which parasites have been found. On examining the latter about a week later, crescents will be found in the blood. In about twenty minutes, or in hot weather in England in five minutes or less, many of these will be seen to become spherical and to get free of the corpuscle in which they were situated. Two varieties may be distinguished—the male in which the pigment is distributed over the whole of the parasite, and

the female in which the pigment is concentrated into a ring or figure of 8. Observe that attached to these spherical bodies small ring-like bodies occur, one to two in number, about as big as a pin's head. These are the so-called 'polar' bodies. They occur in the male and female, and, as seen in stained specimens, consist of little circular masses of chromatin. These changes occur in the tropics very rapidly, so that the examination must be commenced as rapidly as possible.

On watching these spheres, the pigment in some will be seen to be in active motion—this probably indicates the internal changes preparatory to extrusion of flagella. Suddenly one of these spheres is perceived to be oscillating violently, and in a moment three or four or more pale, long processes are emitted. The red cells all around are put in motion by their violence, and it may be only after a time, when the activity has grown less, that the flagella are actually seen. Nodosities will be observed in the flagella, and occasionally a speck of pigment at their extreme end. The flagella, after a time, break off, but they have only once, by MacCallum, been seen penetrating the female gamete.

Under certain unknown conditions the crescents do not become spherical and eventually flagellate, but remain as crescents.

Breathing on the slide, adding a trace of water, etc., have been recommended to produce the change more certainly, but it is probable that the real cause lies in the state of development of the crescent for certain observations, *e.g.*, those of Major Buchanan, I.M.S., shew that there is a certain time after the fever when a maximum number of gametes flagellate.

To Stain Flagellated Bodies.

1. When flagellation is observed the coverglass is forcibly 'smeared' off, and slide and coverglass are then fixed and stained with Romanowsky.

2. A number of rather thick drops of blood are placed on a series of slides. These are inverted over rectangular holes cut in blotting paper, moistened with water, and spread on a sheet of glass. A series of moist chambers is thus made. A dozen or more films are made, and each one is removed at intervals of five minutes, dried (spreading out somewhat if necessary), fixed, and stained with Romanowsky; or dry the thick film, decolourize with water, stain with Romanowsky (without fixing), as in Professor Ross' method of making thick films.

To Determine the Species of Parasite Present

Three forms are recognized—simple tertian, malignant tertian, and quartan. The malignant tertian can, as we shall see, produce a quotidian temperature with only a single generation of parasites. Whether or no there is a true quotidian parasite, one or more, is extremely doubtful.

1. Minute rings, one-sixth to one-seventh the diameter of a red cell, showing the signet ring shape, are characteristic of malignant tertian (Fig. 4).

2. Large rings.—If, when the temperature of the patient is still high, the rings are of considerable size, one-fourth to one-third of the red

cell, they are, probably, simple tertian or quartan. If, on the contrary, the temperature is low and the febrile attack finished, these forms correspond to fully developed malignant tertian parasites.

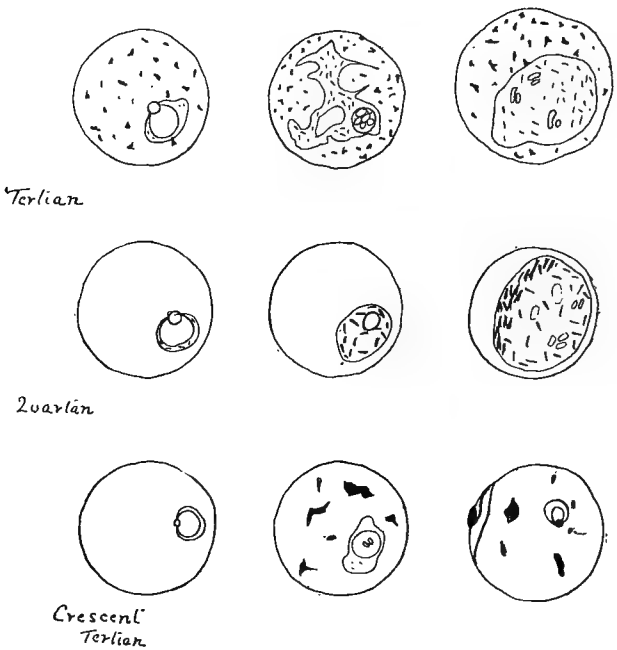


Fig. 4. The three species of Malarial Parasites

The large malignant tertian rings have a characteristic appearance. They are *oval*, with a thicker layer of protoplasm (blue) opposite the nucleus (red).

3. Large forms, with considerable blue protoplasm and with pigment granules, are, probably, simple tertian or quartan.

The tertian parasite is an irregular and flimsy-looking body, and the medium sizes may show several pseudopodia (Fig. 4). Pigment is scattered throughout and is actively motile, while the quartan parasite is oval or globular, of compact appearance, with darker, coarser pigment, shewing but slow motion (Fig. 4).

The *enlargement of the cell* in which the simple tertian lies is also very *characteristic*.

In a well-stained specimen we have the further characteristic differences.

1. *Simple Tertian.*—The cell is dotted all over with fine red granules (SCHÜFFNER'S dots), these cells strike the eye during the microscopic examination and are diagnostic (Fig. 4).

2. *Malignant Tertian.*—In specimens deeply stained with ROMANOWSKY, the malignant tertian parasite also produces changes in the red cell (Fig. 4). These consist of coarse dots or clefts, especially around the parasite. They are few in number and are equally characteristic of this parasite. Their appearance is quite different from SCHÜFFNER'S dots.

MAURER recommends the following method of developing them :—

10 drops of methylene blue (stock solution) + 25 c.c. of tap water.

15 drops of eosin (stock solution) + 25 c.c. of water.

Mix and stain for five minutes; shake actively the whole time.

3. *Quartan.*—The red cell shows no altered staining characters, but it may appear even smaller than normal. The parasite is not irregular in shape, but compact, oval, or globular. (In

the fresh specimen the parasite is very refractile and has a peculiar opaque look) (Fig. 4).

The finding of crescents, is, of course, diagnostic of malignant tertian, but the possibility of a double infection, *e.g.*, simple and malignant tertian, must be borne in mind.

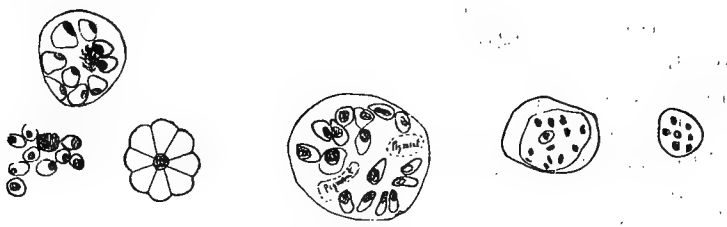


Fig. 5. 1 *Quartan Parasites: segmenting forms*
2. *Simple Tertian* ,,
3. *Malignant Tertian* ,,
4 *Quotidian (after* ZIEMANN) ,,

We have so far described the forms generally encountered during a febrile attack and the means of making a diagnosis, but it is necessary to consider other forms, *e.g.*, the sporulating forms, and more especially the gametes.

Sporulating Forms.—Besides the sporulating forms or segmenting forms, we can recognize also the presegmenting forms, in which the pigment begins to collect into a single mass and the chromatin gets split into a number of fragments. These are even commoner than the final or sporulating forms, in which the segments or spores are arranged around a central mass, though frequently here also the appearances do not correspond with the diagrammatic exactitude of the text-books. The segmenting and presegmenting forms are best seen in a case of regular quartan.

Quartan Sporulating Forms.—The pigment is placed centrally or often laterally, and grouped around it can be seen several, six to eight, chromatin masses. In the presegmenting forms the pigment has not yet condensed into a single block, and the distribution of the chromatin masses is still irregular. In fresh preparations the typical 'daisy' forms can be clearly seen (Fig. 5).

Simple Tertian Sporulating Forms.—Here the whole parasite mass is larger, and fifteen or more chromatin segments can be distinguished (Fig. 5).

Malignant Tertian Sporulating Forms.—Rarely seen in the circulation. There are eight to ten chromatin masses (Fig. 5).

Gametes

Simple Tertian.—The young forms which, under certain unknown conditions, also appear in the circulation are characterized by the fact that the chromatin appears in the centre of the vacuolic area (Ruge), while in the asexual forms (schizonts) it is applied laterally.

The full-grown gametes are much more easily distinguished. The female gamete (♀) is characterized by the possession of much protoplasmic matter, staining deep blue with Romanowsky and little chromatin; in the ♀ the chromatin is laterally placed, and is generally surrounded by a thin vacuolic area, the pigment is black in colour, and is irregularly scattered over the whole protoplasm (Fig. 6).

The male gamete (♂). The chromatin is more voluminous than in the female, it is of a looser

texture, that of the ♀ being compact; the chromatin is centrally placed or extends in a broad band across the cell. The male gamete stains a characteristic greyish-green or greyish-red colour with ROMANOWSKY. It has little blue, so that the pigment is clearly seen, yellowish-brown in colour, while the female stains a deep blue, more deeply than the schizonts (*i.e.*, asexual forms) (Fig. 6).

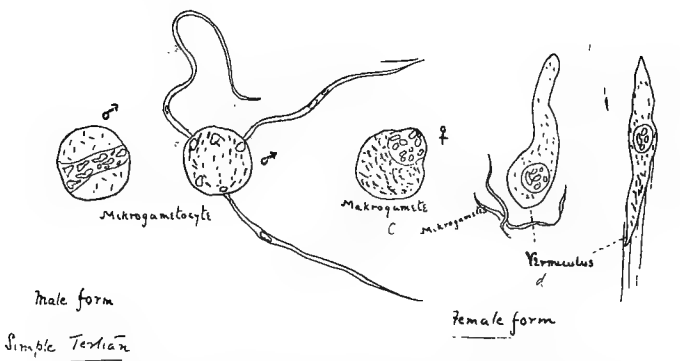


Fig. 6. *Male and Female Gametes (after* SCHAUDINN*)*

Further, there is the difference that asexual forms of a corresponding size would show several portions of chromatin corresponding to pre-segmenting forms.

Malignant Tertian.—The young gametes also occurring in the circulation are characterized according to MAURER :—

(i) By their accurately spherical form.
(ii) The ring is of the same thickness all round.

(iii) The nucleus forms a portion of the ring and does not project in various forms as in the schizonts.

(iv) The absence of 'coarse' stippling in the red cells.

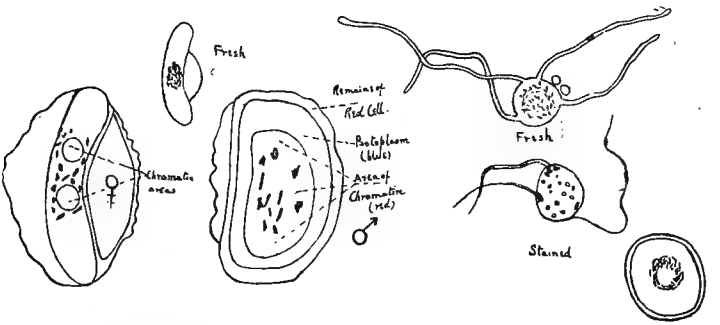


Fig. 7. Malignant Tertian Gametes (partly after MAURER*). Right-hand corner: Spherical body (fresh)*

(v) A circumferential red-staining line soon develops as in the mature gametes.

The adult gametes.—(Fig. 7).

MIKROGAMETOCYTE (♂) MALE CRESCENT

1. The chromatin of the nucleus occurs in an extensive loose network, occupying the greater portion of the parasite.

2. Comparatively little blue staining protoplasm.

3. The pigment is scattered.

4. The shape is somewhat kidney-shaped, shorter and broader than in the ♀.

MAKROGAMETE (♀) FEMALE CRESCENT

1. The chromatin occurs in a compact mass more or less centrally placed.

2. Much more blue staining protoplasm.

3. The pigment is concentrated into a ring around the nucleus or heaped up into mass.

4. Crescentic in shape, longer and narrower than the male.

Staining Reactions of Gametes (Malignant Tertian)

Argutinsky,[1] who has lately studied this question of stippling by means of a modification of the Romanowsky stain comes to the following conclusions. By his method he is unable to obtain stippling of the young asexual forms, which we have ourselves often obtained, and which Maurer also confirms, but he obtains stippling of the red cell infected by the adult gametes (crescents). These forms were also seen by us in West Africa while using a very active haematein stain;[2] but they are certainly not shown by the usual Romanowsky methods in use. Argutinsky's results depend upon his fixative acetic-osmic vapour; while then he differs in not having obtained stippling of cells containing ordinary ring forms, yet his criteria for distinguishing the gametes agree with those generally received. With regard to the stippling in the mikrogametocyte (male) there is a single peripheral row of finer and less intensely stained dots than in the red cell surrounding the makrogamete. The rim of red cell surrounding the parasite is also narrower in the case of the male.

Quartan Gametes.—Presumably similar differences exist as in other forms of the parasite, but they have not yet been described, and, in fact, gametes are often very rare in quartan cases.

Note.—Gametes (crescents) may be but rarely found in the blood of Europeans in the tropics (West Africa).

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1 *C. für Backteriologie*, Bd. xxxiv, S. 144.
2 *Reports to the Malarial Committee.* Harrison & Sons. Series iii, p. 8.

Chapter IV

THE SUBSIDIARY SIGNS OF MALARIA

When patients have taken quinine it is not uncommonly impossible to find parasites in the peripheral blood. Apart from the actual presence of the parasites, one may still derive evidence of malarial infection from

1. The presence of pigmented leucocytes.
2. An alteration in the proportion of the leucocytes.

Pigmented Leucocytes.—Pigmented leucocytes, even in severe malaria, are often very few, and often require for their detection prolonged search in large films. In other cases, however, they are abundant. The presence of very few is quite compatible with a severe malarial infection. For instance, in two cases seen by us only very few were found in the peripheral blood, but in the spleen, post-mortem, enormous numbers occurred. To detect them, it is necessary to make large and good films by the method already described. By following the margins and termination of the film, the majority of leucocytes in the film will have passed beneath the eye, and pigment, if present, is readily seen.

In the vast majority of cases, the pigment will be found in the large mononuclear forms, and

only very rarely indeed in the polymorphonuclear forms. As a rule, a pigmented large mononuclear (Fig. 3) is crowded with granules of pigment, the presence of only a few grains, or a single granular clump, is exceptional. The appearance of the clearly defined yellowish-brown or black pigment granules in the clear protoplasm is so characteristic, that no doubt ought to exist. It should be remembered, however, that in dirty films, specks of dirt may be over a leucocyte, and so resemble pigment. In this case, similar specks will be found lying free. The occurrence of malarial pigment free in the blood has never been seen by us.

Leucocytic Variation.—Often in cases where pigmented leucocytes are difficult to find, there is a very obvious increase in the percentage of the *large mononuclear leucocytes*. This change, which is usually very pronounced in the apyretic periods of an attack of malaria, is, however, most frequently absent during pyretic periods. If, during a period of low temperature, this change is not found, there is a strong presumption that the case is not malarial. If the blood be taken at the height of the fever, a negative result does not exclude malaria, and a further examination should be undertaken, if possible, during an apyretic period. In some cases, the change can be detected even during the pyretic periods, but in these it is always more marked in the apyretic. In some cases, during the course of the fever, no such change occurs, but appears immediately the temperature subsides, and diminishes as convalescence proceeds. Perhaps the cases where this test is of the greatest value, are those where the patient has

already been treated with quinine, and one can scarcely hope, even if the disease be malaria, to find parasites in the blood.

To Make a Differential Count of the Leucocytes

Large films are necessary, especially in malaria where, during the apyretic period, there is a distinct diminution in the *total* number of the white cells. It is important, in making films for leucocyte counting, that the margins and terminal points of the film be regular, and so in a convenient position for examination (Fig. 1). The margin of the film is focussed and passed beneath the objective. By passing along one-half or the whole of the margin of the film, the great majority of the leucocytes in the film are seen. In order to obtain accurate results, one thousand leucocytes should be counted, but a count of three or four hundred is generally sufficient for diagnostic purposes. Counts of a smaller number of leucocytes are valueless, as too great variations will occur.

As a leucocyte is seen, it is marked under the heading, large mononuclear, intermediate, transitional, small mononuclear, polynuclear, eosinophil, as the case may be. As many as ten to twenty or more are mentally noted before making each record in its column.

From the results obtained by blood counts of a considerable number of Europeans living in the tropics, we found that an increase beyond fifteen per cent. of the large mononuclear forms is proof of an actual or recent malarial infection, whereas

with a value of twenty per cent. it is almost always possible, by long search, to find an occasional parasite or pigmented leucocyte. A value of over twenty per cent. probably implies actual infection at the time of observation.

The Normal leucocyte values are :—

Polymorphonuclear leucocytes,	65-70	per cent.
Large mononuclear ,,	4-10	,,
Intermediate ,,		
Small mononuclear or lymphocytes ,,	20-25	,,
Eosinophil ,,	2-4	,,

Other forms of leucocytes, *e.g.*, 'mast' cells, are always in extremely small numbers in health (0·5 per cent.)

Chapter V

THE PARASITE IN THE TISSUES

Tissues may be readily examined for the presence of parasites or pigment in the following way :—Place a minute portion of the tissue on a slide, and with the end of another slide spread it out as evenly and thinly as possible. Dry, fix, and stain in the same way as a blood film. Parasites, if present, are in this way much more easily and clearly seen than in sections. Spleen pulp, bone marrow, kidney, liver, etc., give beautiful results, and in the same way any secretion or fluid can be examined. For certain tissues, *e.g.*, bone marrow, it is advisable to fix in—

Absolute alcohol, 1 part,
Ether . . . 2 parts,

in order to dissolve out the fat present.

To Prepare Tissues

In preparing tissues for examination :—

1. Use as small pieces as possible, *i.e.*, at most five mm. thick.

2. Use plenty of the fixing and hardening fluid because the tissues contain much water, and if the fixing fluid becomes dilute it acts as a macerating agent.

3. See that the separate pieces do not cohere to one another. Place some cotton wool on the bottom of the vessel.

Corked collecting tubes will be found most convenient and will hold ample material. The large masses of tissues sometimes sent home are of far less value to the pathologist than much smaller pieces well fixed and hardened. Always put a label *in* the fluid, with the data written on it in pencil, as well as the outside label.

Fixing

1. Alcohol is on the whole the most useful fixing fluid. Small pieces of tissue should be put directly into absolute alcohol. Larger pieces should be placed in ninety-five per cent. alcohol for two or three days, and then for twenty-four hours in absolute alcohol. Intestine should be spread on filter paper, as also nerves, or other tissue, which it is desired to keep flat. When removing the tissue from the paper, care should be taken that no fibres of the paper adhere, as they may prevent the proper cutting of sections.

For other modes of fixing *vide* appendix.

To Store Tissues

Keep tissues in diluted alcohol (seventy-five per cent. about). If kept long in absolute alcohol, many tissues become very hard.

To Embed Tissues for Section Cutting

Except for very special reasons, embedding in paraffin should always be the method employed. Very general misconceptions exist as to the *time*

and trouble necessary to prepare tissues in this way. It may be pointed out :—

1. That the times usually given for immersion in paraffin and other reagents are unnecessarily long.

2. That the use of two paraffins for embedding, a soft and a hard, is an unnecessary and even harmful procedure.

3. That an elaborate apparatus for the paraffin bath is unnecessary (*vide* later).

4. By using flat and very thin pieces of material, sections of considerable area may be obtained in a minimum of time. It is necessary to cut thin slices of the raw material (1 mm. or less in thickness), and place these upon a small piece of paper or coverglass before placing in the alcohol to harden. The paper keeps the slab from becoming distorted, and enables one to cut sections of the full area of the slab, say two-fifths inch square.

5. By placing minute pieces of tissue (in slabs on paper, if a section of some size is needed) directly into absolute alcohol, fixing, hardening, and dehydration can be accomplished within an hour.

Necessary Apparatus for Paraffin Sections

1. *Cambridge Rocking Microtome.*—The ordinary form is all that is necessary, costing about five pounds. It is convenient to have a ball and socket adjustable holder, which enables one to change the angle of the block without remelting the paraffin.

2. *Razors.*—These may be hollow-ground on one side, or on both, to a varying depth. For

general use a moderately hollow-ground razor is used. Examine the edge under a low power to see if any notches exist, if so they must be ground out on a hone. A 'water of Ayr' stone, as long as possible, should be used and kept absolutely free from grit during use. The stone should be soft, capable of being scratched with a pin, and as a lubricant water or filtered kerosene oil may be used. After honing, the razor should be stropped. On one side of the strop a *minimum* amount of razor paste should be rubbed in and the leather side should be kept scrupulously clean and dry. If the razor is hollow-ground on one side only, it should only be honed on this side.

Examined under the microscope the edge should now present a clear, sharp line. It may be tested on a thin hair, which it should easily cut.

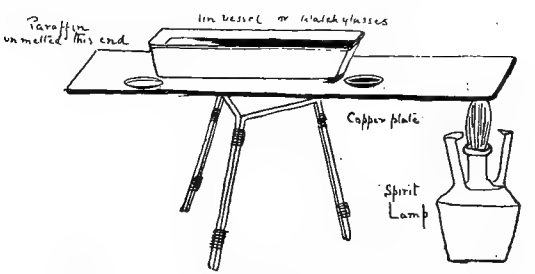


Fig. 8. *Simple Embedding Apparatus*

3. *Embedding Apparatus.*—A slab of metal (copper), 12 × 3 × $\frac{1}{4}$ inches. Heat this at one end, and place the vessel containing the paraffin at a point on the slab where the paraffin is just

kept melted. This is the temperature for embedding. This simple device serves all the purposes of an elaborate paraffin oven (Fig. 8).

4. *Alcohol.*—Absolute alcohol in the tropics has absorbed a good deal of water, and it is necessary to dehydrate it.

Heat crystals of $CuSO_4$ till a white mass is formed. Allow to cool, and place in a tall bottle of alcohol. Allow to settle and decant off alcohol as required. Add fresh anhydrous $CuSO_4$ if a marked blue tint develops, or tie up the anhydrous copper sulphate in a muslin bag, and place in the alcohol pot.

Gelatine may be used to dehydrate alcohol; it must previously be washed free from salts by soaking in water.

5. *Xylol.*—Xylol is the most generally satisfactory agent for displacing the alcohol and allowing the paraffin to permeate the tissue. Chloroform, wood naphtha, turpentine, oil of cloves, and other substances may be used.

6. *Paraffin.*—For use in the tropics, paraffin melting at sixty degrees will scarcely be found too hard. At high altitudes a softer will be required, and the right degree of softness must be determined and produced by mixtures of paraffin melting at 60° C. and paraffin of lower melting point, say 50° C., such as is suitable for use in temperate climates.

To obtain paraffin suitable for use in a given temperature place a block of paraffin in holder and cut thin sections.

(*a*) If the sections curl very much the paraffin is too hard.

(*b*) If the sections formed are forced together (telescoped) the paraffin is too soft.

(*c*) A certain amount of crinkling is usual with thin sections, and can subsequently be got rid of before mounting.

To Embed Tissues

1. *Alcohol.*—Two to three hours, using dehydrated alcohol (*vide* antea) in excess, and changing two to three times. If soft tissues, *e.g.*, liver, spleen, time is unimportant so long as dehydration is complete. If fibrous organs, the least possible time that will ensure dehydration (using thin slabs). Fibrous tissue becomes excessively hard if left too long in alcohol, xylol, or paraffin; thus skin and connective tissue require great care in preparation.

2. *Xylol* (or oil of cloves).—Ten to twenty minutes. When the tissues become transparent they are ready, and should be transferred without delay to melted paraffin.

3. *Paraffin.*—Ten to thirty minutes. If a tin trough be used, the tissues should not be allowed to rest upon the bottom of the trough, but be supported upon a strip of paper kept in place by folding the ends over the edge of the trough. A watch glass generally suffices.

4. Prepare a block for cutting by one of the following methods:—

(i) If the piece of tissue be small, smear a watch glass with glycerine, fill with melted paraffin and add the piece of tissue picked out of the bath with forceps, warmed by passing through the flame.

(ii) Fold a piece of paper, so that by folding a trough of required size is made. If an extra length of paper be left at each end of the trough it can be folded down and holds the rest in position. Fill with freshly melted paraffin and add the piece of tissue picked up with warmed forceps.

(iii) Use metal pieces, supplied with most microtomes, upon a slab of glass.

The following points should be borne in mind :—

(i) Fresh paraffin should be melted for the block, as paraffin frequently melted, or kept melted for long periods, does not form so uniform a mass when cooled as freshly melted paraffin.

(ii) The more rapidly the paraffin is cooled, the more uniform is the resulting mass. It is well therefore, as soon as a well-marked surface crust appears, to plunge the watch glass or trough into cold water.

When cold, cut out a square block with the tissue arranged in the position required for the sections.

5. *Cut sections.*

Note (i) The angle the knife is placed at is important, and must be found by experience.

(ii) It is well to use pads of paper to protect the edge of the razor, where it presses against the iron of the microtome.

(iii) To cut in ribbons, the top and bottom edges of the block must be parallel and horizontal. It is well to dip the block in soft paraffin, or merely to smear the top and bottom surfaces of the block with soft melted paraffin.

(iv) Cut as thin sections as will remain intact.

To Stain and Mount Tissues

1. If the sections are crumpled, float them upon water just hot enough not to melt the paraffin. They will become quite flat. Float the flattened sections on to a clean slide. Remove excess of water, and firmly press a piece of filter or blotting paper over the section. Thoroughly dry by holding a few minutes over the flame (care being taken not to melt the paraffin), or by placing for twenty-hours in a dessicator or warm oven. No further fixative is generally needed. If necessary, the slide may previously be smeared with the merest trace of egg-albumen fixative. It must, in this case, be dipped rapidly into the water and quickly withdrawn. (Appendix.)

2. If the sections are flat they may be placed directly upon a slide slightly smeared with fixative. In this case, celloidin in oil of cloves is the best fixative. (Appendix.)

3. Hold the slide or coverglass with the section over the flame till the paraffin melts. Dissolve off the paraffin with xylol, and then drop alcohol over the section. Place the slide or cover-glass in water.

4. *Stain.*—The best stains for general use are :—

(i) Haematein purissimus, saturated solution in 70 per cent. alcohol 10 cc.
Alum solution (alum 50 grammes, water 1,000 cc.) . . . 50 cc.

Stain for five to twenty minutes, according to the depth of colour of the sections.

(ii) Methylene blue, or gentian violet.

(iii) Counterstain, if desired, with watery eosin. For the detection of pigment it is well to stain a section faintly with eosin alone.

5. Pass through alcohol, oil of cloves, to Canada balsam. In hot moist climates, the cold produced by the evaporation of the alcohol causes dew to be deposited upon the slide. When the xylol or oil of cloves is added, this produces a troublesome milkiness and may spoil the section. To avoid this, all excess should be rapidly wiped up after the use of alcohol, and the oil of cloves added as quickly as possible.

To stain sections with Romanowsky

A —1. Fix and dehydrate small pieces of tissue, one to two millimetres thick, in *absolute* alcohol (one to four hours) Xylol, fifteen minutes. Hard paraffin, twenty minutes

2. Cut sections *as thin as possible.*

3. Stain with the undiluted eosin solution ten to fifteen minutes. Blot off excess.

4 Stain with the diluted methylene blue solution fifteen to twenty minutes Pour off excess.

5. Pass rapidly through 70 per cent alcohol Place at once in water.

6. Treat with one in four hundred acetic acid momentarily, and place at once in distilled water. Examine with a low power, and when the nuclei appear bright red, and the section contains but little blue, blot off excess of water.

7. Allow to dry on the slide

8. Examine by placing oil on the section without a coverglass.

B.—LEISHMAN, before staining, places some fresh blood serum on the section for five minutes. Stain with LEISHMAN'S stain, using two or three lots of fresh stain for one or two hours. Differentiate alternately with two solutions, 1 in 1,500 acetic acid to remove excess of blue, and 1 in 7,000 caustic soda to remove excess of eosin. Watch effect with a low power. Dehydrate rapidly with alcohol. Xylol. Balsam.

Chapter VI

THE MALARIAL PARASITE

Life History

Among the groups into which the protozoa are divided we find such well-known classes as the Sarkodina, *e.g.*, Amoeba Coli, the Mastigophora, possessing flagella, *e.g.*, Trypanosomes, and the Sporozoa. It is these last that chiefly concern us. The Sporozoa include such orders as the Gregarines (*e.g.*, monocystis in the testes of the earth-worm) and the Haemosporidia (which include the malaria parasites of man, and blood parasites of birds, etc.) There is a close relationship between the coccidia and the haemosporidia (malaria parasite), the delevopmental cycles of the two being almost identical. The developmental cycle in the blood (the febrile cycle) of the malaria parasites was first demonstrated by Golgi, the further cycle in the mosquito by Ross. The cycle of Golgi is the asexual cycle, producing auto-infection of the patient ; the cycle of Ross is the sexual cycle, producing a new infection in a healthy subject.

The sexual cycle, it has been thought, commences in the blood when the conditions are unfavourable for the continuance of the asexual cycle, and, in fact, has been taken as a sign that the

patient has already developed an immunity against the fever-producing young parasites (spores). Thus it is well known that in malignant tertian the sexual forms, gametes or crescents, first appear about a week to ten days after the first febrile attack. If this view be true, then it follows that the gametes develop from forms already present in the system, viz., the asexual forms, and possibly the divergence into sexual forms takes place from the youngest form of the parasite, *i.e.*, the spore. But it is possible that the divergence takes place at a stage previous to the youngest form of parasite, *i.e.*, at a stage immediately succeeding the entry of sporozoits into the blood, so that we have from the first asexual and sexual forms present. Sexual development has been supposed to proceed mainly in the internal organs, *e.g.*, bone marrow; but it is being gradually recognized that young forms of gametes are also found in the circulation; the characters of these have already been noted (p. 35). Let us suppose, however, that we are now dealing with fully developed gametes in the blood. We shall proceed to describe the further changes undergone in the mosquito. The male cell is, as we have seen, called the mikrogametocyte; the female cell, the makrogamete. These we can distinguish in the blood. Further flagellation can be observed, *i.e.*, the protrusion of so-called 'flagella,' *i.e.*, mikrogametes or spermatazoa. These 'flagella' break off and fertilize the female cell, the makrogamete, a process which has been seen in halteridium of birds, but only once in man.

This fertilized female cell or egg is known as a Zygote, though it is more convenient to reserve this term for a little later stage, and to call this stage

the Vermiculus or Ookinet (Figs. 6 and 9). Both these terms are suitable ones, for the first describes the fact that the fertilized female becomes worm-like in shape, and the second that the fertilized

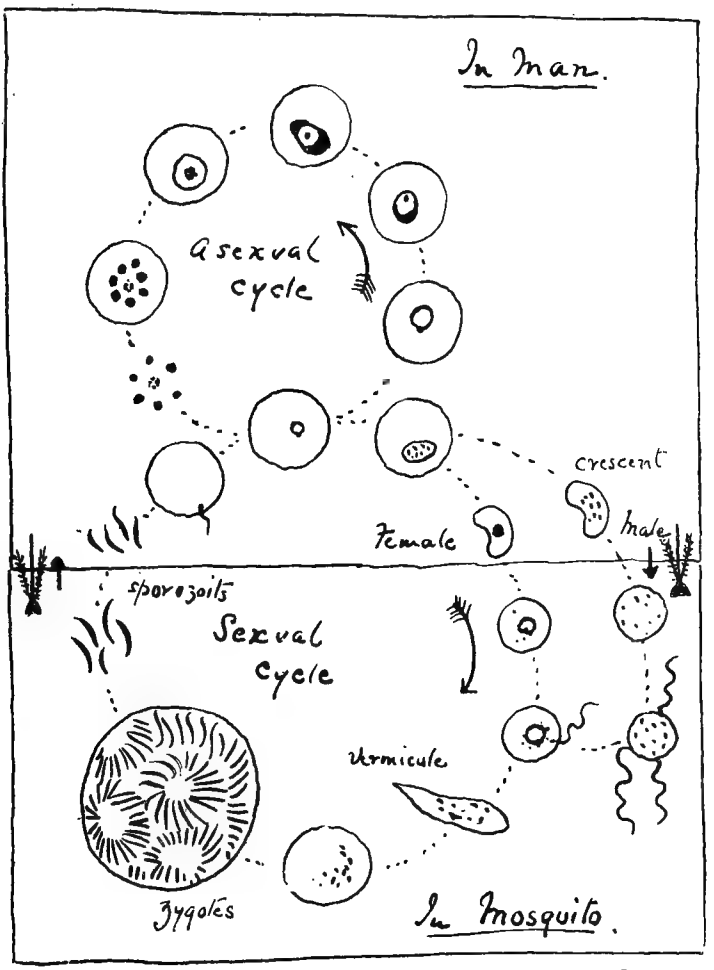


Fig. 9. Life Cycle of the Malaria Parasite in Man and the Mosquito.

egg moves. The vermiculus stage can be seen on the slide in the case of halteridium, but in the case of malaria parasites, only by taking the blood from the stomach of the mosquito after a suitable lapse of time. The vermiculus now finds its way through the epithelium of the stomach, and then lies in the external muscular layers as a spherical or ovoid body, the zygote. A kind of capsule is formed around it by these tissues, and so at this stage it is also called the *Oocyst*. Growth proceeds, and signs of division into several masses appear in the protoplasm. These masses are termed sporoblasts. Then we reach the stage of large zygote (with sporoblasts), and by this time the masses of the sporoblast have undergone division into a number of fine curved thread-like bodies, the sporozoits, so that eventually the large cyst is almost entirely filled with sporozoits. The capsule of the cyst eventually ruptures, and the sporozoits pass from the tissues of the stomach to the thorax, being found at first amidst the muscles, but eventually all collected in the salivary glands. From here they are injected into the blood by the mosquito, and they then attach themselves to and penetrate the red cells (as has been actually observed under the microscope by SCHAUDINN), producing a new infection.

We may briefly summarize these various steps :—

1. Mikrogametocyte, and makrogamete in blood.

2. Development of mikrogametes = flagellation, on the slide and in nature in stomach of an Anopheline.

3. Fertilization of the makrogamete = ovum or copula, on the slide and in nature in mosquito.

4. Vermiculus or ookinet. Only in mosquito stomach.

5. Zygote or oocyst. In stomach wall.

6. Medium or large zygote with sporoblasts.

7. Sporozoits. In salivary glands.

The sexual cycle is known also as sporogony or amphigony, while the asexual cycle is known as schizogony or monogony. These two cycles and their relation to one another are shewn in the figure (Fig. 9).

Further, there is a certain amount of evidence to shew that a gamete (♀) in the blood can undergo a kind of retrogressive development, and give rise by parthenogenesis to young parasites (*i.e.*, schizonts). If this is so, it would explain the supposed function of old attributed to crescents (gametes) of producing relapses.

Chapter VII

MOSQUITOES

Mosquitoes belong to the order of Diptera, or true flies, which are characterized by :—

1. A single pair of membranous wings.
2. Suctorial mouth.
3. Complete metamorphosis.

In all mosquitoes, except the genera, Corethra and Mochlonyx, there is a long piercing proboscis, which is characteristic of the *Culicidae.* Mosquitoes usually are about five mm. in length, but certain species, *e.g.*, Megarhinus, are much larger.

Flies which may be mistaken for mosquitoes are :—

1. *Chironomidae* (Midges), *e.g. Chironomus.*— Costal vein does not extend beyond the tip of the wing. Antennae of male densely feathered. Legs

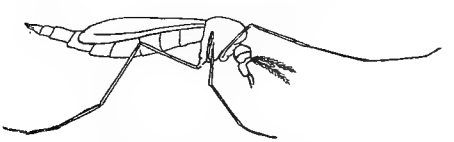


Fig. 10. *Chironomus.*

long and slender (*Vide* Appendix). They do not possess the characteristic proboscis of mosquitoes. The veins of their wings are more complex, and

are *quite devoid of scales.* The absence of scales upon the veins of the wings at once distinguishes these from true mosquitoes (Fig. 10).

Enormous numbers of Chironomidae are found near water, especially sedgy rivers and swamps. They are attracted by light, and are constantly seen around lamps and candles, a position in which true mosquitoes are scarcely ever found.

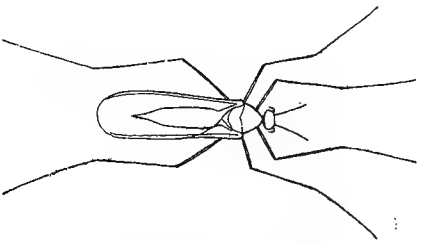


Fig. 11. *Trichocera.*

2. *Tipulidae* (Daddy long-legs).—Some small Tipulidae often possess a considerable superficial resemblance to mosquitoes, as, for example, the winter gnat (Trichocera). When at rest their bodies lie parallel with the surface, and upon it. They have no distinct proboscis (Fig. 11).

3. *Cecidomyidae*, or gall midges.—These have a simple wing venation, and there are no forked cells. In most species the wings and bodies are hairy, not scaled.

4. *Rhyphidae.*—Wings have a discal cell (below the anterior cross vein). They may have spotted wings.

5. *Simulidae*, or sand-flies (sometimes also called midges).—These are minute flies which suck blood voraciously. They have a short and

stout proboscis. The salivary glands are very large in proportion to the size of the fly, and the bite is as severe as that of a mosquito. The males are harmless (Fig. 12).

The larvae of the Simulidae are aquatic, cylindrical in shape, and live in the stems of water plants. The imago hatches beneath the water.

6. *Psychodidae* (or owl midges), *e.g. Phlebotomus*.—Small fluffy-looking flies which suck blood readily. They are most readily detected after feeding, when the abdomen is swollen with blood. They have very hairy wings and body, and a short powerful proboscis (Fig. 12). The larvae are aquatic but can also exist in air.

Fig. 12. Sand Fly (left). Owl Midge (right).

Life History of the Mosquito

In common with all other insects shewing complete metamorphosis, the mosquito passes through four stages :—

The egg.
The larva.
The nymph.
The imago.

The Imago.—The imago is the well-known winged insect. The emergence of the imago may be seen on the surface of almost any collection of foul water. Shortly after hatching, the insect may be seen resting quietly upon the surface of the water, and does not fly away when disturbed, or only very feebly. For some considerable time after hatching (twenty-four hours) the insects refuse to feed.

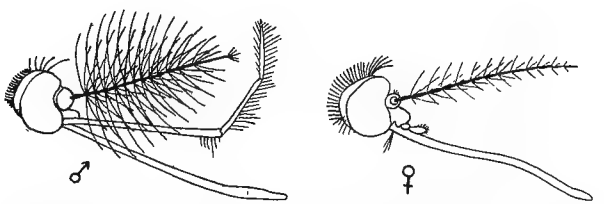


Fig. 13. *Heads of Male* (♂) *and Female* (♀) *Culex.*

In the imago there are marked differences between the male and the female insect.

The Male.—In the male the antennae are markedly plumose. The palps also are long and hairy. The effect is to make the 'head' of the male mosquito very conspicuous (Fig. 13).

The male mosquito, with the exception of certain species, does not feed upon blood, and the proboscis is only used to suck in vegetable juices. The male of *Stegomyia* mosquitoes, however, is said to suck blood like the female.

The Female.—In the female the antennae are inconspicuous and have only short lateral hairs. The palps are also less conspicuous than in the male (Fig. 13).

The female feeds upon blood, and is frequently seen with the stomach distended with blood, more or less digested.

The female is also seen with the abdomen more or less swollen, with the greatly enlarged ovaries, which give a whitish and opaque colour to the mosquito, and often make the insect much more conspicuous in its flight than it otherwise would be.

The commonest species of mosquitoes belong largely to the following genera or closely related forms :—

1. *Anophelina* (sub-family).
2. *Culex.*
3. *Stegomyia.*
4. *Taeniorhynchus* and *Mansonia.*
5. *Uranotaenia.*

The sub-family, *Anophelina*, is in many ways the most distinct of these groups. Not only are the adult insects highly characteristic in appearance, but the ovum and larva are quite unlike those of any other genus. One, indeed, can recognize the *Anophelinae* at a glance merely by their characteristic general appearance, once the peculiarities of this genus are known.

The points which serve to distinguish the *Anophelinae* from other groups of mosquitoes are:—

1. The character upon which the sub-family is founded, viz., the relative length of the palps and proboscis. In both the sub-families, *Culicina* and *Anophelina*, the palps in the male are long plumose structures, as long or longer than the proboscis. In the female of the *Culicinae*, however, the palps are quite short and insignificant structures, whereas in *the female Anophelinae* these are scaled

and as long as the proboscis. An examination of the female proboscis will at once determine whether an insect belongs to the sub-family, *Anophelina*, or one of the other genera (Fig. 14).

2. The wings in nearly all species of *Anophelines* are 'spotted.' The spots can be seen with the naked eye. By the use of the low power of the microscope or an ordinary lens, these spots are seen to be due to the presence of areas of dark scales upon the wing veins, elsewhere covered with light scales.

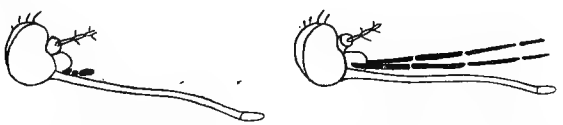


Fig. 14. *Shewing distinction between palpi of Female Anopheline (right) and a Culicine (left).*

There are a few members of the *Anopheline* group which, however, have not spotted wings (*e.g.*, *A. bifurcatus*, and the Indian *A. immaculatus*). Also there are other flies than *Anophelines* which have spots (*e.g.*, *Rhyphus*, *C. mimeticus* (costal spots), the genera *Theobaldia* and *Lutzia*). Nevertheless, as a general practical rule, mosquitoes with *spotted wings* are *Anophelines*.

3. The angle which the proboscis makes with the rest of the body is very different in *Anophelines* from that of other mosquitoes. In *Culex*, *Taeniorhynchus*, or *Stegomyia*, the proboscis forms a distinct angle with the line of the body (*Taeniorhynchus*, forty-five degrees). In the case of *Anophelines*, the proboscis continues on in the line of the body (*P. stephensi*, fifteen degrees). The

result is to give to an *Anopheline* mosquito a peculiar and very characteristic awl-like appearance (Fig. 15).

4. The attitude adopted by *Anophelines* is, as a rule, characteristic. When an *Anopheline* rests upon a wall, its body projects so as to form a distinct angle with it. In some cases the angle assumed is almost a right-angle. In the case of almost all other mosquitoes, the body is held either parallel with the wall, or what is more frequent, the tail approaches the wall, giving the

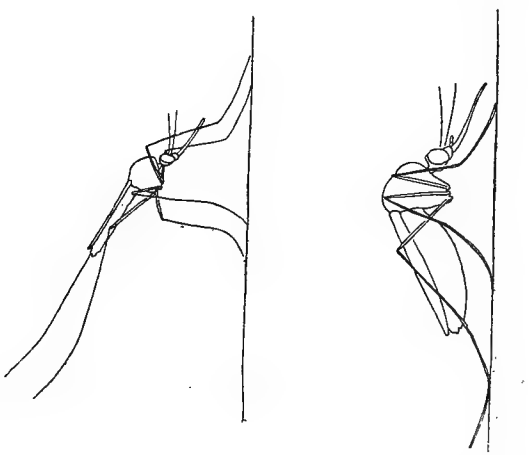


Fig. 15. *Shewing distinction between resting attitude of an Anopheline (left) and Taeniorhynchus (right).*

insect a 'hunchbacked' appearance. This difference is readily seen by any careful observer, and is a practical and useful distinction. A characteristic of an *Anopheline* is that it rests by preference on the first two pairs of legs only, and keeps the

last pair stretched out *stiff* and *straight*, or they slowly oscillate to and fro. Many mosquitoes wave the hind legs, notably *Stegomyia*, but they are held with the tarsi curved backwards.

The exact attitude adopted depends upon the species and the situation, whether a vertical or horizontal surface on which the *Anopheline* is resting. One very common species *(M. culicifacies)* at least, when sitting on a wall, looks exactly like a small brown *Culex*, since it holds its body parallel with the wall as a *Culex* does.

Culex.—Mosquitoes of the genus *Culex* are many of them brown mosquitoes of sober hue, *e.g.*, the common house *Culex*, *C. fatigans*, which is uniformly brown without markings. The genus, however, contains a very large number of species. In *Culex* mosquitoes the attitude when resting is 'hunchback.'

Stegomyia.—The genus *Stegomyia* is of the greatest interest and importance, since it is this form which is concerned in the transmission of yellow fever (*Stegomyia fasciata*).

These mosquitoes are generally black and white, with banded legs and abdomen, and spots on the thorax. They are found in houses, and are most troublesome mosquitoes from their habit of feeding in the day, and their great alertness and persistence. *Stegomyia* are also very common in woods and forests.

Capture of Mosquitoes and Flies

1. Place a lamp upon a sheet of white paper, and note the insects which are attracted by the light. Note insects belonging to the

orders Lepidoptera (moths), Hemiptera (aphides, green flies, etc.), Heteroptera (plant bugs), Neuroptera (caddis flies, stone flies, white ants). Pick out any mosquito-like flies. They will probably belong to the Chironomidae. Note the absence of proboscis, the delicate transparent structure. Note the plumed head of the male (as in the true mosquito) and the less conspicuous antennae of the female. Examine the wings under a strong lens or a low power of the microscope, and note that the wing veins are bare and do not carry any scales. Note that true mosquitoes are not seen around the lamp.

2. Examine, with a light, some wall which has been only dimly illuminated by the lamp, *i.e.*, some wall at the distance of several yards, and note true mosquitoes resting upon this. Capture several of these by placing a tumbler over them, and kill them by puffing in a little tobacco smoke. Observe that they have a distinct proboscis. Observe the 'plumed' male and the female without plumes. Examine the wings under a strong lens or low power objective, and note the scales attached to the wing-veins.

The specimens caught will probably be specimens of *Culex*. If near a swamp or jungly place there may be *Taeniorhynchus*, *Mansonia*, and possibly *Anophelines*. Observe the hunchback attitude in the case of most of the mosquitoes caught. If an *Anopheline* should by chance be caught, note the striking difference in the general appearance, the attitude, and the spots on the wings.

3. Observe in stuffy, furnished rooms, offices, etc., the presence of mosquitoes feeding actively

during the day. Capture some of these. They will probably belong to the genus *Stegomyia*. Note their extreme alertness. Observe that they are black with white bands. Note the habit of waving the hind legs, and that the tarsi of these are kept curved. Ascertain whether the males feed upon blood.

4. Examine stables, huts, outhouses in the early morning.

LITERATURE

The Cambridge Natural History. 'Insects, Part II.' A most useful book for an introductory knowledge of a variety of winged life in the tropics and elsewhere.

Chapter VIII

THE OVUM

Ova are minute bodies one mm. or less in length. When first laid they are white in colour, but become rapidly brown or black. They occur on the surface of water, and if submerged do not

Fig. 16. *Eggs of Stegomyia*

hatch out. Mosquito eggs may be laid by the edge of water, or on floating objects, or upon the water. In the last case, they have some device to

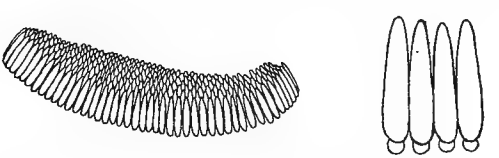


Fig. 17. *Egg Raft and Eggs of Culex*

ensure that they shall float, and not sink and be destroyed. In the case of *Anophelines* and some species of *Stegomyia* (Fig. 16), each ovum lies

separately upon the water, and has air cells which keep it afloat. In the case of *Culex* and *Taeniorhynchus*, hundreds of eggs are cemented together to form rafts, each egg lying perpendicularly, with its larger end pointing downwards. In *Culex*, the

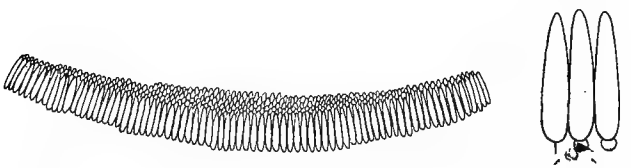


Fig. 18. *Egg Raft and Eggs of Taeniorhynchus*

egg-rafts are broad and roughly oval in shape (Fig. 17). In *Taeniorhynchus*, the egg-raft is extraordinarily elongated, resembling, in shape, a racing skiff (Fig. 18).

The Examination of Ova

Culex.—Examine the surface of some semi-putrid water for egg-rafts of *Culex*. Egg-rafts can almost always be found on the surface of water containing macerating leaves, fruit, etc. They are bodies of a blackish-brown colour, and are readily wafted about by the wind.

1. Note that the raft is boat-shaped, measuring one-fifth to one-third inch in length, and consists of two hundred to four hundred eggs.

2. Note that the separate ova are smooth elongated bodies, about 0·7 to 0·8 mm. in length. Note that there are no floats or other markings as in the case of *Anopheline* ova.

3. Note that one end of the egg is thicker and blunter than the other, and that to the thicker

end is attached a clear transparent globular body (the micropilar apparatus). Note that this body is readily detached, often leaving a spike-like process projecting from the thicker end of the ovum.

4. Make as many observations as possible upon the egg-rafts, *e.g.*, time necessary for hatching of larvae, amount of desiccation they will withstand.

The egg stage in *S. fasciata* lasts twelve to twenty-four hours; in *C. jamaicensis*, twelve hours; in *C. sollicitans*, twelve hours (TAYLOR). The eggs of *Culicidae* have but little resistance to desiccation, but those of *S. fasciata* will hatch after being kept 'dry' for three months.

Anophelinae.—The ova of *Anophelinae* are difficult to detect in nature, but may be seen by the aid of a lens on the margins of small pools, where larvae abound. They are about 0·7 to 1·0 mm. long.

Examination of Anopheline Ova:—

1. Confine some female *Anopheles* as described on p. 120. Endeavour to choose those in which the ovaries are nearly mature (p. 97). Fifty to one hundred and fifty eggs are laid. Remove the piece of paper upon which the ova have been deposited and place this upon a slide. Examine with a low power in strong daylight, and the mirror turned off.

2. Observe the remarkable resemblance of the ova to little boats, and the presence of the two beautiful oval air cells placed upon either side, acting as floats. (These are absent only in one species as yet described, viz., *M. turkhudi*). Observe also the presence of a white frill or a mere ribbed rim around what would be the gunwale of the boat (Fig. 54).

3. Observe that one end of the ovum is always stouter than the other. The stout end contains the head of the embryo, and is the end from which the young larva escapes. Note also that when *Anopheline* eggs are seen at the side of vessels drawn up by capillarity the thick end is at the bottom. Examine the surface of the water remaining in the hollow stopper or receptacle, and observe that the ova of *Anophelines* are laid singly without any cement substance, and float singly or touching one another on the water.

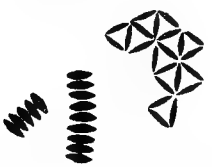


Fig. 19. *Patterns formed by Eggs of Anophelines*

4. Observe star-shaped patterns formed by some species, or the arrangement in parallel groups assumed by the ova of others (Fig. 19). Note that this arrangement is dependent on physical causes (shape of the egg, etc.), and not on the fact that the eggs are laid in such positions. This is readily done by stirring up a number of *Anopheline* ova on water, and noting how they tend to form groups in triangles and star shapes (*Vide* p. 221).

5. Ascertain that *Anopheline* ova, when first laid, are white, but rapidly darken and become black. Observe that *Anopheline* ova are very often laid in heaped-up masses, which eventually become dispersed by waves, etc. Observe that the eggs then form patterns.

6. Place some half-dried mud in a flat dish, and put this inside a piece of mosquito netting in which some *Anophelines* with ripe ovaries are placed. Observe that ova are laid upon the mud.

7. Preserve the mud for forty-eight hours, preventing it from becoming completely dry.

At the end of forty-eight hours or more, remove a few ova to a dry slide, and place under a low power. Allow a drop of water to flow on to the ova. Observe the escape, within a minute or so, of the young larvae, and the fact that a cap-like piece of the egg-shell is pushed off.

8. Observe that *Anophelines* kept in a dry test tube will occasionally lay their eggs on the side of the tube.

9. Note the time when the eggs were laid and the time at which the larvae emerge. This depends greatly on the temperature. It may take two to three days. *Ce. argyrotarsis*, one-and-a-half days (TAYLOR).

10. Remove *Anopheline* ova on paper and allow them to dry, and note that after two or three days (t., 86°-96° F.) at the most, they will not hatch when carefully placed on water.

Stegomyia.—Confine some gravid females of *Stegomyia* mosquitoes.

1. Note that in *S. fasciata* the eggs are laid singly, and much resemble, at first sight, the ova of *Anophelines*. Note that in others the eggs are laid in rafts (*S. notoscripta*).

2. Note that they are irregularly oval, thicker at one end than the other, and have a corrugated surface in which are entangled numerous minute air bubbles.

3. Examine the surface of water left exposed for several days in a tumbler, etc. Note, if *Stegomyia* mosquitoes have ovi-posited, the presence of eggs occurring singly or in parallel groups. Note that the ova are larger than those of *Anophelines*, and that they hatch into *Culex*-like larvae (see *Stegomyia* larvae, p. 85).

Taeniorhynchus.—Examine natural waters, especially small pools with a dense growth of alga, swamp pools, irrigated land, etc., for the egg-rafts of *Taeniorhynchus.*

1. Observe the extreme length and narrowness of the rafts. Note also how small a portion of the raft is submerged.

2. Observe that the ova are arranged as in *Culex* rafts with the thicker end downwards, and that they are smooth and have a micropilar apparatus.

3. Endeavour to obtain the ova of known species of *Taeniorhynchus*, by confining gravid females. Note the shape of the rafts.

Mansonia.—Observe that the eggs have a curious snout-like projection, and that they are laid singly.

Psorophora.—The eggs are large, two mm. long. They occur in patterns like those of *Anophelines.* The eggs are covered with minute prickly scales.

Taylor, in Havana, has made many observations on eggs. He gives the following:—*C. pipiens*, raft 200-400 eggs; egg 0·9 by 0·16 mm. *C. nigritulus*, raft 200-300 eggs; egg 0·6 by 0·14 mm. *U. lowii.* raft 50-75 eggs. *S. fasciata.* Eggs laid singly, about fifty in number.

Chapter IX

THE LARVA AND NYMPH

The Larva

The larvae of mosquitoes, more especially of *Culex*, are well-known objects. They can be seen by holding up to the light almost any specimen of water that has been left undisturbed for some days, but especially water which contains macerating leaves.

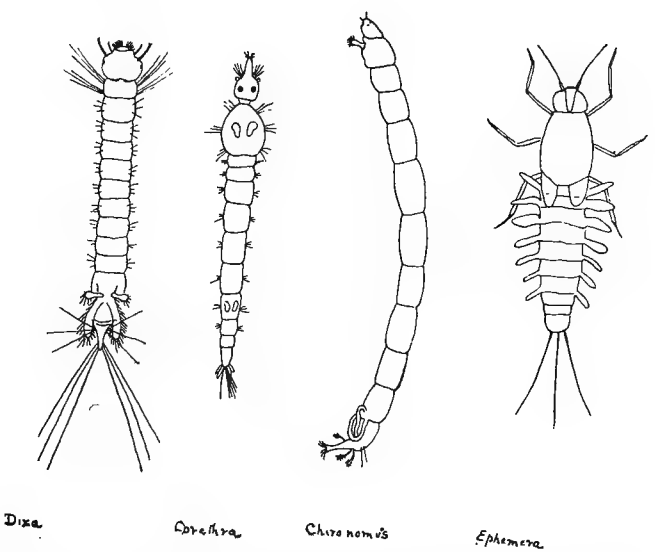


Fig. 20. *Larvae that may be mistaken for Mosquito Larvae*

Larvae which may be mistaken for those of mosquitoes are :

1. *Chironomidae.*—The larva of Chironomus is a red worm-like creature (blood worms). On the prothorax it has a pair of feet armed with hooks (Fig. 20).

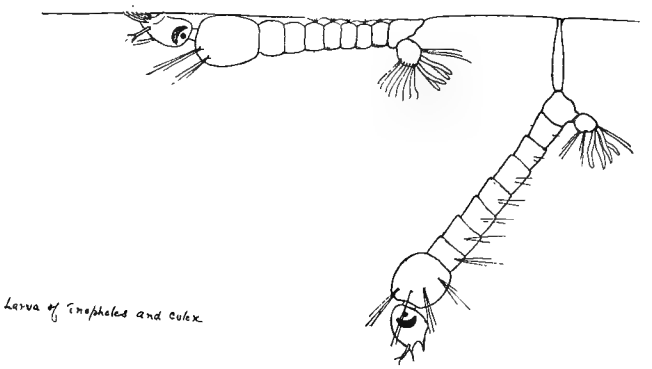


Fig. 21. *Larvae of an Anopheline (left) and Culex (right)*

2. *Ephemeridae* (May-flies).—The larvae of certain small Ephemridae may, at first glance, be mistaken for mosquito larvae. There is no real resemblance, and the triradiate tail of the ephemera larva and the tracheal gills distinguish it (Fig. 20).

3. *Dixidae.*—The larva of Dixa rather closely resembles the larva of *Anophelines*, though not other mosquito larvae (Fig. 20). Ventrally it has pseudo pods on the fourth and fifth segments.

Examination of the Larva

Culex (Fig. 21).—Obtain some *Culex* larvae from any source and place in a glass vessel.

1. Observe the hanging attitude of the larva. Note the angle it makes with the surface of the water, and how this varies in different species. Note, if the larva of *C. concolor* is being examined, that the position is nearly horizontal.

2. Observe the large head, the prominent eyes and projecting antennae.

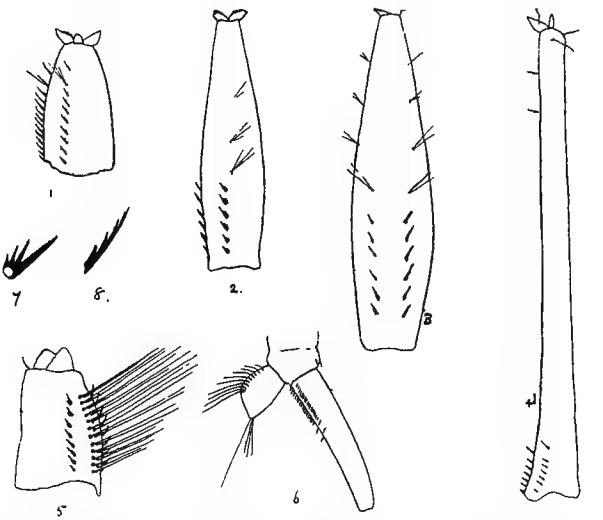


Fig. 22. Respiratory Syphons of Larvae.
(1) *Stegomyia*; (2) *Culex*; (3) *Culex with Large Syphon Tube*; (4) *Taeniorhynchus*; (5) *Cannibal Larva (? C. concolor)*; (6) *Enormous Syphon Tube (one-quarter scale of others, genus undetermined)*; (7) *Spine on Tube seen on the flat*; (8) *Spine seen sideways*

3. Note the long respiratory syphon arising from the eighth abdominal segment.

Place a half-grown larva under a coverglass and examine under one-third inch objective.

1. Make an accurate drawing of the antennae.

2. Carefully observe the length and thickness of the respiratory syphon.

3. Note the absence of palmate hairs.

4. Determine whether the larvae examined are *Culex*, *Stegomyia*, *Taeniorhynchus*, etc.

The characteristics of the larvae of *Culex* appear to be :—

(i) Antennae not markedly curved, or sickle-shaped (Fig. 23).

(ii) The respiratory syphon long, but not extremely slender as in *Taeniorhynchus* (Fig. 22).

5. Determine the relative length and breadth of the various larvae :—

In *Culex*, the length is to the breadth as 4 : 1.
In *Taeniorhynchus* ,, ,, ,, 9-12 : 1.
In *Stegomyia* ,, ,, ,, 2 : 1.

In any particular species there will be deviations from these, but broadly speaking, these differences hold good.

The Larvae of Anophelines

To collect the larvae.—Necessary apparatus :—

1. An ordinary spoon, dessert or table-spoon size is most convenient, but a tea-spoon does well.

2. A white enamel tin or large cup, or an ordinary bath tin.

3. Bottles, specimen tubes, paper, pencils, etc.

Procedure.

1. *By inspection.*—Inspect closely the surface of any small puddles that have been in existence some time. Examine especially small rock puddles,

small shallow pools in 'nallahs' and river-beds, in the dry season.

Examine especially the edges where larvae are fond of resting, with the head facing the open water and the tail touching the bank. Note also how larvae tend to cling to floating twigs, etc. If no larvae are seen, stir up vigorously the bottom of the pool with the spoon. This will dislodge larvae from the edges, etc.

Examine the surface of the pool again and observe the larvae now plainly visible against the muddy water. Wait a few minutes for the appearance of the larger larvae, which remain below longer than the younger forms. Examine carefully for nymphae, which easily escape detection.

Dip out the larvae and nymphae with the spoon as they appear. The thinner the edge of the spoon the less disturbance is caused, and the more readily are larvae removed.

Place the larvae as caught in bottles, tubes, etc. If desirous of labelling these, write in pencil on the paper, and put into the bottle.

2. *By Dipping.*—Choose any water with grassy or weedy edges, *eg.*, the edges of rivers, streams, ditches, lake margins, swamps, etc.

With the least possible disturbance, dip out water from the most sheltered positions, and as close to the vegetation as possible, bringing up water and weeds in the can. Allow the specimen of water to remain a few seconds, and remove any larvae or nymphae as they appear on the top with the spoon.

Examination of Larvae

Anopheline larvae cannot be mistaken for any other mosquito larvae (Fig. 21).

1. When undisturbed they lie flat along the top of the water, and on every segment certain hairs (palmate hairs) actually indent the surface film. Observe that when viewed in certain lights from one side these identations can be plainly seen. The appearance may even be as though the dorsum of the larvae projected from the water. This, however, is not the case.

This appearance is diagnostic of the *Anopheline* larva. One species *(M. turkhudi)* does not, however, rest in this position, but after rising to the surface in a horizontal position slowly sinks until the tail only touches the surface.

Anopheline larvae, which are about to turn into nymphae, also sometimes tend to sink, so that the head is directed obliquely downwards (often seen in *M. rossii*).

One species of *Culex*, at least *(C. concolor)*, adopts a nearly horizontal attitude. The line of indentations of the surface film mentioned above is not, however, seen.

2. When disturbed, *Anopheline* larvae dart into the water, or what is very characteristic, if not greatly disturbed, they pass by a series of wriggling jerks along the surface of the water.

When moving up towards the surface, an *Anopheline* moves in a much more irregular and jerky manner than a *Culex* larva. This is well demonstrated when living larvae in a cell are projected upon the screen of the lantern. The *Culex* larvae are seen rising by a series of regular

lateral sweeps, whereas *Anopheline* larvae exhibit only irregular jerks.

3. When kept in a vessel, *Anopheline* larvae tend to collect round the edges with their heads pointing towards the centre. In this position the body of the larvae is often bent as it lies along the curved surface of the capillarity film at the edge. *Culex* larvae and the larvae of *M. turkhudi* frequently do not go to the side, but remain in the more central portions of the fluid.

4. *Anopheline* larvae, when full grown, possess very small heads in proportion to the size of the larvae (about eight mm. in length). In most of the *Culicidae* the head is very large, with very prominent and large antennae.

5. *Anopheline* larvae have no 'spiracle tube,' the tracheae open into a pit on the dorsum of the eighth abdominal segment. In *Stegomyia*, the spiracle tube is short and thick (Fig. 22), in *Taeniorhynchus* (Fig. 22) very long and slender, but in *Anopheline* larvae alone is this structure absent (Fig. 21).

Procure a considerable number of *Anopheline* larvae, and ascertain the following points :—

1. *The Moulting of Anophelines.*—Note that as *Anopheline* larvae grow in size they cast their skins. Remove a cast skin by floating it upon a slide. Note the perfect nature of the 'skin,' and how all the chitinous structures are represented, even air tubes. Dry the specimen upon the slide and mount in a drop of balsam. Observe how beautifully certain hairs resembling fan-palm leaves are shewn (palmate hairs). (*Vide* p. 235).

2. *The Method of Feeding of Anophelines.*—Observe with a lens the action of the feeding

brushes and the currents they produce on the surface of the water. Note the rotation of the head so that, whilst feeding, the ventral surface of the head is uppermost.

3. *The Food of Larvae.*—Tear a larva to pieces with a needle and remove a small portion of the dark central mass of food material filling the straight alimentary canal. Place in a drop of clean water and crush under a coverglass. Note what organisms form the chief bulk of the food. Note the presence of sand grains—unicellular plants and animals—short lengths of alga, diatoms, etc. Also bacilli.

Determine the common foods of several species of *Anophelines.*

4. *Desiccation of Larvae.*—Celli and Casagrandi have found that *Anopheline* larvae can only resist desiccation at 20° C. for two days, at 35° C. for one day, and 40° C. for two minutes only. Larvae of *Anophelines* stranded on moist mud will live as long as four days, but in the tropics as soon as the mud loses its glistening surface they die.

Culicine Larvae.—The larvae of the *Culicidae*, with the exception of those of *Anophelines* and possibly some other genera, are superficially much alike. The conspicuous hairs and spines, and even the complicated terminal segment, are very similar in the different genera. There are, however, marked differences in some features on closer examination. These differences are mainly to be found in the syphon tube, the antennae, and mental plate, but to a less extent in other structures.

Note differences in naked eye appearance; note the long worm-like *Stegomyia* larva and its wriggling mode of progression; note the transparent and spiny appearance of some larvae (notably *Taeniorhynchus*); note that some larvae adopt a nearly horizontal attitude (*C. concolor* and others), others a vertical attitude (*Stegomyia*), whilst the majority form a small angle with the vertical. Examine larvae under a low objective. Note the head with eyes, large feeding brushes, antennae and various hairs; note the large bunches of hairs arising from the thorax and abdominal segments; note the last segment bearing four large clear papillae and two systems of hairs; note the penultimate segment which carries the syphon tube and some curious claw-like spines.

Note especially the following :—

(i) The syphon tube.
(ii) The antennae.
(iii) The mouth parts.
(iv) The anal papillae.
(v) The hairs of the thorax and abdomen.

The Syphon Tube.—This is formed of a single cylindrical piece of chitin, and contains the origin of the two main tracheae of the body. Note the small flap-like pieces of chitin forming a closing apparatus at the extreme tip. Measure (by eye-piece micrometer) the length and greatest breadth of the syphon tube; note that in different species, and especially in different genera, the syphon tube varies greatly in its measurement. By dividing the length by the breadth a figure may be obtained which is useful, and may be termed the syphonic index number; note that in *Stegomyia* this number is about two. In *Culex*, four to seven. In

Taeniorhynchus, as much as twelve in some cases. Draw accurately by measurement (eye-piece micrometer) a number of syphon tubes of different *Culex* larvae. Note that marked variations in different species exist (Fig. 22).

Note two rows of spines on the posterior aspect of the syphon tube, starting from the base and extending a variable (in different species) distance up the syphon tube; note that they differ in number and length, etc., in different species. The spines appear serrated or compound, according to the angle they are viewed from, and differences may be supposed to exist which depend upon this fact. In some species (certain carnivorous or cannibal larvae) a large fan of hairs projects posteriorly in the medium line from the syphon tube. In certain species the syphon tube is of enormous size, and may attain to one-third the length of the larva.

The Antennae.—Note in the case of most typical *Culex* larvae that the antennae are large conspicuous objects; note a basal, medial, and terminal portion, and a large bunch of feathered hairs arising at the junction of the two first-named portions; note also large single and stout hairs from the more terminal portion; note spines on the body of antenna.

Examine the Antennae of various Larvae.—Note in some cases that the antennae are more rudimentary (*Stegomyia*, *Anopheles*). In the case of *Stegomyia* (as far as described) they are small and spineless, and possess only a small hair arising from a papilla, which may be single or in three or four branches. Make drawings (using eye-

piece micrometer), and note great variation in different genera and species.

The Mouth Parts.—Note the characters of the claw-like mandibles, and especially the exact character of the triangular mental plate, which forms a conspicuous dark triangular body on the under surface of the head (Fig. 23).

Note that in different species the plate varies in appearance, especially in the size and number of notches in its margin. In some species the plate is like a shark's tooth, in others it is comb-like.

The Anal Papillae.—Note the tracheae ramifying in these, the papillae being possibly gill-like in function. In *Megarhinus*, *Toxorhynchites*, *Mucidus*, *Psorophora*, *Lutzia*, *C. concolor*, and *C. tigripes* they are quite rudimentary.

The Large Body Hairs.—These are long in some larvae, much shorter in others; their arrangement is very similar in the different larvae.

5. *Cannabalism of Larvae.*—Add some large *Culex* larvae to a small bottle containing some small larvae or *Anopheles* larvae. The *Anopheles* larvae or small *Culex* larvae will be devoured by the large forms. *Mucidus sp.*, *C. concolor*, and *Psorophora sp.*, are especially cannabalistic.

6. Observe the occurrence in nature of the two forms, *Culex* and *Anopheles*, also what *Culex* larvae are found living together.

7. *The Enemies of Larvae.*—Add small fish, waterbeetles, and their larvae (Dytiscidae, Hydrophilidae), Libellula larvae, Corysca, Nepa, tadpoles, and other water animals, respectively, to a series of wide-mouthed bottles containing equal numbers of larvae. Note the rate at which they

are devoured, if at all. The carnivorous forms Nepa, Corysca, Libellula rapidly devour larvae. Hydrophilidae beetles, tadpoles, etc., do not destroy larvae. Observe that some species of fish are much more active devourers of larvae than others. Note that weeds often protect larvae from being consumed by small fish.

8. Make experiments with different chemical and other bodies, and note the absence or presence of culicidal power.

(a) Note that chemical bodies in solution kill only with difficulty, as a rule, *e.g.*, corrosive sublimate. Ammonia, however (1 in 4,000 of water) will kill mature larvae according to WADDELL.

(b) Note that oils rapidly kill larvae by blocking the air tubes. Treat larvae by pouring a little olive oil upon the water. Stain with osmic acid and note globules of oil within the air tubes.

9. Add some paraffin oil to a small *Anopheline* pool, observe the presence next morning of dead female mosquitoes that have come to lay their eggs. Observe the effect of paraffin on different kinds of natural water, and the great efficacy in some cases and futility in others.

10. Observe that pools covered with Lemna are very frequently, if not always, free from larvae. The action of the Lemna is said to be mechanical.

EXAMINATION OF OTHER LARVAE

Dixa.—In its movement along the surface of the water the larva of *Dixa* resembles *Anopheline* larvae, and this larva also rests horizontally just beneath the surface film.

In *Dixa* there is no globular thorax, and the whole larva is longer and thinner than an *Anopheline* larva (8 mm.) Moreover, *Dixa* larva only indents the surface film at the head and tail, there being no palmate hairs on any of the segments. *Dixa* larvae move very rapidly, and have a habit of climbing above the surface of the water and resting in a loop with the head and tail downwards. When placed in a specimen tube it climbs up the side and becomes lodged in crevices in the cork.

It is found frequently in running water (Fig. 20).

Mochlonyx Larva.—Note absence of palmate hairs on dorsum and presence of respiratory syphon, but less developed than in a *Culicine.* They are extremely voracious. They lie deep in the water.

Corethra Larva.—These are the so-called 'phantom' larvae. They are extremely transparent, and lie horizontally rather deep in the water. The head is smaller than in *Anophelines.* There is no syphon communicating with the external air. They are extremely voracious. Add some *Corethra* larvae to a tumbler containing *Culex* larvae.

Stegomyia.—The larva of *Stegomyia* is rather longer than that of *Culex.* When disturbed it exhibits a rather lashing movement like that of certain small aquatic worms. When at rest at the surface, the attitude of the body is almost vertical The larva, however, spends a good deal of its time browsing at the bottom of the water, and then lies for the most part horizontally.

The head is small in proportion to the rest of the body, and the thorax is less conspicuously marked off from the abdomen than in *Culex.*

The antennae resemble those of *Anophelines* larvae, more than those of *Culex*. The large branched hair of *Culex* is represented by a short inconspicuous simple hair (or as many as three) projecting from the side of the antennae (Fig. 23).

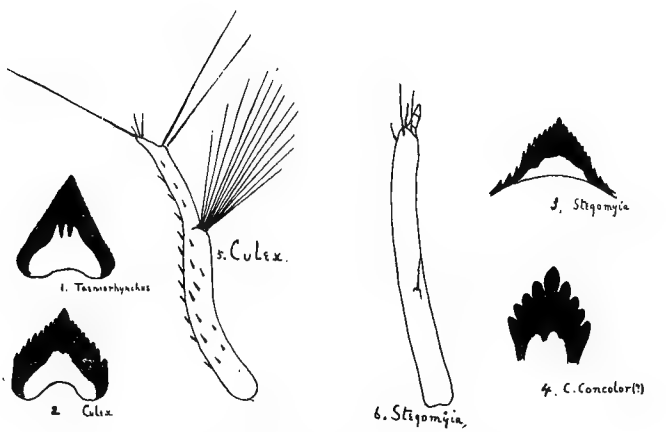


Fig. 23. *Antennae and Mental Plates of Larvae*

The syphon tube is characteristic, being very short and stout (Fig. 22), only twice as long as broad, whereas in *Culex* the syphon tube is four or more times as long as broad (Fig. 22).

1. Examine domestic utensils, disused water pots, and tins containing water. Examine the water which collects in the axils of the banana leaves, collections of water in tree-stumps in the jungle. If larvae are present—

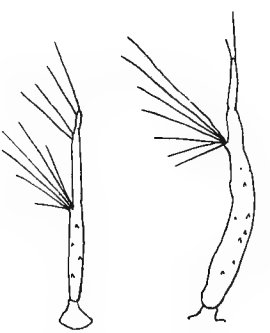
(i) Note the very short and broad spiracle tube and its dark colour. Compare with that of *Culex* and *Taeniorhynchus*.

(ii) The larva is longer and more worm-like than most mosquito larvae. The 'wriggling' motion is also very markedly shewn owing to the length of the body.

Taeniorhynchus.—In natural waters, especially shallow trickling water forming pools, with a dense growth of spirogyra, etc., swamp water and river margins, the larvae of *Taeniorhynchus* will be readily found.

1. Note that the larva lies often embedded in the masses of green spirogyra or other thread-like algae.

2. Note the great transparency of the larva and the frequency with which brilliantly green specimens are found.



Culex Fatigans. Taeniorhynchus
Fig. 23A

Under a low objective note the following, which appear to be characteristic of this genus :—

1. The enormous horn-like and curved antennae (Fig. 23A).

2. The extreme length and slenderness of the syphon tube (Fig. 22).

Psorophora.—The larvae are large, half-an-inch in length. They are extremely cannabalistic.

The Nymph

The nymphae of mosquitoes are extremely characteristic bodies. Wherever a number of fully-developed larvae are found there will generally also be seen numbers of bulbous comma-shaped creatures, having a large globular body (head and thorax) and a small tail, kept more or less tucked in beneath. When disturbed they dart downwards with great speed, but very soon reappear at the surface.

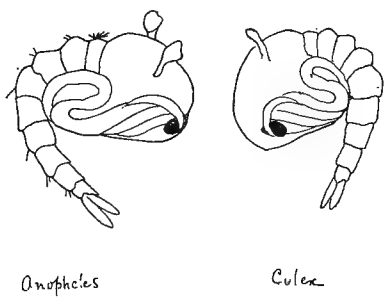


Fig. 24. Nymphae of an Anopheline and Culex

Nymphs are not so easily seen in pools as larvae.

The differences in the nymphae of different genera of the *Culicidae* is not nearly so great as in the case of the larvae.

By keeping under observation a number of nymphae, some will be seen to become less inclined for active movement, and the abdominal segments (tail) may be extended horizontally. Soon after these changes the adult insect emerges through a crack in the chitin of the back of the thorax. The process as seen in *Anopheles* is very fully described by NUTTALL and SHIPLEY.[1]

EXAMINATION OF NYMPHAE

1. Note the effect of tapping the glass vessel and the rapidity with which the nymphs regain the surface.

2. Observe that when first they appear the nymphs are light in colour, but darken very considerably later.

3. Note that just before the hatching of mosquitoes the nymph lies with the tail extended, and that silvery marks may be seen, due to air lying under the chitin.

4. Observe the emergence of the imago.

Examine the nymphs of *Anophelines*, *Culex*, *Taeniorhynchus*, etc., and observe that to the naked eye they are very similar.

1. Note that the nymphae of *Anophelines* lie less vertically in the water than those of *Culex*.

2. Observe that the nymphs of *Anophelines* are more elongated antero-posteriorly and compressed laterally than those of *Culex* and *Taeniorhynchus*.

3. Observe the very large nymphs of some common species of *Taeniorhynchus* and the great length of air tubes which are directed straight forwards in a very characteristic manner.

1. *Journal Hygiene*, vol. 1, part II.

Place nymphae in drops of water on a slide and examine the air syphons. Note—

1. In *Anophelines* the syphons have a square truncated end, and are proportionally much shorter than in *Culex*, and project from about the middle of the thorax (Figs. 24 and 25).

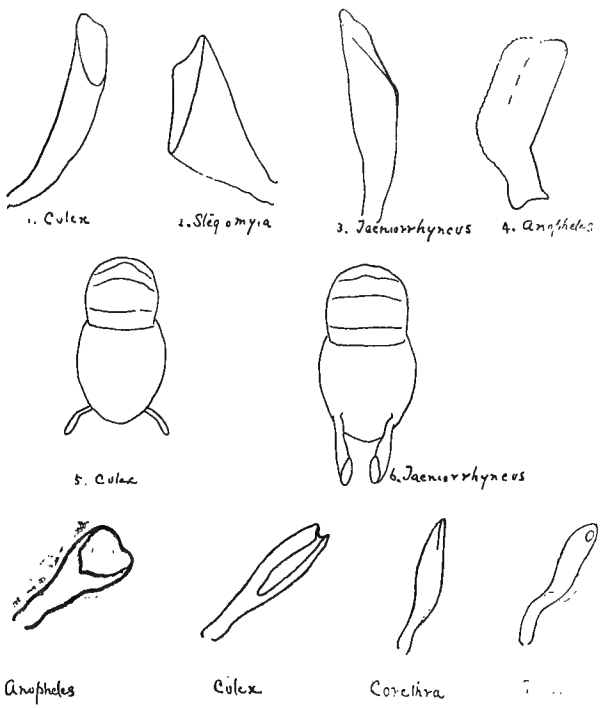


Fig. 25. Nymphal Syphon Tubes

2. In *Culex* the syphons are long and narrow, and have a slit-like opening, and project from the posterior portion of the thorax (Figs. 24 and 25).

3. In *Stegomyia* the syphons are broadly triangular, and are characteristic. Note the marked contrast in appearance to those of *Culex* (Fig. 25A).

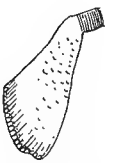


Fig. 25A. Nymphal Syphon Tube of Stegomyia

Examine the nymphs of *Corethra* and *Mochlonyx* when they are encountered.

(*a*) Note in *Corethra* the pointed syphons and the straight tail (Figs. 25 and 26).

(*b*) Note in *Mochlonyx* the *Culex*-like nymph and the thin rounded and pointed syphons (Fig. 25).

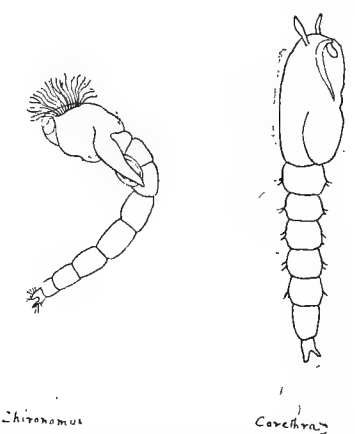


Fig. 26. Nymphs of Chironomus and Corethra

Examine the bottoms of pools of polluted water, and note in the mud the brilliant red nymphs and larvae of *Chironomus* (Fig. 26).

1. Observe that the *Chironomus* nymph has a large globular body (head and thorax) and bears a general resemblance to mosquito nymphs.

2. Note, however, the presence of the conspicuous white feathery gills which form tufts at the side of the head.

3. Note that the *Chironomus* nymph and larvae do not rise to the surface to breathe as do those of mosquitoes.

4. Note the curious rhythmic bending movement of the larvae and nymph of *Chironomus* which, when they are present in numbers, give the mud at the bottom of the pool a curious appearance.

LITERATURE

Miall. *Aquatic Insects.*

Chapter X

TO CAPTURE, PRESERVE ALIVE, AND EXPERIMENTALLY FEED MOSQUITOES

To Capture Anophelines

Necessary Apparatus.—One or two small collecting tubes (Fig. 27), a clean and perfectly dry bottle (whiskey bottle), some cotton wool.

To Detect Anophelines

Choose a suitable native village, *i.e.*, in Africa, any bush village; or in India, any village near a nallah or other source of *Anopheline* larvae.

Determine whether *Anophelines* exist in any of the following situations:—

1. In the dark corners of sheds, cow-houses, or other out-houses.
2. Under the eaves (in darkish parts) of the huts.
3. In the huts themselves, hanging to straws, stalactites of soot, etc., etc.
4. Any other likely situations, *e.g.*, collections of dry grass; in the undergrowth in the bush (capture in this situation is difficult).

Procedure.—If, on inspection, none of the insects can be detected by careful scrutiny (the

most concentrated attention is, as a rule, needed), the thatch should be carefully disturbed with the hand or a short stick.

Observe carefully any insects which fly out, and note where they settle. Choose especially portions of the thatch which are not too dark to prevent one seeing clearly, but are not too much exposed to light.

Train, if possible, one or more intelligent natives to detect the insects and to collect them, as shortly described. It is a good thing, if even only a very few *Anophelines* have been found by a personal inspection, to offer a small reward to any persons in the village who will undertake to collect them. One or two tubes should be left for this purpose.

To Detect Culex

Examine the walls of houses, out-houses, and native huts. Especially examine clothes hung up in native huts. Many specimens of *Culex*, resting in their characteristic hunchback attitude, will probably be detected. Especially on dark clothing, old blankets, inside leather boots or boxes.

Mosquitoes seem especially fond of the smell (?) of leather.

To Detect Taeniorhynchus

These are best caught by sitting, with a light, near a marsh or grassy land. A wall, or tent, or cloth hung up should be at hand, and kept slightly illuminated with a lamp. They may be captured as they settle upon the sheet or upon oneself.

To Capture Mosquitoes

1. Place a collecting tube *very slowly* over the mosquito.

2. Insert a finger underneath, and so rapidly block the tube; or a piece of cardboard or wool may be carefully slipped underneath.

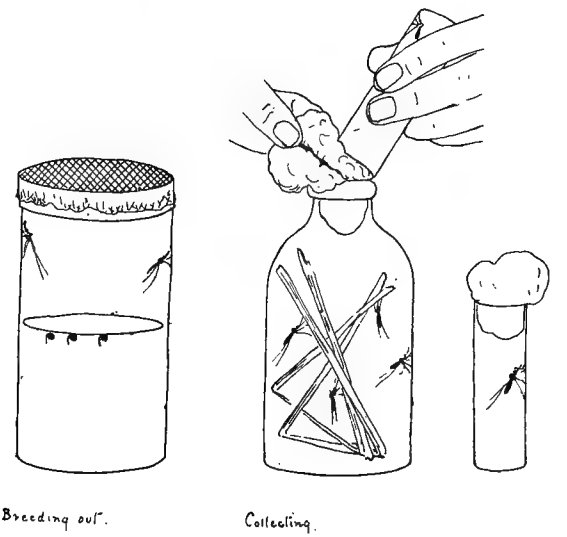


Fig. 27. Method of Collecting and Breeding-out Mosquitoes

3. Place a plug of cotton wool in the mouth of the tube.

4. Transfer to the large bottle by placing the tube over the mouth of the bottle and withdrawing carefully the cotton wool. Keep the bottle closed with a plug of cotton wool.

5. Capture as many specimens as required, and transfer them as caught to the bottle.

A wide-mouth bottle, over which a piece of stout paper has been tied, with a small trap-door cut slightly larger than the tube, may be used. This has the advantage that mosquitoes do not so easily fly out into the tube during the act of transference. It has the disadvantage that the paper tears, and the mosquitoes are more likely to escape through accidental circumstances.

To Breed Out Mosquitoes

(Fig. 27)

Collect a number of full-grown larvae and nymphae of both *Anophelines* and *Culex*.

1. Separate the nymphae from the larvae and place them in a jar or wide-mouthed bottle half-full of water, leaving room for the insects when hatched. Cover the jar with a piece of thick cardboard or a lid, the central portion of which is replaced by mosquito netting.

2. Place the larvae where they will receive plenty of light, but will not be subject to great heat.

3. Remove the nymphae as they are seen at the end of each day.

To Keep Mosquitoes Alive

The length of time mosquitoes remain alive in captivity depends almost entirely upon the suitability of the conditions under which they are kept.

Except for special purposes, mosquitoes (especially *Anophelines*) should *not* be kept in open spaces, *i.e.*, frames covered with mosquito netting.

Procure several 'chutney jars' with hollow

glass stoppers. These can be obtained generally from the native bazaar in India for a few annas (Fig. 28). This form of jar is very convenient, but any other jar will serve.

Cut a piece of thick cardboard so that it will, when forced down into the jar, remain supported on the shoulders of the jar.

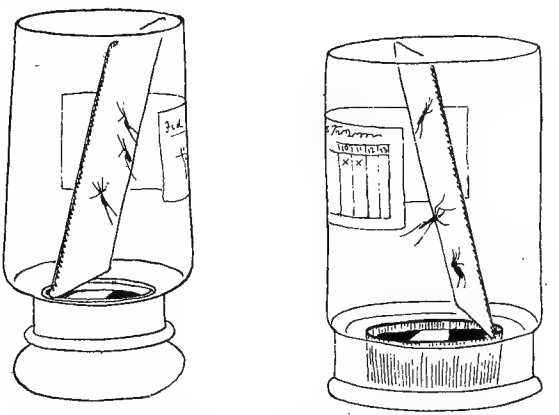


Fig. 28. Method of keeping Mosquitoes alive

Fill the stopper nearly to the brim with water. Cut a thin slice of cork and place it on the surface of the water. Upon the cork place a piece of clean white paper. The paper should not quite occupy the whole of the space in the mouth of the stopper.

1. Invert the chutney jar (prepared as above) over the top of a jar in which some mosquitoes have hatched. Remove the cardboard and gently tap the glass. The mosquitoes will fly upwards into the chutney jar. Place the chutney jar containing mosquitoes upon its stopper prepared as above.

Place the whole, after labelling, *in a dark cupboard* or other convenient place (incubator).

At the end of the first day or so, the males will be found dead upon the piece of paper, and can be removed. On the second night after hatching, most of the insects will feed, and the jar is ready for use.

2. Place the inverted (chutney) jar, prepared with cardboard as above, over a bottle in which *Anophelines*, caught in a village or elsewhere, have been placed. Remove the cotton plug and shake the bottle gently to drive the insects out. Replace jar upon the prepared stopper. Place in a dark spot. Next morning remove the stopper and remove any dead mosquitoes and ova by taking out the piece of paper.

On the second night after the mosquitoes have been collected, the bottle is ready for feeding experiments. On the third day, generally, the mosquitoes have no longer any blood remaining in the mid-gut, and are ready for dissection.

The glands of any mosquitoes that may die before this may of course be dissected, if desired, on the chance of finding sporozoits.

In the use of village-caught *Anophelines*, it must be borne in mind that any subject upon which they are fed is liable to a fresh infection. In the case of natives (who sleep without hesitation in any village), the employment of village-caught mosquitoes cannot, however, be very prejudicial.

The advantages of the above way of keeping mosquitoes are :—

1. The mosquitoes will keep alive longer than in any other way known to us.

2. The immense convenience in feeding.

3. Any mosquitoes that may have died in the night can be recovered, and are not dried up.

4. It is an extremely convenient way of obtaining and examining the ova.

5. Mosquitoes which have become feeble are given the best possible chance of living, and will be found resting all day on the piece of paper.

If boxes and net-covered frames be used, an enormous mortality usually results. The dead bodies dry up and get lost in the folds of netting, or, unless special precautions are taken, are eaten up by ants.

If chutney jars with hollow stoppers cannot be procured :—

Procure any form of wide-mouthed jar or bottle, such as a prune jar, preserved fruit bottle, etc. Insert a piece of stout cardboard as before.

1. Prepare the metal top of a screw-top bottle or some other suitable small receptacle with water, cork, and paper as above. Place upon a square piece of very stout cardboard or wood. Invert the jar over this (Fig. 28).

2. Prepare a saucer by adding a few tea-spoonfuls of water and placing on this cork and paper. Invert the jar over the saucer. This is rather more convenient than the last mentioned method, as mosquitoes are less liable to escape in the process of lifting the bottle.

To Feed Mosquitoes

Select a bottle in which the mosquitoes (twenty to thirty, or at least a dozen, in each bottle) are ready for feeding, *i.e.*, the second evening after hatching or collecting. Lift the bottle from the

stopper, first disturbing any mosquitoes which may be resting on the stopper, and place it mouth downwards on the table.

Slip underneath the mouth of the bottle a small piece of mosquito netting of rather a fine mesh. Tie this around the neck of the bottle with twine. The bottle is then ready for feeding.

Fig. 28A. *Method of Feeding Mosquitoes* ½

At, or shortly after dark, take as many bottles as may be desired to the ward or dormitory. Slightly damp the forearm (or the calf) of the patient, and, turning the bottle right side up, let the patient's arm rest upon the mouth of the bottle (Fig. 28A).

In from a few minutes to half-an-hour or more the bottle will be noticed to have splashes of blood upon the bottom and sides (in the case of *Anophelines* only). If possible wait till all the *Anophelines* have fed.

Remove the bottle, invert upon a table, untie the twine, and remove the netting. Replace the bottle upon the prepared stopper.

Repeat the process every night, allowing the mosquitoes to feed by preference on the same case throughout. Where it is uncertain which of several cases may or may not have mature sexual forms in the blood, a bottle may be fed on alternate nights on the different cases.

Add clean water and a fresh piece of paper each time the bottle is used.

To Prepare Fed Mosquitoes for Dissection

After having fed the mosquitoes in a bottle for a certain number of days, place it apart from others, and allow it to remain undisturbed (merely changing the water, etc.) for several days.

Ascertain each day whether the mosquitoes have completely got rid of the blood in the mid-gut. When they are quite free from any dark colouration of the ventral aspect of the abdomen they are ready for dissection.

N.B.—If chloroform, and especially if tobacco smoke is used to kill the mosquito, it is essential to well wash the jars before again keeping mosquitoes in them.

To Feed Mosquitoes on Birds, etc.

1. Prepare a framework of wood and book-binder's cardboard. Cover two sides with card-board. Cover one end with netting drawn tight, and to the other attach a 'sleeve' of netting. Catch or breed out a number of *Culex* (*e.g.*, *Culex fatigans*), and place in the frame. Keep the frame in a dark place, and place a saucer of water in it.

Before placing the bird in the cage, a small bag of netting should be tied around its head, as it then remains perfectly quiet, and further, the legs may be fastened. Small birds, such as sparrows, should be carefully treated, as, otherwise, they are very liable to succumb. Pigeons should be treated in the same way, if necessary.

2. Mosquitoes may be fed singly on pigeons and other large birds by placing the end of the test tube, in which the mosquito is confined, against an area of skin denuded of feathers.

Feeding Experiments on Birds

1. Feed a number of *Culex*, *e.g.*, *C. fatigans*, on sparrows (in which have been detected proteosoma in the blood), by placing these for a time in the mosquito cage.

After feeding one or two days, place those mosquitoes, which obviously have fed and are gorged with blood, in a prepared chutney jar, and keep until ready for dissection.

Note (i) the zygotes of proteosoma which generally occur in large numbers in the stomach wall, and in which very coarse and dark pigment is seen.

(ii) Feed some *Anophelines* on proteosoma sparrows, and note that no zygotes are formed.

(iii) Feed some *Taeniorhynchus* on proteosoma sparrows, and note the negative result.

(iv) Feed some *Culex* upon pigeons containing halteridium, and note negative result.

Sparrows containing halteridium so frequently (in India) contain proteosoma that, even if the latter is not observed under the microscope, it is difficult to be sure of their absence.

Chapter XI

DISSECTION AND EXAMINATION OF MOSQUITOES FOR THE MALARIAL PARASITE

Dissection of Mosquitoes

Necessary Apparatus.

1. Slides and coverglasses.
2. Two needles, preferably the straight surgical needles described for making blood films, as they have a cutting edge.
3. Some salt solution, 0·5 grammes per cent.
4. It is convenient to have a board, twelve by three inches, covered half with white and half with black paper.

Some mosquitoes are caught by slipping over the top of the jar used for feeding another empty jar of the same size, and they may be kept alive in a dark cupboard for two or three days, until their stomachs are quite free from blood (seen by the complete disappearance of black from the ventral portion of the abdomen).

A few specimens are killed by chloroform or tobacco smoke, or by 'concussion' simply, in a test tube.

1. Observe (if a gravid female) two whitish areas on either side of the hinder portion of the abdomen (ripening ovaries). If the blood in the stomach be not digested, a dark mass will be seen

in front of these, and possibly the extreme anterior portion of the abdomen will appear transparent (air containing oesophageal diverticulum). (Fig. 29.)

To Dissect Out the Mid-Gut (Stomach)

1. Pull off, with forceps, the legs and wings (and remove most of the scales by a few strokes of a small camel hair brush).

2. Place a drop of salt solution on a slide, and place the slide on a light background.

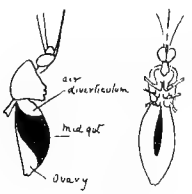


Fig. 29

Turn the mosquito upon its back and with a needle, held in the left hand, transfix the thorax.

Carry the mosquito transfixed on the needle to the slide, and lower the tip of the abdomen into the drop of salt solution.

Keeping the transfixing needle in position, make with the other needle a nick upon either side between the sixth and seventh abdominal segments, which point corresponds to the division between the mid and hind-guts. After thus loosening the last few segments, place the point of the needle upon them, slowly dragging them away from the rest of the abdomen.

3. After separating the segments a very short distance, remove the preparation to a dark background. Again draw apart and note the white viscera stretching between them. Make

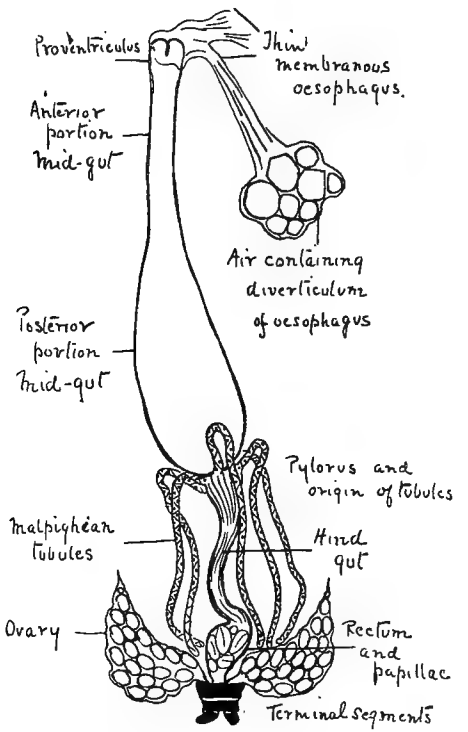


Fig. 30. Dissection of the Viscera of a Mosquito

steady traction until a central, rather transparent body is alone left between the two portions of abdomen.

Cut across the anterior attachments of the mid-gut.

4. Draw the body of the mosquito away from the separated segments; the mid-gut and sundry other viscera will be left attached to the latter floating in the salt solution.

Observe that when the tension is relieved, the structure last to leave the abdomen of the mosquito assumes a saccular appearance. This is the mid-gut.

The Viscera

(Fig. 30)

1. Unless the mosquito is newly hatched, note two opaque white oval bodies (the ovaries) attached to the separated segments. If the ovaries are near maturity, masses of white ova are seen.

2. *The Mid-gut.*—This extends from the level of the first pair of legs to the posterior border of the sixth abdominal segment.

(i) An anterior narrow portion resembling an oesophagus.

(ii) A posterior dilated portion at the level of the sixth (and fifth) abdominal segments in which, if the last meal of blood is not quite digested, a black mass will be seen. If any blood remains in this portion, *i.e.*, 'the stomach,' discard the specimen for one kept longer without food.

(iii) At the commencement of the mid-gut a ring-like, thickened portion (the proventriculus). It acts as a valve between the oesophagus and mid-gut (Fig. 30).

3. Passing between the mid-gut and the separated segments, note five brilliantly white threads—the malpighian tubules (Fig 30).

4. Between the malpighian tubules the transparent intestine which may exhibit active peristalsis (Fig. 30).

5. Attached to the proventriculus an exceedingly delicate membrane, the dilated oesophagus and three diverticula of the same, which usually contain gas bubbles. SCHAUDINN has shewn by adding Baryta water to these bubbles that they are really carbonic acid gas. Further, also, the bacteria in these diverticula produce enzymes which are the cause of the 'irritation' of the bite, as may be shewn by rubbing a diverticulum into a scratch on the skin. The salivary secretion as has been generally supposed has not this property.

The ventral diverticulum extends as far back as the fifth abdominal segment.

TO PREPARE THE MID-GUT FOR EXAMINATION

1. Cut (by pressing with the needle) across the intestine and malpighian tubes just below the termination of the saccular mid-gut. This will separate the mid-gut from the rest of the viscera.

Remove everything from the slide but the mid-gut. Remove excess of fluid, and see that no ova or extraneous matters are left upon the slide. Add a small drop of clean salt solution, and place a thin coverglass upon the preparation. The mid-gut will flatten out considerably. Remove with filter paper applied to the edge of the coverglass any excess of fluid. Examine under one-third inch objective and afterwards under one-twelfth.

If the mid-gut has been removed *in toto*, and the preparation not too much compressed, the following appearances are seen :—

1. The narrow anterior portion of the mid-gut, with the calyx-like proventriculus at its free end.

2. If a portion of the extremely thin membrane of the true oesophagus or its diverticula be included in the preparation, it will probably be seen to exhibit peculiar markings, due probably to muscular fibres in the membrane, but resembling rather closely sporozoits. It is essential that this structure should be recognized when seen, and that the resemblance of its markings to sporozoits should not lead the beginner astray (Fig. 32).

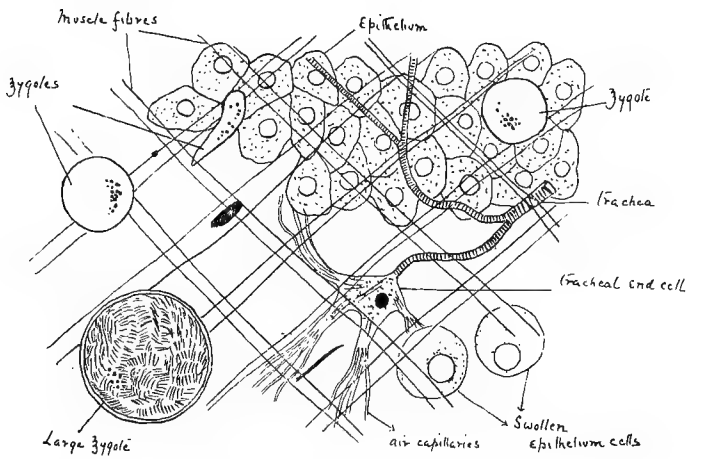


Fig. 31. *Microscopic appearance of Mid-gut, shewing Cell Structure and Zygotes*

3. The expanded posterior portion of the mid-gut. This body forms the main mass of the

preparation, and is all important in relation to malarial studies.

The following appearances are seen in a good preparation :—

1. Well-defined tubes with spiral lining (air tubes or tracheae). Note that these branch and ramify upon the surface of the mid-gut and malpighian tubes (Fig. 31).

2. Large muscular fibres, together with elastic fibres, forming a check pattern. Note that they are circular and longitudinal (external). Note that at the edge of the viscus they are seen in optical section (Fig. 31).

3. Large cells with large nuclei and granular protoplasm (epithelium of mid-gut). Note some *in situ* forming a single layer of polygonal epithelium, and others detached and in process of being carried along by fluid streaming from interior of mid-gut. Note that in some places these cells are undergoing vacuolization with dancing of the protoplasm granules (Fig. 31).

4. Note any contents of the stomach—
   (i) Remains of blood.
   (ii) Crystals of various kinds.
   (iii) Gregarines, flagellates, bacteria, etc.

5. Note that in focussing downwards one passes through a double thickness of wall. Note that the air tubes are focussed on the upper and lower surfaces of the preparation, and the epithelium and crystals in the middle.

6. Trace several of the finer air tubes to their apparent termination, and note that when they lose their spiral lining they are continued as very fine transparent tubules (air capillaries).

Note that at the point of breaking up, one can generally make out large stellate cells (tracheal cells). (Fig. 31.)

7. Observe in some preparations, large oval cells of brownish colour lying upon the outer surface of the stomach. Note that they are rather opaque, and contain a certain amount of diffuse yellowish pigment. They are so called pericardial cells (see Fig. 32).

8. Observe, in most preparations, one or more large clear cells with a small nucleus and filled with oil globules (cells of the fat body) (Fig 32.) These lie upon the stomach and, in common with the last named cells, are accidental in this situation.

The Examination of the Mid-Gut for the Zygote or Oocyst Stage of the Malarial Parasite

(The examination of the stomach blood for flagellating and the motile or vermicule forms is deferred to a later Chapter).

Obtain a number of *Anophelines* (not *M. rossii*) from some native quarter (see p. 92), or better, those specially fed. Keep these alive for two or three days until no blood remains in the mid-gut (for methods of keeping alive, see p. 95).

Prepare the mid-gut as described above. A considerable number may prove negative, but a variable percentage will be positive. Examine with one-twelfth inch.

Carefully note the presence of small collections of pigment of the nature of *malarial pigment*. By careful focussing, the younger forms may be

seen as clear oval or round bodies, 6-7 μ, in which the distinct and clearly defined pigment occurs. The more advanced forms can scarcely be missed. It is necessary to bear in mind the normal structures and the fact that, until the parasite reaches a considerable size and has a very sharply defined cyst wall, pigment, of the characters belonging to the species of parasite concerned, is present.

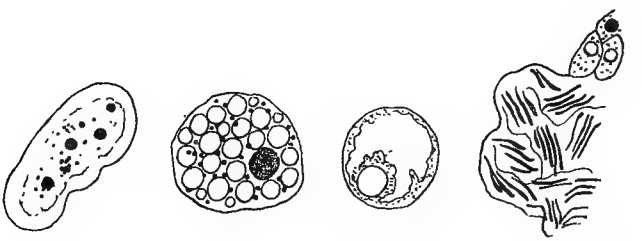


Fig. 32. (Left to right) Pericardial Cell, Fat Body Cell, Swollen Epithelium Cell, Thin Membrane shewing Sporozoit-like appearance

1. Zygotes of crescent tertian shew, when young, a clump of pigment resembling black pepper grains (Fig. 33).

2. Zygotes of simple tertian shew yellowish or golden pigment in wisps (Fig. 33).

3. Zygotes of quartan shew rather coarse pigment in a clump (Fig. 33).

The older zygotes (40-60 μ) are indistinguishable as regards the species of parasite concerned. They may shew :—

1. A very clear and distinct cyst wall (adventitious).

2. The formation of sporoblasts.

PLATE II

Fig. 1.—Young zygotes of the benign tertian parasite.

Fig. 2.—Young zygotes of the malignant tertian parasite.

Fig. 3.—Mature zygote, outside epithelium of mid-gut (Section, haematein).

Fig. 4.—Transverse section of salivary gland shewing sporozoits *in situ* (haematein).

Fig. 5.—Sporozoits from the salivary gland (Romanowsky stain).

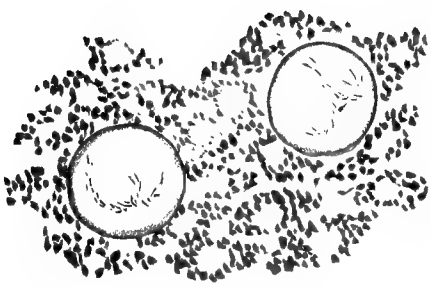


FIG. 1.

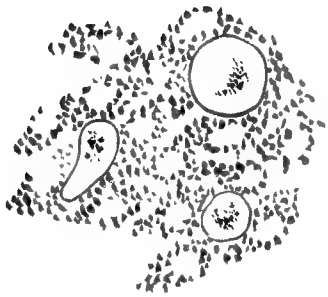


FIG. 2

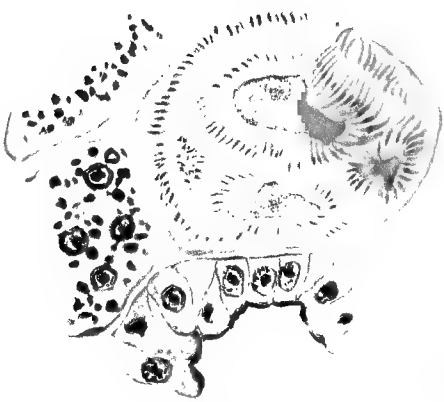


FIG. 3.

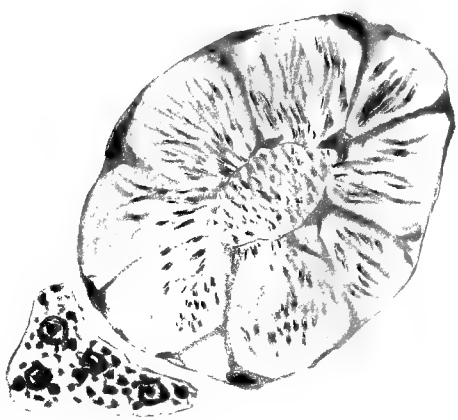


FIG 4.

FIG. 5

3. In still more developed forms, the sporoblasts are seen to be surrounded by a radiating arrangement of young sporozoits or blasts (Figs. 9 and 31).

4. Fully developed forms are large cysts packed with many hundreds of fine sickle-shaped bodies, and, if they are ruptured, these latter escape into the surrounding fluid, and are readily distinguished as sporozoits (Fig. 37).

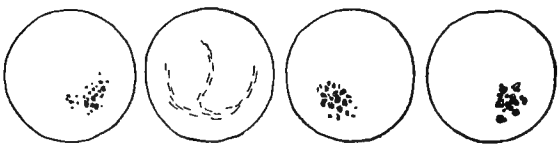


Fig. 33. *(Left to Right) Zygotes of Malignant Tertian, Simple Tertian, Quartan, and Proteosoma*

To Make Permanent Preparations of Zygotes

Method 1.—In case of a specimen shewing zygotes, place a large drop of two per cent. formalin on one side of the coverglass, and draw this through by filter paper placed on the other side. Repeat several times. Remove excess of the formalin with filter paper. Ring edges of coverglass with black varnish or Canada balsam.

The zygotes will retain their appearance as seen in the fresh specimen.

Method 2.—Run formalin through as in Method 1. When an excess of fluid is present, slide off the coverglass. The flattened mid-gut will probably remain attached to the coverglass.

Wash in water, and stain lightly with methylene blue. Wash in water, and allow to dry. Warm gently to ensure complete dryness, and place the coverglass, mid-gut downwards, upon a drop of balsam upon a slide.

The muscular fibres and other structures of the mid-gut will be well exhibited. The zygotes will be stained rather a dark blue. If not too darkly stained, the pigment of the zygotes will have the appearance it had in the fresh specimen.

3. Mount directly in glycerine.

4. For more minute histological examination, imbed the stomachs in paraffin or the whole mosquito (*vide* p. 123).

To Dissect Out the Salivary Glands

This is quite a simple proceeding if it be remembered where they lie. They are intra-thoracic structures, and they commence at the hinder portion of the neck and end opposite the first pair of legs. They lie ventrally, in fact, roughly speaking, they lie just above the origin of the first pair of legs (Fig. 34).

The simplest and most rapid method, and the one that hardly ever fails, is the following :—

1. Place the mosquito in a drop of salt solution on its right side, with the head pointing towards you, as you dissect.

2. Place the needle of the left hand on the thorax to steady it, and place the needle of the right hand on the back of the head and make steady *gentle* traction.

3. If done carefully, it will be seen that the head has pulled out a little mass of white tissue

from the thorax (the dissection is best done on a dull-black surface).

4. Examine the piece of tissue under a half-inch lens. (The diaphragm should be as nearly closed as possible). The glands will be seen hanging on to the neck as finger-like, transparent, *glistening* bodies. Muscle has a greyish look, and even the fat body is not so refractile as the glands.

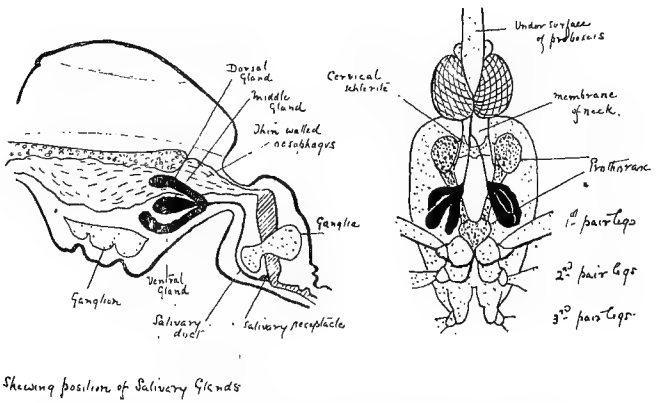


Fig. 34. *Shewing position of Salivary Glands*

5. Now place one needle on the head, and with the other make a transverse cut between the head and the attached portion of the glands.

6. Examine again the now separated glands. Generally all six are with certainty got in this way.

7. If the glands are not found on the neck, proceed with the dissection by Method 2.

8. When dissected out in this way, they are generally quite free from surrounding tissues,

but if found necessary they can be teased out further and placed in fresh drops of salt solution.

9. At all stages of the dissection make sure that the glands are really present and that they have not floated to the side or stuck to the needles.

10. By this certain and rapid method, as many as one hundred glands may be dissected out, put under a coverglass, and examined microscopically in a day's work.

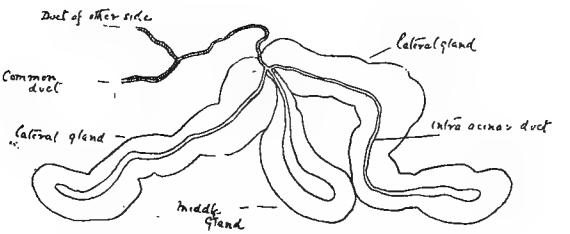


Fig. 35. The Salivary Glands of one side

Method 2.—Consists in isolating, by a series of cuts, the anterior ventral portion of the thorax in which the glands lie.

1. Make a cut obliquely in an antero-posterior direction, so as to sever the main mass of thoracic muscle.

2. Make a cut at right angles to this, passing just behind the attachment of the first pair of legs.

3. Cut through the neck.

4. The glands lie in the portion thus isolated. Considerable teasing out is still required to isolate them from the surrounding tissues.

Examine each portion of tissue separated out, and remove to a fresh clear drop of salt solution.

Remember that in examining under the microscope the apparent right hand is really the left, and *vice versâ*.

This method, which is longer than Method 1, requires more dissection and teasing out in order to isolate the glands cleanly, and, as we have said, may still be followed, even if No. 1 has failed ; but our experience has been that Method 1 is learned at once without any difficulty.

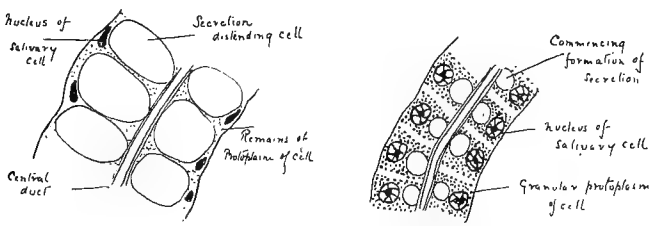


Fig. 36. *Microscopic Structure of Salivary Acinus and that of a Newly-Hatched Mosquito (right)*

Ascertain that the glands of either side consist of three acini, the ducts of which join almost immediately after leaving the acinus to form a single long duct.

1. Observe that of the three glands of each side (Figs. 35 and 38) :—

(i) Two are highly refractile, and the cells in these are very distinct and clearly defined (lateral glands).

(ii) One is much less refractile, and the component cells are much less easily defined (central gland).

2. Observe that each acinus has a duct

running through its whole length, and that the secretory cells form a single row around this.

3. Observe that each secretory cell has a large mass of clear secretion within it, forming the chief bulk of the cell, and that the nucleus is flattened and pushed to the periphery (Fig. 36). Pressure tends to force the secretion out of the cell in viscid looking droplets. The secretion of the lateral glands is far more refractile than that of the central (Fig. 38).

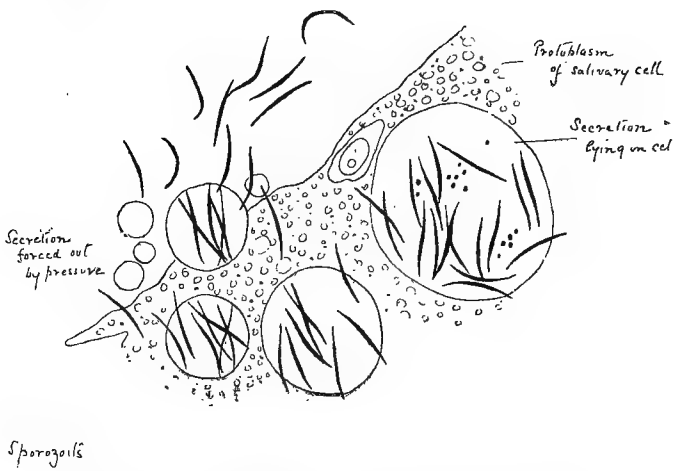


Fig. 37. *Sporozoits in the Salivary Gland (Fresh preparation)*

4. Ascertain that the duct formed by the junction of the three intra-acinar ducts joins, eventually, the similar duct from the other side, to form a common salivary duct which passes into the salivary receptacle. The duct is thick walled, and is lined with a spiral thread resembling a tracheal tube.

Examination of the Sporozoit Form of the Malarial Parasite

Obtain a number of *Anophelines* (not *M. rossii*) from a native quarter (five per cent. to twenty per cent. or more have sporozoits in the glands), or *Anophelines* fed for twelve days or more at a temperature of 80° F. Prepare specimens of the glands, as described above. Having placed one or more lobes under a low power, press with the point of a needle on the coverglass, so that the gland is ruptured, and the secretion poured out as droplets into the surrounding fluid.

Examine with one-sixth inch. If sporozoits are present they are generally very numerous, and large numbers of fine, very distinct curved rods, will be easily seen with this power, lying throughout the fluid around the gland and packed in large numbers in the substance of the gland. Finally, examine with one-twelfth inch (Fig. 37).

The sporozoits have a mean length of 14 μ, and vary between 10 μ and 20 μ, and are 1-2 μ in width.

Examination of Motion of Sporozoits

Dissect out the glands and, when isolated cleanly, transfer to a drop of human serum, previously got ready by allowing blood to clot in a small tube. Three kinds of motion may be observed :—

1. Formation of curves.
2. Formation of ring-formed contractions.
3. Locomotion. Forward motion.

Penetration of Red Cell by Sporozoits.—This has not been seen in case of sporozoits of the

salivary glands, but has been observed twice by SCHAUDINN in the case of sporozoits from a ruptured cyst in the stomach.

TO PREPARE PERMANENT PREPARATIONS OF SPOROZOITS

Pressing firmly upon the coverglass, draw it along the slide, so that a film is made on coverglass and slide.

Dry by rapidly waving the slide and the coverglass in the air. Fix both in alcohol, and stain with ROMANOWSKY. Wash, dry, and examine without coverglass with an oil immersion.

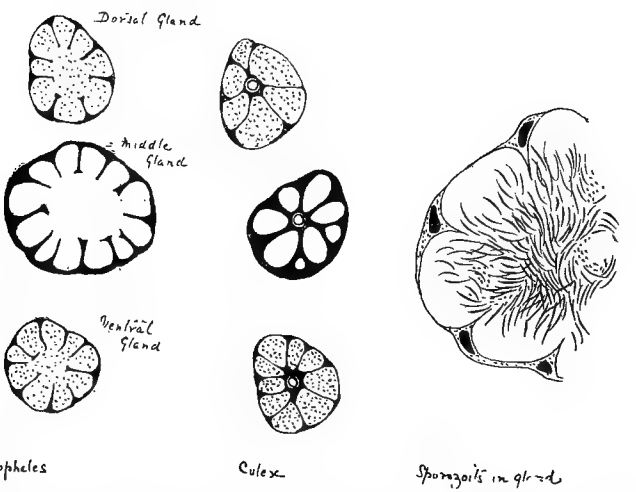


Fig. 38. *Sections of Salivary Glands, shewing differences between the Central and Lateral Glands and between those of Anophelines and Culex, also Sporozoits in the Glands of an Anopheline*

The sporozoits appear as fusiform bodies with a central red mass of chromatin. They are about 14 μ in length, with one end often more pointed than the other.

Wash off the oil with xylol, dry and label.

The Reproductive System

To Examine the Spermatheca (Fig. 39).—By pressing with the edge of the triangular needle cut off the extreme tip of the abdomen—the last or eighth segment only. Place this in a very small drop of salt solution, and tease the fragment carefully apart. A small black granule (In *Culex*, three) will be seen. Isolate this as much as possible from other tissue and cover with a coverglass.

Observe, under a low power, a brown chitinous ball. Press firmly on the coverglass so as to rupture it. Examine under one-twelfth inch.

Observe the masses of fine hair-like actively motile bodies, if (as is probably the case) the mosquito has been fertilized. Isolate some of these; they possess the characters of spermatozoa (Fig. 39).

Examine the spermatheca of a mosquito newly hatched; it does not contain spermatozoa.

To Examine the Ovaries

Examine the ovaries of a number of mosquitoes caught in the room, etc.

Observe that when the ovaries nearly reach maturity they are readily detected as white areas

on either side of the posterior part of the abdomen, and that when fully developed they occupy the whole of the lateral and dorsal portions of this.

Drag off the last few segments of the abdomen in a drop of salt solution, and allow the ovaries to float out in this. Observe that they are pyriform bodies, the apex being above (Fig. 39).

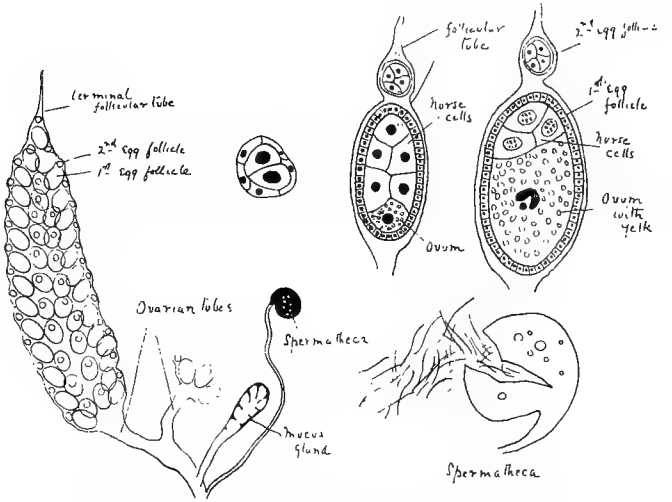


Fig. 39. *Spermatheca, with Spermatozoa Structure of Ovaries*

Ascertain that each ovary consists of a large number of follicular tubes, commencing as fine threads and ending in the oviduct. Observe especially the follicular tube forming the apex of the ovary, as here, this is most readily made out.

Ascertain that each follicular tube contains several egg follicles, the lowest of which is the most advanced in development.

Note that these latter show various stages of development in the different mosquitoes. Note that each consists of an outer layer of small cubical cells (follicular epithelium) and an inner mass composed of from four to eight large cells. Note the first appearance of the yelk as small oil globules in the lowest cell (the ovum), and how this increases until it nearly fills the follicle by encroaching on the other cells (nurse cells).

Observe in the mature ovum that the outer covering is formed of the original layer of outer cells (follicular epithelium).

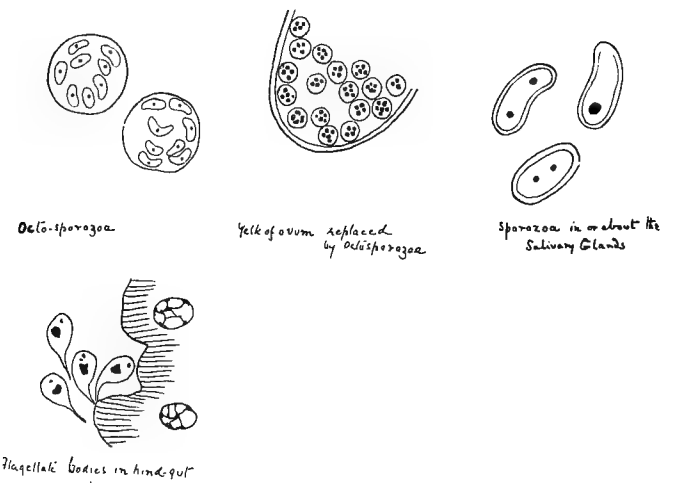


Fig. 40. *Protozoa other than the Malarial Parasite found in Anophelines*

The following organisms may be found in mosquitoes apart from the various stages of the malarial parasite :—

1. Encysted Trematodes, mostly found in the tissues, near the neck ; also free in the stomach.

2. Nematodes in the thorax or abdominal cavity.

3. Sporozoa. (*a*) Sausage-shaped bodies in masses, sometimes in close connexion with the salivary glands (Fig. 40).

(*b*) Octosporozoa, consisting of eight small sausage-shaped bodies in small cysts, enormous numbers of which replace the yelk of the ovum (Fig. 40).

(*c*) Flagellate bodies in the rectum and hind-gut, frequently in enormous numbers, and shewing, when stained, large numbers of developmental forms (*Vide* under *T. noctuae*).

(*d*) Gregarines. Occasionally on the outside of the stomach or encysted in the malpighian tubes. In the larvae, the worm-like gregarine will be found actively motile in the malpighian tubules.

4. The developing embryos of filariae. These are seen as sausage-shaped bodies with a terminal spike in the dissection of the salivary glands. In sections of mosquitoes they are seen in the muscles, especially in those of the thorax.

5. The developed embryos of filariae in the labium or about the base of the neck.

6. In the oesophageal diverticula masses of micro-organisms and sporozoa (*Nosema*)? will be found.

To Cut Sections of Mosquitoes

1. Kill some *Anophelines* by tobacco smoke or chloroform, or allow them to fall directly into absolute alcohol, by pouring a few drops into the tube containing them. Avoid, if possible, mosquitoes containing blood, as the blood becomes very hard.

2. Allow to remain in alcohol fifteen minutes to harden somewhat.

3. Remove one by one. Cut off with a fine scissors the legs and wings. Make a minute incision into both the thorax and abdomen, *e.g.*, holding the mosquito carefully between the finger and thumb, slice off with a sharp razor a portion of the dorsum of the thorax, and a minute portion of one side of the abdomen. This allows more perfect penetration of fluids.

4. Replace in absolute alcohol[1] (some authors recommend boiling in alcohol, as the air in the tracheae is expelled, and the alcohol then penetrates completely), allow to remain one to two hours. This, which is to ensure complete dehydration, is the most important of operations, and upon it depends the success of the embedding. Make two or three changes, using alcohol to which $CuSo_4$ has been added.

5. Remove from alcohol and place for ten minutes or so in xylol. When the thorax becomes transparent, the mosquito should be removed, as too long a time in xylol produces much hardening.

6. Place for ten minutes in paraffin (*vide* p. 46) kept melted in a watch glass on the heated metal slab.

7. Smear a clean watch glass with a little glycerine, and fill with paraffin heated somewhat over melting point; transfer the specimen to this with a warm forceps to avoid cooling the paraffin.

8. Arrange the specimen as required. As soon as the surface of the paraffin has set, plunge the whole mass into water, as paraffin rapidly cooled is more homogenous.

1. For finer work pass through thirty, fifty, and seventy-five per cent. to absolute. See, however, examination of separate organs.

If the watch glass has been smeared with glycerine it will be easy to remove the block.

9. Cut out the specimen, arrange as desired for cutting transversely, vertically, or horizontally. Take care that the top and bottom edges of the block are parallel. Smear these with a little melted soft paraffin (made by melting a little hard paraffin with vaseline). This gives an unbroken ribbon of sections.

To Mount Sections of Mosquitoes

Unless the sections are thick it is necessary to flatten them.

Heat some water in a dish a little below the melting point of the paraffin. Test the right temperature by dropping a section on the surface. If it melts, the water is too hot. If it does not flatten out, the water is not hot enough.

Drop upon the surface a ribbon of five or six sections; it will become perfectly flat if the temperature is right.

1. Smear some slides with solution of pyroxylon in oil of cloves (Appendix), taking care to avoid an excess. Lower the slide rapidly into the water, and with the aid of a needle draw it out again with the sections lying flat upon it.

2. Press lightly, but firmly, between smooth blotting paper. Protect from dust, and allow to dry for twenty-four hours, or dry more rapidly over the flame, but avoid melting the paraffin.

When dry, hold the slide a few seconds over the flame, this drives off the oil of cloves and melts the paraffin.

Pour over the slide first xylol, then alcohol, and finally place in water.

Mosquito tissues are so delicate that in mounting it is difficult to avoid the separation of portions or the whole of the section. This is especially so in the case of the chitin, which frequently breaks away.

OBREGGIA's method is recommended as giving excellent results :—

1. A slide is smeared with a thin film of a mixture, consisting of two parts of commercial liquid glucose and one part of a solution of dextrin (dextrin, 16 oz. ; water, 17½ oz. ; thymol, 15 grains).
2. Place the sections directly on this layer on the slides.
3. Heat at 40° C. for some hours until the glucose becomes hard.
4. Dissolve off the paraffin in xylol and pass through absolute alcohol. If the alcohol is not quite absolute, the glucose will dissolve and the film come off.
5. Dry.
6. Pour on thin film of photoxylin, and allow to dry until edges of film become slightly crumpled.
7. Put slide in water. Sections come away in the film of photoxylin.
8. Stain *in situ*, dehydrate and clear in carbol xylol (xylol, 3 parts ; absolute phenol, 1 part). Mount in balsam.

To Stain.—The best stain for general use in sections is haematein (*vide* p. 50).

THE EXAMINATION OF SEPARATE ORGANS

Remove the mid-gut or the ovaries or other organs desired, and place in a saturated solution

of corrosive sublimate (saturated whilst hot and allowed to cool).

As this is a watery solution, practically no shrinking occurs. It, unfortunately, cannot be used for the whole mosquito, as watery solutions will not penetrate the tissues.

Allow to remain in corrosive solution ten minutes. Place in large volume of water for twelve hours, and in weak watery iodine solution (sherry colour) for one hour. Pass through 30 per cent., 50 per cent., 75 per cent., to absolute alcohol. Allow to remain in absolute alcohol fifteen minutes. Transfer to xylol, three minutes; paraffin two minutes. Cut in ribbons and treat as already described.

To Cut Sections of the Salivary Glands

Embed only the head and front half of the thorax. Cut sections as near as possible horizontally, this will give sections in the length of the glands, and both sides at the same time.

Stain with various stains: Heidenhain's haematoxylin, gold chloride, etc. Much work remains to be done upon the structure and nature of the glands, and the method by which the sporozoits reach the salivary cells.

LITERATURE:

Grassi. *The Studies of a Zoologist* (in German or Italian).

Chapter XII

INTERNAL ANATOMY OF MOSQUITOES

The Alimentary Canal

The alimentary canal is specialized on account of the blood-sucking habits of the mosquito. It differs from many insects in not possessing any caecal diverticula of the mid-gut. It also differs in the possession of five malpighian tubules, these being in insects usually even in number (Fig. 30).

The parts of the alimentary canals are as follows :—

The mouth	The fore-gut.
The pharynx with pumping organ	
The oesophagus	
The oesophageal diverticula	
The homologue of the proventriculus	The mid-gut.
The stomach (so-called)	
The pylorus	
The pyloric dilatation	The hind-gut.
The small intestine	
The colon	
The rectum with rectal papillae	

The mouth, pharynx, and oesophagus are ectodermal in origin, and both the mouth and pharynx are lined with chitin. The hind-gut is also ectodermal in origin; it does not possess, however, any portion lined with chitin. The

mid-gut is the true digestive portion of the tract.

The Pharynx.—The pharynx, which is lined throughout its extent with chitin, passes upwards and backwards through the ganglionic ring formed by the supra and infra-oesophageal ganglia and their commissures. At first it is narrow, but posteriorly becomes a large chamber (the pumping organ). (Fig. 43A).

The pumping organ occupies with its muscles a large portion of the head behind the level of the cerebral ganglia. In the state of rest its lumen is triradiate in transverse section. The walls are formed of three large and thick chitinous plates,one placed on either side and one superiorly. Into each of these plates powerful muscles are inserted. Those of the superior plate consist of two muscular masses, taking their origin from the occiput. Those of the lateral plates consist on each side of a single large muscular mass arising from the lateral portions of the head. The plates are connected by thin non-chitinous membrane, and their edges are rolled so that they form a spring capable of returning to their original position so soon as the separating force of the muscles ceases.

Posteriorly, where the pharynx becomes very narrow, a sharp bend occurs and a valvular action is produced. The whole forms a very powerful suctorial apparatus.

The Oesophagus.—Immediately beyond the pumping organ the chitinous layer ceases, and the rest of the fore-gut is formed of excessively thin membrane. At the junction of the two portions a sharp bend occurs, and the floor projects so as to form a valvular flap.

The thin-walled oesophagus is a large dilated sac, whose walls are supported by surrounding structures. Into the posterior wall of the dilated and thin-walled oesophagus projects the papilla-like anterior portion of the mid-gut.

The Diverticula of the Oesophagus.—From the oesophagus two or three diverticula, similar in nature to the oesophagus, extend backwards. Of these, one is of great size, and usually contains gas. This most usually extends into the abdomen, and is a prominent object in dissections and sections. In the newly-hatched mosquito it is small, but rapidly becomes large enough to extend into the abdomen (Fig. 30).

The Homologue of the Proventiculus.—There is no true proventriculus as in many insects. There is, however, an interesting fold of the fore-gut into the mid-gut which represents this organ. The muscular bundles are here increased, and the whole forms a valvular muscular organ (Fig. 30).

The Mechanism of Feeding.—The powerful pumping action which must result from a drawing asunder of the three large chitinous plates of the pumping organ is very evident. These plates, also, when drawn apart must, by reason of their spring-like shape, revert to their original positions close together, without any muscular aid. Posteriorly the valve-like arrangement mentioned before prevents regurgitation. Further, when the blood reaches the junction of the oesophagus and mid-gut the invaginated portion is withdrawn, and is distended by the entering blood into a distinct 'crop.' The valvular function is suspended and the blood flows onward.

The Mid-gut.—The mid-gut extends from th

proventriculus to the origin of the malpighian tubes. It consists of two portions which merge into one another—an anterior narrow portion, and a large dilated posterior portion, which becomes greatly distended after feeding. Unlike most insects there are no caecal appendages in the mosquito. Posteriorly there is a marked constriction, with strong muscular bundles, which forms a very marked pylorus (Fig. 30).

The anterior narrow portion of the mid-gut lies in the thorax, and does not become distended with blood. The posterior portion when fully dilated fills the greater portion of the abdomen, the viscera being pushed into the last few segments.

The Hind-gut.—The hind-gut is short and passes in one or two bends from the pylorus to the anus. Immediately beyond the pylorus there is a considerable dilatation which is poorly supplied with muscular fibres: into this open the five malpighian tubules. For a short distance beyond this the lumen is narrow (small intestine), but becomes gradually larger (colon). At the termination of the colon there is a slight constriction, after which the canal dilates again to form the rectum (Fig. 30).

Into the rectum project six solid growths, the so-called rectal glands, which are, however, papillae. Posteriorly the rectum ends in the anus close above the gynaephoric canal.

The appendages of the alimentary canal are:—

The Salivary Glands.—The salivary glands consist of six tubular acini lying three upon either side. Those of one side lie generally one above the other in the long axis of the body, their

anterior ends lying close against the prosternum, where the ducts coming from each acinus unite to form a single duct. The upper and middle acini generally lie with their distal ends close to the proventriculus. The lower acinus passes towards the thoracic ganglion. Occasionally, an acinus becomes bifid at a short distance from its termination. A common abnormality also, is a small accessory acinus near the proximal end of an acinus. A duct can be seen traversing almost the entire length of each acinus. Shortly after leaving the acinus, the three unite to form a single duct. The duct of each side passes up into the neck, and lies close to the nerve cords passing between the thoracic and the cerebral ganglia. Beneath, and in contact with the lower surface of the suboesophageal ganglion, the ducts of each side unite to form a common salivary duct which passes forwards and enters the chitinous first portion of the alimentary canal close to the base of the proboscis (Fig. 34).

The Malpighian Tubules.—These are five in number and open into the first portion of the hind-gut immediately beyond the pylorus. Their blind ends are held in position in the neighbourhood of the rectum by tracheal branches. They pass forwards in loops above their origin, so that, in transverse section, as many as ten may be seen cut across.

The Muscular System

The chief muscular masses in the mosquito are contained in the thorax. They are chiefly muscles moving the wings and legs.

Wing Muscles.—There are two large muscular

masses on either side of the thorax, passing from the dorsal to the ventral body wall. Between these bundles there is a space, in the lower portion of which lies the alimentary canal, main air tubes, and other structures. The upper portion of the space is occupied by a second series of large muscular bundles, passing from the front to the back of the thorax. Neither of these large masses of muscle are inserted directly into the wings, the up and down movement of the wings being caused by alterations in the shape of the thorax, consequent on the contractions of the vertical and horizontal fibres, respectively.

There are, however, a few fibres arising from the lateral portions of the thorax, and inserted about the base of the wings.

Leg Muscles.—These occupy but little space in the thorax. They rise, to a large extent, from the internal processes of the exoskeleton (apodemes), and are inserted into neighbouring portions of the limbs. They arise, also, from one segment of a limb and are inserted into another.

The Muscles of the Body Segments.—These arise from one segment and are inserted into the next. They are arranged dorsally and ventrally in lateral groups throughout the abdomen.

A small muscle is also situated on each side, passing vertically from the tergum to the sternum. These on contracting flatten the abdomen.

Muscles in Association with the Alimentary Canal.—Several important muscular masses are connected with the large chitinous pumping organ. A pair of muscles arises from the occipital region of the exoskeleton, and is inserted into the upper

plate of the organ. A large muscle arises on each side, and is inserted into each of the lateral plates.

In the thorax, a small muscular band rises from the neighbourhood of the first pair of legs, and passes upwards close to and outside the salivary glands of each side. The contraction of this band must exert pressure upon the salivary glands.

Anteriorly and posteriorly small muscular bundles pass from the dilated portion of the mid-gut to the abdominal wall.

The Tracheal System

Respiration is entirely carried on by tracheae. These take their origin from external openings—the spiracles—and eventually terminate in minute capillaries in the actual tissues of the insect. In the Culicidae there is no development of large or multiple air sacs in connexion with the tracheal system, as in many insects.

The spiracles are placed both in the thorax and in the abdomen. The thoracic spiracles are two in number, situated in the meso-thoracic and meta-thoracic segment, respectively. Of these the anterior one is the largest in the body. The second thoracic spiracle is also much larger than the abdominal spiracles. The abdominal spiracles are situated in the pleural membrane, one in each segment (Fig. 42).

The Tracheae.—Very large tracheae pass inwards from the anterior thoracic spiracles.

1. A large branch passes forwards towards the neck and gives off a branch which passes down on either side of the middle line to the two anterior coxae and the salivary glands. The main branch

continues on through the neck, and supplies the head with numerous large branches.

2. A large branch passes upwards and backwards along the edge of the meso-scutum, and gives off branches which supply the wing muscles. A smaller branch also passes forwards and supplies the muscles of the thorax.

3. The largest trachea in the body (main trachea) passes downwards, backwards, and inwards, so as to lie on either side of the anterior portion of the alimentary canal. Numerous branches are given off from this trunk to the thoracic muscles, the alimentary canal, and legs. Posteriorly the trunk is continuous with a trachea passing forwards from the second thoracic spiracle, thus forming on either side a large tracheal loop.

Large tracheae also pass inwards from the posterior thoracic spiracles.

1. Branches pass forwards and join in a loop with the main trachea, also backwards to join the abdominal system.

2. Branches pass downwards to the meta-thorax and posterior pair of legs.

3. Branches pass inwards to the muscles and mid-gut.

From each abdominal spiracle a short thick trunk passes inwards, which gives rise to the following branches :—

A dorsal branch ramifying beneath the tergum and joining the branch of the opposite side.

A sternal branch supplying the sternal plate and muscles, also joining the branch of the other side.

Loop branches passing to the trunks, anterior and posterior.

Branches passing inwards and supplying viscera. Branches from the first, second, third, and fourth abdominal tracheae supply mainly the mid-gut, those from the fourth and fifth the ovaries, those from the sixth and seventh the genital organs.

The Vascular System. –As in most insects where the respiratory system ramifies throughout the whole body, the vascular system is not well developed. A dorsal vessel or heart and an anterior prolongation of this (aorta) are the only closed blood-vessels. Apart from the dorsal vessel the blood circulates in large blood spaces, which lie between the lobes of the fat-body and among the muscles and viscera.

The dorsal vessel passes close beneath the tergal plates throughout the abdomen. It is very thin walled, and is not provided with valves. The upper portion is attached to the dorsum at intervals by suspensory fibres (muscular), so that a festooned appearance is given in longitudinal section. There is, however, no true division into compartments. Laterally large cells (pericardial cells) are arranged throughout its entire extent, and fibres of a muscular nature (alary muscle) pass from the body wall and end in branches in close connexion with the dorsal vessel.

At the first abdominal segment the dorsal vessel dips down beneath the mesophragma, lying as it does so in direct contact with the cuticule. In the thorax it again arches upwards, and lies between the lower portions of the antero-posterior wing muscles close above the anterior portion of the mid-gut.

In the anterior third of the thorax it divides into two smaller portions which pass outwards, and coming in contact with the salivary ducts enter the neck.

Blood spaces without definite walls occur throughout the body. The thorax especially contains large spaces among the muscles, and the complex fat-body which lies between and supports the organ is everywhere bathed with blood fluid.

The Nervous System

The ganglionic system in the *Culicidae* is considerably developed. The head ganglia are large and complex. The thoracic ganglia are large and compressed so as to form a large ganglionic mass. The ganglia of this system are as follows :—

(*a*) Lying around the pharynx is a ganglionic ring composed of large supra and infra oesophageal ganglia with their commissures. From these, large nerves go to the eyes, antennae, and mouth parts.

(*b*) In the thorax, lying below the oesophagael diverticulum and close to the sterna, is a large compound ganglion showing evidence of its origin from the conjoined ganglia. Between this and the head ganglia are two long slender nerve cords, which pass in the neck in close relation with the salivary ducts. From the thoracic ganglion large nerves pass to the limbs, and posteriorly nerve cords connect it with the first abdominal ganglion.

(*c*) The abdominal ganglia lie with their connecting commissures close upon the abdominal

sterna. The last ganglion lies just below the junction of the oviducts to form the common oviduct. A large nerve passes from it among the viscera of the last few segments.

The Visceral System.—Small ganglia connected with the main ganglionic system occur in connexion with the viscera. The most important of these are two small groups of large nerve cells lying in front of and above the thoracic ganglion, with the middle portion of which they are connected by nerves. They lie laterally beneath the oesophageal diverticulum and anterior portion of the mid-gut, and are not far removed from the salivary glands. Another small ganglion occurs above and in front of the proventriculus (Fig. 34).

The Reproductive System

The organs of the reproductive system are :—

1. Ovaries.
2. Oviducts and common oviduct.
3. Mucus gland and duct.
4. Spermathecae and ducts.

The ovaries occupy a variable position dependent upon the state of their development. In the newly-hatched mosquito they are small bodies lying in the fourth and fifth abdominal segments close by the posterior portion of the mid-gut, and attached to the body wall by numerous tracheae. As they enlarge they push the mid-gut, hind-gut, and malpighian tubes towards the ventrum, so that eventually the ovaries occupy nearly the whole of the posterior portion of the abdomen. Each ovary consists of very many follicular tubes, each containing egg follicles in different stages of

development. In the mature ovary the lower follicles have in every tube become the large completely-formed egg (Fig. 39).

The oviducts are muscular tubes passing from the ovaries. They join beneath the rectum to form the common oviduct, which is still more abundantly supplied with muscle fibres, and which eventually opens beneath the anus.

The spermatheca is a chitinous sac, which in the impregnated female is filled with a mass of spermatozoa. Its duct is long and twisted and opens into the common oviduct near its termination. (In *Culex* there are three spermathecae.)

The mucus gland, globular or ovoid in shape, opens by a short duct into the same region.

The Fat-body.—The adipose tissue is disposed in two ways.

1. As a general lining to the body wall, being nearly everywhere present directly beneath the cuticle, and

2. As lobular masses lying in among the organs and muscles. Thus a large pad lies over the compound thoracic ganglion, and sends processes which lie in among the salivary glands and other viscera. Other smaller masses lie in the head and abdomen.

Histology

Methods.—The examination of the fresh tissues frequently reveals structures not easily seen in fixed preparations. The tissues are best dissected out in normal saline of low tonicity, 0·3 or 0·4 per cent., as insect juices have a lower isotonic point than those of mammals. Better preparations of

both tissues and included parasites are usually to be obtained by the use of fixed tissues. Several tissues (including the salivary glands and mid-gut) may, when dissected out, be spread by means of the edge of a slide or cover-glass, and rapidly dried. These, fixed and stained, give beautiful preparations of sporozoits, as well as certain parasites in the mid-gut, hind-gut, etc.

For fixing mosquitoes as a whole, watery solutions are not generally so good as alcohol, on account of the difficulty of penetration from the nature of the exoskeleton and the large amount of air contained in insect tissues; very good results are obtained by fixing and hardening in absolute alcohol, and proceeding at once to embed in paraffin. It is best, so soon as considerable hardening has taken place, to make a minute incision into both the thorax and abdomen. For fixing portions or isolated organs of mosquitoes saturated solution of perchloride has advantages over alcohol and fixes the cells of the mid-gut extremely well. It does not penetrate, however, well into undissected mosquitoes. Picric acid gives good results with isolated organs. The changes in the mid-gut cells during digestion are well shown.

Both *Culicines* and *Anophelines*, but especially the latter, cut readily in paraffin or celloidin. For staining smear preparations and sections haematein gives very good results; sporocysts and sporozoits, as well as the normal tissues, are well stained.

The stellate cells in connexion with the tracheal endings upon the mid-gut, etc., are frequently well shown by gold chloride (Fig. 32).

Heidenhain's haematoxylin gives good results with the salivary glands, and also the muscle fibres in connexion with the alimentary canal.

The Histology of the Alimentary Canal and Appendages

The epithelial lining differs considerably in the mid-gut from either the fore-gut or hind-gut. In the mid-gut the possession of a marked striated border by the epithelial cells is characteristic. The muscular fibres of the alimentary canal are striated throughout.

The Fore-gut.—The anterior portion of the fore-gut is lined by chitin and does not differ from the cuticle in structure. It consists of a single layer of cubical cells of small size. The oesophageal dilatation and its diverticula resemble one another in structure. In the adult mosquito they consist of an extremely delicate membrane formed of a single layer of flattened cells, with externally some scattered muscular fibres. In fresh preparations peculiar wrinklings of this membrane are seen, which may appear like bundles of sporozoits. A similar appearance is seen in the dilated portion of the hind-gut just beyond the pylorus.

In the pupa, the oesophageal diverticulum is seen passing backwards as a narrow tubular organ lying beneath the mid-gut. It is in this stage lined with well-marked cubical epithelium. In a freshly-hatched mosquito this organ is frequently undistended, and shows a narrow lumen surrounded by a single layer of large cells. These cells retain very little trace of protoplasm, which, however, may still be present in fine strands, and

around the nucleus, which is pushed to the outer portion of the cell.

In the majority of mosquitoes the walls of the oesophageal diverticulum are crowded with micro-organisms and bodies which appear to be protozoal in nature.

The Mid-gut.—There is but little structural difference between the narrow anterior portion of the mid-gut, which lies in the thorax, and the posterior dilated portion, which lies in the abdomen. In many insects there are caecal tubes or pouches opening into the anterior portion of the mid-gut. These are, however, quite absent in the adult mosquito. The main thickness of the wall consists of epithelium; external to this is a thin coat of muscle fibres (Fig. 32).

The epithelium consists of a single layer of large cells, which are columnar in the undistended organ, but become flat and pavement-like when the organ is full of blood. They have a finely-reticulated protoplasm, which stains more deeply towards the free border. Stained with HEIDENHAIN'S haematoxylin, alcohol-hardened specimens are seen to contain numerous stained granules, collected especially in the outer portion of the cell. These are especially abundant in the anterior portion of the mid-gut. They have also, very frequently, a number of small clear vacuoles (droplets), which become more frequent and of larger size towards the free border of the cell. The most marked feature of the cell is the clear striated border which is present in all the cells of the mid-gut, but absent in all other portions of the alimentary canal. The striated border is best marked in the undistended organ, and becomes

almost invisible in the fully distended state, when the cells are much flattened.

The nucleus of these cells is large and centrally situated. The chromatin is arranged in small stellate masses arranged circumferentially and centrally, and connected with one another by fine threads of chromatin. There is a body which stains less deeply, generally to be made out (karyosome) in the centre of the nucleus.

Occasionally young cells are to be seen near the basement membrane.

The muscular coat is very thin. It consists of an open mesh-work of long muscular fibres running longitudinally and circularly. In the large posterior portion of the mid-gut, these fibres form a very regular series of large square or rhomboidal meshes. In the narrow anterior portion they are more closely approximated, so that the muscular layer here is more evident in sections.

The individual muscle fibres are very long, fusiform, striated fibres. On the outer surface of the mid-gut lie numerous large branched cells in which the small tracheae end, and from which bundles of minute structureless air tubes pass into the wall of the mid-gut. These cells are frequently well shown in gold chloride specimens. Similar cells occur throughout the viscera in connexion with the tracheal endings (See 'Tracheal Endings').

The Homologue of the Proventriculus.—Mention has been made of a fold occurring at the anterior extremity of the mid-gut. This consists of an invagination of a portion of the fore-gut into the mid-gut. The mid-gut is also folded in with the portion of fore-gut, so that in this region there is

a double thickness of mid-gut wall as well as the fore-gut. There is an increase in the muscular fibres of the mid-gut at this point, especially the circular fibres, so that a very distinct mass is formed homologous to the proventriculus of many insects. There is no chitinous development, however, and the structure would appear to act only as a muscular sphincter (Fig. 30).

The Hind-gut.—The nature of the epithelium and arrangement of the muscular fibres differs somewhat in different portions of the hind-gut. Structurally the small and large intestine are similar, whilst the dilatation beyond the pylorus, and especially the rectum, differs from these.

The dilatation which occurs at the origin of the malpighian tubules is thin-walled and poorly supplied with muscle fibres. The cells lining it are small and flattened.

The intestine is lined with a single layer of large cubical cells; external to these is a muscular coat. The cells of the intestine have large nuclei which have a similar, though more open, arrangement of the chromatin than the nuclei of the mid-gut. The protoplasm is finely reticular, and stains less deeply than the cells of the mid-gut. Stained with HEIDENHAIN'S haematoxylin, no granules are present as in the cells of the mid-gut. They have no striated border.

In the rectum the cells become small and flattened. There are here, however, bodies usually termed rectal glands. These are papillae covered with a single layer of much hypertrophied cells resembling those lining the small intestine and colon.

The muscular system of the hind-gut is very

similar to that of the mid-gut, consisting of very large fusiform, straited cells arranged circularly and longitudinally. The circular fibres in the small intestine lie outside the longitudinal, and pass spirally around the mid-gut. Towards the termination of the intestine longitudinal fibres also lie outside the circular. In the rectum and extending throughout the hind-gut and mid-gut in the Culicidae, there are, in a large proportion of specimens, swarms of a flagellate organism (Fig. 40).

The Salivary Glands.—The salivary acini lie in a cleft in the fat-body, which latter comes in close contact with the glands. Each gland acinus consists of a single layer of large cells, limited externally by a delicate sheath (basement membrane) and internally by the intra-glandular duct wall.

In *Anophelines* the intra-glandular duct becomes larger as it approaches the termination of the acinus, and forms a large cavity.

In *Culicines* the duct remains of the same diameter throughout the acinus, and terminates abruptly near the end of the acinus without any dilatation.

In both *Culicines* and *Anophelines* there are two types of gland acinus. These are recognizable both in the fresh gland and in fixed specimens. From their appearance in the latter they may be termed

(1) The granular type.
(2) The clear or colloid-like type.

The Granular Type—The greater portion of the acinus consists of cells whose nucleus and protoplasm has been pushed to the outer portion

of the cell by a large mass of secretion, which occupies almost the whole of the cell. In the fresh gland this secretion appears as a clear, refractile substance, and can, by pressure, be made to exude from the cell in refractile globules. In specimens hardened in alcohol, this clear secretion appears as a granular mass, occupying the greater portion of the cell. It stains faintly with haematein, and shows under high powers (one-sixteenth oil immersion) a coarse reticulum and isolated globules, an appearance probably due to the precipitation or coagulation of the secretion by the alcohol. Considerable variations exist, however, in the appearance of this granular secretion, both in the different mosquitoes and in different parts of the same gland. In *Anophelines* the greater portion of the gland contains cells densely crowded with granular material. Very frequently, however, the terminal portion contains cells in which only a few large globular masses exist (Fig. 38).

The protoplasm of the cell occupies, in the fully-matured gland, only the extreme periphery, and the nucleus, which is much degenerated, is pushed to the outer portion of the cell, and usually lies in the angular interval left at the base of two or more contiguous cells. In the granular type of gland this disappearance of the protoplasm and nucleus from view is more pronounced than in the clear type of gland.

The Clear or Colloid-like Type.—Of the last-mentioned type there are two acini upon either side; of the present type there is but a single acinus upon either side, which usually lies between the two acini of granular type (Fig. 38).

In the fresh gland the cell outlines are not so

distinct as in the granular type, and the secretion, when extended by pressure, is much less refractive. In alcohol-hardened specimens, the acinar cells contain a large mass of clear, homogenous secretion which, as in the last-mentioned type, fills almost the entire cell, and pushes the protoplasm and nucleus to the periphery.

In the clear type, however, the protoplasm is always in greater amount than is the case with the granular type, and the nucleus never becomes so greatly degenerated. The clear, homogeneous secretion stains readily with haematein, and may even stain quite deeply. With HEIDENHAIN'S haematoxylin it frequently becomes almost black. It resembles very much in appearance colloid substance as it is seen in the mammalian thyroid.

In *Anophelines* this substance also distends the central duct space within the acinus. In this situation an appearance is sometimes produced which resembles faintly-stained sporozoits, but which is a normal condition.

The Maturation of the Glands.—In freshly-hatched mosquitoes both types of acinus consist of large glandular cells arranged round the lumen. These contain a large, centrally situated nucleus, and have protoplasm containing a large number of coarse granules, staining with haematein. In the portion of the cell nearest the lumen a vacuole of varying size is situated. This is the commencement of the large mass of secretion which, in the mature gland, occupies the entire cell. In the granular type of acinus the vacuole contains granules ; in the clear type it resembles the colloid-like secretion (Fig. 36).

Further Variations in the Cells of the Salivary

Acini.—In the granular type of gland the greater portion of the acinus is composed of cells of the character described above. A portion, however, usually exists which differs considerably in structure. This portion adjoins the duct, and may, in *Anophelines*, reach as much as one-quarter of the entire gland in length. In this portion of the gland the cells are much smaller than those containing the granular secretion, so that the diameter of the acinus is much less here, and a sudden increase takes place when the portion containing the granular secretion is reached. The cells lying towards the duct differ from those lying towards the acinar end of this portion. There is, however, no line of demarcation between them, the one gradually becoming changed into the other. In the centre of each cell is a clear body, pushing the nucleus and protoplasm to the outer portion of the cell. Towards the duct end in the centre of this clear substance is a darker portion continuous with the duct lumen. As the cells come to lie nearer the distal portion, this central dark lumen becomes obliterated. This structure, though present in *Anophelines*, may be absent in *Culex*. In certain *Culex* another variation in the gland cells frequently occurs. The portion of the gland lying close to the duct, instead of being less in diameter is greater. The cells composing this portion are columnar in shape, with centrally situated nuclei and no contained secretion.

In certain specimens it is not uncommon to find cells occupying a peripheral position, and not approaching the lumen, which contain a substance resembling the colloid-like secretion of the clear type of gland.

Changes after Feeding.—Very little change occurs in the glands after feeding. They are for the most part still quite full of secretion. Probably a very small amount only of secretion is used with each puncture.

The Ducts.—The intra-acinar ducts vary in *Culicines* and *Anophelines*. In *Culicines* they remain narrow and tubular throughout the entire length of the gland. In *Anophelines* they become large spaces in both types of acini, but especially in the clear type. The duct is lined throughout by a clear homogeneous skeletal material, which is continuous with a similar substance dividing the cells of the gland from one another. Into the duct the secretion-filled cell opens by means of a small opening.

The duct, after leaving the acinus, consists of a thick-walled tube, with a central spiral thread resembling the spirals in the trachea. The wall is homogeneous, but contains many nuclei.

The Malpighian Tubules.—The malpighian tubules are tubular bodies with caecal ends, which open into the hind-gut. The cells are extremely large, being, next to the pericardial cells, the largest in the body. Each cell contains a large nucleus, and contains numerous large granules, which stain feebly with haematein, but powerfully with HEIDENHAIN'S haematoxylin. Numerous fatty granules are also present. Each cell is wrapped round a central lumen, the cells being arranged alternately, so that a zig-zag appearance is given in section. The inner portion of each cell is markedly striated, the lumen being thus bounded by a striated area. In relation with these tubules, a large number of tracheae and tracheal end-cells exist.

In certain conditions the malpighian tubule cells may be found quite free from granules, though otherwise unchanged. This change occurs in mosquitoes with large numbers of a flagellate organism (previously noted) in the rectum and hind-gut.

The Muscular System.—The muscular fibres of the mosquito are without exception striated. Those of the wings differ in structure very much from those of the limbs and body segments. The muscle fibres of the alimentary canal are large fusiform cells, with a single large nucleus with some surrounding protoplasm. The muscle fibres in connexion with the heart are much branched.

Many of the fibres contain a very marked sarcolemma and space between this latter and the fibre. This space is usually seen occupied by extremely delicate branching threads, which stain feebly with haematein.

In the pupae there exist some large cells of peculiar nature in association with the sheaths of the muscle fibres.

The structure of insect muscle is described in many works on histology, and does not need repetition here.

The Tracheal System.—The larger tracheal vessels consist of a single layer of flattened cells with an inner chitinous layer. In smaller tubes the cells embrace the entire vessel, the nucleus frequently being bent around the lumen. The cells of the tracheal vessels contain numerous small clear vacuoles (chitin formation). The chitinous lining possesses a thickening in the form of a spiral thread, which may become unwound and lie stretched as a wavy thread in fresh preparations.

The smaller tubes contain the spiral thread until they become from 2 to 5 μ in diameter. They then divide to form bundles of excessively minute air capillaries, which enter among the tissue cells. The division into capillaries takes place in the substance of large branched cells situated at the termination of the tracheal vessels. The cells often appear cribriform in section from the number of air capillaries. These cribriform cells in connexion with the tracheal endings are well seen in the mid-gut and malpighian tubules. They are, however, seen best of all in the undeveloped ovary of the newly-hatched mosquito, which is extremely rich in bundles of capillary air tubes.

The Vascular System.—The dorsal vessel is a delicate walled tube composed of longitudinal and oblique fibres with a nucleated inner layer. The fibres may be traced directly from the terminations of the branched alary muscle fibres. The alary fibres break up into fibres which pass in close connexion with the large pericardial cells, and eventually form (1) fibres passing into the dorsal vessel as longitudinal fibres, (2) fibres joining in an anastomosis in connexion with the floor of the dorsal vessel.

The pericardial cells are extremely large cells lying on either side of the dorsal vessel throughout its whole extent. They are by far the largest cells in the mosquito, varying from 30 μ to 50 μ in longitudinal diameter. They are elongate or pear-shape in form, and contain several nuclei. The nuclei usually show signs of degeneration. The peripheral portion of the cell stains more deeply than the central portion, which contains the

nuclei and small stained granules. There are a considerable number of masses of a light yellowish pigment resembling that found in the large visceral ganglia cells. The fibres from the branches of the alary muscles pass over and around the pericardial cells to reach the dorsal vessel. From their structure and situation the pericardial cells appear to be of the nature of ganglion cells (Fig. 32).

The Fat-body.—The fat-body, both where it occurs as a portion of the body wall and where it lies as free lobulated masses, consists of cells containing numerous oil globules. The cells are of considerable size, and their borders may be frequently traced as polygonal areas. The nuclei are oval in shape with a central mass of chromatin and chromatin threads. Besides oil globules the cells contain granules staining with haematein, and minute droplets of a highly refractile, dark substance, which gives the appearance of pigment. These droplets are larger in amount in old mosquitoes than in those freshly hatched (Fig. 32).

The Nervous System.—The ganglia of the ganglionic system consist of an outer portion of nerve cells and an inner portion of non-medullated nerve fibres. Considerable complexity exists in the larger ganglia, especially the head ganglia.

The ganglia of the visceral system differ greatly from those of the ganglionic system. The ganglion cells are few in number and of large size. They possess clear reticular protoplasm, a little denser around the periphery than in the centre. Around the inner margin of the denser peripheral portion small stained points are arranged. In the centre a variable number of granules of yellowish pigment exist.

The Reproductive System.—Each ovary consists of a large number of follicular tubes whose lower ends open into the ovarian tube, and whose upper ends terminate in a delicate supporting filament (terminal filament). The apex of the ovary is formed of a single follicular tube, whose filament is attached to the fat-body of the fourth segment.

Around the whole ovary there is a delicate nucleated sheath.

Each follicular tube contains one or more egg-follicles in different stages of development. In the freshly-hatched mosquito each follicular tube contains an undeveloped egg-follicle. As this develops, a second and a third undeveloped follicle appear above it, which again undergo development into mature eggs. The follicle at first consists of two to four large cells, with large nuclei surrounded by a single layer of smaller epithelial cells (Fig. 39).

The central cells then increase in size and number, so that many very large cells are contained in the now enlarged follicle. The surrounding epithelial cells also become larger, and rapidly increase in number so as to form a layer of regular cubical cells surrounding the follicle. The central cell nearest the ovarian tube is the ovum, the rest are nurse cells, and eventually disappear. Both the ovum and the nurse cells increase greatly in size. The nurse cells have clear protoplasm and extremely large nuclei, which exhibit karyokinetic figures. The ovum contains very numerous yolk granules, which occupy the whole of its substance, except a thin coating of granular protoplasm. Still later this thin

external layer can only with difficulty be made out (Fig. 39).

The nucleus of the ovum undergoes very pronounced changes. It appears as an irregular mass, staining uniformly with nuclear stains. This mass becomes more and more distorted and broken up, and eventually disappears. It may frequently, however, be seen as irregular masses of staining material even in the mature egg. A portion of the nucleus is seen very early to be separated off from the rest, often surrounded by the latter. This portion (female pronucleus) is small and difficult to detect in sections in the more mature ovum. As the ovum increases still more rapidly in bulk, the nurse cells become crowded into the distal portion of the follicle and eventually disappear, so that, in the mature egg, no trace of them is to be seen. The epithelial layer surrounding the follicle becomes much flattened, and forms eventually a covering to the egg (chorion). The outer portion of this covering (exochorion) is transparent, and marked with oblique parallel markings. Over the proximal end, *i.e.*, the end lying towards the ovarian tube, the chorion forms a globular mass ornamented with rows of pits. This is the micropylar apparatus through which the spermatozoa penetrate the ovum.

Frequently in *Anophelines* a large portion or the whole of the adult ovum consists of a mass of sporozoa. These consist of numerous small cysts, each containing eight round or crescent-shaped bodies,each with a central chromatin spot (Fig. 40).

The ovarian tube arises in the centre of the ovary, and receives on all sides the follicular

tubes. It is lined with a single layer of small cubical epithelium. After passing out of the ovary, a considerable number of striated muscular fibres are arranged in a loose network around it, and pass from it to surrounding structures. There are also muscular fibres in the ovary itself in connexion with the ovarian tube and egg-follicles.

The spermatheca consists of a chitinous sac, with large cells lying externally. These resemble the cells of the cuticle, and contain droplets. They do not cover the whole of the surface of the spermatheca. The contents of the spermatheca in the fertilized insect consist of a mass of spermatozoa, which, in the fresh state, may be seen revolving with great rapidity within the sac. The spermatozoa have a narrow, slightly-curved head and a long tail. The duct of the spermatheca is narrow and thick-walled, and contains muscular fibres. Certain large cells lie in connexion with the duct externally. The mucus gland contains cells filled with secretion. There are small nuclei in connexion with the intra-acinar duct (Fig. 39).

Chapter XIII

TO COLLECT AND PRESERVE MOSQUITOES

How to Collect Mosquitoes

Mosquitoes may be collected in two way :—

1. By capturing the adult flies.
2. By breeding out from larvae and nymphae.

1. Search in the daytime in houses, huts, and out-houses, at the base of large trees, amidst brushwood, and other dark or shaded places. *Anophelines*, however, are rarely caught except in huts and out-houses. They are especially fond of cow-sheds and the darker portions of the eaves of huts.

Some species of mosquitoes may be caught by sitting with a light near a white wall or suspended sheet, or inside a tent, near a jungle or marsh. *Culex* and *Taeniorhynchus* may be found sitting on the surface *just beyond* the brightly illuminated area. *Anophelines* are rarely caught in this way, but, one species at least *(My. barbirostris)*, appears to be attracted by light, and was caught by us on an illuminated sheet at night, near swampy land.

In searching for adult *Anophelines*, as many places as possible should be examined, as the distribution of some species is very local.

If the captured insects appear to have fully matured ovaries, some of these should be placed in bottles, as previously described (p. 97), and allowed to lay their eggs.

If care is taken to place only one species in a bottle, the characters of the ovum may be noted, in addition to the adult insect.

Some of the ova should be placed in fresh water, and an attempt made to determine the characters of the larva (p. 73), when it has hatched out and is sufficiently grown.

2. *Breeding out.*—Full-grown larvae, and especially nymphae, are collected. These are collected from every possible source. Scarcely any water will be found free from some form of mosquito larvae. Even strongly brackish waters, containing over one per cent. of salt, often contain large numbers.

Examine water from the following sources :—

(i) Domestic utensils, cisterns, tins, pots, calabashes, in which there has been water for three or four days. The larvae of *Stegomyia*, *Culex*, etc., and only rarely *Anophelines*, will be found.

(ii) Cess pits, pools full of decaying leaves, etc., sewage ditches. Note larvae of certain species of *Culex*, etc.

(iii) Observe presence of the larvae of *Stegomyia* and *Culex* in the water which collects in the axils of banana leaves and other plants. Also, occasionally, *Anophelines* in large collections of water of this kind.

(iv) Puddles of all kinds, with and without algae, ponds, tanks, swamps, rice fields, ditches, canals, rivers, streams, lake margins, and wells,

and observe that in all, *Anopheline*, as well as *Culicine* larvae, may abound.

Note that in waters covered with certain species of *Lemna*, *Anophelines* are rarely found.

(v) Place any larvae (nearly adult specimens if possible,) and nymphae in collecting tubes with a note as to where they have been obtained. If it is necessary to cork the tubes, some air space should be left and the corks loosened as often as possible. Larvae, as a rule, survive carriage in small collecting tubes better than they do in bottles or larger vessels. They may be carried long distances, *e.g.*, in a train or on horseback, provided that occasionally the tube is uncorked and they are allowed breathing time. Larvae survive rough treatment better than pupae, and when apparently dead, may often be revived by floating out on the surface of the water. Examine the larvae (Chap. IX), and roughly divide them into as many groups as possible, observing the main characters of each.

(vi) Place each variety in small bottles, over the neck of which a piece of mosquito netting must be tied as soon as the larvae have turned into nymphae.

When the adult insect has hatched out, note its attitude and any other special features.

To Kill Mosquitoes

1. A mosquito that has just hatched out from the nympha should not be killed for some hours until its exoskeleton has hardened. If it is killed immediately, the wings on drying will shrivel, and possibly the whole insect become distorted.

2. Allow the insect or insects to escape into a clean, dry, glass vessel.

Pour some chloroform on a pellet of wool and place under the jar, but take care that the mosquitoes do not get wetted by the chloroform. Leave them exposed to the vapour of chloroform some little time after apparent death. Tobacco smoke may also be used for killing.

After killing, turn the mosquitoes out upon a sheet of clean paper.

To Mount Mosquitoes

Necessary apparatus—

Fine silver pins. No. 20.
Thin cardboard or thick paper.
Large entomological or ordinary pins.
Specimen tubes with corks.

1. Prepare a disc by cutting with scissors a circular piece of Bristol board (or very thick paper). The diameter should be slightly less than that of the specimen tube.

2. Push a fine 'silver' pin two-thirds of its length through the centre of this.

3. Place the mosquito upon its back on a clean sheet of paper. (In this and other manipulations use a pin for moving or steadying the mosquito.)

4. Take the head of the fine pin in the finger and thumb, or hold it near the head end with a pair of forceps. Endeavour to place the point of the pin exactly in the centre of the origin of the legs, which all arise very close together from the under surface of the thorax. Bear in mind, that the more the insect is touched the more

scales are rubbed off, and that a crookedly mounted specimen is better than a 'rubbed' one.

5. Push the pin steadily through the thorax, so that it emerges as near the centre of the dorsum of the thorax as possible. [Practise mounting by forcing the fine pin through without aid from the other hand.]

6. Having transfixed the mosquito, force the point of the pin one millimetre beyond the back, by pressing it against the smooth surface of a cork or tissue paper. The pin should not be pushed through too far, as it prevents the lens of the microscope being brought near enough for examination.

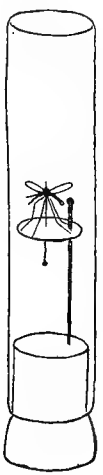


Fig. 41. *Authors' Method of Preserving Mosquitoes*

7. Placing the disk against a cork, pass carefully through the edge a large entomological pin. This is passed in the reverse direction to the fine pin. Force three-quarters of the length

of the large pin through the cardboard disk, and then firmly press the point into the cork of a specimen tube, so that when the tube is corked the mosquito is inside (Fig. 41).

In damp climates, it may be necessary to carefully dry the tube and insect in a dessicator (over sulphuric acid or lime), or by placing in the sun or warm place to prevent mould. This, however, is but seldom required. Mites are rarely seen in insects preserved in tubes as described.

Write any information, *e.g.*, locality, date or reference number *upon the outer surface of the cork* and on the edge of the cardboard disc. For transmission, all that is necessary is to pack the tubes in wool in a box and send by post. Packed in this way they are far more secure than when mounted in the ordinary way in an entomological box. Mosquitoes for the British Museum should be addressed :—

The Director
The British Museum
(Natural History)
Cromwell Road, London, S.W.

Endeavour always to send both male and female, at least two of each, and, what is of the greatest possible importance for the advance of our knowledge of mosquito classification, the careful description of ova and larvae.

Note.—If from any cause it is impossible to pin and mount mosquitoes in this way they may instead be simply placed between layers of tissue paper in a pill box, etc. This is far better than placing them in any fluid such as spirit, by which treatment they are rendered useless for identification.

To Mount Portions of Mosquitoes Permanently

1.—*Wings*.—Clip off one or both wings as near as possible to the thorax, so as to avoid cutting the base of the wing itself.

2. Allow the severed wing to fall in the centre of a clean glass slide. See that the dorsal surface of the wing is upwards.

3. Place one or two very minute drops of *thick* Canada balsam at some distance from the wing, but within the area of a coverglass. This is merely to hold the coverglass firmly in place; the balsam must not be allowed to touch the wing.

4. Press a coverglass firmly down on the Canada balsam. If it is desired, the coverglass may be ringed with paraffin or some material which will not run by capillarity beneath the coverglass.

Legs.—Mount the legs of one side in order in a similar way.

Mount the palps and proboscis.

Mount (*a*) The male ungues.
(*b*) Scales from head, scutellum, etc.
(*c*) Wing denuded of scales.

In Canada balsam, by placing a drop of balsam on these and mounting in the ordinary way.

Chapter XIV

ANOPHELINAE. EXTERNAL ANATOMY OF THE IMAGO

The Head

The head is composed mainly of the two large compound eyes. These meet below and approach one another very closely above.

Parts of the head. The following are the usual names for the different regions of the head (Fig. 42).

1. The nape: the extreme back of the head.
2. The occiput: the portion behind the eyes.
3. The vertex: the space between the eyes.
4. The frons: the space in front of the eyes.
5. The gena: the side of the head below the eyes.

The frons is triangular in shape, with one angle directed downwards. From the upper two angles arise the antennae, and from the lower projects the clypeus, lying over the base of the proboscis.

The Clypeus.—Projects over the base of the proboscis as a prolongation of the frons. The character of the clypeus is of specific importance. It is

1. Hairy in *Culex.*
2. Scaly in *Stegomyia.*
3. Nude in *Joblotia.*

The Antennae.—Consists of fourteen to sixteen segments, of which the basal one is large and globular. The plumose antennae of the male readily distinguishes it from the female.

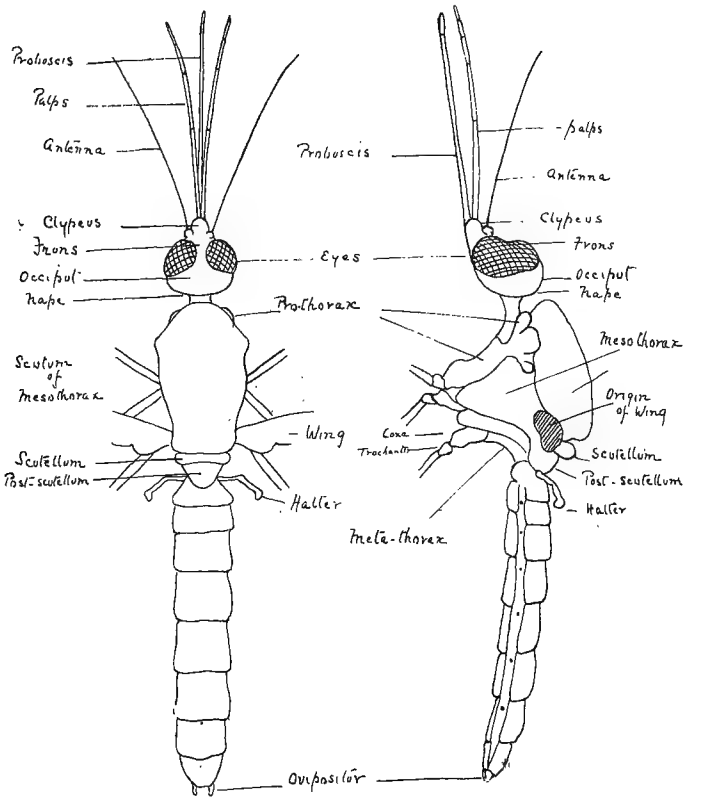


Fig. 42 *External Anatomy of Female Anopheles*

The Proboscis.—The proboscis consists of the very highly specialized mouth parts, ensheathed

in the lower lip or labium. The proboscis consists of (Fig. 43) :—

1. The labium, forming the sheath.
2. The labrum and epipharynx, or upper lip
3. The hypopharynx, or tongue
4. Two mandibles
5. Two maxillae
6. Two maxillary palps.

(2–5: forming the stylets.)

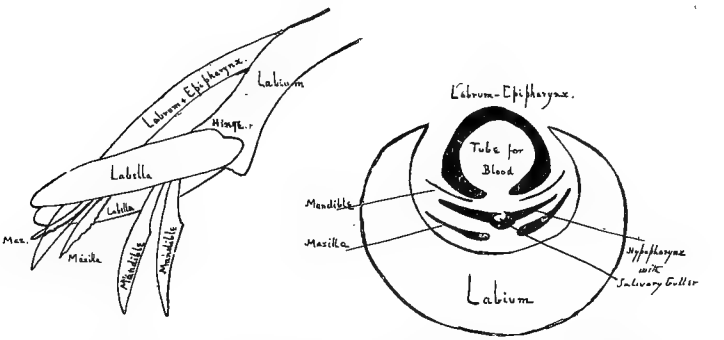


Fig. 43. *The Proboscis (Labium and Stylets), after* NUTTALL *and* SHIPLEY. *Right hand, cross section of Proboscis The Palpi are not shown*

The Labium.—The labium forms the thick and scaly proboscis as usually seen. On its dorsal surface it is hollowed out, and in this hollow run, as in a sheath, the piercing mouth parts or stylets (Fig. 43). The labium itself does not penetrate the skin, but becomes sharply bent during the act of biting, just as when a cane walking stick is pushed against the ground. This may easily be seen if a mosquito is watched during the process of biting.

The Labellae.—Attached to the end of the labium by a hinge joint on either side are two leaf-like processes, the labellae (Fig. 43). It is through the angle made by the two labellae, that the stylets pass, as a billiard cue, between the first and second fingers (NUTTALL and SHIPLEY).

The labium proper stops short at the point of junction of the labellae, but is continued on its upper surface as a blunt point covered with fine hairs (DUTTON). We may liken it to a pen continued on beyond the penholder, the junction of pen and penholder being the point at which the labellae are hinged on.

Dutton's Membrane.—The area between the end of the labium proper and the extreme tip is covered by an extremely thin membrane (DUTTON). In the act of biting, when the labellae are separated, this membrane is somewhat stretched, and applied to the skin.

THE ESCAPE OF THE FILARIAL EMBRYO

It has been shewn by LOW and JAMES that the filarial embryo occurred in the proboscis, according to LOW among the stylets. According to DUTTON, the embryo lies really in the tissue of the fleshy labium, moreover with its head at the level of the membrane described above, and that it is by the rupture of this excessively thin membrane that the embryo escapes. GRASSI and NOE think that the embryo escapes through the middle of the bent-up labium through a rupture at this point, but DUTTON's explanation seems more likely.

The *epipharynx* is the central tube through which the blood is sucked. Its point slopes off

somewhat like the tip of a hypodermic needle. In cross section it has the shape of an Ω, the completion of the tube being formed by the apposition below of the hypopharynx. The labrum is blended with the epipharynx, but does not extend to the tip.

The *hypopharynx* is a thin, flat two-edged lamella closely applied to the under surface of the epipharynx. It is pierced by the salivary duct down which the salivary secretion and sporozoits pass. The opening of the duct is continued as a groove reaching almost to the tip of the hypopharynx.

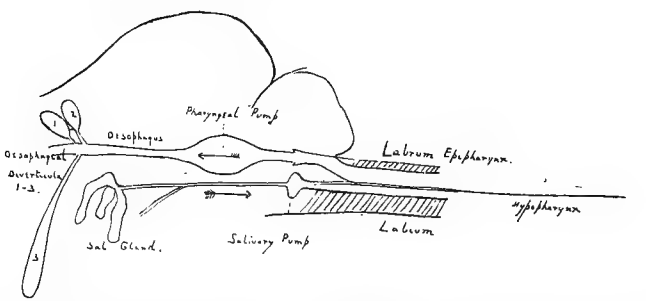


Fig. 43A. *Showing relation of Pharyngeal and Salivary Pumps to the Proboscis*

The *mandibles* are very fine chitinous rods in cross section, crescentic in shape. At the tip of the mandibles are about thirty serrations, though in certain species of *Culex* these appear to be absent.

The mandibles are closely applied to the sides of the epipharynx.

The *maxillae* are stouter than the mandibles, and fit around the outer side of these and the

hypopharynx. They have about twelve serrations at the extremity, coarser than those of the mandibles. In some culices, papillae replace the serrations.

The Maxillary Palps.—These lie upon either side and somewhat dorsally to the proboscis. In the act of biting they take no part, but are then separated from and lie at right angles to the proboscis. Differences in the palpi are of both specific and generic importance.

The expanded ends of the palpi in the male *Anophelines* are even more conspicuous than the plumose antennae.

The Prothorax.—The main portion of the thorax is mesothoracic; anteriorly, however, there is a collar-like piece of chitin, the prothorax. To this are attached two moveable bodies, the patagia.

The prothorax is of importance in classification, *e.g.*, in the new genus of the *Anophelinae Stethomyia* the prothoracic lobes are mammillated.

The Mesothorax (Fig. 42).—The *scutum* of the mesothorax forms the large globular mass of the thorax. Behind the scutum, and just behind the origin of the wings, is a transverse bar of chitin, the *scutellum*. Behind the scutellum is a convex triangular area extending as far as the first abdominal segment, the *post-scutellum* (Fig. 42).

The scutellum and post-scutellum are of importance in classification. Thus the scutellum, with its 'posterior border bristles,' is often of specific value, whilst the post-scullem may be—

1. Bare. *Culex* and *Anophelinae.*
2. With hairs. *Wyeomyia.*
3. With scales and hairs. *Joblotia.*

The Wing

The wings shew :—

1. An anterior straight, thick, and strong border or costa.

2. A posterior curved and thin border, carrying a fringe.

3. Two small folds at the base of the wing (squama and alula).

4. Nervures, or veins.

The *costa* in *Anophelines* is generally covered in part with white, and in part with black, scales (spotted).

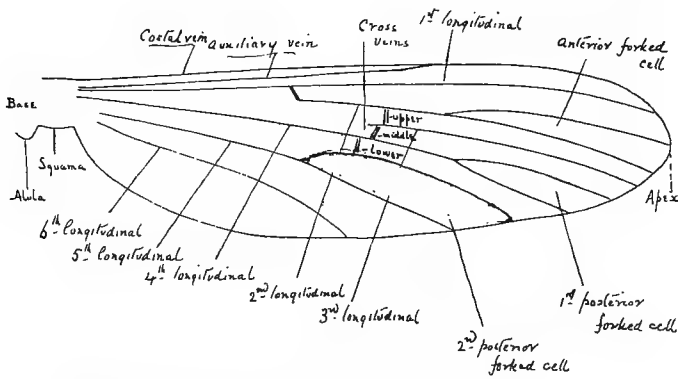


Fig. 44. Wing of Anophelinae :—
Upper = Supernumary cross-vein
Lower = Posterior cross-vein
Anterior forked cell = First forked cell
First posterior forked cell = Second posterior cell
Second posterior forked cell = Anal cell

The *fringe* in *Anophelines* has most frequently light and darker portions, the number and position of which are of specific importance (Fig. 51).

The Nervures.—The nervuration of the wing is of considerable importance. Several nomenclatures are in use. That used in the accompanying diagram is, however, the simplest (Fig. 44).

In classification, the relative position of the apices of the two forked cells are frequently used. Also the relative positions of the point where the auxiliary vein cuts the costal vein, and the point where the fifth vein cuts the posterior margin. As a rule, the position of this first point is much nearer the base than that of the second point, but in a few instances, *e.g.*, *My. sinensis*, they almost coincide.

Also the positions of the upper, middle, and cross veins. It will be found, however, that even in the same species there is no constancy in these latter, and they can hardly be given as of specific importance as has been done. DÖNITZ has made the same criticism, and indeed, finds that the position in each wing of the same mosquito may be different.

THE LEGS

These consist of the following segments :—

1. Coxa and trochanter. Small pieces at the origin of the legs (Fig. 42).
2. Femur.
3. Tibia.
4. Tarsus, consisting of five segments, the last of which carries the claw or ungues.

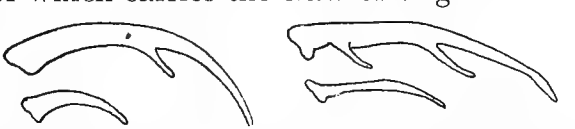


Fig. 45. *Fore Ungues of M. funesta* (♂) *the larger Uniserrate.*
Fore Ungues of M. rossii (♂) *the larger Biserrate.*
(*After* THEOBALD)

The Ungues.—The ungues vary in the male and female and in the different legs. They may be simple, uniserrated or biserrated (rarely triserrated). (Fig. 45.) They are of specific value (THEOBALD).

THE ABDOMEN

The abdomen consists of nine segments. To the ninth segment are attached the genitalia.

The genitalia are variously shaped lobed appendages. In the male they are provided at their free end with claspers. The claspers in the male are of specific value (Fig. 46).

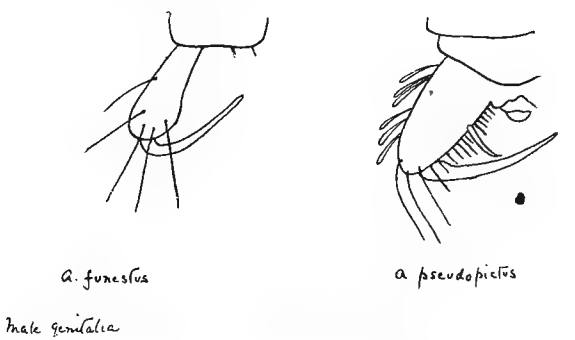


Fig. 46. Male Genitalia

Technique;—

1. The constituent parts of the proboscis may be readily separated by dropping the living mosquito into alcohol (CHRISTY).
2. The study of the chitinous parts is much facilitated by soaking in oil of cloves.

Chapter XV

CLASSIFICATION AND IDENTIFICATION OF THE CULICIDAE

Scales

Theobald has attached to scale structure the greatest importance from the point of view of generic and specific classification. Hence it is necessary to consider somewhat in detail these structures. Theobald gives the following :—

Head Scales.—Three forms of scale occur (Fig. 47).

1. Narrow curved scales.
2. Upright forked scales.
3. Flat scales overlying one another like the tiles of a roof.

No. 1, 2, and 3 scales found, *e.g.*, *Culex.*

No. 2 and 3 scales only, found, *e.g.*, *Stegomyia.*

No. 3 scales only, found, *e.g.*, *Megarhinus.*
Toxorhynchites.

Thoracic Scales.—Theobald describes five forms (Fig. 47).

1. *Narrow Hair-like Curved Scales.*—They often form a dense feltwork over the mesothorax.

2. *Narrow Curved Scales.*—They may occur all over the mesothorax and scutellum, or at the sides of the scutum and in front of the scutellum.

3. *Spindle-Shaped Scales.*—These lie scattered about, and never form a complete covering.

4. *Flat Scales* like those on the head. They cover the scutellum in *Stegomyia*, whereas in *Culex* the scutellar scales are of the narrow curved type.

5. *Long Twisted Scales.*—Characteristic of *Mucidus*, a genus of mouldy-looking mosquitoes.

Abdominal Scales.—The scales covering the abdomen in all *Culicidae*, except the *Anophelinae*, are overlapping flat scales. In the *Anophelinae* they are not found, except to some extent in *Myzorhynchus* and *Nyssorhynchus*. In the genus *Aldrichia*, however, of *Anophelina*, the abdomen is covered with flat scales as in *Culex*.

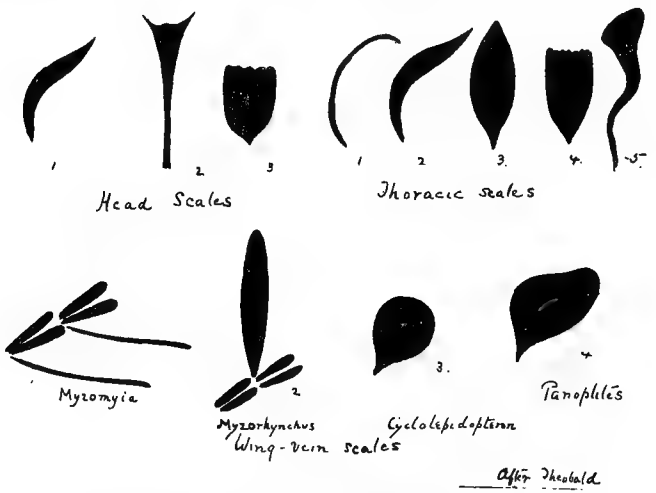


Fig. 47. *Varieties of Scales (after* THEOBALD). *Panoplites = Mansonia*

Wing Scales.—Scales clothe the veins, except the cross veins. Flat scales are arranged in a double row along each vein.

Many, or in some species all, of the veins have also lateral scales.

The lateral scales are very variable in shape, *e.g.*,

1. In *Mansonia* they are broad asymmetrical flat scales.

2. In *Aedomyia* the scales are similar.

3. In *Mucidus* the wing scales are quite characteristic, being pyriform or inflated and half-dark, half-white.

4. In *Megarhinus* the scales may be azure green or blue.

The Wing Fringes consist of—

1. Long narrow-pointed scales attached to the edge of the wing by a narrow stalk.

2. Smaller scales similar in shape.

3. Border scales. Small flat scales.

Leg Scales.—The legs are covered with flat scales in nearly all *Culices*.

1. In *Sabethes* the scales are hair-like and occur in tufts.

2. In *Mucidus*, *Psorophora*, the scales are elongated and project from the legs.

The sub-family *Anophelina* contains, as we shall see, twelve genera, the *Culicinae* twenty-five, and the *Aedeomyina* seventeen. When we consider, further, the large number of species in some of these genera, *e.g.*, *Culex*, it is impossible to attempt here to describe each mosquito, however briefly. Considering the great importance, however, of the *Anophelinae*, we shall attempt to give the characteristic specific points for each of the species, as an aid to a detailed examination by means of THEOBALD'S monograph. With regard to the other sub-families we shall attempt only to give characteristics of each genus.

The *Culicidae* are divided into the following

sub-families, based mainly upon the length of the palpi in male and female :—

1. Palpi long in both sexes, as long as the proboscis in the female *Anophelina*

2. Palpi long in both sexes, shorter than the proboscis in the female *Megarhinina*

3. Palpi short in female, long in male— *Culicina*

4. Palpi short in female, long in male. Post-scutellum with hairs (chaetae) and scales, *Joblotina* [= *Trichoprosopina*

5. Seven (not six) longitudinal veins with scales *Heptaphlebomyina*

6. Palpi very short in female and male— *Aedeomyina*

7. Proboscis short, not formed for piercing *Corethrina*

Sub-Family Anophelina (*vide* next Chapter)

Sub-Family Megarhinina

Genus 1. *Megarhinus*.—First sub-marginal cell much smaller than second posterior cell. Palpi

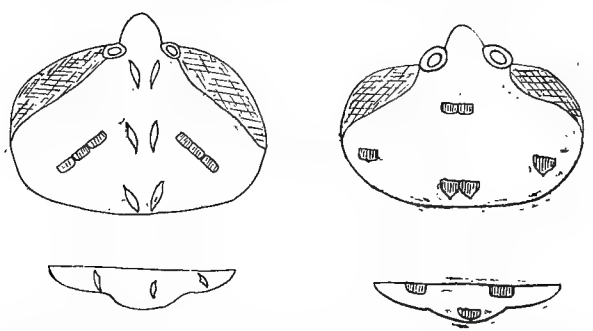


Fig. 48. *Head and Scutellar Scales of Aedes (left) Megarhinus (right)*

five-jointed in ♀ (*M. purpureus* only four). Readily recognized: (i) by their large size, often called 'elephant mosquitoes'; (ii) by their brilliant metallic colours; (iii) by a caudal tuft of hairs on each side of the abdomen; (iv) by the long and curved proboscis; (v) head is clothed with flat scales only (Fig. 48).

They may be found resting on the trunk of trees in the forest, also in houses in the bush. Species, about six.

Genus 2. *Toxorhynchites.*—Palpi much shorter than proboscis in ♀, three-jointed. Supernumerary cross-vein nearer the apex of the wing than the mid cross-vein. Species, four.

Sub-Family Culicina

First sub-marginal cell equal to or longer than the second posterior cell.

Genus 1. *Janthinosoma.*—*Hind* legs densely scaled, giving a characteristic appearance. Species, five.

Genus 2. *Psorophora.*—Characterized by (i) great length of ♂ palpi, five-jointed; (ii) densely long scaled legs; (iii) posterior cross-vein a little nearer the base than the mid; (iv) proboscis curved in ♀. Species, four.

Genus 3. *Mucidus.*—Easily recognized by their curious mouldy appearance. Posterior cross-vein nearer apex of wing than mid. Wing scales large, pyriform, parti-coloured. Head and thoracic scales long and twisted, expanded at the apex. Legs densely scaled with projecting scales. Species, five.

Genus 4. *Desvoidea = Armigeres.*—Head, flat scales, a few upright-forked. Differs from *Stegomyia*, (i) is longer, with unbanded tarsi and abdomen. ♂ palpi, untufted. ♀ palpi, very pointed and provided with bristles only. Species, two.

Genus 5. *Stegomyia.* — Head completely clothed with broad flat scales (Fig. 49) and a few upright forked. Palpi four-jointed in the ♀, five-jointed in the ♂. *Scutellar scales flat*, mostly black and white mosquitoes with banded legs and abdomen. Species, twenty.

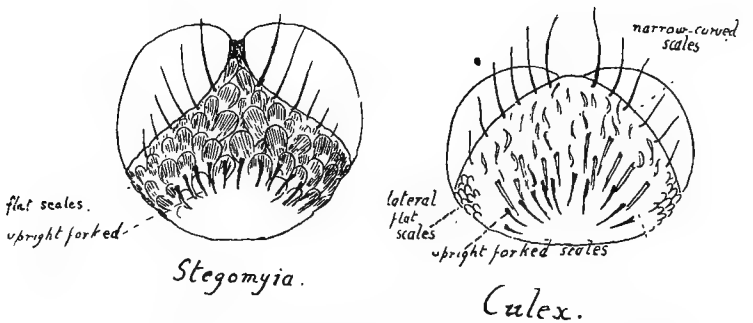


Fig. 49. *Head scales of Stegomyia (left) and Culex (right)*

S. fasciata.—Transmits yellow fever.

1. Tarsi *basally* banded white.
2. Proboscis unbanded.
3. Thorax. A pure white broad curved band on each side, and two median pale parallel lines.
4. Ungues of ♀ toothed.

Genus 6. *Theobaldia.*—Palpi in ♂ clubbed as in the *Anophelinae.* Palpi in ♀ five-jointed, apical joint mammilliform, wings in both sexes densely

scaled, collected into spots, thus forming a *spotted wing* group of mosquitoes. Species, five.

Genus 7. *Lutzia.*—Resembles *Theobaldia.*

Palpi in ♀ three-jointed, apical joint not mammilliform.

Palpi in ♂ not clubbed, three-jointed.

Wings spotted by scales similar to those of *Taeniorhynchus.* Species, one.

Genus 8. *Culex.*—Head scales: *narrow curved and upright forked*; laterally flat scales (Fig 49). Palpi in ♀, three-jointed. Third palpal joint usually as long or longer than the other two.

Wing: first sub-marginal cell longer and narrower than the second posterior. Posterior cross-vein nearer the base than the mid wing-veins. Scales small, lateral ones linear.

Scutellum: *narrow curved or spindle scales* (Fig. 49).

C. mimeticus has spotted wings. Species, very numerous.

Genus 9. *Gilesia.*—Related to *Culex* and *Stegomyia.*

(i) Scutellum *with small flat scales*, some spindle scales.

(ii) Head: broad, flat, spindle scales.

(iii) Basal joint of antennae, hairy and scaly.

(iv) Claws short and thick with a blunt tooth.

(v) Wing scales like those of *Taeniorhynchus.* Species, one.

Genus 10. *Lasioconops.*—

(i) Head scales as in *Culex.* Basal joint of antennae, a few scales.

(ii) Abdomen: *large projecting flat scales with deeply dentate apices*, giving these mosquitoes a ragged appearance. Species, one.

Genus 11. *Melanoconion.*—Distinguished from *Culex* by the dense broad scales on the costa and apex, and by the black spine-like scales along the upper border. Small dark mosquitoes. Species, six.

Genus 12. *Grabhamia.*—Allied to *Culex* and *Taeniorhynchus*. Palpi in ♀ four-jointed. Apical joint minute. Penultimate long and thick. Wing scales not so long or dense as in *Taeniorhynchus*. Scales mottled. Wings short and stumpy. Legs mottled and spotted. Species, ten.

Genus 13. *Acartomyia.*—Allied to *Culex* and *Grabhamia*. Distinguished from *Grabhamia* by having *flat irregularly disposed scales* all over the head, from *Culex* in the ♂ palpi. Two terminal segments and the apex of the antipenultimate swollen. Terminal segment club-shaped. Ragged appearance of head, well marked. Species, one.

Genus 14. *Taeniorhynchus.*—Palpi five-jointed in ♂, the fifth segment minute. Characterized by the wing scales. They are *thick elongated scales ending with a broad sloping convexity or blunt point;* median linear scales often absent, proboscis usually banded. Species, about sixteen.

Genus 15. *Mansonia* = *Panoplites.*—Palpi four-jointed in ♂, more than one-third the length of the proboscis. Characterized by *wings densely scaled along the veins with broad asymmetrical flat scales. No median scales.* The genus resembles *Aedeomyia*, but the palpi in the ♂ are long in members of this genus, short in the *Aedomyina*. Species, eight.

Genus 16. *Finlaya.*—Three ventral abdominal scale tufts. Scutellum, four median bristles. Wing scales, large and broad, pyriform. Species, two.

Genus 17. *Howardia.*—Resembles *Aedes*, but scutellum has only four bristles. Palpi, four segments, apical, one minute, not mammilliform. Species, two.

Genus 18. *Skusea.*—Head, *flat scales only*. Anterior and posterior forked cells densely scaled. Palpi in ♀, three segments. Scutellum, six bristles and narrow curved scales. Species, three.

Genus 19. *Katageiomyia.*—Differs from *Stegomyia* in having (1) narrow-curved scales on back of head; (2) narrow-curved scales on lateral lobes of scutellum. Species, one.

Genus 20. *Macleaya.*—Differs from *Stegomyia* in having (1) narrow-curved scales on the centre of the head, and (2) on the lateral lobes of the scutellum. Species, one.

Genus 21. *Hodgesia.*—Resembles *Stegomyia.* Differs in having the lateral vein scales long and almost overlapping those of neighbouring veins. Apices of scales have marked lateral spines. Palpi very short (one-jointed ?), possibly an *Aedeomyine.* Species, one.

Genus 22. *Scutomyia.*—Differs from *Stegomyia* in having narrow-curved scales on head. From *Macleaya*, in having scutellum entirely clothed with flat scales. From *Leicesteria*, in having all scutellar scales flat.

Genus 23. *Danielsia.*—Distinguished from *Macleaya* and *Scutomyia* by the narrow-curved scutellar scales, and from *Katageiomyia* by the long male palpi.

Genus 24. *Eretmapodites.*—Head, flat and

upright forked scales. Scutellum with flat scales on mid lobe. Palpi in ♀ four-jointed, in ♂ five-jointed, long, thin, and hairless. Last two hind tarsi in ♂ densely scaled, forming a paddle in one species. Species two.

Genus 25. *Hulecoetomyia.*—Head a marked median area of narrow-curved scales extending backwards. Scutellum with a rosette of flat and somewhat spindle-shaped scales on mid-lobe. The scutellar scales are *rounded* apically not pointed. Distinguished from *Stegomyia* by head and scutellar scales. Species, one. Malay.

Genus 26. *Leicesteria.*—Unpublished.

Sub-Family Joblotina

Genus 1. *Joblotia.*—Metanotum (= Post-scutellum) *with a tuft of chaetae and with flat scales.* Clypeus and base of antennae bristly. Second long vein carried nearly to the base of the wing. Second posterior fork cell (anal cell) very large. Mid cross-vein nearer the apex than the anterior (supernumerary). Wings densely scaled; scales shorter than in *Taeniorhynchus.* Species, one.

Sub-Family Heptaphlebomyina

Genus 1. *Heptaphlebomyia.*—Like *Culex*, but has a distinct scaled seventh vein. Species, one.

Sub-Family Aedeomyina

Genus 1. *Deinocerites* = [*Brachiomyia*].—Characterized by the ♀ antennae. Much longer than the proboscis. *Second segment as long as the*

three terminal segments. Antennae scaled. Antennae in ♂ pilose and longer than the whole body. Species, two.

Genus 2. *Aedes*.—Head, narrow curved scales form a *broad median line only*. Other scales flat. Scutellum, *narrow curved scales*, six bristles. Palpi in ♀, four segments, apical segment minute, mammilliform. Traces of a fifth segment. Species, two (Fig. 48).

Genus 3. *Aedimorphus*.—Head, mostly flat scales, narrow curved behind. Scutellum, *flat scales*, eight (?) bristles. Has no flat thoracic scales as *Uranotaenia*; probably a Culicine. Species, one.

Genus 4. *Verrallina*.—Head as in *Skusea*. Palpi, two segments only (trace of a third), apical segment large. Scutellum, four bristles and narrow curved scales. Species, three.

Genus 5. *Ficalbia*.—Intermediate between last two and next genus. Head scales, *no narrow curved*, almost entirely flat. Scutellum, *flat scales* as in *Uranotaenia*, but *thoracic scales narrow curved*. Palpi, two segments. Species, two.

Genus 6. *Uranotaenia*.—Head, *flat scales*, upright forked may or may not be present. Scutellum *flat scales*. Thorax, *narrow curved and flat scales*. Wings, small forked cells. Metallic scales at the base of the wings. Related to *Aedes*, but more brilliant (metallic) and stouter mosquitoes. Species, fourteen.

Genus 7. *Mimomyia*.—Resembles *Uranotaenia*. Has no flat scutellar or thoracic scales. Forked cells larger than *Uranotaenia*. No metallic scales at the base of the wings. Species, two.

Genus 8. *Aedeomyia*.—Allied to *Aedes*. Distinguished by (i) head scales upright, fan-shaped;

clypeus scaly; (ii) thorax, broad, flat spindle scales; (iii) scutellum, broad flat scales; (iv) legs, densely scaled; (v) wings, densely scaled as in *Mansonia*, also with long lateral scales. Species, three.

Genus 9. *Haemagogus.*—Related to *Aedes*, but palpi five segments. Head covered with flat scales. Brilliant metallic (blue) mosquitoes. Species, two.

Genus 10. *Wyeomyia.*—*Chaetae on the post-scutellum.* Head, flat scales. Thorax, spindle and flat scales. Scutellum, flat scales. Palpi short. *Proboscis not as long as whole body.* Species, two.

Genus 11. *Phoniomyia.*—Resembles *Wyeomyia*, but distinguished by (i) wing scales broad, lateral scales as in *Taeniorhynchus*; (ii) proboscis longer than the whole body. Species, two.

Genus 12. *Dendromyia.*—Resembles *Wyeomyia*, distinguished by (i) scutellar scales small, flat, rounded apically; (ii) wings more densely scaled than in *Phoniomyia*, scales *Taeniorhynchus*-like; (iii) proboscis moderately long. Species, five.

Genus 13. *Runchomyia.*—Allied to *Dendromyia.* Characterized by (i) frons projecting as a blunt spine; (ii) proboscis as long as the body in ♀; (iii) ventral apical tuft of bristles; (iv) wings covered with rather broad scales. Species, one.

Genus 14. *Sabethes.*—Distinguished from *Wyeomyia* by the *asymmetrical wing scales.* One or more *legs with dense paddle-like structures* in both sexes. Mid cross-vein nearer the apex than the anterior. Posterior nearer the apex than the mid in the ♂. Third long vein carried through into the basal cell. Brilliant metallic mosquitoes. Species, four.

Genus 15. *Sabethoides.*—Closely resembles *Sabethes.* Distinguished (i) by much smaller palpi; (ii) unpaddled legs. Species, one.

Genus 16. *Goeldia.*—Post-scutellum with *chaetae and scales.* Wing scales as in *Runchomyia*, dense, elongated. Wing venation as in *Culex.* Proboscis short and thick; not as long as body. Palpi in ♂ one-third length of the proboscis. In ♀ quite short. Species, one.

Genus 17. *Limatus.*—Characterized by the proboscis bent in the middle; densely scaled at the bend. Species, one.

Sub-Family Corethrina

Genus 1. *Corethra.*—First tarsal segment longer than the second tarsal.

Genus 2. *Mochlonyx.*—First tarsal segment shorter than the first tarsal.

LITERATURE

A Monograph of the Culicidae. Vols. I-III. F. V. Theobald. *Brit. Mus. Nat. Hist.* The data in this chapter have been taken almost entirely from Theobald's work.

Chapter XVI

THE CLASSIFICATION AND IDENTIFICATION OF THE ANOPHELINAE

It is by no means an easy matter to fix definitely the *species* of an *Anopheline*, and yet the identification of species is essential in connexion with malarial studies. It does not suffice merely to ascertain that *Anophelines* are present in any given locality, but it must be clearly made out what the *species* are. It is, indeed, only by accurately observing the relation of *Anophelines* to malaria that we can hope for the explanation of many of the difficulties surrounding the anomalous distribution of endemic malaria. In a later chapter the extreme importance of the species of *Anophelines* in this connexion will be evident. In the description of the habits of *Anophelines*, their breeding places, occurrence apart from man, etc., it is no longer sufficient to ascribe these to the whole genus, but they must be ascribed to the actual species involved. The study of the *Anophelines* from the point of view of classification and identification is, therefore, of importance.

Classification of Anophelinae

Theobald has sub-divided the old genus *Anopheles* into twelve new genera. These genera

comprise over eighty different species. The assigning of an *Anopheles* (old sense) to its proper genus, simplifies, therefore, very much the ultimate determination of the particular species. THEOBALD'S classification is the following :—

Thorax and abdomen with hair-like curved scales	Prothoracic lobes simple ; no flat head scales	Wing scales lanceolate	*Anopheles*
		Wings scales mostly long and narrow-	*Myzomyia*
		Wing scales partly large and inflated	*Cyclolepidopteron*
	Prothoracic lobes mammillated : median flat scales	Wing scales lanceolate	*Stethomyia*
Thorax with narrow curved scales ; abdomen hairy	Wing scales small, lanceolate or narrowed		*Pyretophorus*
Thorax with hair-like curved scales ; some narrow curved ones in front ; abdomen with apical lateral scale tufts and scaly venter, no ventral tuft			*Arribalzagia*
Thorax with hair-like curved scales ; abdominal scales on venter only with a distinct ventral apical tuft, no lateral tufts			*-Myzorhynchus*

Thorax and abdomen with true scales	Abdominal scales with dorsal patches of small or narrow flat scales; thoracic narrow curved or spindle-shaped	*Nyssorhynchus*
	Abdomen nearly completely scaled with irregular scales and with lateral tufts	*Cellia*
	Abdomen completely scaled with large flat scales, as in *Culex*	*Aldrichia*

These features are shewn in the accompanying diagram (Fig. 50). The thoracic scales in *Cyclolepidopteron* are not sufficiently hair-like (THEOBALD).

Place the mounted or unmounted mosquito under a low power of the microscope, and determine carefully the characters of the hairs or scales on the head, thorax and abdomen. By this means the *Anopheline* is assigned to its proper genus.

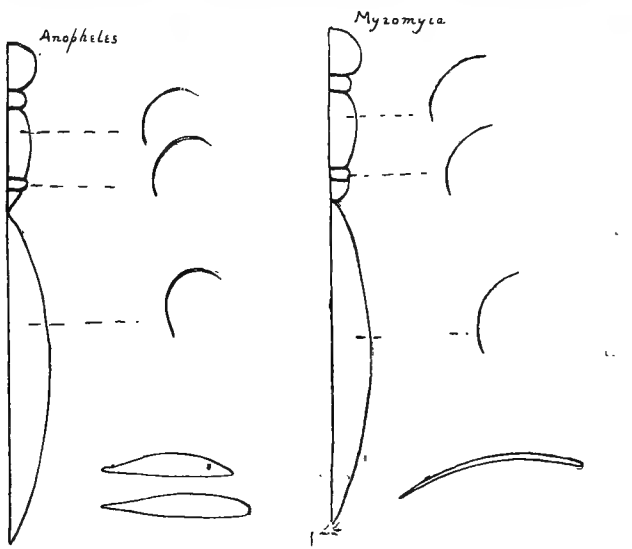


Fig. 50 *Thoracic, Scutellar, Abdominal, and Wing Scales of the Anophelinae* (after THEOBALD)

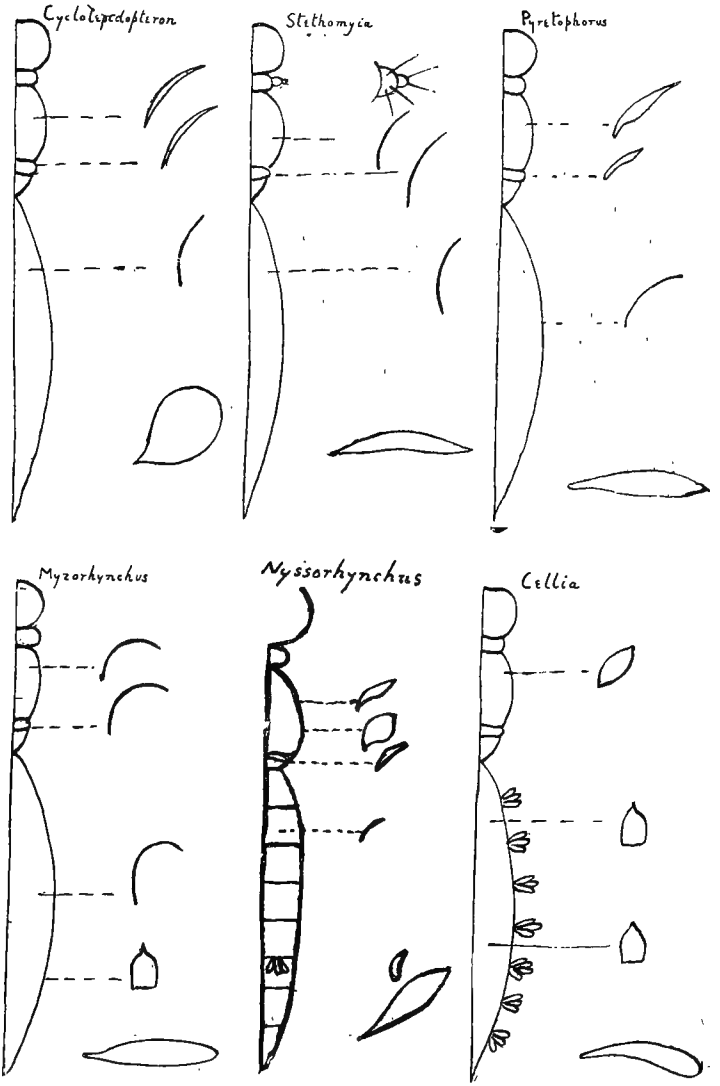


Fig. 50 (*contd.*)—*Thoracic, Scutellar, Abdominal, and Wing Scales of the Anophelinae* (*after* THEOBALD)

The Differentiation of Species

Many features are of value in determining the species.

1. *The Wings* :—

(*a*) They may shew areas of dark scales on the costa, auxiliary, and first longitudinal veins, producing the main spots of the wing.

(*b*) Small areas of scales on the second to sixth long veins; less dark and distinct than the large costal spots.

(*c*) Pale areas on the wing fringe.

(*a*) The markings on the wing are fairly constant in each species, but variations occur, so that the spot may be longer or shorter, giving the wing a darker or lighter aspect in the same species. Thus in *N. stephensi* the following variations in the second costal spot may be encountered (Fig. 51). Especially does this variation occur in the wing of males. The costal spots may also be confluent. They may depart from their typical shape, as is frequently seen in the T spot of *M. rossii*.

(*b*) The smaller spots on the wing field along the course of the veins are also useful for determining species. Thus *M. leucophyrus* has six spots on the sixth long vein, while *M. elegans* has only four. The extent to which the third longitudinal vein is scaled is also of specific importance (Fig. 52).

(*c*) The wing fringe has at the points, where the long veins cut the margin, a variable number of light areas. Thus *A. punctipennis* has only one pale area, while *A. pseudo-punctipennis* has many. Another example of this means of distinguishing species is given in the figure (Fig. 51).

2. *Leg Markings* :—

(*a*) Uniformly coloured as in the second division of *Myzomyia*.

(*b*) Speckled or banded, chiefly in the genera *Nyssorhynchus* and *Cellia*. The banding of the legs is of great importance in distinguishing the

species (Fig. 53); thus (1) banded tarsi, *e.g.*, *N. maculatus*; (2) tarsi pure white, *e.g.*, *N. fuliginosus*, *N. jamesii*.

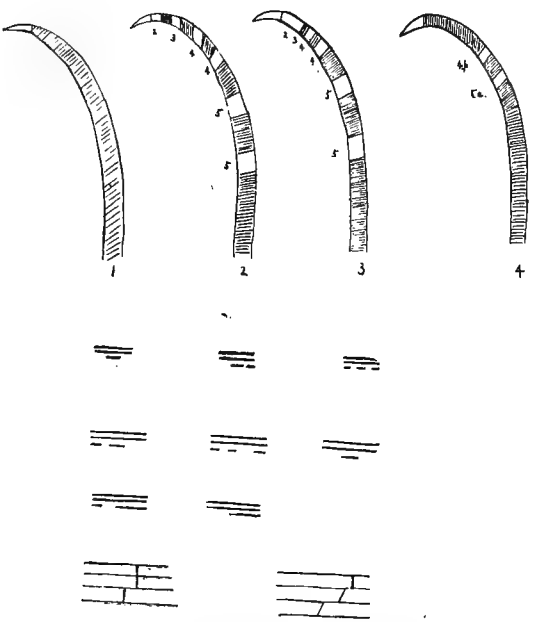


Fig. 51.—*Wing fringes of* (1) *M. rhodesiensis*, (2) *M. funestus*, (3) *M. listoni*, (4) *M. culicifacies*

Variations in Wing Spots of M. rossii, N. stephensi, and P costalis

Variations of Cross-veins of M. rossii

3. *Palpi*:—Similarly the bands or collection of white scales on the palpi is a convenient means of separating the members of a particular genus (Figs. 52, 53). It should not be forgotten that in these two characteristics there is a certain amount

of variation, possibly seasonal, and a slight difference in the bands on the palpi and legs is not sufficient in itself to constitute a difference in species.

Other characteristics that are useful for the determination of species are the male genitalia, and the character of the ungues in the male, whether having one or more teeth. The position of the cross-veins has also been used, but this is so variable in the same species that it has little value.

Characters of the Larvae and Ova.—In the *Anophelina*, as in the rest of the *Culicidae*, this is a most important means of differentiation. Mosquitoes that otherwise are almost indistinguishable are readily separated by their larvae being different.

One precaution must be taken. It must be quite certain that it is the larva of the mosquito in question that is being examined. The easiest way to make sure of this is to carefully examine the larva first, and then to hatch out the mosquito and then examine it. The examination of the larvae is considered later.

Genus 1. *Anopheles.*—Wings unspotted or slightly spotted. Mostly belong to temperate climes or hill districts.

A. bifurcatus and *A. maculipennis* transmit malaria. *A. punctipennis* has experimentally given negative results.

Costa Uniform, Wings spotted.

1. *A. maculipennis.*—Wing with four spots; apex of first tarsal joint spotted. Europe.

2. *A. crucians.*—White spots on brown

veins. Three black spots on sixth vein. Costa uniformly dark; tarsi unbanded. North America.

3. *A. eiseni.*—Resembles *A. maculipennis.* Apices of hind tarsi, yellowish. Gautemala.

Costa Spotted

4. *A. punctipennis.*—Two yellow spots, one at the apex; the second on the apical third. One fringe spot. North America.

5. *A. pseudo-punctipennis.*—Wings as in previous species; but wing fringe with several yellow spots. North America.

6. *A. punctipennis* (Var. A).—Three costal spots.

7. *A. franciscanus.*—Costa, a pure yellow apical spot; a second spot about middle of costa.

8. *A. gigas.*—Costa, two large black spots. Length, five to six mm. A large hill species. India.

9. *A. lindesayii.*—Costa black, apical white spot. Femora have a characteristic broad median white band. A hill species. India.

Wings Unspotted

10. *A. bifurcatus.*—Thorax. Golden hairs arranged so as to leave two broad bare lines on the front. *Abdominal hairs golden.* Europe.

A. walkeri is regarded by THEOBALD as identical with this.

11. *A. algeriensis.*—Lateral vein scales longer and finer than in *A. bifurcatus.* Anterior and posterior cross-veins in same line in both sexes. In *A. bifurcatus* the posterior is internal in ♀, the anterior in ♂.

12. *A. nigripes.*—A black mosquito. No bands on tarsus. Europe. America.

13. *A. immaculatus.*—Ash-grey in colour. Slight apical bandings to tarsi. Palpi and proboscis lighter at apex. Ennur, Madras.

14. *A. aitkenii.*—Uniformly dark. No markings on palpi or legs. Bombay Presidency.

15. *A. stigmaticus.*—Light brown; tarsi unbanded. Australia.

16. *A. annulipalpis.*—Tarsi banded. Last tarsus pure white. S. America.

Genus *Myzomyia.*—To this genus belong those species which are associated in the tropics with the most severe endemic malaria, *e.g.*, *M. funesta* in Africa and *M. listoni* and *M. culicifacies* in India. The group includes, however, several species, one at least of which has, as far as our knowledge extends, no power of transmitting malaria in nature, viz., *M. rossii.*

The malaria transmitters form a natural group: they are small, dark mosquitoes, with unbanded legs, and they breed in fresh natural waters, *e.g.*, streams, river beds, etc.; whereas we also have in the group domestic mosquitoes, *i.e.*, those that breed in foul pools about houses. *M. rossii* is the type of this class.

Whether in this genus any others than the three mentioned above convey malaria there are at present no facts to shew, and the larval characters of only the Indian species are at present known.

The type species is *M. funesta*, which is a typical spring and fresh-water breeder. It is noteworthy that *M. funesta* is associated with a higher malarial endemicity than *P. costalis*, which is a typical domestic mosquito breeding in foul pools.

Group I

Small dark mosquitoes breeding in natural waters.

1. *M. funesta.*—Costa: six white spots. Basal spots with pale interruption. Wing fringe: pale spots at ends of all the veins, except sixth. Palpi: three bands, the basal one further from the middle one than the apical. A variable species: third long vein may be dark. Resembles *listoni* and *rhodesiensis* (Fig. 51).

2. *M. listoni.—Third long vein light.* Wing fringe, four or more light spots (Fig. 51). Palpi, two broad apical bands further apart than in *funesta*, one narrow basal. Basal portion of costa uniformly black (characteristic). Attitude, *Anopheline*-like. Associated with high endemic index in the Duars, Bengal. Larval characters: antennae with simple hair. Clypeal hairs simple. Palmate hairs on thorax and on all abdominal segments.

3. *M. aconita.* — *aconita* = unspeckled, because at the commencement of the third long vein the usual dark spot is absent. Palpi, four bands. Costa, four spots, light interruption in basal spot. Fringe, several pale areas. Anterior forked cell much longer and narrower than posterior. Differs from *listoni* in palpi. Sumatra, Java.

4. *M. culicifacies.—Third longitudinal vein dark.* Wing fringe, three spots at most. Palpi, three equal bands, two at the joints, one at the apex. *Attitude, Culex-like.* Associated with high endemic index of malaria in the Punjab and Madras. Larval characters as in *M. listoni.* Ova: floats do not touch margin of upper surface (Type 1) (Fig. 54).

5. *M. leptomeres.*—Base of first long vein white. Anterior forked cell much longer and narrower than posterior. Costa, two spots, thus differing from *Hebes*. Fringe, pale areas at all the veins.

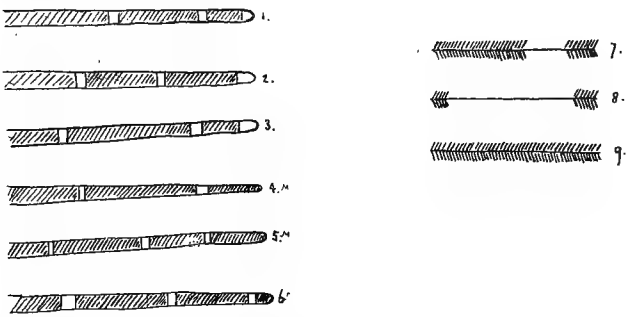


Fig. 52

Palpi of M. funesta (1), *M. listoni* (2), *M. culicifacies* (3), *M. rhodesiensis* (4), *M. hispaniola* (5), *M. turkhudi* (6), *Third Long Veins of M. funesta* (7), *M. listoni* (8), *M. culicifacies* (9)

6. *M. hebes.*—*Hebes* = inconspicuous, a small species resembling *rhodesiensis*. Wing costa, four spots; wing fringe, seven light areas. Vein six, one long spot. Palpi, first and second segments covered with white scales. End of third segment is dark, fourth segment quite white. Distinguished from *M. rhodesiensis* by palpi and wing fringe. East Africa.

Group II

Larger species than the above. Generally lighter. Wings not so covered with dark scales.

Habits: Domestic species breeding in foul puddles, etc., near houses

Larval characters (*M. rossii*) : Antenna without branched lateral hairs. Clypeal hairs simple. Palmate hairs second to seventh segments, and often rudimentary hairs on thorax and first and second abdominal segments. Ova (type 2), *i.e.*, floats, touching margin of anterior surface.

7. *M. albirostris.*—Characterized by the banded proboscis; pale scaled to about half its length. *N. deceptor* has also a banded proboscis. Malaysia. Length, two to five mm.

8. *M. longipalpis.*—Palpi long, thin; three narrow white rings; wing costa, black, four almost equal yellow spots; wings mostly brown scaled; hind legs only, banded; narrow apical and basal yellow bands. British Central Africa. Length, three mm.

9. *M. ludlowii.*—Palpi, apex broad white band; a second small one close to it; a third basal band. Wing costa, four large spots, one or two small basal. Legs, femora, tibiae and metatarsi, especially in hind legs, *spotted* with yellow. Tarsi, broad apical and basal pale banding, especially in hind legs. Philippine Isles. Length, four to five mm.

10. *M. rossii.*—Probably = *A. vagus* (Dönitz). Sumatra. Palpi, somewhat like those of *M. ludlowii*, but easily distinguished; the apical white band is broader; the second band is much nearer the base than in *M. ludlowii*, so that the black area between is longer. Wings, four spots, and some basal spots. The second large spot has the characteristic *T. shape*, but is very variable. Tarsi, slight pale apical and basal bands to some of tarsi. India, Malaysia.

11. M. *lützii*.—Characterized by the linear ornamentation on the thorax, and marked bands (five in number) on the fore and mid metatarsi. Wings, three distinct pale spots; two smaller ones, 3 to 3·5 mm. Rio de Janeiro.

12. *M. elegans*.—Possibly = *M. leucosphyra* (Dönitz). Leukos = white, sphyron = ankle-joint. Palpi, four white bands. Wing costa, four large black-scaled areas, three small. Wing fringe, six pale interruptions. Legs speckled with white scales. Femora and tibiae speckled in hind legs. Characterized by a large tibio-metatarsal band on the hind legs. Resembles *N. stephensi*; differs in the palpi; has four, not three, spots on the sixth longitudinal vein. Differs from *N. leucosphyrus* in having four, not six, spots on the sixth vein. Possibly belongs to *Nyssorhynchus* genus. India.

13. *M. tesselatum*.—Costa, four large, four small spots. Fore tarsi apically and basally banded. Mid and hind tarsi apically only. Thorax, two dark spots in front and a dark area near the scutellum. Malay.

14. *M. punctulata*.—Costa, four large spots and numerous dark and white spots. Malay; closely resembles the former.

15. *M. leucosphyra*.—Related to the two former. Distinguished by prominent tibio-metatarsal band and by the prominent median dark spot on costa. Sumatra.

16. *M. impuncta*.—Costa, four small dark spots. Fringe spotted. Sixth vein, three spots. Relationship doubtful; not fully described. Egypt.

Group III

Medium size dark mosquitoes. Apex of palpi black.

17. *M. turkhudi.*—Palpi, *apices black*, the band not so broad as in Hispaniola; third long vein mostly dark, but varies; pale interruption in basal costal spot. India. Larvae resemble *Culex*. Ova, very peculiar, type 3 (*vide* p. 222).

18. *M. hispaniola* (Theo.) Spain. Third longitudinal vein, mostly pale yellow, except at the base and apex. Wing fringe with spots, except where lower branch of fifth and sixth join the costa. Basal portion of costa uniformly black.

19. *M. rhodesiensis* (Theo.) Rhodesia.—Third longitudinal vein dark. Palps with only two conspicuous bands. The palpi are much longer and thinner than in *M. funesta*. The veins are all dusky scaled. Base of the costa black. In *M. funesta* there is a white interruption. Wings, costa three small white spots and a yellow apical spot. *Fringe unspotted*, except an apical spot (Fig. 51).

Genus *Cycloleppteron.*—Wings with numerous large inflated scales; collected in patches or irregularly disposed.

Larval Characters (Theobald).—Antenna without lateral branched hair. Clypeal hairs simple. Palmate hairs, six pairs. Lanceolate.

1. *C. grabhamii.*—Palpi unbanded. Jamaica.

2. *C. mediopunctatus.*—Palpi banded, black and gold. Brazil.

Genus *Stethomyia*(στηθος = breast).—Head with a median patch of flat scales. Palpi very thin.

1. *S. nimba.*—Wings unspotted. Thorax brilliant silvery median band. S. America.

2. S. *fragilis*.—Wings unspotted. Thorax greenish-brown. Malay.

Genus *Pyretophorus* (πυρετοφορος = fever producing).

1. *P. superpictus*. — Larva has branched frontal hairs. Wing costa, four distinct spots and additional basal spots. Legs dark brown with apical white tarsal bands. Palpi, apical white band and two narrower bands, the second slightly nearer the first than the second. Europe and Mashonaland.

2. *P. costalis*.—Wing costa, four large and two small spots. On the first long vein there are two broken spots, under the two middle large spots, giving a pattern only found in *P. marshallii* besides. This arrangement is, however, variable. Femora and tibiae mottled with yellow. Tarsal banding involves to some extent both sides of the joints. Palpi, three narrow bands at the joints. West Africa, Uganda.

P. costalis v. melas. Pale costal spots are absent, but the arrangement on the first long vein is the same. *P. costalis* conveys malaria.

3. *P. cinereus*.—Wings, three white spots on the black costa; wing fringe brown, with yellowish patches. Palpi, four white rings; legs very thin, *jet black*; *apex of femora and tibiae* pure white spot; apices of fore and hind metatarsi, minute apical bands; length five mm. South Africa. B.C.A.

4. *P. marshallii*. — Wing markings very similar to *P. costalis*, distinguished by the palpi; two broad apical bands; one small basal one; apex white. Mashonaland.

5. *P. jeyporensis*.—Costa black, two large

white spots on the apical half, and two small ones at the base. Fringe spotted. Palpi black, with three white bands, the broadest apical. Apex white. Madras.

6. *P. chaudoyei.*—Wing, six black costal spots ; legs unbanded, a pale knee and tibial spot on the hind legs. Palpi, apex black and with three narrow white bands. Algeria.

7. *P. palestinensis.*—Wings, five large black costal spots and five yellowish ones of unequal length. Legs brown, a pale spot at junction of tibiae and femora, and tibiae and metatarsi. Palpi, three pale bands, the apex white. Differs from *P. chaudoyei* in the form of the large costal spot in the apical half of the sixth long vein being dark, and in presence of a deep brown medium thoracic line. Resembles closely *P. superpictus*, but the legs are unbanded ; spotted wing fringe, and uniserrated large fore ungues in the male.

8. *P. minimus.*—Wings, three nearly equal creamy spots and an apical spot. Fringe, spotted except at the sixth vein, thus distinguished from *P. superpictus*. Legs, no trace of banding or pale knee spots. Mid ungues straight ; fore ungues curved. Hong Kong.

9. *P. atratipes.*—Clypeus, trilobed, costa uniformly black, six spots on the veins. Australia.

10. *P. merus.*—Resembles *P. cinereus*, but distinguished by the spotted and banded femora and tibiae, also by its broader fringe spots.

11. *P. pitchfordi.*—Three main costal spots, two basal interruptions. Sixth vein two spots. Thorax broad white median band. Zululand.

Genus *Arribalzagia.*—Related to *Myzorhynchus*, but has no distinct lateral scale tufts.

1. *A. Maculipes.*—Hind and mid legs much banded and speckled. Almost certainly transmits malaria (LUTZ).

Genus *Myzorhynchus.*—μύζω to suck, ῥύγχος, proboscis.

Palpi densely scaled in the ♀, also the proboscis. These are 'wild' mosquitoes found in situations remote from the dwellings of man. They breed in swamps and large bodies of water, especially those containing weeds. They do not usually frequent houses. *M. sinensis* is, however, attracted by light. They feed readily on human blood when occasion offers.

(A) Palpi *unbanded.*—Last hind tarsus brown:

1. *M. barbirostris*, one fringe spot. India, Malaysia.

1A. *M. pseudo-barbirostris.* — Distinguished from former by its speckled femora and tibiae. Philippine isles.

2. *M. bancroftii*, several fringe spots. Australia.

3. *M. umbrosus*, no fringe spot, only one costal spot. Malaysia.

Last hind tarsus white:

4. *M. albotaeniatus*, other hind tarsi much banded. Malay.

Last two hind tarsi white:

5. *M. coustani.* Madagascar.

(B) Palpi *banded.*—Last hind tarsus brown:

6. *M. sinensis*, wing fringe, one pale spot. China.

Palpi banded, last hind tarsus brown, wing fringe unspotted. Apex of palpi white:

7. *M. vanus*, costa two yellow spots, wings distinctly spotted. India, Malay, Philippines, etc.

8. *M. pseudopictus*, wings without prominent spots. Europe.
9. *M. minutus*, wings, two white costal spots. Punjab.

Apex of palpi black:

10. *M. nigerrimus*. India.

(C) Palpi banded, last hind tarsi white:

11. *M. mauritianus*, two hind tarsi white.
12. *M. paludis*, three hind tarsi white. Africa.
13. *M. ziemanni*.—Two and two-thirds hind tarsi white. Africa.

Genus *Nyssorhynchus*.—νύσσω, to puncture, 'bite,' ῥύγχος, proboscis.

Mosquitoes mostly with legs spotted and banded, or one or more tarsal segments pure white. They are both domestic and wild mosquitoes. They breed chiefly in pools with algae, and in lakes. *N. Stephensi* will, however, breed in pots and tins.

A. Legs *unspotted*. Larva with outer pair of clypeal hairs markedly branched. Leaflets of palmate hairs with long filament.

1. *N. fuliginosus*. — Probably = *leucopus*, Dönitz. Costa, four large and one or more small pale spots. Femora, pale band near the apex. Hind tarsi, three and one-fifth pure white. Palpi, two narrow white bands, apex white (Fig. 53). India.

2. *N. karwari*.—Legs not speckled, one and one-fourth hind tarsal joints white. In fore and mid legs, tarsal joints, except fourth and fifth, have apical white band. In hind legs, tibia, first and second tarsus, have apical bands, third and fourth have both apical and basal bands; the fifth is white. Palps, four white bands, two terminal

broad and equal, two basal narrow, apex white. India.

B. Legs markedly *spotted*. Larva with simple or slightly branched frontal hairs. *Filaments* of *palmate* leaflets *very short*.

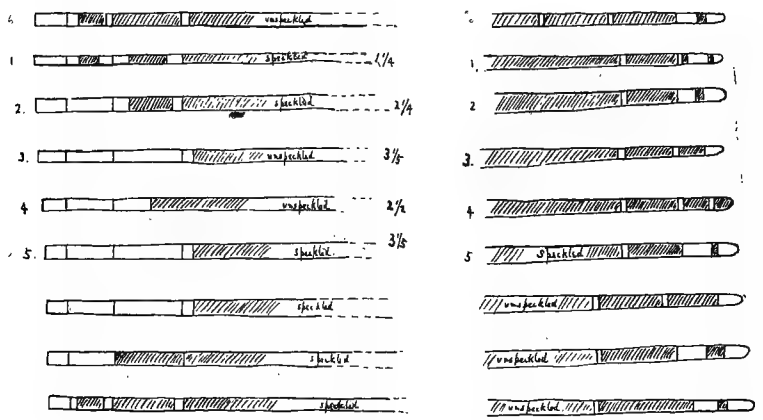


Fig. 53

Hind Tarsi and Palpi of N. karwari (6), *N. maculata* (1), *N. theobaldi* (2), *N. fuliginosus* (3), *N. fuliginosus* (*nagpurensis*) (4), *N. maculipalpis* (5), *N. jamesii* (7), *N. pretoriensis* (8), *N. willmori* (9)

3. *N. stephensi*.—Syn = *A. metaboles*, THEOBALD. Tarsus without any segment of hind leg white. Legs brown, speckled with white; joints of fore and hind tarsi with apical spots. Wing costa, four broad prominent black spots and two smaller basal ones. The third largest spot has three typical spots beneath it on the first long vein. Fringe dark, with pale areas. Palpi, two broad apical white bands, one narrow basal; white scales between the last two bands. India.

4. *N. maculatus.*—Resembles *N. stephensi*, but is easily distinguished by tarsi. Wings, costa four large and two small basal spots. Under the third largest spot are three black spots on the first long vein. Legs, with femora, tibiae, and metatarsi with broken creamy bands and spots. Fore and mid tarsi with narrow yellow bands. Hind tarsi with broad white ones. *Last segment pure white.* Palpi, four bands, two unequal white apical bands, then a small white one, and a second towards the base.

5. *N. theobaldi.*—Wings, jet black with the costa interrupted by five white spots and an apical spot. Legs, brindled with white scales and a large sub-apical white patch on the femora. Two and a quarter hind tarsi pure white, then a black band, then a small white one. Palpi, three white bands, apex white, two apical bands equal, a third narrow.

A Nagpur variety which THEOBALD considers may be a distinct species has two-and-a-half hind tarsi white and the tips of the palpi black. India.

6. *N. maculipalpis* = *A. jamesii* in *Reports to Royal Society*, STEPHENS and CHRISTOPHERS. Wing, costa black with five white spots. Legs, black spotted with white, last three hind tarsi pure white, and apex of next. Palpi, two broad white bands, one apical, a third narrow one towards the base. The rest of the palpi spotted with white. Length, 5·5 mm. India, Africa.

N. maculipalpis, v. Indiensis.—Hind legs not quite so banded as in the type. Some variation in wing markings.

7. *N. jamesii.*—Costa, four large and two

small dark spots. Legs brown, fore femora and tibiae more or less *spotted*. Hind legs, femora and tibiae with an apical white spot, last three tarsi white, and apex of next (Fig. 53). The first tarsal segment of the fore leg has an indistinct median band. Palpi black, with white rings and white apical joint, closely related to *A. fuliginosus*, but easily distinguished. Length, 3 to 3·5 mm. *Cp. A. maculipalpis*, length, 5·5 mm.

8. *N. pretoriensis*.—Clypeal hairs of larva simple. Palps not mottled, otherwise like *N. maculipalpis*. The two white apical bands are further apart. Second hind tarsus has a small black patch near its base. Metatarsus, mottled with white and black, and has a broad white apical band like the first tarsal. The last two hind tarsi only white.

9. *N. deceptor* (Dönitz). Sumatra. *Deceptor* because very like *M. punctulata* or *leucosphyra*. Terminal half of *proboscis* white. Terminal half of palpi white, with a narrow black ring at the commencement of the third and fourth joint. The ring around the light end of the second joint, possessed by *M. punctulatus*, is wanting. Legs similarly marked as in *leucophyrus*, excepting the hind legs, which have only a small light spot at the end of the tibia, and not a broad white band.

10. *N. willmori* (James). Punjab, Kashmir. Wings, four large and three small black areas. Palpi, three white bands, the two terminal ones are equal and broad, the third narrow and basal. Legs, dark brown, thickly speckled with white spots. The last tarsal segment of hind leg pure white.

11. *N. annulipes*. Femora and tibiae banded.

Tarsi have apical and basal bands. Palpi: apices of last three segments banded. First and second segments have white scales. Australia.

12. *N. masteri.* Distinguished from former by the proboscis having pale apex in ♀. It is also smaller. Australia.

13. *N. philippinensis.* Pale spot at apex of tibiae. Three and a fifth hind tarsi white. Palpi golden brown, three bands.

14. *N. nivipes.* Wing resembles that of *N. stephensi*, but legs not speckled. Resembles *N. maculatus* but has three and a fifth hind tarsi white. Malay.

Genus *Cellia.*—Wings densely scaled. Palpi of ♀ densely scaled. Easily recognized by the dense coating of irregular scales.

Larvae (*C. pulcherrima*). — Antennal hair simple. Clypeal hairs, outer pair branched. Ova, type 2.

Last hind tarsi white:

1. *C. pulcherrima*, $3\frac{3}{4}$ white. Punjab.
2. *C. bigotii*, 3. Chili.
3. *C. pharoensis*, $1[\frac{1}{3}]$. Egypt, Gambia.
4. *C. argyrotarsis*, $\frac{1}{2}$. Palpi, three bands, deep black basal band to last tarsus. Acts as a host for *F. nocturna* (VINCENT). West Indies.
5. *C. albipes*, $\frac{1}{2}$. Palpi, two bands. West Indies, Brazil.

Last hind tarsi yellow:

6. *C. kochii*, 3. Malay.

Last hind tarsus black:

7. *C. squamosa.* Africa.

Genus *Aldrichia.*—Wings much as in *Myzomyia*, for which genus it was originally mistaken.

1. *Al. error.*—Resembles *M. rossii.* Easily distinguished by the abdominal scales. India.

Genus *Lophoscelomyia.*—Resembles *Nyssorhynchus*, but differs in having (1) long curved hair-like scales on thorax instead of narrow curved and spindle scales; (2) dense apical tufts on the hind femora in both sexes.

1. *L. asiatica.*—Very small. Wings, five black spots and four yellow costal spots. Malay.

Genus *Christya.*—Thorax, hair-like scales and narrow curved laterally. Wings, dense lanceolate scales. Abdomen with characteristic dense long lateral apical tufts of hair-like scales.

1. *Ch. implexa.*—Fore femora with white spots and a prominent pale band. Hind tarsi black, apex of leg white. Uganda.

Addenda

A. wellcomei:—Palpi, basal third black, apical two-thirds ochreous. Apical band almost white, and a second white band. Wings resemble those of *A. gigas.* Costa, black with two yellow spots on apical half. Fringe, yellow spots at junction of all the veins. Sudan.

M. Nili. Resembles *M. funesta.* It is darker. Palpi have only one small apical band. Palpi and proboscis much shorter than the body. Sudan.

Chapter XVII

THE HABITS OF ANOPHELINES

Geographical Distribution

This is as yet far too imperfectly known for a close consideration of the subject to be of much value. We may consider, however, that the *Anophelinae* of some portions of Africa and some portions of India are known with a sufficient degree of exactitude to make a comparison of interest. The following is a complete list of the known *Anophelinae* :—

Europe

A. maculipennis
A. bifurcatus
A. nigripes
Pyr. superpictus
Mym. hispaniola (and Teneriffe)
Myzo. pseudopictus

Palestine

Pyr. palestinensis
M. pseudopictus
A. maculipennis
P. superpictus

North America

A. maculipennis (? European species)
A. nigripes
A. pseudo-punctipennis
A. bifurcatus
A. punctipennis
A. crucians
A. franciscanus

South America and West Indies

C. argyrotarsis
C. bigotii
Mym. lutzii
Arri. maculipes
A. eiseni
Cy. grabhami
Cyclo. medio-punctatus
Steth. nimba
C. albipes
A. annulipalpis

Africa

Mym. funesta
Myzo. paludis
Mym. rhodesiensis
C. squamosas
Pyret. superpictus (West Coast and Mashonaland)
Pyret. costalis
C. pharoensis (Gambia, Egypt)
Mym. longipalpis
Pyret. marshallii (Mashonaland)
P. pitchfordi (Zululand)
Ch. implexa (Uganda)
M. coustani (Madagascar)
A. welcomei (Sudan and Aden)
Pyret. cinereus (S. Africa)
Mym. mauritianas
Mym. hebes (E. A. and S. W. A.)
Pyret. merus
Nys. maculipalpis
Nys. pretoriensis
Myzo. barbirostris
A. algeriensis
Mym. impunctas (Egypt)
Pyret. chaudoyei (Sahara)
M. zeimanni (Cameroons)
M. rossii (Egypt ?)

India

Myzo. nigerrimus
Myzo. barbirostris
Nysso. maculipalpis
Nysso. jamesii
Nysso. stephensi
Nysso. theobaldi
Nysso. maculatus
Nysso. willmori
Nysso. karwari
Nysso. fuliginosus
Mym. rossii (everywhere)
Mym. listoni
Mym. leptomeres
M. indiensis
Mym. culicifacies
Mym. turkhudi
Mym. elegans
Myzo. minutus
Myzo. varus
Ce. pulcherrima (Punjab)
A. lindesayii (hill species)
A. immaculatus (very rare)
A. aitkenii (Goa)
A. gigas (hill species)
Ald. error
P. jeyporensis

Malaysia

Mym. aconita
Mym. ludlowii
Mym. albirostris
Mym. punctulata
Mym. kochii
Mym. rossii
Mym. listoni
Mym. vara
Mym. minuta
Myzo. barbirostris
Myzo. sinensis (China)
Nysso. maculatus
M. tesselata
S. fragilis
L. asiatica
P. minimus (China)
Nysso. deceptor
Mym. umbrosa
Mym. albotaeniata
Myzo. plumiger or [64] (Dönitz)
Mym. tenebrosa (Dönitz)
Mym. leucosphyra (Dönitz)
Nyss. leucopus (Dönitz)
Pyret. gracilis (Dönitz)
Mym. vaga (Dönitz)
N. nivipes

Australia

Myzorhynchus bancroftii	N. masteri
A. stigmaticus	P. atratipes
N. annulipes	

Philippine Isles

M. pseudo-barbirostris	N. philippinensis

Seasonal Prevalence.—Few observations have been made on this point. It would appear that there is one simple explanation which, at least in part, will account for the prevalence of a particular species at a particular time, and its appearance or disappearance at others. We have already shewn how selective the *Anophelinae* are in their choice of breeding-grounds, consequently, if at any time, *e.g.*, the dry season, a suitable breeding-ground does not exist, a particular species or genus of the *Anophelinae* may be absent.

Thus we found in Nagpur (India, C.P.), during the dry season, in those places where shallow puddles had dried up, *Mym. rossii* was rare, but it abounded wherever puddles still remained. Where weedy lakes existed, *Nyss. fuliginosus* was common, elsewhere rare. Now these conditions are directly dependent on the rainy season, and where vast areas of weedy swamp are formed during the rains, then *M. nigerrimus* prevailed, to disappear when the swamps dried up. In temperate climes, the temperature is, no doubt, an important factor, the onset of the cold weather causing a general hibernation. We may quote the following observations :—

Nyssorhynchus pretoriensis.—This species first appeared February 10, and gradually became more prevalent, superseding the other common

species *(Pyret. cinereus)* in April (THEOBALD, *Monograph of the Culicidae*, p. 99).

Pyretopherus chaudoyei.—'In the winter up to June one only sees *C. pipiens*. These then disappear and *Pyret. chaudoyei* appears' (THEOBALD, p. 70).

The incidence of *Pyret. chaudoyei* is said to be accompanied by the recrudescence of severe malaria, but it ought also to be definitely shewn that sporozoits are present in this species, and presumably that the sporozoit rate increases with the outbreak of malaria.

THE HIBERNATION OF ANOPHELINES

1. *Hibernation of the Adult Insects.* ANNETT and DUTTON describe the finding of *A. maculipennis* during the winter in England in cellars, lumber-rooms, and other cold places, but not in stables where the temperature is higher.

They observed the following points:—

(i) The attitude is peculiar, the insect lying quite flat upon the surface with its legs spread out. In this position the under surface of the thorax touches, or nearly touches, the wall.

(ii) Only females are found, and these are always fertilized, and have the spermatheca filled with spermatoza.

(iii) The insects are difficult to arouse, and very sluggish in any movements they make.

(iv) They do not feed unless the temperature is raised. If kept at a low temperature (provided the air is moist) they remain for weeks without feeding.

(v) If roused by raising the temperature they

feed readily, and the ovaries rapidly develop. Eggs are laid, and, in most cases, the female dies after their deposition.

The adults of *A. bifurcatus* do not hibernate, or only rarely.

2. *Hibernation of the Larva.*—The larvae of certain *Anophelines*, *e.g.*, *A. bifurcatus*, appear able to resist low temperatures, and are found even when parts of the water are frozen over. Under these circumstances they grow extremely slowly, if at all.

So also in the tropics, different species tide over the 'cold weather' in different ways. Thus JAMES found that *M. culicifacies* hibernated by means of larvae only, little or no growth occurring in these (t. 55° F. about); whereas *Ce. pulcherrima* and *N. fuliginosus* laid eggs which developed into pupae and imagines.

3. *Hibernation of Eggs.*—There is a certain amount of evidence to shew that eggs can survive for some months in moist earth, exposed to frost, etc. For young larvae have been found in fresh pools in the winter, under conditions that made it unlikely that the eggs had been deposited there on the appearance of water. The resistance of eggs to drying under a tropical sun is, however, practically nil.

MODE OF DISPERSAL OF ANOPHELINAE

There is no evidence existing at present to show that mosquitoes habitually disperse any considerable distance from their breeding-grounds. In fact, the evidence is completely against such a dispersal, and, broadly speaking, the *Anophelinae*

remain where they were developed, and in the native huts where they find abundant food.

That various accidental modes of distribution occur is equally certain, *e.g.* :—

1. On trains, boats, and even ocean-going steamers, they may be carried long distances, *e.g.*, from West Africa and South America to England, but it remains to be shewn that *Anophelinae*, thus introduced, ever effect a permanent habitation, even when the removal by this means is from one portion of the tropics to another.

2. Locally, streams and canals may carry larvae and ova long distances, perhaps miles.

3. *Winds.*—The maximum distance that the *Anophelinae* can be carried in this way is quite uncertain. Nearly all of the excessive distances that have been given as possible flights refer to *Culex*. It appears certain, moreover, that the *Anophelinae* dislike wind and seek shelter from it.

4. *Trees, Plantations, 'Bush' Jungle.*—These elements undoubtedly hinder the flight of *Anophelines*, and, on the contrary, open spaces promote their diffusion. It is necessary to bear this fact in mind, where a belt of jungle screens off a source of *Anophelines* (larvae), which may find an opportunity of becoming infected later.

'Domestic' and 'Wild' Species of Anopheles

Anophelines are mostly found in association with native dwellings where there is abundance of food (blood). *Anophelines* are also generally abundant where cattle are kept.

Certain species are distinctly 'domestic' in their habits, *e.g.*, *Mym. rossii*, *Pyr. costalis*, *Nyss.*

stephensi, and others. They are found resting in the daytime in the thatch of huts, and they breed close at hand in the nearest puddle. They may, however, fly up to half-a-mile if there are no breeding places closer.

Other species are not peculiar to houses, but are also found breeding in streams and pools in the jungle far from habitations. Such species are *Nyss. maculatus*, *Nyss. theobaldi.*

The mosquitoes of the genus *Myzorhynchus*, on the contrary, are 'wild' *Anophelines*. They are only occasionally found in houses. They breed in extensive bodies of water, swamps, rivers, jungle pools, etc. It is *Anophelines* of this type which chiefly frequent one's tent when this is pitched in remote and especially in swampy jungle. The more common species of these wild *Anophelines* are *M. barbirostris*, *M. sinensis*, *M. paludis.*

Nature of Food

The normal food of the female (domestic) *Anophelines* is blood. In nature they appear to feed every night, the stomach never becoming empty. In *Anophelines* caught under natural conditions, the stomach contents generally shew blood in two or three stages of digestion.

Female *Anophelines* readily drink water, especially if they have been kept for some time in a dry bottle. It seems doubtful whether vegetable juices form an important article of food as appears to be the case with some of the *Culicidae.* Male *Anophelines* can be seen feeding upon banana and other fruit juices, but are, notwithstanding, found dead about the second or third day of captivity.

Under some conditions the females do not feed upon blood, *e.g.*, *A. maculipennis* in England (THEOBALD).

BANCROFT states that *Nyss. annulipes* will live for a month on dates, but only for three days on bananas.

TIME OF FEEDING

The usual time for feeding of *Anophelines* is after dark, more especially in the early night and before dawn. Occasionally some *Anophelines* may be found biting in broad daylight, and ANNETT and DUTTON state that *Anophelines* feed readily in certain parts of Nigeria by day. Possibly certain species feed more readily by day than others.

We have ourselves seen on rare occasions *M. rossii* attempting to feed in the daytime, and GRAY, B.C.A., says 'that *Ce. albipes* when disturbed will bite at any time of the day or night.'

On the whole, however, the *Anophelinae* are strictly nocturnal in their habits. Nor do they hover around lamps as has been supposed. Of *A. bifurcatus*, BLANCHARD states that it bites fiercely at dusk, but at night practically not at all. At dawn, however, it begins again, and it bites at all times in shady places, outhouses, etc.

DISTANCE OF FLIGHT

The *maximum* distance that *Anophelines* can fly requires further study. In questions of flight, the species of mosquito should always be noted. Observations upon the flight of mosquitoes have, so far, been vague and uncritical. With regard to *Anophelines* on ships, it must be borne in mind that they have not necessarily come from the land

on the night upon which they appear, but may have come on board when the ship was in port or even have been bred on board. In certain villages in India studied by us, *Mym. culicifacies*, *Nyss. stephensi*, and *Nyss. fuliginosus* were always present in abundance, if there were extensive breeding-grounds within quarter-of-a-mile. Where villages were distant half-a-mile from extensive breeding-grounds, they contained few or no *Anophelines*. The only exceptions to this rule were when breeding-places had only recently dried up. In the case of the above species they undoubtedly fly fairly readily quarter-of-a-mile, but half-a-mile appears to be beyond the normal distance of flight.

Relation to Colour, Odour of Objects Etc.

Anyone who, in the tropics, has left his wardrobe open at sunrise and then closed it, and again examined it some time later, will have often observed the well-known fact that, on his white clothes, few or no mosquitoes are resting, but that on his blue serge clothes there may be dozens. He will have noted, too, that outside his mosquito net it is on the shady side that the mosquitoes remain longest, until from here also they fly away as the fierce sun rises.

He will have noted, too, that *Anophelines* as well as *Culicines* have a predilection for certain smells. Old boots and blacking attract them strongly, and the leather of a saddle room is their favourite haunt. *Anophelines*, too, much prefer the odoriferous skin of the native to that of the European, as experiments made by us in Sierra Leone clearly shewed.

Nuttall and Shipley have made some laboratory experiments on the influence of colour, and find that navy blue is the colour most preferred by *A. maculipennis*, and yellow the one most shunned.

As, however, the *Anophelinae* at least are nocturnal in their habits, and prefer biting unclothed portions of the body, the colour of one's clothing will not be much protection. If white or yellow socks can prevent the persistent attacks of *Stegomyia*, it would indeed be a practical boon. To various trees and plants has been ascribed a repellent effect upon mosquitoes. None of these statements has, so far, borne a critical examination.

Length of Life of Mosquitoes

The length of life of mosquitoes, under suitable conditions, is probably considerable; several weeks to months. In captivity they may, if suitably housed and constantly fed, be kept alive for days, weeks, and even months. A mosquito kept some time in captivity becomes infirm, and readily falls into the water whilst laying its eggs. It also finds difficulty in hanging on to smooth glass, and even though a rough surface is supplied the insect is constantly found on the bottom of the cage resting in a horizontal position. After laying eggs, such infirm mosquitoes generally die the same night. In nature, *Anophelines* certainly remain alive in huts for one or two months and possibly longer. After the drying up of all breeding-places, the winged *Anophelines* do not much diminish in number for several weeks. If the drying up continues, the numbers gradually diminish, but specimens may be caught up to two months or more afterwards.

'Aestivation' of Mosquitoes

In very hot and dry countries, the *Anophelines* which remain through the dry season appear to exhibit some peculiarities in their habits :—

1. Unlike hibernating mosquitoes, they feed regularly and are found full of blood.

2. The ovaries are in the majority large and the ova fully developed.

3. They do not lay their eggs even when 'test pools' are made near the houses in which they abound.

The Significance of Breeding-Places in the Dry Season.—It is usual in hot and dry climates to find in the dry season at most a few breeding-places. It is a mistake to conclude that these represent the distribution of *Anophelines* in the area in question. Under such conditions careful search will demonstrate that *Anophelines* are present in small numbers in nearly every hut. After the first downpour of 'the rains,' young larvae will be found, in from three to four days, in puddles throughout the district, shewing that numbers of *Anophelines* have survived the period of drought which may have been two months or more. One cannot, therefore, by measures directed solely against a few pools that remain in the dry season, hope to effectually get rid of the *Anophelines* in a district.

The Male Anopheline

Little is known as to the habits of the male *Anophelines*. When they are found in numbers it is probably a sign that breeding is going on at the time. At times the number of males caught in native huts exceeds that of the females. Copula-

tion is said only to occur after the first meal of blood. ANNETT and DUTTON describe many hundreds of male *Anophelines* dancing together in midge-like fashion in the villages at dusk.

FECUNDATION

Fecundation takes place it is thought generally on the wing. It is also effected in captivity when the *Anophelinae* are confined in test tubes. In the case of *Stegomyia*, LOW describes fertilization as taking place soon after the flies emerge from the pupae.

Proportion of Males to Females of Reared Insects. One is often struck by the large proportion of males among mosquitoes artificially raised, a troublesome fact when feeding experiments are being conducted. According to BERKELEY, if the larvae are kept supplied with abundant food the proportion of males is much reduced.

Chapter XVIII

ANOPHELINAE—THE OVUM

The Ovum

Anophelines in captivity generally lay their eggs on some floating object, but also upon the surface of the water. When laid on a solid object, and even when laid on the water, the eggs are deposited in a piled up mass. Later, the ova, if on water, often form very regular and beautiful patterns. Brick-red masses of eggs are sometimes laid. These do not develop further.

Observe (i) the arrangement in equilateral triangles and star patterns (Fig. 19).

(ii) The arrangement in rows of eggs lying side by side.

Both patterns are dependent upon the shape of the individual ovum; ova belonging to type 1 forming stars, and ova belonging to type 2, rows.

The number of ova varies, but is usually about one hundred. The size of the ovum varies with different species from about 0·6 to 1·0 mm.

Duration of Egg Stage.—Temperature is no doubt an important factor. Thus the egg stage in *A. maculipennis* lasts from two to four days, whereas in *Ce argyrotarsis* it is one-and-a-half days in Havana. In *M. rossi* it is about forty-eight hours.

Anopheline ova (with one exception as yet described) are boat shaped, with an approximately

flat upper surface and a deeply convex lower surface. One end which contains the head of the embryo is blunter and broader than the other. During the act of hatching this end is forced open by the escaping larvae.

1. *The Upper Surface*—Observe that the upper surface is generally granular or tuberculated in appearance. At either extremity it is continuous with the pointed ends of the ovum, and in this position there are usually several small polygonal areas. The width of the upper surface and the extent to which it is encroached upon by the floats varies in different species.

2. *The Lower Surface.*—The lower surface is generally smooth and dark grey. In damaged ova a silvery membrane will be seen partly detached, shewing a deep shiny-black surface beneath. The silvery membrane is the outer covering of the egg, and formed by the layer of follicular epithelium (Fig. 39). In some species the lower surface is marked with silvery lines forming a reticular pattern.

3. *The Floats.*—Occupying about the middle third of the side of the ovum is a remarkable structure—the float. This consists of a very delicate membrane continuous with the chitinous cuticle covering the whole ovum and containing air cells.

The floats are generally oval in shape and shew regular transverse corrugations. The shape and position of the floats vary considerably in the different species.

4. *The Frill.*—Around the margin of the upper surface (forming the gunwale of the boat) there is in some species a gleaming white frill-like

structure. This is striated in appearance, but portions of it may (in some species) be free from striations. In other species the appearance is rather that of a white striated rim. In all species of *Anopheles* ova yet described, a striated frill or rim is present. The width and extent of the frill vary in different species.

Type 1.—Ova have the upper surface very narrow, with the lateral floats not touching the margin (Fig. 54 : 1).

The species with ova of this type are—

M. barbirostris — *M. culicifacies*
M. sinensis — *M. listoni*
A. bifurcatus

Type 2.—Ova having a more or less broad upper surface, with the lateral floats touching the margin (Fig. 54 : 2, 3, 4, and 6).

Species having ova of this type are—

M. rossii — *N. fuliginosus*
Ce. pulcherrima — *N. stephensi*
A. maculipennis — *A. algeriensis*

Type 3.—Ova with no floats, and with upper surface rudimentary (Fig. 54 : 5).

One species only as yet described has ova of this type, viz. :—

M. turkhudi

Species having ova of the first type have in all cases been species breeding in either open natural waters or running streams.

Species with ova of the second type are in general found breeding in pools.

The only ova as yet systematically described are those of the Indian *Anophelines*. Further observations will probably add further types to the above.

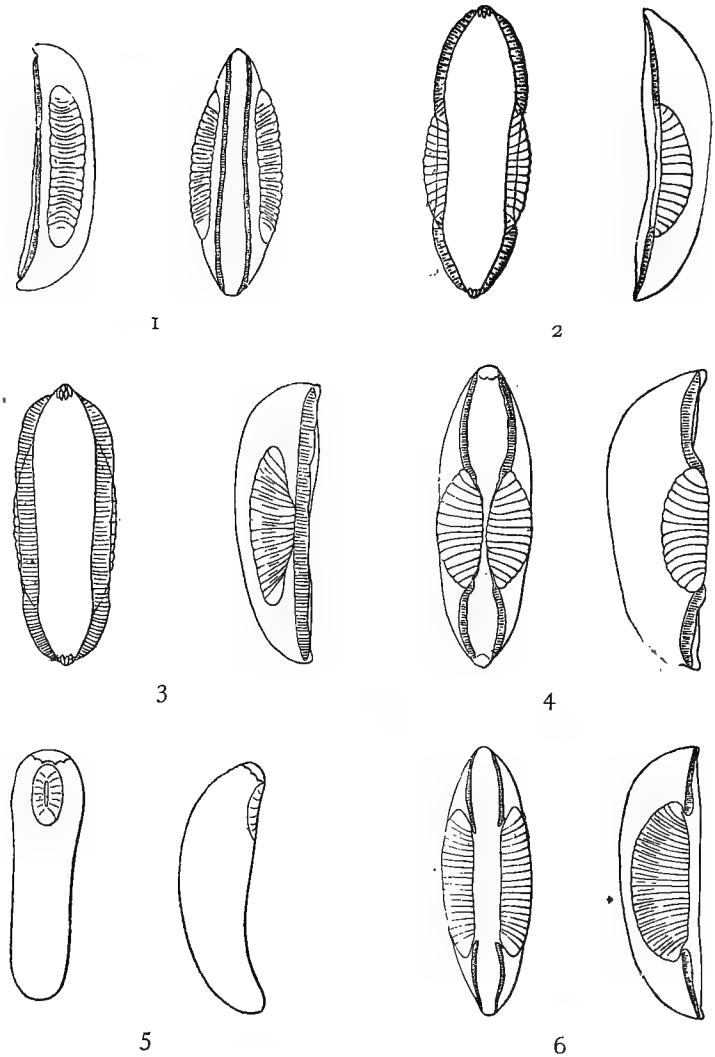


Fig. 54. *Ova of Anophelinae*

1. *M. culicifacies* 2. *C. pulcherrima* 3. *M. rossii*
4. *N. stephensi* 5. *M. turkhudi* 6. *N. maculipalpis*

Within each type great variation usually exists in the different species. The following are the most notable variations found :—

1. *The Frill.*—The width. The continuity of the frill around the whole of the margin of the upper surface or its replacement in the middle third by the floats. The extent of striation of the frill. The presence of a striated rim only.

2. *The Floats.*—The position, placed forwards and encroaching on the upper surface, or laterally situated. The shape, oval, globular, or scallop-shell.

3. *The Lower Surface.*—Whether ornamented or not with silvery reticulated pattern.

The following is a brief résumé of the characters of the ova of *Anopheles*, as far as these have been described :—

Type 1. *M. sinensis*, sub-sp. *nigerrimus*

Ovum.—Upper surface very narrow. Floats do not touch margin of upper surface. Lower surface of ovum ornamented with polygonal markings.

M. barbirostris

Ovum.—Upper surface very narrow. Floats do not touch margin of upper surface. Lower surface of ovum ornamented with polygonal markings.

M. culicifacies

Ovum.—Upper surface very narrow. Floats do not touch margin of upper surface. Lower surface not ornamented. A short but distinct fringe is continued around margin of upper surface.

M. listoni

Ovum.—Upper surface very narrow. Floats do not touch margin of upper surface. Lower surface not ornamented. A small fringe passes around margin of upper surface.

Type 2. *M. rossii*

Ovum.—Upper surface broad. Fringe very well developed and striated throughout whole length. Floats scallop-shell shape and touch margin of anterior surface. Lower surface not ornamented.

Ce. pulcherrima

Ovum.—Upper surface broad. Floats touch margin of upper surface. Fringe well developed around margin of upper surface. Striations are not present in that portion of the fringe lying over the floats. Lower surface not ornamented.

N. fuliginosus

Ovum.—Upper surface moderately broad. Floats touch margin of upper surface. Floats long and narrow. Fringe around upper surface only indicated by white border. Lower surface not ornamented.

N. maculipalpis

Ovum.—The upper surface is rather narrow. The floats are rather short and oval, and are placed far forwards as in the ovum of *N. stephensi*, though less markedly so. The fringe is fairly developed, but is not continued over the floats.

N. stephensi

Ovum.—Upper surface broad, except in central portion where encroached upon by floats. Floats placed on margin of upper surface so that they touch, or nearly touch, one another in middle line. Floats short and almost globular. Fringe not well developed. Lower surface not ornamented.

N. theobaldi

Ovum.—As the females of this species have only been very occasionally caught by us in houses, we have not been able to describe the ovum as deposited by the insect. Fully developed ova removed from a bred specimen showed, however, that the ovum resembled that of *N. maculipalpis*. The floats were rather short and situated far forwards as in *N. stephensi*. The fringe is fairly developed, but does not pass over the floats.

Type 3. *M. turkhudi*

M. turkhudi is a very aberrant type, so far as the ovum and larva are concerned. Both the ovum and larva approach to the characters of the *Culex* ovum and larva. The eggs were laid upon a floating object. When placed upon water they sank. They were laid in the heaped-up manner sometimes adopted by *Anophelines*, especially *M. rossii* and *N. maculipalpis*. The chief characters of the ovum are:—

1. No separation of an upper surface as in all other *Anopheline* ova. At the thicker end of the ovum there is an oval area about a quarter the length of the whole egg. This is glistening

white and striated, and probably represents the upper surface of other *Anopheline* ova.

2. There are no floats or any markings representing them.

3. There is a pale area at the thicker end of the egg with a scalloped edge.

4. The ovum is otherwise without markings.

It is obvious that the characters of the ovum are of considerable importance in the classification of *Anophelines*, and every care should be taken to describe these in as great detail as possible.

In making drawings of the ova of *Anophelines*, it is convenient to use an eyepiece micrometer.

To Mount Ova

No thoroughly satisfactory method is known to us, but although imperfect, any of the following methods will give specimens in which some, at least, of the ova preserve most of their characteristics.

1. Place the eggs on a slide which has been made slightly sticky with balsam, and then mount them in a drop of balsam and place a cover-glass over them.

2. Mount in two per cent. formalin solution and ring the coverglass with balsam or shellac.

3. Mount in glycerine and ring the specimen.

4. Mount in a drop of cedar-wood oil.

Chapter XIX

ANOPHELINAE—THE LARVA AND NYMPH

The Larva

The larva of *Anophelines* when first hatched out are minute characteristic creatures, with very black heads and transparent bodies. They move with a very active wriggling movement. They can, even at this stage, be distinguished from the larvae of *Culex*, especially with the aid of a lens, as they take up a horizontal position. At first the heads of all species are dark and very conspicuous. After a certain number of days, however, the head becomes lighter in colour, and characteristic markings can be made out on the dorsum. When first hatched the head is very large in proportion to the thorax, later, it is smaller, and finally, it is the thorax which is the larger.

During the first few days the palmate hairs are simple lanceolate structures, and cannot be used as specific characters.

Duration of Larval Stage.—This is determined by at least two factors. (1) *Food.*—Thus larvae kept in tap-water in the laboratory grow very slowly, if at all; (2) *temperature.*—Thus the larval stage of *A. maculipennis* varies from sixteen to twenty-two days at air temperatures of 68°-78° F., while in the tropics the time is much shorter, *e.g.*, twelve days for *Ce. Argyrotarsis* in Havana, and eleven days for *M. rossii*, where the temperature of the water varied from 96°-102° F.

Examination of the Full-Grown Larva.—Cover the larvae in a drop of water, with a coverglass, and examine with one-fourth or one-sixth objective; the following points can be readily made out. Observe that old larvae are often almost totally enveloped in vorticellae or other infusoria.

1. *The Head.*—The head is globular in shape, and is for the most part enclosed in a hard and continuous chitinous case. Anteriorly, there are the rather complicated mouth parts. Posteriorly, there is an opening into which the neck is inserted, around this is a pigmented border resembling a collar. There is a gap in this dark border in the middle line posteriorly, and here two diverging bands of chitin form a 'V' on the back of the head. Grouped around this 'V' mark are more or less continuous patches of pigment, which shew differences in their arrangement, to some extent, specific.

2. *The Antennae.*—Arise from two prominent lateral protrusions, they are freely movable at their articulation. Each antenna is a rod-shaped unjointed body. At its terminatian are two leaf-shaped bodies, and a branched hair arises between the leaflets. The antenna is covered with small spines, which are particularly developed in pairs along the inner border. In most species of *Anophelines* a hair can be made out arising from a papilla situated at the junction of the proximal and middle third of the antennae.

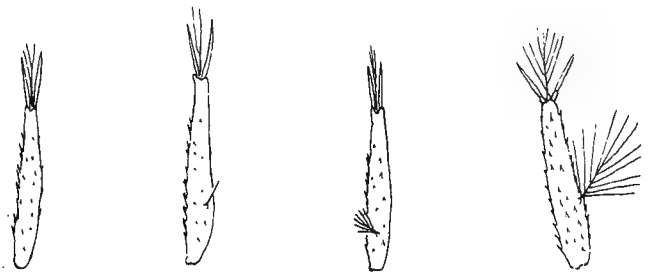
This hair is of specific importance.

(i) In the majority of *Anophelines* it is simple and unbranched.

(ii) In *A. lindesayii*, *M. nigerrimus*, *M. barbirostris* it is branched, and in the last two very large and conspicuous. (Fig. 55).

3. *The Eyes.* The eyes are situated laterally, and can be seen both from the dorsal and ventral surface. Their size and appearance vary with the age of the larva. In the full-grown larva a crescentic compound eye is seen on either side, and behind this a single pigment mass (simple eye). The compound eye is absent in the first stages, and becomes more prominent as the larva approaches maturity.

4. *The Mouth Parts.*



1 2 3 4

Fig. 55. Lateral Hairs of Antennae

1. *M. rossii.* 2. *N. stephensi.* 3. *A. lindesayii* 4. *M. nigerrimus*

(*a*) Two very conspicuous bodies resembling somewhat shaving brushes are protruded or withdrawn under the overhanging clypeus. These are the feeding-brushes, and are employed in collecting the minute food particles on which the larva feeds.

(*b*) On either side of the mouth is a broad blade-like structure carrying several leaflets and some hairs (maxillary palps).

(*c*) Below the feeding brushes, and not so easily visible, are two stout bodies with comb-like projections (mandibles).

(*d*) In the middle inferior line lies a conical toothed structure. The under lip of Meinert (Fig. 23).

(*e*) In the fully-grown larva a snout-like process covered with short hairs projects forwards in the middle line between the brushes.

The front portion of the head projects between the antennae as a semi-circular smooth area. In front of this is a protrusion overhanging the base of the brushes (the clypeus).

5. *The Clypeal Hairs.*—These are four or six in number. Two spring from the extreme front of the clypeus near the middle line ; two from the outer corner of the clypeus immediately over the feeding-brushes, and two usually very small and not always present behind the origin of the others.

The clypeal hairs are best seen when the feeding brushes are retracted. They must not be confounded with certain other hairs on the larval head. These are :—

(i) Six large branched hairs arising from the prominence lying between the bases of the antennae.

(ii) Four similar branched hairs, but smaller, situated further back (NUTTALL and SHIPLEY).

NOTE.—ED. and ET. SERGENT describe variations in these hairs in *A. algeriensis*. In eighteen out of forty-six examined both were simple. In three out of forty-six both were slightly branched. In twenty-five out of forty-six the central hair had two or three small terminal branches.

The hairs exhibit great variation in different species, but are quite constant in the one species. A minute description of these hairs is of great importance in describing the specific characters of the larva.

Clypeal Hairs of Larvae :—

(i) The four anterior hairs may be quite simple and unbranched. *M. rossii*, *N. stephensi*, *M. culicifacies*, *M. listoni*, *M. turkhudi*, *A. bifurcatus*.

(ii) All four anterior hairs may shew small lateral branches. *P. jeyporensis*.

In *A. maculipennis* all four hairs are branched, the outer pair form distinct tufts.

(iii) The outer pair may be markedly branched, *e.g.*, *Ce. pulcherrima* and *M. pseudopictus*.

(iv) The outer pair may be developed into a close tuft (cockade), *e.g.*, *M. barbirostris*, *A. punctipennis*.

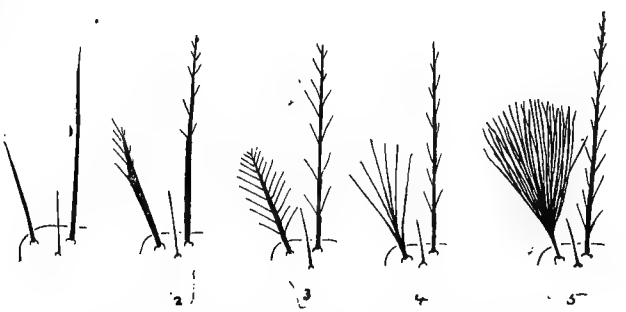


Fig. 56. *Clypeal Hairs of Larvae*
1. *M. rossii*, *N. stephensi*, *M. culicifacies*, *M. listoni*
2. *N. maculipalpis*. 3. *P jeyporensis*. 4. *Ce. pulcherrima*
5. *M. sinensis*, *M. barbirostris*

The two hairs situated behind these may, instead of being very short and inconspicuous, be long and prominent, *e.g.*, *M. turkhudi*.

6. *The Thorax.*—The thorax in the adult larva is large and globular. In the young larva it is not so broad as the head, but becomes proportionately larger as the larva advances in age. Numerous hairs arise from the front and sides of the thorax. None of these vary perceptibly in different species. A number of large hairs arising from papillae on the lower surface are capable of being used almost as a means of progression when the larva is in very shallow water.

(*a*) Observe on the dorsum of the thorax a short but extremely stout and strong hair, unlike the others, projecting outwards and forwards.

(*b*) A flap-like body may, with careful focussing, be seen lying at the base of the most anterior hairs on either side.

(*c*) In some species of *Anophelines* a single pair of palmate hairs, similar to those on the abdominal segments, are found upon the thorax. In others they are rudimentary or absent. The presence of well-developed palmate hairs on the thorax is of specific importance.

(i) It is well developed and functionally active in *M. culicifacies*, *M. listoni*, *P. jeyporensis.*

(ii) It is rudimentary or absent in all other larvae as yet described.

7. *The Abdomen.*—The first seven segments are very similar in shape. The eighth carries the opening of the air-tube, and the ninth some curious papillae and large hairs.

Each of the first two segments carries on each side a pair of long feathered hairs. The third carries a single similar hair. On the other segments there are much smaller and unfeathered hairs. On all the segments there are groups of

small hairs which do not appear to vary and which are not of sufficient importance to describe here. None of the above structures appear to vary in different species.

Skeleton Larvae

Examination of Palmate Hairs.—The most important appendages of the abdominal segments are certain small fan or palm-leaf-shaped hairs attached by a short stalk to the outer dorsal portions of certain of the segments (Fig. 57A). The number of segments bearing well-developed palmate hairs varies in different species.

Place the larva under a coverglass in a drop of water or use a permanently mounted larva.

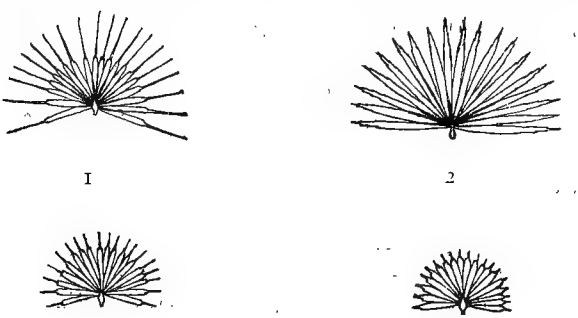


Fig. 57A. *Palmate Hairs of Larvae:*
1. *M. rossii.* 2. *M. nigerrimus.* 3. *M. listoni* 4. *N. maculatus*

1. Determine the number of segments which carry distinct and large palmate hairs and those carrying ill-developed ones.

2. Carefully cut out with a needle two or three central abdominal segments. Crush these.

The following arrangement of these hairs is found in Indian species of larvae, the only larvae as yet systematically described.

1. Fully developed hairs on all segments (one to seven) and on the thorax.

P. jeyporensis
M. listoni
M. culicifacies

2. Fully developed hairs on the second to seventh, or third to seventh segments. Rudimentary hairs on the second or even first abdominal segments and on the thorax.

N. stephensi
N. maculatus
N. theobaldi

3. Palmate hairs confined to the third, fourth, fifth, sixth, and seventh segments.

M. sinensis
M. barbirostris
A. maculipennis (NUTTALL and SHIPLEY)

4. Palmate hairs confined to the fourth, fifth, and sixth segments.

M. turkhudi

The Leaflets.—In the well-grown larvae each palmate hair consists, as a rule, of nineteen or twenty leaflets arising close together from a short stalk, and forming a semi-circular fan. When collapsed, as is the case when the larvae is beneath the surface, these hairs are inconspicuous. When, however, the larvae takes up its characteristic attitude at the surface of the water, these spread out fan-like, and are very striking objects under the microscope. In the freshly hatched larva, the separate leaflets appear to be folded together, so that the hair has the appearance of a single

lanceolate structure. About the third day, the hairs are seen with seven to eight uniformly lanceolate leaves. Very soon after this, they take on the characters seen in the hairs of the mature larva.

In the mature larva the leaflets shew much variation in the different species. In most species, the leaflets terminate rather suddenly in a number of jagged points or notches, whilst the central portion continues as a more or less fine filament.

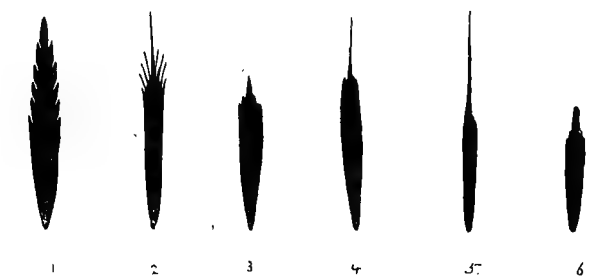


Fig. 57B. *Leaflets of Palmate Hairs*

1. *M. sinensis, M. barbirostris.* 2. *A. lindesayii*
3. *N. theobaldi, N. stephensi.* 4. *M. listoni, M. culicifacies*
5. *M. rossii.* 6. *M. turkhudi*

The character of the notching and the relative length of the filament to the leaflet are of specific importance. The following types of leaflets are known :—

1. The leaflets are unbrokenly lanceolate in shape, with saw-like notches along the edge of the outer half. There is no distinct terminal filament.

M. sinensis
M. barbirostris

2. The filament is long and filamentous.

M. rossii
M. culicifacies
M. listoni
N. fuliginosus

Further differences are seen in the case of most of the above species. In *M. rossii* the filament is as long as the leaflet, and there is scarcely any notching where the two join. In *N. theobaldi*, the notching is well marked (Fig. 57B).

3. The filament is very short, a mere spike-like process.

N. stephensi
N. maculata
N. theobaldi
N. maculipalpis

The Stigmatic Syphon.—The eighth segment bears the stigmatic opening. This is a large quadrilateral space, with hard comb-like chitinous processes on either side. These have the teeth projecting backwards, and are capable of being approximated so as to obliterate the cavity. Into the anterior portion of the space, under cover of a lip-like process, the two main air tubes open.

The ninth segment is cylindrical in shape, and is chiefly notable from the fact that it carries four large transparent papillae well supplied with air tubes and certain long curved hairs. Of the hairs one series projects downwards so as to resemble a rudder. The others project posteriorly. There does not appear to be much variation in the different species.

Examination of the Larva

1. Some features, *e.g.*, feeding, are conveniently studied by placing the larvae in a drop of water in a watch glass.

2. For examining under a high power, the activity of the larva must be restrained by a cover-glass.

3. Permanent preparations may be made at once by placing in strong formalin, then alcohol, then oil of cloves, then balsam (*vide* p. 250).

4. Beautiful preparations of the palmate hairs, etc., are got by mounting the larval skeleton thrown off at the time of pupation.

Pupation

Just before this process the larva becomes quieter. The attitude also frequently alters, becoming a hanging one, somewhat like that of a *Culex* larva.

In this condition larvae are very readily killed by agitating the water (and it is difficult to carry larvae in this stage without killing them).

The change into the nympha is very sudden. A few rapid motions and the larval skin is cast off, leaving the characteristic nympha.

The Nympha

This stage in the tropics usually lasts about forty-eight hours. When first the larval coat is cast the nympha is light in colour, and may be readily overlooked. Later, the nympha becomes darker, and towards the end and immediately prior to the emergence of the imago, *silvery patches*

due to collections of air are seen beneath the cuticle.

Pupae taken out of the water and kept on moist blotting-paper will still develop into winged insects (NUTTALL and SHIPLEY).

Egg to Imago.—The developmental cycle for *A. maculipennis* is about thirty days at a temperature of 20°-25° C. In the tropics it is much less. Thus the *minimum* time for *Ce. argyrotarsis*, *M. rossii*, *M. culicifacies* is fourteen days.

Chapter XX

THE BREEDING-PLACES OF ANOPHELINES

Larvae should be sought for in the most diverse situations and, after being examined and described, be allowed to hatch out. New species of *Anophelines* are often obtained in this way. It is the case in India, and almost certainly will be found to be so in other countries, that certain kinds of breeding-places are preferred by certain species.* A collection of larvae made from shallow puddles will be found to yield quite a different set of species to one made from a streamlet or pool full of vegetation, even though close to the puddles (Fig. 58).

The following tabular statement gives the more common situations of *Anopheline* breeding-places and the species, as far as known, found in each. It is obvious that a great deal of work yet remains to be done.

1.	Foul puddles near habitations	M. rossii
2.	Clean puddles without much alga and often turbid with suspended matter :—	
	(*a*) Pits and puddles near houses	M. rossii P costalis

* Thus *M. lutzi* is said to breed only in the water collected in the leaves of the parasitic *Bromeliaceae*, and *N. annulipes* is said to breed in the sea.

(*b*) Roadside puddles	N. maculatus P. costalis
(*c*) Cattle footmarks	N. stephensi
(*d*) Shallow, muddy sheets of water	M. rossii
(*e*) Pools in sandy river beds	M. culicifacies
(*f*) Large pools in quarries, etc.	M. turkhudi
3. Puddles and pools with much alga. Common in stream beds, water trickling over rocks	N. fuliginosus N. maculatus
4. Earthenware vessels, empty paraffin tins, boats, water barrels	N. stephensi P. costalis
5. Wells, springs	N. stephensi P. costalis A. lindesayii
6. Swamps.	
(*a*) Deep water with much aquatic vegetation	M. sinensis M. barbirostris M. paludis
(*b*) Rice-fields, wet cultivation of all kinds	M. rossii P. jeyporensis N. maculatus N. maculipalpis A. lindesayii
7. Running water	
(*a*) Swiftly flowing streams	M. listoni
(*b*) Sluggish irrigation channels, ditches, muddy trickles, edges of rivers	M. funesta M. culicifacies N. maculipalpis
(*c*) With much weed and alga	M. sinensis M. barbirostris N. fuliginosus N. maculatus N. theobaldi
(*d*) Stony and shallow	M. culicifacies N. theobaldi M. turkhudi

8. Lakes with weedy margins — N. fuliginosus
9. Hill species — A. lindesayii, N. maculatus, A gigas

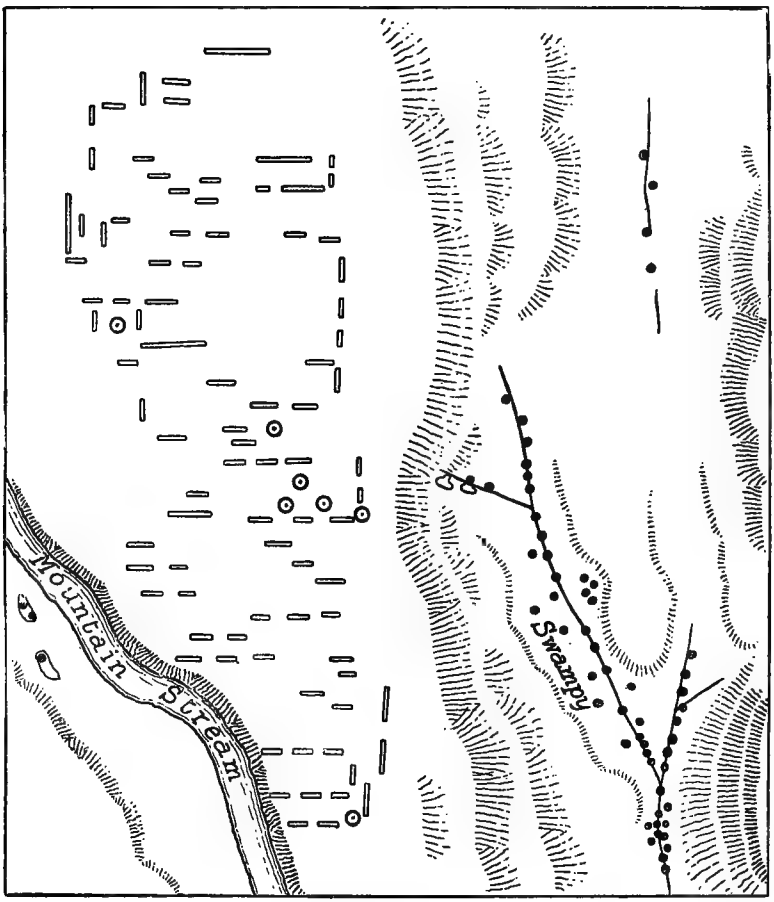


Fig. 58. *Portion of Coolie Lines on a Tea Plantation, to shew different breeding-places of M. rossii, M. listoni, and N. maculatus*

⊙ = *M. rossii.* ● = *M. listoni and N. maculatus*

Chapter XXI

THE IDENTIFICATION OF ANOPHELINE LARVAE

1. *Naked Eye Characters.*—Some larvae may be identified by the naked eye. The distinction, however, between most species is insufficient to allow of separation by this means.

2. Observe that the colour of larvae is not dependent on species but on the nature of the food, amount of light they have been exposed to in nature, the colour of the water, and other general conditions.

3. The most distinctive of *Anopheles* larvae are those of *M. sinensis* and *M. barbirostris*. These are very large larvae, most frequently black, or black speckled with white, but also brown or vivid green in colour. One of their characteristics is a peculiar 'stick-like' appearance, and the assumption of a bent or contorted attitude.

The larvae of *M. turkhudi* can be detected by their attitude, which is almost *Culex*-like. Larvae about to change into nymphae, however, also frequently adopt this position.

Naked eye examination always requires verification by the microscope.

(A) Larvae may be bred from ova deposited by females of a known species. To successfully accomplish this requires a good deal of care.

1. Remove the paper upon which the ova have been laid (p. 96), and place in a small bottle containing some filtered fresh water from a pool or rain puddle.

2. Place in a good light, but take care that the sun, by the focussing action of the glass, does not heat the water, otherwise the larvae will be killed.

3. When the larvae are hatched, transfer them (after a day or two) to a larger vessel of fresh water containing some weed. When the fresh natural appearance of the water disappears, more fresh water from a pool should be added.

4. By keeping larvae in a not too porous earthenware vessel, they may be placed with impunity all day in the direct sun. It is necessary, however, to watch carefully, to guard against desiccation and consequent death of the larvae.

Larvae kept in flat, partially glazed earthenware vessels, with a certain amount of mud, and placed in the sun, develop more quickly than those kept in bottles.

It is of course necessary to make certain that foreign ova or young larvae are not introduced with the fresh water.

Some larvae are exceedingly difficult to rear artificially, notably those of *M. barbirostris* and *M. sinensis*. They remain for long periods without perceptibly increasing in size, and frequently die.

(B) An alternative and less tedious way is to examine nearly adult larvae found in nature, and to observe, after accurately noting the larval characters, what species of *Anopheles* eventually hatches out.

By examining the larva on a slide without a coverglass, the main characters may be noted without in any way damaging the larva, which later becomes a nymph and eventually an imago. As a rule, however, many specimens of the same species are found together. By a preliminary examination, larvae shewing the same characters may be sorted out, and some specimens afterwards mounted and subjected to a more detailed examination, whilst the rest are allowed to hatch out in due course.

The characteristics of the larvae which are of specific importance are, as we have seen—

1. The antennae.
2. The clypeal hairs.
3. The leaflets of the palmate hairs.
4. The segments carrying palmate hairs.

By means of these characters most species of *Anopheline* larvae can be identified. So far as Indian *Anophelines* are concerned, the following characters hold good :—

Type 1.—Larvae with the external pair of clypeal hairs converted into a cockade-like tuft (Fig. 56).

Species having larvae of this type are—

M. barbirostris
M. sinensis, sub-sp. *nigerrimus*

Larvae of this type also have a large branched hair upon the antenna, and the leaflets of the palmate hairs differ markedly from all other larvae (Fig. 57B).

Type 2.—Larvae with the external frontal

hairs branched but not developed into tufts (Fig. 56). *N. fuliginosus*
Ce. pulcherrima

Type 3.—Larvae with the external pair of frontal hairs simple and unbranched, and with palmate hairs on every abdominal segment and on the thorax (Fig. 56).

M. culicifacies
M. listoni

Type 4.—Larvae with the external pair of frontal hairs simple and unbranched, but with no developed palmate hairs on thorax or first abdominal segment (Fig. 56).

M. rossii
N. stephensi

Type 5.—Larvae with two large additional hairs placed behind those already mentioned. Also with first three abdominal segments free from palmate hairs. *M. turkhudi*

Larva of M. barbirostris.—Antenna with large branched hair. External pair of frontal hairs developed into cockades. Palmate hairs on second to seventh abdominal segments. Leaflets of palmate hairs lanceolate in shape and deeply serrated in outer half. Head of larva without pigmented markings.

Larva of M. sinensis.—Antenna with large branched hair. External pair of frontal hairs developed into cockades. Palmate hairs (?). Leaflets of palmate hairs lanceolate with serrations in outer half. Head of larva without pigmented markings.

The habits of both these species, *M. barbirostris* and *M. sinensis*, sub-sp. *nigerrimus*, are very similar.

The larvae are found in water with much aquatic vegetation—rivers, lakes, ponds, and swamps. They are only caught singly, but are generally widespread in their occurrence where large bodies of water are present.

Larva of N. fuliginosus.—Antenna without large branched hair. External pair of frontal hairs branched (branches usually six in number). Palmate hairs on second to seventh abdominal segments. Leaflets of palmate hairs with very marked 'shoulder' at origin of terminal filament. Terminal filament from one-half to two-thirds the length of basal portion. Head of larva with distinctive markings.

Larva of M. culicifacies.—Antenna without large branched hair. Frontal hairs all unbranched. Palmate hairs on first to seventh abdominal segments, and a pair of fairly developed ones upon the thorax. Palmate hairs with terminal filament nearly as long as basal portion. Head with markings.

Larva of M. listoni.—Antenna without large branched hair. Frontal hairs simple. Palmate hairs on all segments, and very well-developed pair on thorax. The palmate hairs in this species are very large. The terminal filament is nearly as long as basal portion.

Larva of Ce. pulcherrima.—Antenna without large branched hair. Outer pair of frontal hairs branched (six branches). Palmate hairs on second to seventh abdominal segments. Filament of palmate hair nearly as long as basal portion. Head markings present.

Nature of breeding-place unknown.

Larva of M. rossii.—Antenna without large branched hair. Frontal hairs unbranched. Palmate hairs second to seventh abdominal segments. Terminal filament of palmate hair very long; often longer than basal portion. The 'shoulder' at the origin of the filament is very slightly marked. There are markings upon the head (Fig. 57A).

Breeds nearly always in small pools near houses. These pools are frequently foul and nearly always muddy. The female lays her eggs very readily in captivity.

Larva of N. maculipalpis.—Antenna without large branched lateral hair. Frontal hairs are peculiar and show a condition intermediate between the branched hairs of *M. barbirostris*, *N. fuliginosus*, and the unbranched hairs of *M. rossii* and other species (Fig. 56). Palmate hairs on second to seventh segments. Leaflets of palmate hairs have very short filaments. The notching at the termination of the leaflet is not so marked as in *N. theobaldi*.

Larva of N. theobaldi (GILES).—Antenna without large branched lateral hair. Frontal hairs unbranched. Palmate hairs on second to seventh segments. Leaflets of palmate hairs have very short filaments. There are marked notches at the ending of the leaflet in the filament (Fig. 57B).

The larvae of this species frequent especially sluggish streams with much growth of alga. They were found by us in Nagpur in association with *N. fuliginosus*, *M. barbirostris*, and *M. listoni*.

Larva of M. turkhudi.—The larva is *Culex*-like in some of its characters, though undoubtedly much more nearly related to the *Anopheline* type.

The full-grown larva is distinguished by the adoption of the slightly hanging attitude. The chief characters of the larva are :—

1. Two large additional frontal hairs are developed, which reach as far forward as the longest of the hairs described in other larvae.

2. The shape of the head differs from that of the ordinary *Anopheline* larva.

3. The palmate hairs are only represented on two or three abdominal segments, namely, the fourth, fifth, and sixth. They are absent on the first three abdominal segments.

4. The palmate hairs are small and poorly developed. The leaflets are irregular and the terminal filament blunt.

This species must be looked upon as a form which in its egg and larval stages has lost many of the characteristics of *Anopheline* eggs and larvae, and has approached in these stages the characters of the eggs and larvae of *Culex*.

Classification of Indian Anophelines According to Larval Characteristics *

Antenna with lateral branched hair		Tufted frontal hairs	M. sinensis	Leaflets of palmate hairs lanceolate and serrated
			M. barbirostris	Ditto
		Simple frontal hairs	A. lindesayii	Leaflets of palmate hairs shewing regular and deep notching (Fig. 57B)
Antennae without branched lateral hair	Simple unbranched clypeal hairs	Filaments of leaflets long	M. rossii	Palmate hairs well developed on third to seventh segments.
			M. culicifacies	Palmate hairs on all segments and on thorax
			M. listoni	Palmate hairs very large on all segments and on thorax
		Filaments of leaflets short	N. stephensi N. maculatus N. theobaldi	
	Feathered clypeal hairs	Filaments short	N. maculipalpis	
		Filaments long	P. jeyporensis	Palmate hairs very large on all segments and thorax
	Branched clypeal hairs		N. fuliginosus	Filaments of leaflets long
			Ce. pulcherrimus	
	Two posterior hairs large and conspicuous		M. turkhudi	Palmate hairs on fourth, fifth, and sixth segments only; filaments short, leaflets rudimentary

* The Larvae of the remaining Indian species are, so far, undescribed.

To Mount Larvae

1. Place a drop of formalin in a hollow ground slide. The drop must be just sufficient to fill the cell when the coverglass is in position.

By means of a pipette or spoon take up a larva and, removing the excess of water, allow the larva to float off into the drop of formalin.

Place the coverglass in position, avoiding air bubbles, and ring with Canada balsam, etc.

It is important that no air bubbles are included, as a white deposit forms around them.

If too much formalin has been added, the excess must be carefully removed before ringing. If hollow ground slides are not available, a ring of balsam may be made on the slide and allowed to become somewhat hard. Fill the cavity with formalin, place the larva therein, and cover carefully with a coverglass. Avoid excess of fluid or air bubbles. It is best to allow the Canada balsam to be just soft enough to stick to the coverglass.

Larvae mounted in this way retain their characters very well, and the clypeal and palmate hairs can be examined with ease.

2. *To Mount in Balsam.*—If placed in alcohol, oil of cloves or xylol, and balsam in the ordinary way, the shrinkage of the soft parts and even of the hairs is very great.

On no account touch the larvae with forceps, and only occasionally, and with the utmost care, with a needle point.

Place a number of larvae in a covered watch-glass containing formalin. Leave for twenty-four hours at least.

Lift each larva carefully by means of a strip of cigarette paper. Drain off the excess of formalin, and place with the greatest care in absolute alcohol. Allow the specimen to remain for at least ten minutes in alcohol.

Remove with cigarette paper to a watch glass containing oil of cloves. With cigarette paper transfer to a slide. Remove excess of oil of cloves, mount in a large drop of balsam, taking care that the dorsum of the larva is upwards.

If great care is taken not to detach the hairs by handling, the larval characters are beautifully displayed in this way.

Chapter XXII

THE RELATION OF SPECIES OF ANOPHELINAE TO MALARIAL ENDEMICITY

Species undoubtedly play an important part in the development of blood parasites in the mosquito.

Proteosoma, for instance, develops in certain species of *Culex*, *e.g.*, *C. nemorusus* was used by Koch in Europe. It does not, however, develop in certain species of *Taeniorhynchus* (S. P. James).

The malaria parasite does not develop in species of *Culex*, *Taeniorhynchus*, *Stegomyia*, or other blood-sucking flies, *e.g.*, *Phlebotomus*, *Simulium*, etc. In the case of *Culex fatigans* placed under absolutely identical conditions with *Anopheles*, no sign of zygote formation occurs on the second or third day.

Similarly with regard to filaria, it is only in certain species of *Culicidae* that certain species of filaria will develop, thus *Ce. argyrotarsis* is an efficient host for *F. nocturna*, but inefficient for *F. demarquaii*.

The *malarial endemicity* or *endemic index* may be defined as the percentage of infected children (under ten years of age) in any district, and represents the liability of immigrants to contract malaria.

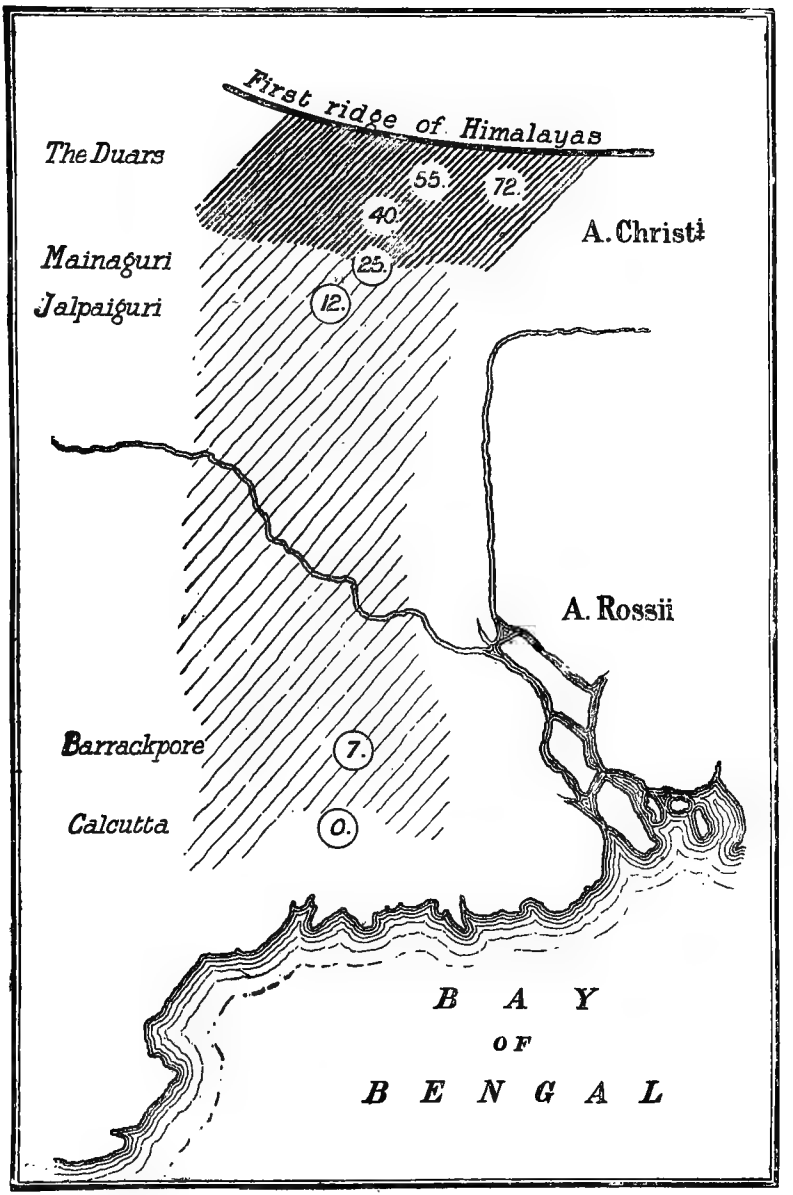


Fig. 59. Shewing variations in Malarial Endemicity

It is well known in a general way that in one country malaria is more intense than in another, but here we have a means of exactly measuring this difference, and, moreover, in the different parts of any particular district. We may illustrate this by the differences we found in Bengal in an extent of country where, as far as we could judge, the climatic conditions were practically identical, yet we find in the environs of Calcutta the endemic index is 0, while in the Duars (at the foot of the Himalayas) it is as high as seventy-two (Fig. 59). We found, however, that there was one important matter in which the Duars differed from Calcutta, and that was in its *Anopheline* fauna. Whereas in Calcutta *M. rossii* was the predominant species, in the Duars *M. listoni* was the commonest *Anopheline*.

Again, in the Jeypore district (Madras), we had a district of uniformly high endemic index, fifty to one hundred, and here we found an *Anopheline*, *P. jeyporensis*, which we had not encountered elsewhere, so that the view seemed tenable that the high endemicity of these districts was dependent on their special *Anopheline* fauna. To test to what extent species was concerned in determining endemicity, we then made use of another more exact method, viz., determining by dissection whether any difference occurred amongst the different species in the percentage of infected specimens: we were able to carry this out in the case of *M. rossii* and *M. culicifacies*. We caught these species in the same huts in the same villages at the same time, and determined by actual dissection the percentage of glands infected with sporozoits. The results were most striking, and

fully confirmed our previous idea, based on more general considerations of the importance of species. They were as follows :—

I. Mian Mir (Punjab)

	Number dissected	Number with sporozoits	Percentage
M. culicifacies	259	12	4·6
M. rossii	496	0	0

II. Ennur (Madras)

	Number dissected	Number with sporozoits	Percentage
M. culicifacies	69	6	8·6
M. rossii	364	0	0

Undoubtedly then, under natural conditions, the species is here a very important factor.

Again, under artificial conditions (feeding experiments), we found that there was a difference in the number of zygotes found in the stomach as the result of feeding.

The species which appeared to be most active were :—

M. culicifacies
N. stephensi
N. theobaldi

Those in which zygote formation seemed less abundant were :—

M. rossii
M. turkhudi
M. barbirostris

It should be noted, however, that in these experiments *M. rossii* became infected, while in nature it has never been found infected by us.

There are, moreover, many considerations which lead to the conclusion that in nature all species of *Anopheline* are not equally concerned in the transmission of malaria.

We may have countless numbers of *M. rossii*, as in Calcutta (environs), and get a malarial index of 0, and this appears to hold good in Madras, Bombay, and, as far as our observations go, universally. On the other hand, where we find *M. listoni*, *M. culicifacies*, *P. jeyporensis*, in India, we have a high endemic index.

The group of mosquitoes, those associated with intense malaria, are small dark mosquitoes with unbanded legs (*Myzomyia*, group 1).

M. funesta and P. costalis in Africa

The former mosquito is, like *M. listoni*, which it closely resembles, a breeder in clean waters, streams, springs, etc., while *P. costalis* is found breeding in shallow pools about houses and frequents towns (in Africa), which *M. funesta* does not.

M. funesta was found by us to be infected in the Lagos hinterland to the extent of twenty-five to fifty per cent.

P. costalis, in Lagos itself, contained only three per cent. of sporozoits.

It is important then to determine precisely the species in a district and to determine the percentage of infection sporozoits.

A. maculipennis and *A. punctipennis* in America. Both these species were fed on the same case of malignant tertian malaria by HIRSHBERG, and kept at the same temperature—30° C.

	Number fed	Number infected
A. maculipennis	48	8
A. punctipennis	58	0

Similar results (unpublished) have been obtained in Japan.

Anophelinae that are known to transmit malaria.—

Although we have over eighty species, it has been determined, only in a very few cases, which of these actually do transmit malaria in nature. Thus we know that the following species do :—

Europe.—*A. maculipennis* (mainly); *A. bifurcatus* (less concerned).
P. superpictus and *M. pseudopictus* (in some parts especially).
Africa.—*M. funesta*, *P. costalis*, *A. maculipennis* (Algeria).
N. America.—*A. maculipennis*.
W. Indies.—*Ce. albipes* (according to PAJOS).
India.—*M. listoni*, *M. culicifacies*, *N. maculatus* (?).

LITERATURE

Stephens and Christophers. *Malarial Reports to the Royal Society*. Series VI and VII. Harrison and Sons, London.

Chapter XXIII

TO MAKE A MALARIAL SURVEY

Endemic Malaria

The clue to the epidemiology of malaria in the tropics is to be found in the infection of the native population of a country. The malaria of Europeans is merely the result of their exposure to infection from this source. Investigation into the natural history of malaria, therefore, resolves itself largely into the study of native or endemic malaria. It has always been recognized that in a particular country certain districts are more malarial than others. It was not, however, till Koch used the percentage of infected children as the test of the malarial intensity of a place that accurate measurement of this became possible.

To Investigate the Endemic Malaria of a District

(A) *The Breeding-Places of Anophelines*—

1. Examine all collections of water within half-a-mile. Stir up the mud of small puddles, and use a dipper where the water is weedy or difficult of access. Examine wells, 'chatties,' streams, and swamps, as well as pools of every description. Take specimens of larvae from each, placing in specimen tubes and labelling.

2. Determine the species of the larvae collected.

3. Make a map of the neighbourhood, noting—

(*a*) All breeding grounds.

(*b*) What species are found breeding in those examined (Fig. 60).

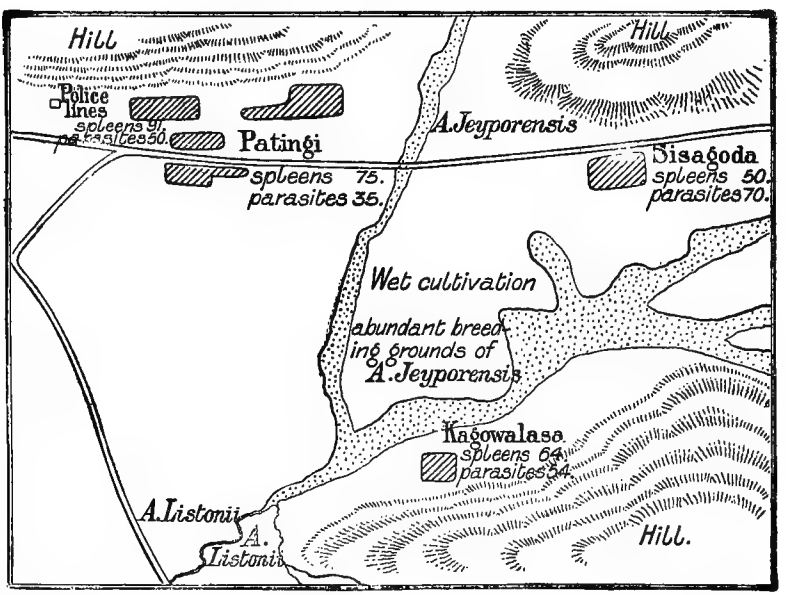


Fig. 60. *Map shewing how to make a Malarial Survey*

(B) *The Presence of Winged Anophelines*—

1. Search in outhouses, under eaves, etc., as described in Chap. XIII, for *Anophelines*. Determine the species, note relative numbers of each species on map. The relation of *Anophelines* to native dwellings will probably be evident.

2. In the dry season the search for *Anophelines* may be negative, and there may be no breeding-places. Make in the most sheltered places small cement pools, and keep these filled with water. After a certain number of days they may contain young *Anopheline* larvae if the adults are present in the houses. (It is necessary to be sure one's water supply does not contain young larvae or eggs). The absence of the larvae in the pools does not necessarily mean, however, that adult *Anophelines* are not present in the houses (see choice of breeding-grounds by different species of *Anophelines*).

Note the result in the case of each test pool.

3. In the conditions just described observe the pools made by the first shower of rain of the on-coming 'rains.' Note after three days have passed the presence of larvae in many of these. Note the presence of these on the map. The distribution of *Anophelines* at the end of the DRY season will usually be found to correspond to that of native huts.

The Prevalence of Malaria

If we proceed to ascertain to what extent malaria prevails in a district we may attempt to do so in several ways.

1. We may consult hospital statistics and returns of death from malaria. This method is open to such grave error that it is extremely doubtful whether it is worth the labour bestowed upon it.

2. We may determine to what extent enlargement of the spleen occurs. This method has been largely used.

Precautions Necessary in Applying the Spleen Test

1. *The Age of the Individuals Examined.*—The enlargement of the spleen due to ordinary malarial infection tends to disappear once the individual has ceased to suffer from malarial infection. In very malarious countries, where each individual, after childhood, has become highly immune, the adult population usually shew no splenic enlargement (Tropical Africa).

In less malarious regions the adults have not become highly immunized, and a certain number of them will be found with enlarged spleens and malarial infection. The use then of the percentage of adults with enlarged spleens is not a reliable method of determining the real intensity of malaria.

In children, the spleen enlargement appears to require a certain time to become apparent, and it takes a certain time to disappear, as the malarial infection disappears with ensuing immunity.

In the examination of children for splenic enlargement and the presence of parasites in their blood, we found :—

(i) In the early ages, one to two years, the number infected is usually in excess of those shewing splenic enlargement.

(ii) Above two years, the spleen rate is usually somewhat in excess of the parasite rate.

(iii) Above ten years, the spleen rate is usually considerably in excess of the parasite rate.

In the use of a spleen census one should then avoid a mixed adult and child count, and children

between two years and ten years of age should be chosen.

2. *The District in Question.*—It seems clear that the comparison of the malaria of widely different regions by means of the percentage of enlarged spleens in the children is not possible. We have, however, found that in Bengal, the parasite rate and the spleen rate in children varied proportionally, the spleen rate was, however, nearly always about double that of the parasite rate.

3. *Time of Year. Seasonal Variations.*—We may determine by actual blood examination how many individuals have parasites in the peripheral circulation. By the use of the parasite rate in children up to ten years of age we get a definite and true index of endemicity which may be used in the comparison of one locality with another.

4. To the last method we would add, as a complimentary one, the determination of the percentage of infected *Anopheles* as giving the actual risk of infection in a district.

The Determination of the Endemic Index of a Place

1. Place a number of cleaned slides in a slide box. Take a straight surgical needle, paper and pencil.

2. Choose any village or quarter of a town. Get the assistance of a native with local influence, the native magistrate in an Indian bustee, the chief in an African village. Instruct him to muster the children of the village. The free display of 'pice,' half-pence, etc., will greatly aid one,

and by palpating a few spleens previously to taking blood specimens the children will come readily. It is well first to take the blood of one or two adults or big boys so as to allay fears. In all cases it will be found best to take for granted the willingness of the child, and if the operation is quickly and quietly performed there is little objection, especially when each receives payment.

3. Make dry blood films by the method described in the early part of the book.

4. At the same time a spleen census may with advantage be made.

On examining the films determine :—

(i) Number shewing parasites or pigmented leucocytes in the blood.

(ii) The species of each parasite present and the percentage value for each if the numbers are large enough.

To Determine the Infection in the Anopheles

(The Sporozoit Rate)

1. Collect as large a number of *Anophelines* as convenient from the village in and around which the previous observations have been made.

2. Dissect as many specimens as possible, noting in each case the species dissected, and noting in which species, if any, sporozoits are found.

In many cases the sporozoit rate is extraordinarily low, *e.g.*, two per cent., although *Anophelines* are abundant and the malarial index

is not low. In others, especially in African bush stations, the percentage may reach fifty per cent.

3. Leave specimens not dissected for several days and examine the mid-gut for zygotes.

Malarial Infection of Europeans

Although malaria is an infectious disease, and can arise only from an original human source, yet in the tropics we can no longer consider the origin of infection as occasional and due to the presence of other cases of 'fever.' In the tropics, and especially in Africa, we are dealing with a disease which is a normal condition of childhood, and which, with the coincident infection of *Anophelines*, is the usual accompaniment of every native hut.

European malaria in the tropics is, indeed, chiefly dependent on two factors—

1. The degree of exposure to native malaria, *i.e.*, the proximity to native dwellings.

2. The endemic index of the native dwellings in question.

To Investigate European Malaria

1. Examine the blood of as many Europeans as possible. Enquire *carefully* whether the person is taking quinine at the time, also take the temperature.

(i) The number shewing parasites or crescents.

(ii) The presence of pigmented leucocytes.

(iii) The presence of an increase of the large mononuclear leucocytes.

In every case make a differential count of the leucocytes and keep the record.

Observe especially, any community of Europeans shewing a larger percentage than usual of malarial infection. Note the conditions under which these are living, and note also the probable greater prevalence of blackwater fever in these communities, *e.g.*, Roman Catholic Fathers, West African miners, railway communities, Europeans in poor circumstances living in the slums of native towns, etc., Syrian hawkers, etc. Note those communities habitually taking quinine.

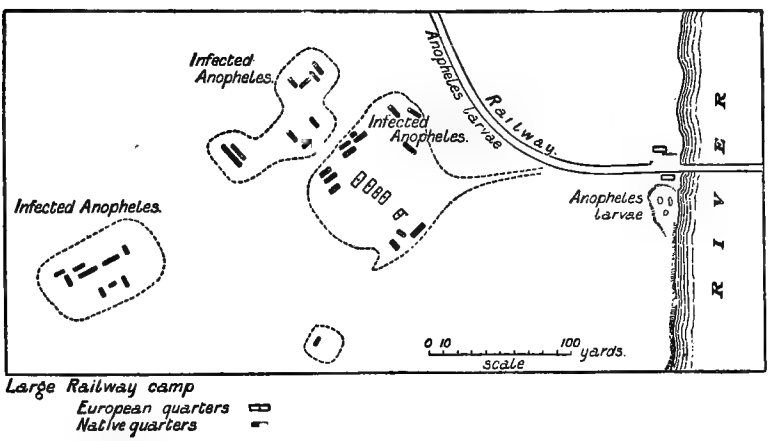


Fig. 61. *Shews how Europeans are infected with Malaria from the native (children)*

2. Note the usual relation between the degree of ill-health and the proximity of native huts. Make a map shewing European dwellings and shewing huts and hovels in relation with these (Fig. 61).

3. Make a thorough investigation of the conditions in these huts.

(i) The percentage of infected children in each group.

(ii) The degree of infection of the adults.

(iii) Roughly estimate the number of *Anopheles* present, whether swarming, abundant, scanty, or impossible to detect by search. In the latter case make several 'test pools.'

(iv) Determine the species present and the relative numbers of each.

(v) Determine the sporozoit rate for each species.

(vi) Carefully map all breeding-places, noting what larvae are found.

4. Capture as many *Anophelines* as possible in the European houses, especially in the morning, and by looking within the nets. Determine the species, sporozoit rate, and from where probably derived. Examine the ovaries and spermatheca, and note whether freshly hatched or impregnated females are chiefly found. Note the presence or absence of males.

In investigating the malaria of any such settlement, native and European, continue the observations if possible throughout the year. Make observations on—

1. Seasonal variations in the endemic index (percentage of infected children).

2. Seasonal variations in the number of cases among Europeans.

3. Prevalence of any particular species of *Anophelines* at any time of the year.

4. Distance of flight of *Anophelines* from breeding-grounds, etc.

5. Sporozoit rate of *Anophelines* at different times of the year.

6. Examine especially the conditions where *Anophelines*, breeding-places, native huts, opportunity for constant importation of malaria and numerous susceptible children exist, and yet there is a complete absence of endemic malaria. In Africa it will probably be impossible to find such places, but they occur in India.

Endemic Areas of a Country

The map (p. 253) shews how the endemicity of large areas of a country is a very variable one. When opportunity offers, the endemic index should be determined for each locality, and, as far as possible, all the other facts detailed above. But the simple taking of the blood of a number of children (under ten) in any village gives at once valuable information as to malaria of the district, information which often is quite unsuspected. Thus, as is shewn in the map, the endemic index of Calcutta is 0, that is to say, in the immediate environs (not in the town itself) where practically the condition is one of a number of isolated villages, there is no malaria among the native children. At Jalpaiguri the figure is low, twelve per cent., but on reaching the foot of the Himalayas,we find the extremely high figure seventy-two per cent. In this case we were able among other differences to find a different species of *Anopheles*, which, as we have seen, is undoubtedly an important factor.

In other cases, however, all the conditions may be apparently identical, but within a distance of even ten miles we may get a change from an endemic index of 0 (Madras) to ninety (Ennur).

These differences hold good in other countries, *e.g.*, in Italy. Here the mortality from malaria in the north is comparatively trifling, while in the south and the islands it is severe.

Here the difference may be due to differences in climate, but this explanation does not suffice in the examples in India we have mentioned.

Again, we have great irregularities in the distribution of the species of parasite. The quartan, for instance, in the Duars (Bengal) is exceedingly common amongst the native children, but in Lahore it is rare.

Similar differences have been noted in Algeria, where over large areas the quartan parasite is extremely rare, yet in a few localities it occurs in seventy per cent. of cases (BILLET).

So in India, as a whole, we have certain small areas where malaria is intense, *e.g.*, the Duars, Jeypore (Madras), and Kanara (Bombay) (CHRISTY), where we also find blackwater fever; yet in others, as in the Central Provinces, where apparently all the conditions are favourable, we have only a moderate intensity.

We require, then, to examine carefully the endemic indices over large areas in order to get an accurate idea of the variations in endemic malaria. The instances we have given will shew how erroneous it is to say broadly, 'such and such a country is highly malarial,' for while this may be true of one district it might be quite untrue of another.

Further, after having established these broad data, it will be necessary to make a close survey of each individual district in order to endeavour to explain the factors at work.

Chapter XXIV

CLINICAL STUDY OF MALARIA

Enumeration of Red Cells

In blood counting, much practice only can give accurate results; inaccurate results are misleading and useless. For comparative purposes, counts should be made always at the same time, if possible, to obviate the effect of food, etc.

For diluting the blood, 0·9 per cent. salt solution may be used, or preferably, Toison's fluid, which has the following formula:—

H_2O .	160 c.c.
Glycerin .	30 c.c.
Sodium sulphate	8 grammes
Sodium chloride	1 gramme
Methyl. violet (or other stain sufficient to colour the nuclei of the leucocytes)	

The diluting fluid must always be poured into a watch glass, and it should not be sucked up out of the stock solution.

No pressure must be used to make the blood drop exude from the finger.

Blood is then sucked up to the mark 1 on the pipette, and the end of the pipette carefully wiped before plunging it into the Toison's fluid. The Toison is sucked up to the mark 101 exactly. The pipette is then rotated between finger and thumb,

so as to ensure thorough mixing. After rejecting the first few drops, which consist of diluting fluid simply, a small drop of the mixture is blown on to the counting chamber, and the coverglass applied as rapidly as possible. No fluid should escape into the moat or under the coverglass. When NEWTON'S rings are seen between the coverglass and side of the chamber, the former is in its right, closely-applied position.

In counting the corpuscles in each square, include, of those that touch or overlap the sides, only those on the left hand and top side or right hand and bottom side. Count at least 1,000 cells, the error is then only about two per cent.

The number of corpuscles per $mm.^3$

$$= \frac{\text{No. of corpuscles counted} \times \text{dilution (100)} \times 4{,}000}{\text{Number of squares counted.}}$$

The normal average values are for man 5,000,000, for woman 4,500,000.

TOTAL LEUCOCYTE COUNT

The leucocytes may also be counted at the same time as the red cells, *i.e.*, from the pipette used for the red. This method has the advantage that all errors effect both counts equally, and the true ratio of red to white may still be got. Now for counting the leucocytes much larger fields are necessary than in the case of the red, as for every five hundred red cells there is only one white, so that counting chambers especially ruled are often sold for this purpose, but their use is unnecessary and may be obviated by the following method.

We require simply to determine the area of the whole microscope field with a given eye-piece, objective and given length of tube.

1. One division of a Thoma Zeiss square = ·05 millimetre.

2. So that if the diameter happened to cover eight divisions the diameter would be ·4 millimetres, or the radius ·2 millimetres.

3. The *area* of the field will therefore be πr^2, and the corresponding volume of blood $\pi r^2 \times \frac{1}{10}$ ($\frac{1}{10}$ = depth between coverglass and chamber, *i.e.*, $\pi r^2 \frac{1}{10} = \frac{1}{79{\cdot}51}$ cubic millimetres.

4. So that to get the number of leucocytes in the cubic millimetre we must multiply by 79·51, but it would be much more convenient to multiply by 100. In that case where $\pi r^2 \times \frac{1}{10} = \frac{1}{100}$, $r = {\cdot}1785$ millimetres, or diameter of field is ·357 millimetres. We require, therefore, to arrange our microscope so that the diameter is of this value, and this is readily done.

Two observations only are necessary.

1. Draw the tube of the microscope out so that diameter of field covers exactly eight divisions of the Thoma Zeiss chamber; note length of tube. Let this = x.

2. Draw tube out so that diameter covers exactly seven divisions. Note length of tube = y.

3. Therefore an increase in tube length of $y - x$ reduces diameter from eight divisions (·4 millimetre) to seven divisions (·35 millimetre); difference = ·05 millimetre.

4. Required to calculate what increase in length will cause reduction from ·4 to ·357 (difference = ·043).

$$\text{This will be } \frac{y - x}{{\cdot}05} \times {\cdot}043$$

This calculation is made, once for all, by the observer for his microscope, and the tube is drawn out the required amount, using, of course, the same eye-piece and objective.

To find total number of leucocytes per mm.^{3}—

$$\frac{\text{Total number counted} \times 100 \times \text{dilution (100)}}{\text{Number of microscope fields counted}}$$

Count one hundred if possible.

The leucocytes may also be counted in the special pipette for white cells, but here again the method of counting by using the whole microscope field should be used. If the ' white ' counter is used, the diluting fluid should be acetic acid 0·3 per cent. Sufficient gentian violet or methyl. violet is added to this to colour the nuclei.

To Clean Pipettes

For any accuracy of observation the pipettes should be scrupulously clean. There should not be the slightest tendency for the glass ball to stick to the sides. After a count has been made, the rubber tube is removed and the contents ejected by blowing from the pointed end.

1. Suck up dilute acetic acid so that all traces of stain are removed.
2. Suck up several lots of clean water to remove the acid.
3. Then absolute alcohol two to three times to remove the water.
4. Then ether two to three times to remove the alcohol.
5. Finally, blow hot air through with a syringe, the glass barrel of which may be heated in a flame (or simply *suck* air through).

These procedures take a very short time, and it is a satisfaction to know that the pipette has been put away perfectly clean and ready for the next observation.

The Estimation of the Haemoglobin

Gower's haemoglobinometer is the simplest and best. In sucking up the blood take care not to hold the tube too vertical, as the blood readily flows out from the rather large calibre of the tube. Order from a good maker, as several inferior instruments are on the market. The round form of tube is more easy to manipulate than the flat.

The standard of comparison in this apparatus is pikro-carmine gelatine, the colour of which corresponds to a one per cent. watery solution of normal blood.

All blood counting apparatus, etc., can be got from T. Hawskley, 357 Oxford Street, London, W.

Dare's haemoglobinometer is accurate. It possesses the advantage of dispensing with a pipette. It costs £4.

To Count Platelets

Diluting fluid: glycerine saturated with dahlia, and two per cent. saline solution, take equal parts of these.

Or better, a freshly-made five per cent. solution in water of crystallized sodium metaphosphate. Dilute the exuding drop of blood with five to ten times its bulk of fluid. The exact amount need not be known. Count the ratio of platelets to red cells in an ordinary coverglass preparation.

The ratio of platelets to red cells is 1:8 about. The absolute value per mm.^{3} 635,000 about.

Differential Counting of Leucocytes (*vide* page 41).

The Leucocytes in Malaria

We shall consider (1) the total leucocytes, (2) the percentage value of each kind.

The Total Leucocytes.—We may take 10,000 as the normal value per mm.^{3}, and as 5,000,000 is the normal value for red cells, the proportion of white to red is

$$\frac{WC}{RC} = \frac{10,000}{5,000,000} = \frac{1}{500}$$

Now, in malaria, we may find two conditions, either that the total number of leucocytes is considerably below the normal value, 10,000, *i.e.*, there is leucopenia or hypoleucocytosis, or that the total number is much above 10,000, *i.e.*, leucocytosis. If there is leucopenia, say, for instance, the total number is 5,000 instead of 10,000, then $\frac{WC}{RC} = \frac{5,000}{5,000,000} = \frac{1}{1,000}$, *i.e.*, the fraction $\frac{WC}{RC}$ is smaller than normal.

If, on the contrary, the total leucocytes are 20,000 instead of 10,000, *i.e.*, leucocytosis, then $\frac{WC}{RC} = \frac{20,000}{5,000,000} = \frac{1}{250}$, *i.e.*, the fraction $\frac{WC}{RC}$ is greater than normal.

It is this ratio $\frac{WC}{RC}$ that it is important to determine, for unless the red cells are counted as well as the white, little value attaches to the leucocytic value.

Turning now to malaria, we find that we get changes of the following kinds :—

(1) 11 a.m., rigor. Red cells = 2,900,000. White cells 10,000 $\frac{WC}{RC} = \frac{1}{290}$

i.e., leucocytosis.

(2) 11.30 a.m., rigor completed $\frac{WC}{RC}=\frac{1}{764}$

i.e., leucopenia.

(3) 2 p.m., temperature 38·2° $\frac{WC}{RC}=\frac{1}{968}$

i.e., increased leucopenia.

The leucocytosis was, in this case, quite transient, followed by a marked leucopenia.

During the course of an attack, we may have changes of this kind :—

1. Some days before the attack and before parasites appear in the blood, instead of $\frac{WC}{RC}=\frac{1}{500}$ $\frac{WC}{RC}=\frac{1}{1,000}$ *i.e.*, a leucopenia.

2. During the shivering attack and height of the pyrexia, the condition changes to one of leucocytosis, so that

$$\frac{WC}{RC}=\frac{1}{300}, \frac{1}{200}, \text{ or even } \frac{1}{90}$$

3. This leucocytosis may not last long, but is followed again by a marked *leucopenia* which is at its maximum before the onset of the next attack.

$$\frac{WC}{RC} \text{ instead of } \frac{1}{500} \text{ may be } \frac{1}{800}$$

Billet (Fig. 62), who has traced out hourly the relation of the leucocyte curve to the temperature curve, has shewn that in regular curves of the tertian or quartan type, the leucocytic curve follows closely the variations in the temperature. Thus, before the febrile attack in a quartan, there may be a leucopenia represented by $\frac{WC}{RC}=\frac{1}{1200}$ at the time of the attack, however, there is a leucocytosis of $\frac{WC}{RC}=\frac{1}{200}$.

This gradually disappears, passing through the normal value $\frac{1}{500}$, and again reaching a marked *leucopenia* before the next attack. The variations are of the same kind in irregular temperatures, the leucocytosis corresponding to the rise of temperature, and the leucopenia to the apyretic intervals.

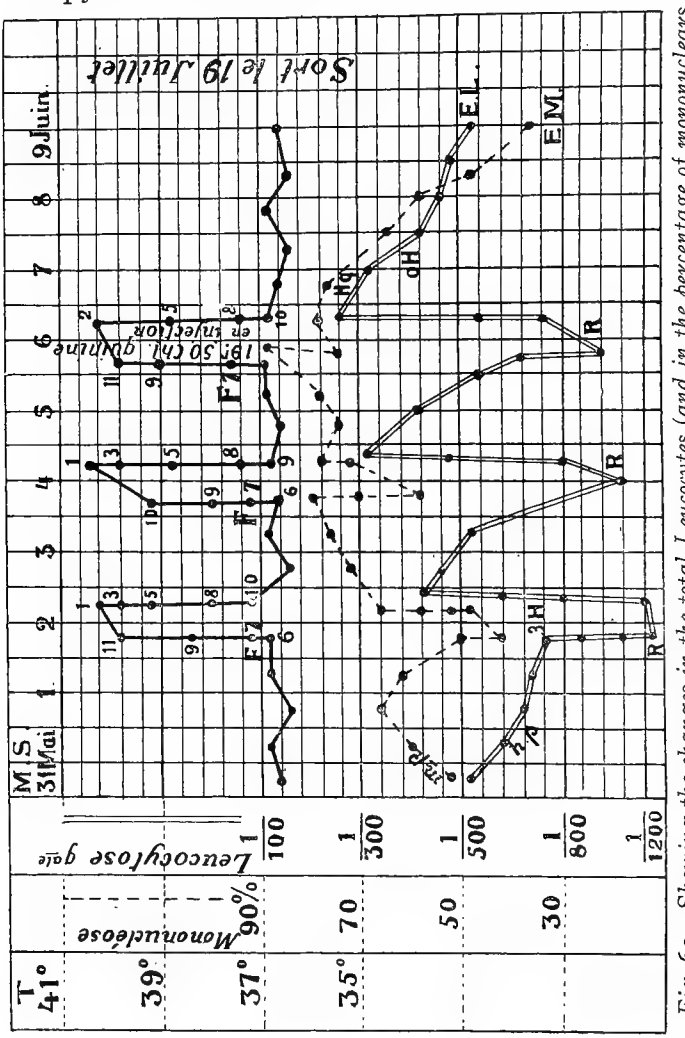


Fig. 62. Shewing the changes in the total Leucocytes (and in the percentage of mononuclears large and small) in a case of simple tertian fever. The double line curve = that of total Leucocytes. (After Billet)

The Percentage Value of the Leucocytes.—If we now make a differential count in a stained specimen we shall be able to ascertain what change, if any, there is in the relative percentage of the different kinds (for normal values *vide* p. 42).

1. The main *characteristic* change is that there is an increase in the percentage of large mononuclears, so that at times they may even outnumber the polynuclear.

2. The change is especially well-marked in the periods of apyrexia (*i.e.*, when there is a leucopenia). When there is a leucocytosis the increase in the mononuclears may not be apparent.

As examples of this leucocytic change, we may give the following :—

(i)	Small mononuclear	18·1 per cent.
	Large mononuclear and transitional	31·4 ,,
	Polynuclear	50·2 ,,
	Eosinophil	0·4 ,,

A fatal case of malignant tertian (BASTIANELLI).

(ii)	Small mononuclear	19·1 per cent.
	Large mononuclear and transitional	41·0 ,,
	Polynuclear	39·0 ,,
	Eosinophil	0·6 ,,

A fatal case of comatose malignant tertian (BASTIANELLI).

* PANSE.—Malignant tertian fever, t. 37·2° C

(iii)	Small mononuclear	18·1 per cent.
	Large mononuclear and transitional	26·4 ,,
	Polynuclear	55·3 ,,

* *Zeitschrift für Hygiene*, 1903 p. 592.

t. 97·6° F. Malignant tertian :

(iv)	Small mononuclear	14·8 per cent.
	Large mononuclear and transitional	46·7 „
	Polynuclear	38·5 „

The figures are by no means always as high as this, but, as we have already said (p. 41), we consider a value above fifteen per cent. as diagnostic of malaria. The higher values are appreciated at once by an inspection of the slide where the large mononuclears seem to occur in every field, and may be pigmented. For the low values a careful count is required.

An increase in the large mononuclears has been found in one case of human trypanosomiasis. This has not, so far, been confirmed, but if it is, it can but slightly effect the value of the counts in malaria as a diagnostic means, for the clinical features of trypanosomiasis, so far as known, are extremely characteristic, and the chance of an *European* being infected with the disease does not appear to be great, the two known cases having occurred in tropical Africa. Further, together with the increase of the mononuclears in malaria there are, if thorough search is made, also pigmented leucocytes to be found. The *relative* count of malaria is of great assistance in at least two conditions, (1) in those cases where quinine has been taken, (2) where consequently the diagnosis is uncertain and the question of typhoid fever arises. As we shall now see, the *relative* count in typhoid is quite different from that of malaria.

Typhoid Fever

During the first week (of uncomplicated cases) the leucocytes are normal.

During the second week there is a *leucopenia*, *e.g.*, 2,000, and the leucopenia is in proportion to the severity of the disease.

During the third and fourth weeks the *leucopenia* is still more marked, though also a leucocytosis may be found without any apparent cause.

Relative Leucocyte Values.—During the third, fourth, and fifth weeks the mononuclears, *large and small*, may reach the values of forty to sixty per cent., and among these the proportion of *small* mononuclears is very striking.

Pneumonia

There is very early a leucocytosis, *e.g.*, 25,000, four hours after the initial chill. The maximum occurs, as a rule, just before the crisis. The number may fall from a high value to normal in twenty-four hours. Leucocytosis is said to bear a relation to the amount of exudation (*i.e.*, lobes involved).

Relative count—

Large and small mononuclear	2 to 4 per cent.
Polynuclear	90 to 95 „
Eosinophil	rare.

The Widal Reaction in Typhoid

While we consider, it is not going too far to say that typhoid and malaria can be readily distinguished by the leucocytic count, yet seeing that

in the WIDAL reaction we have an easy means of diagnosing typhoid, the application of this test is of the greatest service in those cases where the diagnosis of malaria or typhoid remains doubtful. We shall describe briefly how the test is carried out. There is no necessity for specially constructed bulbs or graduated pipettes, as often thought.

1. Draw out a piece of glass tubing, so as to make a pipette, having a fine end about the diameter of a hypodermic needle.

2. Collect enough blood to fill the pipette to the height of about half-an-inch. The blood will readily flow in if the pipette is held sloping downwards. Seal off the fine end in a flame. Centrifugalize, if convenient, but abundance of serum can be got without by allowing to clot.

3. *To Dilute the Serum.*—Draw out a piece of glass tubing into a long fine filament; take a piece about six inches long; make an ink mark about half-an-inch from the end of the tube; insert this marked end into the tube containing serum (and clot) and allow serum to flow up to the ink mark; then let a distinct bubble of air follow (the size of this bubble does not signify); next allow broth to flow up to ink mark; repeat this procedure until nine drops of broth are in the tube; these are now each separated by an air bubble, and also by a bubble from the serum. The dilution is now one in ten.

Blow out all the drops on to a slide or watch glass, and mix by sucking up and blowing out a few times.

4. Take a drop of the diluted serum in a fresh piece of tubing, make a mark as before, and then allow broth to flow up; this gives a dilution

of one in twenty, a second drop one in thirty, third drop one in forty, and finally a drop of typhoid emulsion; this gives a dilution of serum of one in fifty, containing typhoid bacilli. The whole process of dilution takes less than five minutes.

5. *Typhoid Emulsion.*—A bacillus should be used that is known to be active. Take a fresh over-night agar culture and make a fairly thick emulsion in broth or salt solution.

6. *Dilution and Time Reaction.*—A dilution of one in fifty with a time limit of half-an-hour may be used. With a less diluted serum the time limit must be less.

7. Whatever time limit and dilution be used, it is very necessary to perform controls from time to time with a variety of other cases to make sure that the agglutination, if produced, is not produced by normal sera.

The Isotonic Point or Tonicity of the Blood

If a drop of blood is allowed to drop into a one per cent. solution of salt in a small test tube and stirred up, the uniformly turbid solution will eventually become clear when the corpuscles have settled at the bottom and the supernatant fluid will be unchanged; if, on the contrary, we add another drop of blood to a little water in a test tube the whole drop is immediately *laked*, and we have resulting a solution of haemoglobin. The former solution of salt is called hypertonic, the latter solution of water hypotonic. Now, if we start with such a hypertonic solution, one per cent. salt, and proceed gradually to dilute it, we shall

eventually reach a strength where the hypotonic, *i.e.*, haemolysing effect begins to appear. The strength of salt solution just above this where no change occurs is the isotonic point for the particular blood in question. This point then gives us information as to the resistance to a haemolytic action of the corpuscles. The blood in various diseases is found to vary in regard to the strength of salt required to prevent haemolysis. So that if a normal blood is unchanged by a 0·5 per cent. salt solution, whereas an abnormal requires 0·6 per cent. to protect it, the latter blood is described as having a *less* resistance than the former, but it has a *higher* isotonic point.

The determination of the isotonic point then gives us a more definite notion of the state of the blood in disease than does a mere determination of the haemoglobin. The isotonic point of human blood is about 0·41 per cent. salt solution.

To Determine the Isotonic Point

1. Measure out one c.c. of each salt solution of descending strengths, 0·43 per cent., 0·41 per cent., 0·39 per cent., etc., into four small test tubes and one c.c. of water into a fifth tube.

2. Add to each the amount of blood contained in two divisions of the stem of a Thoma-Zeiss pipette (the whole stem contains ten divisions).

3. Allow to stand for some time. Some of the solutions will have haemoglobin in solution.

4. The amount of haemoglobin in each tube can be estimated by adding the amount of a normal blood in two divisions to one c.c. of water. Call

this = 100 per cent. Dilute this solution, so that a number of tubes equal to ninety, eighty, seventy, etc., per cent. are got. Compare the tubes containing the salt solutions directly with these.

In malaria, the resistance of the blood is markedly lowered, thus, whereas in a control normal blood a 0·41 per cent. salt solution gave no haemolysis; in the case of two malaria patients, the haemolysis was equal to twenty-five per cent. and forty per cent., respectively.

In blackwater fever, on the contrary, a raised resistance of the blood may be found.

Clinical Study of Malaria

The Urine.—While not proposing here to consider the general reactions of the urine in malaria, for which we must refer the reader to any standard text-book, yet we think it useful to consider some points which are of more particular interest. It is especially in blackwater that we still require complete analyses of the urine, and more especially in those who are constantly subject to malarial attacks and are at the same time taking quinine. It is possible that such analyses might give us indications which would enable us to avert the danger of an attack of blackwater fever and to determine when quinine should not be given. We have not considered here the method of examining the urine by 'cryoscopy,' as it is not at present a practical clinical method, but its possibilities should not be forgotten.

Albuminuria.—The occurrence of albuminuria in malaria varies according to the particular country; thus in Rome it is uncommon, in Senegal,

on the contrary, exceedingly common. This is an illustration of the often neglected fact that tropical malaria differs in many ways from malaria of temperate climes.

Filter the urine if morphological constituents are present, as is the case in blackwater fever, through two thicknesses of filter paper, or add some calcined magnesia, then filter. Place some urine in a urine glass and, with a pipette reaching to the bottom, allow half as much nitric acid to slowly trickle in (SIMON). A white cloud at the junction layer indicates *serum albumin* (globulin or peptones). Urea nitrate crystals will often separate out at this junction layer.

Serum Globulin.—Make the urine alkaline with ammonia ; filter off any precipitated phosphates ; to the urine add an equal volume of saturated solution of ammonia sulphate. A precipitate indicates globulins ; or the formation of the precipitate may be seen at the junction layer. Test filtrate for albumin by adding excess of acetic acid and boiling.

Albumoses.—Acidify the urine with acetic acid; add an equal volume of a saturated solution of salt ; boil ; if a precipitate occurs (albumen) filter hot. Albumoses separate out on cooling ; or to the hot filtrate add caustic soda solution, then dilute copper solution gradually ; a red colour signifies albumoses.

NOTE.—Urines rich in urobilin (*e.g.*, malaria and blackwater fever) will give this biuret reaction.

In presence of urobilin : to ten c.c. of urine add eight grammes of powdered ammonium sulphate until dissolved ; boil for a few seconds ; the albumoses are precipitated on the sides of the

test tube; pour off the urine, and wash the precipitate with alcohol, then chloroform; dissolve in water and apply the biuret test. Test the alcoholic extract for urobilin.

Nucleo-Albumens.—Filter the urine carefully; boil to remove albumen; then add gradually excess of strong acetic acid. A turbidity indicates nucleo-albumens.

Blood (Haemoglobin, etc.)

1. *Examine Spectroscopically* (Fig. 63).—If the bands of methaemoglobin or oxyhaemoglobin are seen, confirm by adding ammonium sulphide when the bands of reduced haemoglobin are got.

2. *Heller's Test.*—Make the urine strongly alkaline with caustic soda; boil; the precipitate in the presence of haemoglobin is bright red; confirm by dissolving the filtered precipitate in acetic acid, a red solution is formed (spectroscopically this gives the characteristic bands of haemachromogen).

3. *Guaiacum Test.*—Equal parts of tincture of guaiacum and oil of turpentine (which has been exposed to the air) are taken; add slowly to the urine. A blue ring is formed at the junction layer.

Methaemoglobin

The urine in blackwater fever when examined early, most frequently contains blood pigment in this form, later oxyhaemoglobin. This, according to Hoppe-Seyler, also holds good for every urine with haemoglobin in solution.

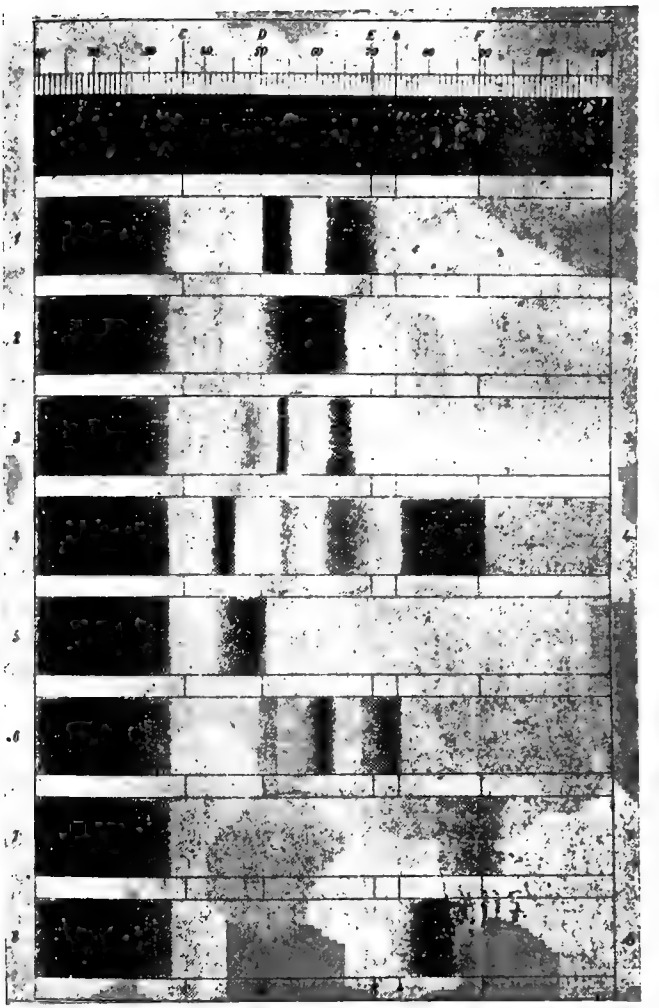


Fig. 63. *Spectra of* (1) *Oxyhaemoglobin ;* (2) *Haemoglobin ;* (3) *Alkaline Methaemoglobin ;* (4) *Methaemoglobin in Neutral or Acid Solution ;* (5) *Alkaline Haematin ;* (6) *Reduced Haematin Haemachromogen ;* (7) *Urobilin ;* (8) *Zinc-Urobilin*

The Characters of Methaemoglobin are :—

In *acid* solution the oxyhaemoglobin bands are weak or invisible. There is a band *between* C and D, nearer the former. The band of acid haematin is similar in position. It is, however, close to C.

In *alkaline* solution the acid band disappears, and a faint band on the red side of D takes its place (compare with alkaline haematin).

Reduced by ammonium sulphide, the bands of reduced haemoglobin are got. It differs from oxyhaemoglobin in its chemical reactions by the fact that it is precipitated by basic or neutral lead acetate solution, whereas oxyhaemoglobin is not.

Detection :—

1. In presence of oxyhaemoglobin. Ppt. with basic lead acetate ; filter, decompose the precipitate with carbonate of soda solution ; examine for the bands of alk-methaemoglobin.

2. In presence of urobilin. Proceed in the same way.

3. In presence of bile pigment. Precipitate these by making the solution alkaline with ammonia after adding $CaCl_2$.

4. In neutral solutions its spectrum is identical with that of haematin in natural solutions (Neubauer and Vogel). Reduced by $(NH_4)_2S$, methaemoglobin is changed to reduced haemoglobin and haematin to reduced haematin, the bands of which are easily recognized.

Urobilin

Frequently occurs in the urine in jaundice instead of bile pigment.

According to HAYEM, it is associated with methaemoglobinaemia. Its occurrence in blackwater fever is very common, occasionally before the attacks, but more constantly after the oxyhaemoglobin has disappeared or together with it.

Characteristics :—

1. In *acid* urine a band near F occurs, between 88 and 101.

2. In *alkaline* urine a band between 81 and 95.

3. Make the urine strongly alkaline with ammonia filter, add $ZnCl_2$ solution, but not sufficient to form a permanent precipitate.

A green fluorescence occurs, and the much clearer band nearer 'b' than the acid band.

Detection :—

1. If oxyhaemoglobin is present. Precipitate the urobilin with *basic* lead acetate, then acidify the precipitate, when the urobilin goes into solution.

2. If methaemoglobin is present. *Neutralize* the urine with carbonate of soda; precipitate the methaemoglobin with neutral lead acetate. Filter; test the filtrate for urobilin.

BILE PIGMENTS

Where urobilin is present, as in blackwater, the colour of the foam on shaking the urine, the staining of the filter paper, etc., cannot be regarded as satisfactory texts.

Detection :—

1. *Gmelin-Rosenbach Test.*—Filter the urine through filter paper (Swedish). Dry; apply a drop of nitric acid (fuming) to this, a play of colours is got.

2. *Huppert's Test.*—Precipitate the urine with $BaCl_2$. Filter; wash the residue off the filter (perforated) with acidulated H_2SO_4 alcohol. Boil. A bright green colour indicates bilirubin.

3. *Smith's Test.*—To ten c.c. of the urine add two c.c. of dilute tincture of iodine (tincture of iodine 1, alcohol 10). A green ring forms at the junction zone.

Bilirubin and Haematoidin (in Urinary Sediment)

1. Bilirubin crystals form yellowish-brown rhomboidal plates or needles.

Easily soluble in $CHCl_3$. Gives Gmelin's reaction, green, under the microscope.

2. Haematoidin, dark-red in colour or greenish if impure, with nitric acid they give a transient blue.

According to Hoppe-Seyler, however, they are identical.

Haematoporphyrin

Occurs in urine as alkaline haematoporphyrin (Fig. 64). In urate sediments a similar form occurs. It is soluble in chloroform, giving bands similar to those of oxyhaemoglobin, but acid converts this into acid haematoporphyrin bands. Solutions have a brilliant red fluorescence. It is found in the urine in toxic conditions, such as chronic sulphonal poisoning. It is precipitated by lead acetate, while oxyhaemoglobin is not.

Sugar

Before testing for sugar, boil to remove all proteids.

Reduction of copper solution is effected by bile pigments. Reduction occurs also in patients taking salicylic acid, sulphonal, and quinine (SIMON), so that it may be necessary to use—

1. *Fermentation Test* or

2. *Phenyl-Hydrazine Test.*—Take a pinch of pure phenyl-hydrazine, ten drops glacial acetic acid, one c.c. of a saturated solution of common

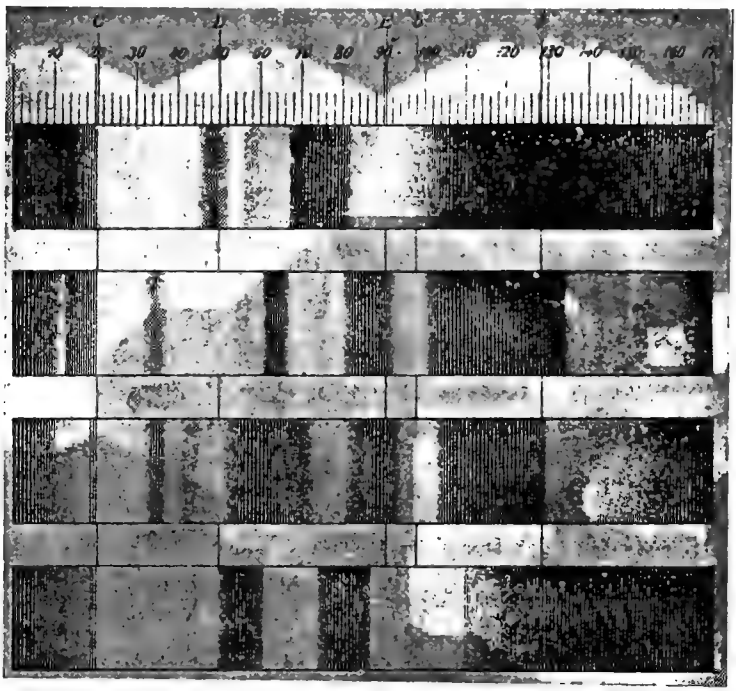


Fig. 64. *Spectra of* (1) *Haematoporphyrin* (*in Acid Solution*); (2) *Haematoporphyrin in Alkaline Solution*; (3) *in Neutral Solution*; (4) *Haematoporphyrin* (*urate-sediments*)

salt; add three c.c. of urine; boil for three minutes; cool; crystals separate out in a few minutes up to one hour. This is an exceedingly delicate test.

The Detection of Quinine in the Urine*

The detection of quinine in the urine is of importance in connexion with the property that this drug has of inducing attacks of haemoglobinuria (blackwater fever) in patients resident in regions where malaria is especially virulent, and where generally the parasite form is the malignant tertian associated with an extremely high endemic index of native (children) malaria.

Two hundred c.c. of urine are acidified with some drops of sulphuric acid. A spoonful of solid picric acid is then added. The solution is allowed to stand for an hour and then filtered. The solution should be quite clear and should give with a saturated solution of picric acid no turbidity. If there is difficulty in getting a clear filtrate add a trace of egg albumen and filter again. The half-dry residue is then digested in an Erlenmeyer flask with fifty c.c. of 3·0 per cent. soda solution for half-an-hour on the water bath. Now add sixty c.c. chloroform; shake for two hours in a shaking apparatus. The solution of chloroform is now removed by means of a separating funnel and collected in a weighed flask. The flask should have a long neck to prevent spurting. Evaporate in a water bath and dry at 120° C. The residue is quinine. The experimental error is only one to two per cent.

* Kleine. *Zeitschrift fur Hygiene.* Bd. xxxviii, H. 3, s. 460.

Determination of the Periodicity of Parasite Development

The inspection of a temperature chart is not in itself sufficient to determine the cycle of development of a parasite. Thus, as is well known, a quotidian temperature chart may be produced by a double tertian (simple) or by a triple quartan infection. If then, in the case of the double tertian, we made microscopical examinations at definite intervals for forty-eight hours, we should find in the blood at any particular time parasites in *two* phases of development corresponding to each cycle. The accompanying chart shews how, in the case of what proved to be the malignant tertian parasite, we were able to establish the cycle of development. We proceeded to make blood examinations at frequent intervals (four hours). We found that at any particular time parasites of various sizes might be found, but by counting several hundred parasites in each film and estimating their size with a micrometer we found that at any particular time there was a preponderance of parasites of one size. Thus, at ten p.m. on the 2nd, there are numerous small forms, *i.e.*, about one-seventh to one-eighth of a red cell in diameter, and it is not till ten p.m. (about) on the 4th that the same condition of blood is found again, accordingly the parasite had a developmental cycle of forty-eight hours (approximately). And, further, we determined the periods taken to develop from small forms to largest forms in the peripheral blood (about eighteen hours) and the disappearance of these and the reappearance of numerous youngest parasites (about thirty hours).

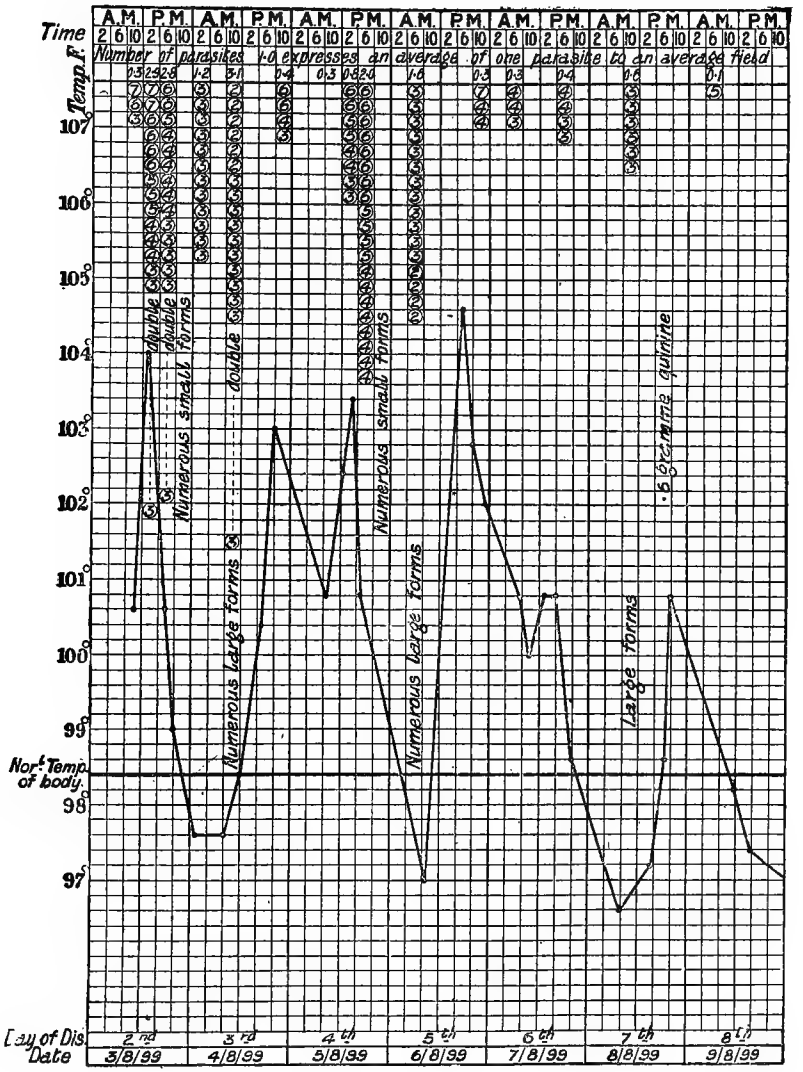


Fig. 65. *Illustrating method of determining the developmental Cycle of a Parasite. (The figures within the circles represent the size of the Parasites—thus 7 signifies the Parasite is one-seventh the diameter of a red cell)*

So that by determining these three periods we were able to conclude that the parasite was the malignant tertian.

In order then to determine the cycle of a parasite it is necessary :—

1. To estimate the size and percentage of parasites of each size at any particular time, *e.g.*, starting with the onset of the attack.

2. To follow each group to its period of maximum development in the circulation.

3. To estimate the time between this period and the next appearance of young forms.

4. To estimate the time between the appearance of an outburst of young forms (No. 1) and a second similar outburst (No. 4).

The interval between one and four should be equal to the sum of the intervals of periods two and three. It is more accurate to use a micrometer scale for measuring, but the estimation can be made with considerable accuracy without.

If we are dealing with three generations of parasites as in a triple quartan the principle is precisely the same, though it may require careful observation to separate the different groups, though in this particular case the process is facilitated by the presence of segmenting and pre-segmenting bodies which are easily counted. In order then to establish a parasite cycle, repeated observations at definite intervals are necessary, and also the temperature should be carefully recorded every four hours or two hours as considerable variations may otherwise escape observation.

Simple Tertian

Examined during the commencement of apyrexia, young parasites are found one-fifth to one-ninth the size of the red cell. The corpuscles may be slightly larger than normal. On the following day the parasites occupy one-half to two-thirds of the corpuscle, much pigmented, but not so actively motile as the smaller ones. The red cells are much enlarged at the end of the second day; presegmenting forms are found. Division into four to six or more parts may take place six hours before an attack, but true fission forms are only found two to three hours before an attack. These forms have sixteen to twenty spores; so that, here again, the multiplication of a group of parasites may be shown, microscopically, to coincide with a febrile attack. But, as opposed to quartan, the actual number of fission forms in the peripheral circulation is small; they are far more numerous in the spleen.

Double Tertian

The interval between the development of the two groups of parasites is about twenty-four hours. So that if a blood examination be made just before an attack, fission forms will be found, and parasites about half-grown. Here again, by following out the development periodically, these latter forms will be found to sporulate on the next day.

Quartan Parasite

If the blood be examined as soon as apyrexia sets in, the corpuscles will contain young parasites

one-fifth to one-sixth as large as the red cells; growth is progressive during the apyrexia, and six to ten hours before the next attack 'presegmenting' forms will be found. The parasite now fills the cell which is not enlarged. The pigment is arranged in radiating bands. The next stage is the concentration of the pigment in a central mass, and it is seen in fresh or stained specimens that the cytoplasm is divided into eight to ten segments or oval bodies (daisy form). This segmentation is nearly coincident with the next attack, but parasites in complete fission may be found five to six hours earlier. As the temperature rises these sporulating forms disappear and, again, young forms are found in the red cells. The quartan then goes through all its stages in the peripheral circulation.

Double Quartan

Two groups of parasites going through the above regular cycle, with about a day's interval, may be followed in the blood.

Triple Quartan

There are three distinct groups sporulating on successive days.

Malignant Tertian (Tropical)

During the pyrexia, small forms, one-eighth [one-fifth] of the red cell, may be found. *They persist during the pyrexia, i.e.*, for the greater part fo a day. The parasites at this stage may be extraordinarily few.

During the apyrexia, forms one-fourth to one-third of a red cell are found, and the parasites are found in greatest numbers. Fission and presegmenting forms are extremely rare in the tropics. During the next attack the young forms are again found, and so the time of the cycle, as we have shown above, may be deduced, and may be controlled by observation of intermediate stages.

Quotidian

Parasites have been described which complete their development in twenty-four hours (about). Thus, at the pyrexia young forms occur. During the apyretic interval large forms and presegmenting forms, and, again, at the next attack young forms, thus developing in twenty-four hours. As we have stated above, to establish accurately this cycle three periods would have to be traced :—

No. 1. (? Twelve hours) from young forms to largest forms.

No. 2. (? Twelve hours) from largest forms to young forms.

No. 3. Twenty-four hours from young forms to young forms.

While some consider that the quotidian temperature is due to the fact that the malignant tertian has a very variable period of development, viz., twenty-four to forty-eight hours, and, in fact, all intermediate times, others consider that with one generation of parasites there is a second accumulation of young forms in sufficient quantity to produce a quotidian attack.

In quotidian fever, due to the malignant tertian parasite, the characteristic febrile attack,

with its preliminary pseudo-crisis, is lost. The attack instead of lasting about a day lasts a few hours only, as in the simple tertian, and instead of a pseudo-crisis there is a true crisis, but the young parasites, as in the malignant tertian attack, are still coming into the circulation, and there follows a rise which replaces the apyretic day in the ordinary malignant tertian.

According to MAURER, in the case of the quotidian chart produced *by one generation of malignant tertian parasites* we have the febrile attack produced by the division of the majority of the segmenting forms, and then a fall to normal occurs; when, however, there is a sufficient accumulation of young forms arising from the same generation there is again a rise giving the quotidian chart. As we have seen, the young forms of the malignant tertian parasite persist during the pyrexia. If, however, by any means they are destroyed or cease appearing temporarily during the day of pyrexia, we should get a fall to normal, and then as soon as this inhibiting cause was removed, again a rise, giving a quotidian chart produced by the malignant tertian parasite.

IRREGULAR TEMPERATURES

Besides the typical malignant tertian temperature chart and the quotidian chart, various irregular temperatures may occur, due solely to the malignant tertian parasite. Such charts are not at all uncommon in first attacks in the tropics, and may be followed by charts with regular curves.

The malignant tertian parasite has a developmental cycle of about forty-eight hours, and it seems

more likely that these irregular charts are produced by an irregular irruption of young forms into the circulation than that the parasite has a variable time of development. If we suppose that young fission forms exist in the internal organs, but do not commence their growth in the circulating red cells, but come into the circulation irregularly, then we should have still a constant time of development, but an inconstant time at which the development started. If, however, a quotidian parasite exists, there should be no difficulty, as we have stated above, in determining the fact by a series of measurements at fixed intervals.

Action of Quinine

The data of different investigators into the absorption and elimination of quinine exhibit considerable differences dependent upon the different conditions of experiment and the mode of estimation employed. The following statements must therefore be received with caution :—

1. *According to* Kerner *the elimination of*—

Quinine hydrochlorate begins in fifteen minutes and ends in forty-eight hours.

Sulphate (neutral) begins in thirty minutes and ends in forty-eight hours.

Sulphate (basic) begins in forty-five minutes and ends in sixty hours.

2. *Mode of Administration*—

Per os, quinine appears in the urine in thirty to fifty minutes.

Per rectum, quinine appears in the urine in eighteen to twenty minutes.

Subcutaneously, quinine appears in the urine in twelve to twenty minutes.

3. *Duration of Elimination*—

According to GAROFALO it lasts one-and-a-half to seven and three-quarter hours.

According to DIETL it lasts forty-eight hours.

According to BYASSON it lasts seventy-two hours.

According to PERSONNE it lasts eight days.

4. *The Acme of Elimination*—

According to THAU and KERNER after the first six hours.

According to GAROFALO after the first one-and-a-half to four hours.

According to KLEINE after the first three to six hours.

5. *Hypodermic Injection.*—GAROFALO states that the elimination is rapid, and that larger doses can be accumulated in the blood in a shorter time by this method than by doses given by the mouth, while KLEINE states that the absorption by this method is slow. KLEINE's figures will be given below.

6. *Amount of Quinine Eliminated*—

WELITSCHOWSKI	100	per cent. about.
KERNER	95	,,
BYASSON	75	,,
KLEINE	9·27	,, (*vide* later)
PERSONNE	16	,,
MERKEL	13	,,
MARIANI, during first day	18·7	per cent.
,, second ,,	6·3	,,
,, third ,,	1·3	,,
,, fourth ,,	0·7	,,
Total	27	per cent.

7. KLEINE's data as to the amount eliminated in twenty-four hours :—

Per os Administration—

(i) 25·34 per cent.
(ii) 19·71 „
(iii) 27·29 „
(iv) 9·67 „

This low value, No. 4, is explained by the fact that the quinine was given on a full stomach, whereas in the three other results the quinine had been given to the patient fasting.

Per Clysma— (i) 17·66 per cent.
(ii) 17·15 „
(iii) 17·84 „

Subcutaneously—(i) 11·37 „
(ii) 9·70 „
(iii) 15·32 „

Now, although proportionately a smaller amount is excreted in this way (and this is possibly in conformity with the clinical experience that ringing in the ears and other unpleasant symptoms of quinine are generally absent after subcutaneous injection), yet it is probable that the excretion is a more prolonged one than by the other methods, for deposits of quinine can still be found at the site of injection some weeks later, and so the undoubted efficacy of this mode of treament may really be due to its prolonged action (and elimination).

MARIANI's results also shew that after an injection of quinine into the muscles of a rabbit, about twenty-four hours later, half the amount could still be extracted from the muscles. KLEINE and MARIANI's results shew that a full stomach inhibits markedly the absorption of quinine, so also any catarrhal state is prejudicial.

8. *Action of Quinine on Parasites.*—Quinine although it does not prevent fission yet destroys the young ring forms.

As is well pointed out by MARCHIAFAVA and BIGNAMI, the ensuing attack may still lack nothing in severity, although parasites are exceedingly scanty.

Although this may be considered as the typical action of quinine, yet there are cases, as anybody who has observed really severe cases of tropical fever, *e.g.*, in West Africa, well knows, in which quinine has not always this inhibitory effect.

In such cases the number of parasites may be exceedingly small or even absent, and yet the severity of the symptoms persist. To those cases where with severe symptoms and yet an absence of parasites and to those cases where other factors promote the rapid disappearance of parasites we shall refer in the succeeding section.

1. *On Young Parasites* (malignant tertain).—When quinine is given at the time of their first appearance in the circulation, the parasites continue in the circulation for a variable period of time depending upon the amount of quinine and probably on other unknown factors. Although parasites are still found yet their growth is arrested, and the outburst is not followed by large forms, presegmenting, and eventually fission forms. It must be noted that quinine may have no such inhibitory effect at all.

2. *On Large Parasites.*—The parasites still go on developing as far as presegmenting and segmenting forms, but generally there is no subsequent production of young forms.

3. *On Presegmenting and Segmenting Forms.* These are, as we have said, rarely found in the circulation, but if quinine is given at the time that corresponds to this stage, the subsequent effect is that very few young rings appear at the next attack.

Quinine Haemoglobinuria

Between this phenomenon and blackwater fever there is, in our opinion, practically no difference. It is apparently true that cases of blackwater fever do rarely occur in which no quinine has been previously administered, and in which we have the exciting cause of 'chill,' other drugs, 'exertion,' etc., but it does not effect the position that quinine, *not necessarily* in large doses, is the common cause of this phenomenon. We cannot here enter into the evidence for this fact, but must refer to the literature of the subject.

Blackwater fever is then a quinine intoxication, but it is something more. It occurs only in those who have previously suffered from malaria, and, in fact, there is considerable evidence to shew that it occurs frequently in *direct association* with a malarial infection. It has often been denied that blackwater fever is malarial at all, on account of the scarcity or frequent absence of parasites, but, as we shall shew on page 308, this depends upon when the examination is made. Regarding the haemoglobinuria attack:—

1. The haemoglobinuria follows the administration of quinine after a certain variable interval,

two to three frequently, five to six or possibly twenty-four hours.

2. The amount of quinine does not determine whether the haemoglobinuria is slight or severe.

3. After haemoglobinuria has been produced by quinine, a second administration does not necessarily produce a second attack of haemoglobinuria.

These facts clearly shew that it is not the quinine, *per se*, but a condition of blood in the particular malarial patient which is the determining factor whether quinine will produce an attack.

This is further borne out by the well-known fact that the aborigines rarely, if ever, suffer from haemoglobinuria, but it is in Europeans subjected to unnatural climatic conditions and subjected to virulent malaria that the disease is most frequently found.

We would only add, finally, that it is quite illogical to abstain from quinine in malaria, on the contrary, its *adequate* administration would prevent the occurrence of these attacks.

As we have already said, an accurate study of the urine in these cases and in allied cases of malaria where quinine produces urobilinuria is necessary.

Especially important is the study of the urine and the blood in the prehaemoglobinuric state. It would, of course, involve an accurate study of all possible subjects of the disease, and more especially those who had already had an attack.

Post-Mortem Changes in Malaria (Marchiafava and Bignami)

Brain :—

1. Punctiform haemorrhages of the meninges.

2. Punctiform haemorrhages of the white substance of the brain.

3. The brain capillaries may contain nearly every red cell infected. Sporulating forms are especially common.

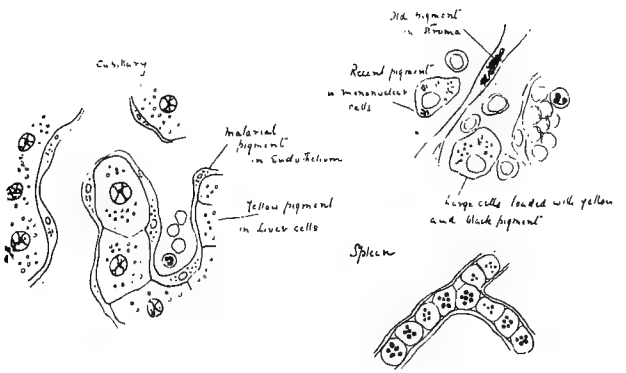


Fig. 66, *Shewing deposition of Pigment in Liver (left), Spleen (right), and Sporulating Parasites in Brain Capillary (bottom)*

4. The capillary endothelium may show fatty degeneration, together with pigmentation, and sometimes parasites.

5. Similar appearances are also found in the vessel of the pia mater.

Lungs :—

1. Large pigmented mononuclears in the capillaries, but especially in the veins; in the lungs especially phagocytosis is proceeding.

2. There is a terminal infection with the diplococcus pneumoniae.

Spleen.—The trabeculae of the pulp are distended by infected red cells, and pigmented large mononuclears are abundant. The malpighian follicles, on the contrary, are non-pigmented.

Liver.—Endothelium of capillaries is swollen and pigmented. Pigment is also found in KUPFER'S cells. The liver cells contain only haemosiderin, not melanin. Pigmentation is most intense around the central veins.

Kidneys.—Pigmentation is much less marked. Changes may occur in the epithelium of the tubules, independent of the presence of parasites.

Bone Marrow.—Parasites and melanin, free, and in large mononuclear, leucocytes and macrophages are found. Crescents may be found here when absent or scanty elsewhere, as in the spleen and brain; it is consequently supposed that they principally develop here.

In cases of malaria of long standing the yellow marrow becomes red.

Stomach and Intestines.—In malaria with choleraic or haemorrhagic symptoms, parasites may abound in the capillaries of the villi.

CHRONIC MALARIA

Spleen.—As is well known, the spleen may in these cases fill the whole abdomen. Dilatation of the various lacunae occurs with a thickening of the splenic reticulum. The pigment tends to become deposited eventually in the connective tissue surrounding the follicles. The splenic septa become thickened.

Liver.—The pigment is found mainly in the *periphery* of the lobules, and pigment in the form of blocks in the perivascular connective tissue.

The capillaries are much dilated, and the epithelium contains blocks of pigment. Atrophy of the liver cells and their nuclei occurs.

Bone Marrow.—The marrow of the long bones is usually red, due to a large development of haematoblastic tissue. Normoblasts are common.

Pigment disappears rapidly from the bone marrow.

LITERATURE

Marchiafava and Bignami. *Twentieth Century Practice of Medicine.* Malaria. Vol. XIX. S. Low, Marston and Co. This comprehensive and learned treatise is incomparably the best in the English language, dealing with all aspects of malaria and also blackwater fever.

Chapter XXV

BLACKWATER FEVER

Diagnosis.—Attention should be paid to the following points :—

(1) *Haemoglobinuria.*—The colour of the urine may vary from a very light red to a dark porter colour.

(2) *Jaundice.*—Varying from a pale lemon yellow to a deep bronze.

(3) *Constitutional disturbance.* — Slight, or extremely severe, with a high temperature, vomiting of green bile, sudden anaemia, pain over kidneys and gall bladder, collapse.

We believe that careful observation of these points will reveal the existence of blackwater fever in many places where it is not supposed to exist.

Examination of the Blood in Blackwater Fever

1. Note the difficulty in obtaining a full-sized drop of blood.

2. Observe the 'thin' nature of the blood drop, its 'oily' nature, and the difficulty with which it adheres to the slide. These properties are best seen in severe cases.

3. Collect a specimen in a fine pipette and

allow the serum to separate. Observe whether the serum is yellow (cholaemia) or reddish (haemoglobinaemia), using the spectroscope if necessary.

4. To some of patient's serum add normal blood. Observe whether there is any haemolysis (using a haemocytometer if necessary).

5. Determine tonicity of patient's blood. Rate of coagulation approximately by placing several drops on a glass slide.

6. Count the red and white cells. The red cells are, as a rule, quite normal in shape.

7. Determine the amount of haemoglobin.

8. Make films every two hours if possible (*as early as possible*), noting accurately the time and temperature at which the films are made.

9. Examine films for parasites; if these are absent, search carefully several large films for pigmented leucocytes, as these, as also in ordinary malaria, may require long search.

10. Make careful differential counts of the leucocytes, especially when the temperature is falling, as it is then that the mononuclear increase is most marked. When the temperature is raised (*e.g.*, 103° to 105°) the polynuclears may reach ninety per cent.

11. Observe presence of normoblasts, megaloblasts, various abnormal staining reactions, *e.g.*, polychromatophilia of the red cell, especially during recovery.

12. Make careful blood counts immediately before and after administering quinine when no haemoglobinuria results. According to PANSE* there may result a blood destruction due to the

*Panse. *Zeitschrift fur Hygiene*, 1903, s. 1.

quinine, which does not shew itself as haemoglobinuria.

Microscopical investigations in this disease are frequently negative as regards malaria parasites, but it is all important when the examination is made, as the following analysis of over one hundred cases microscopically examined shows :—

Parasites are present the day *before* the attack in ninety-five per cent. of cases.

Parasites are present the day *of* the attack in seventy per cent. of cases.

Parasites are present the day *after* the attack in twenty per cent. of cases.

In a series of cases examined by ourselves in British Central Africa we found malaria parasites only in 12·5 per cent., but, as we have already shown, we have two further tests for a malarial infection :—

(1) The increase in the percentage of large mononuclear leucocytes.

(2) The presence of pigmented large mononuclear leucocytes.

By using these tests we were able to prove that 93·7 per cent., not 12·5 per cent., of our cases were due to a malarial infection.

Further, in the only case of blackwater fever seen by us *before* the onset of haemoglobinuria, *parasites were present* in abundance, afterwards they rapidly disappeared.

For the details of the proof we must refer to the original papers, where also the causative action of quinine is discussed. That quinine is the factor which, in the large majority of cases, determines the onset of haemoglobinuria appears to us equally certain.

Examination of the Urine in Blackwater Fever

1. Before the attack (if possible) examine for albumen, urobilin, reducing bodies, etc.

2. Examine so-called 'high-coloured' urines. As a rule these do not shew bile pigment.

3. Examine urine during an attack for methaemoglobin (or haematin), oxyhaemoglobin, urobilin, bile pigment (unusual), bilirubin crystals, haemoglobin casts, granular or hyaline casts, blood cells (rare).

4. Centrifugalize the urine. Examine the clear layer (as in 3), and make films of the sediment.

The sediment may contain hyaline and granular casts stained with haemoglobin. The mass of the sediment, however, consists of masses of haemoglobin of a yellowish-red colour.

Urobilinuria

As we have indicated elsewhere, the occurrence of urobilin may be an important indication in cases where a susceptibility to quinine haemoglobinuria exists: thus in Murri's case, a girl had haemoglobinuria eight times between August 3, 1894, and April 6, 1895, following upon the administration eight times of small doses of quinine. From 1895 to 1897, the girl remained well. On March 27, 1897, she was given 0·5 grammes of quinine, to see whether her disposition to quinine poisoning still remained. The result was fever, vomiting of bile, etc., albuminuria, peptonuria, and urobilinuria (not haemoglobinuria).

A. PLEHN, in a recent paper, points out a peculiar property of the urine sometimes observed in blackwater cases. On boiling the urine and allowing to stand for some time, a bright purple colour appears.

We have observed that blackwater urines made alkaline with potash, and then boiled produce a purple colour, giving the bands of haemochromogen (reduced haematin), shewing that the urine itself contained reducing bodies.

Whether PLEHN's purple colour is the same we cannot say.

POST-MORTEM EXAMINATION

1. Make smear preparations of spleen, kidney, liver, bone marrow, brain, etc. Examine for parasites and pigmented leucocytes. Parasites are generally absent, but pigmented leucocytes may occur in large numbers in the spleen. Fine pigment is also found in the liver in endothelial capillary cells (Fig. 66).

2. Cut sections, especially of brain tissue, as parasites may be found in the capillaries and nowhere else.

Spleen.—Malarial pigment (melanin) occurs in large mononuclear cells and in giant cells (macrophages). Melanin may also occur in the stroma or even beneath the capsule.

Liver.—Melanin occurs in endothelial cells, and especially in macrophages. Yellow pigment (haemosiderin) occurs in the liver cells, also, to a certain extent, in the same situations as melanin. Apply the iron reaction (*vide* Appendix) to the sections. Haemosiderin gives the blue colour, melanin does not.

Kidney.—Necrosis and desquamation of the epithelium of the convoluted tubes. The straight tubules are blocked with masses of granular matter, staining dark red with eosin. Interstitial nephritis usually not present.

Bone marrow.—Evidence of malarial infection (pigment). Proliferation of normoblasts.

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Chapter XXVI

THE HAEMOCYTOZOA—*Continued*

The haemocytozoa, or endoglobular haematazoa, are divided by LAVERAN into three genera :

1. Genus *Haemamoeba.*
2. Genus *Piroplasma.*
3. Genus *Haemogregarina.*

The genus *haemamoeba* includes the malaria parasites with which we have already fully dealt. We now proceed to the other members of the genus, and then to the other two genera, which go to make up the endoglobular haematazoa.

GENUS HAEMAMOEBA

HAEMAMOEBAE IN BIRDS

1. *H. relicta (Proteosoma grassii).*—Discovered by GRASSI in the blood of birds in Italy. In certain regions sparrows and goldfinches are commonly infected. Sparrows are frequently infected in India. In Africa numerous small birds were examined by us, but proteosoma was never found (only halteridium). Transmission from one bird to another by inoculation is readily effected. Canaries are extremely susceptible. Pigeons, among other birds, are immune. Birds that have recovered from an infection have acquired a well-marked immunity against a subsequent inoculation

The parasite is closely allied to the malaria parasite, and is especially suitable for the study of the exogenous mosquito cycle.

Endogenous Cycle (Fig. 67).—The parasite in its earliest stage is unpigmented. Coincident with growth a grain or two of pigment appears, and the characteristic property of the parasite shows itself, viz., the displacement of the nucleus of the red cell, so that the nucleus may take up a position at right angles and away from the normal one. All stages of development up to segmenting forms are found in the blood at the same time, so that no cycle of development can here be followed; nor is there any intermission in the clinical symptoms (temperature, etc.) of infected birds.

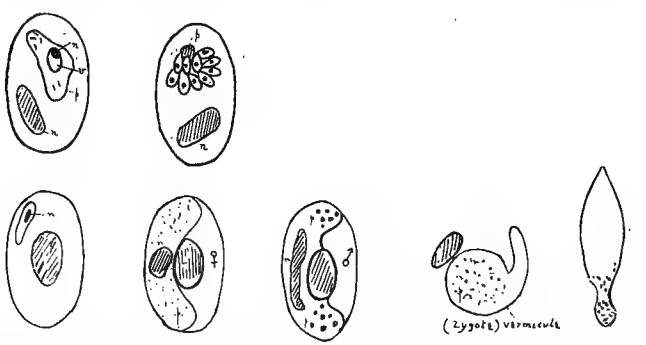

Fig. 67. (*Upper line*) *Proteosoma shewing medium-size Parasite and Segmenting Form.* (*Lower line*) *Halteridium young form, Female and Male Gametes, and Vermicule*

Exogenous Cycle.—Besides the asexual, sexual forms occur in the blood. They are spherical hyaline bodies of two varieties, characterized in stained specimens by the same general differences

which distinguish the male and female gametes of the malaria parasite.

(i) The male cell possesses a mass of compact chromatin and faintly staining protoplasm.

(ii) The female cell possesses but little chromatin, but stains deep blue (ROMANOWSKY).

Flagellation.—(i) This can be observed in a simple wet film preparation. Make stained specimens according to the method given on p. 31, or

(ii) Use artificial serum (bird's serum, one part; salt solution, 0·6 per cent., nine parts), and to this add a trace of bird's blood. Make a series of hanging drops in moist chambers. Dry, fix, and stain, from time to time, according to stage of development, observed microscopically.

Further stages of development (vermiculi) have not been observed on the slide.

Development of Vermiculi.—(i) Determine what species of *Culex* is the suitable one for the process of development. *C. nemorosus* was used by KOCH, in Italy. *C. fatigans* is also a carrier.

(ii) Collect the *Culex* that have fed on sparrows, etc., roosting at night in trees. The *Culex* can be caught in large numbers in shaded drains, under bridges, in outhouses, etc., and excellent material is in this way easily got. Identify the species of *Culex* that is infected.

(iii) For the method of feeding mosquitoes on birds' blood, *vide* p. 102.

Twelve to fifteen hours.—Vermiculi in all stages of development are found in the stomach; a conical projection arises from the fertilized gamete. This gradually elongates, forming a long, curved, oval body, the complete vermiculus. The

protoplasm is vacuolated, and a nucleus (chromatin) is readily shown by staining (ROMANOWSKY).

The proteosoma vermiculi are larger and more slender than those of halteridium.

Development of Zygotes (one to two days).—The vermiculi have disappeared, but in the stomach wall are now found transparent, spherical, pigmented bodies.

Three to four days.—The zygotes have increased in size, and sporoblasts appear in their interior. In the larger forms, signs of further division are seen (striation), formation of sporozoits.

Development of Sporozoits (nine to ten days).—By this time the sporozoits have reached the salivary glands. Somewhat earlier they can still be found amidst the thoracic muscle. Earlier still, they can be pressed out of the ripe oocysts in the stomach wall. The sporozoits occupy chiefly the middle lobe of the gland (KOCH).

Black Spores are found in the larger zygotes. They also occur free in the thoracic region (or, possibly, in the gland substance). They are brownish-black, curved, sausage-shaped bodies, suggesting a mycelial nature. It is believed by GRASSI that they are degenerated sporozoits, as they are found within the large sporoblast cysts. We have, however, found them in or about the salivary glands in *Myzomyia rossii*.

2. *H. danilewskyi* (Halteridium).—Occurs almost exclusively in the blood of 'passerine' birds. Pigeons are very commonly infected, also sparrows, finches, 'paddy' birds, parrots, etc.

The parasite is characterized by its peculiar curved halter shape, embracing the oval nucleus

of the red cell without any displacement of the latter (Fig. 67). Young forms are occasionally seen, but whether these are young sexual or asexual forms is not determined. Segmenting forms and those corresponding to an asexual cycle, as in proteosoma, are unknown.

Two varieties of parasites, the male and female gametes, are easily distinguished.

(i) Note that the male gamete has a clear hyaline appearance. On staining (ROMANOWSKY) a central mass of chromatin is distinguished, while the protoplasm is a faint blue. Five or more oval pigment grains are placed generally at either extremity.

(ii) In fresh specimens the female gamete is finely granular, and the pigment is frequently scattered throughout. On staining, a small amount of chromatin is shewn, while the protoplasm takes on a deep blue colour.

Flagellation.—Select an infected bird that shews numerous gametes in each field. Proceed in the same way as in proteosoma. The gametes first become spherical and then escape from the red cell. The pigment of the male gamete displays violent movement, and in a few minutes four to eight flagella are extended. The motion of these is at first so rapid that they cannot be distinguished, but the corpuscles in the neighbourhood are seen moving. In a few minutes one or more breaks off, and if, fortunately, a female gamete is in the same field, the loose flagellum (mikrogamete) can be seen entering the female. The pigment of the latter shews active movements at this stage.

Vermiculi.—The formation can readily be

observed on the slide. A conical projection forms at one point of the fertilized gamete (copula). This elongates slowly and gets curved, forming an egg-shaped or spindle-shaped mass. The conical portion eventually separates, leaving behind the remains of the cell with the pigment. The vermiculus is thus at first unpigmented, but later again it is pigmented (KOCH). In the fresh specimen the protoplasm appears vacuolated, and has a nucleus which is readily stained by ROMANOWSKY stain.

Note that the vermiculus (or ookinet) shews forward, rotatory, and peristaltic motions. The further development of the vermiculi is completely unknown.

Post-mortem. — Pigment is found in the kidney, intestine, bone marrow, liver, and especially the spleen. The brain, on the contrary, is almost entirely free from it.

It is probable that the halteridia of all birds are not of the same species. Inoculation from one bird to another is extremely difficult, if not impossible. This may be due to the fact that the parasites in the blood are in all sexual forms. In monkeys we appear to have a parallel condition, viz., gametes only in the blood, the asexual forms being unknown.

HAEMAMOEBAE IN MONKEYS

3. *H. kochi.*—These haemamoebae occur in monkeys. The forms usually met with are sexual forms. Asexual forms resembling young malaria parasites are very rare. Flagellation can be seen in fresh specimens. The parasites

in the fresh film are spherical pale bodies containing brownish-yellow pigment. On staining, two types can be distinguished. The male (mikrogametocyte), pale homogeneous blue with much chromatin; the female, deep blue, granular, with little chromatin.

No temperature changes occur in the infected animals. The infection is not transmissible by inoculation (*cp.* halteridium).

Post-mortem.—The spleen is pigmented, the capsule thickened. Pigment also occurs in the marrow.*

Haemamoebae in Bats

Dionisi has described in bats parasites which have a general resemblance to malarial parasites, but almost certainly have no real relation thereto. He distinguishes the following forms:—

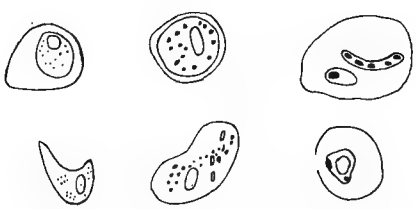

Fig. 68. (1) *H. murinus* (*above*) *medium size*; (*below*) *free form* (2) *H. melanipherus* (*above*) *medium size*; (*below*) *large Ovoid form.* (3) *H. vesperuginis* (*above*) *irregular form*; (*below*) *Ring form.* (*After* Dionisi)

4. *H. melanipherus* (polychromophilus melanipherus).—This occurs in the blood of *Miniopterus schreibersii*, and is so called on account of

*Kossel. *Zeitschrift fur Hygiene.* Bd. XXXII.

its staining reactions with ROMANOWSKY, and because it is pigmented. It somewhat resembles the quartan parasite (Fig. 68).

5. *H. murinus* (polychromophilus murinus).—Found in the blood of *Vespertilio murinus*. It is also pigmented. It shews like the former a variety of 'polychrome' effects with ROMANOWSKY. In this, as in the former, DIONISI figures a great variety of forms (Fig. 68).

6. *H. vesperuginis* (achromaticus vesperuginis).—In the blood of *Vesperugo noctula*. The young forms resemble those of the malignant tertian parasite (Fig. 68). It occurs in large numbers in the blood, but forms no pigment during its development. It produces considerable anaemia and degenerative changes in the red cell.

How bats are infected is quite unknown.

The parasite 'find' differs according to whether the animal is hibernating or not.

HAEMAMOEBAE IN CATTLE

7. [*H. bovis*].—Parasites in the blood of cattle, described by KOLLE in South Africa. They have a general resemblance to malaria parasites, but are quite distinct from *Piroplasma bovis*. They produce remittent fever and severe anaemia, but not haemoglobinuria. KOLLE also describes pigment in red cells (independently of parasites), but what this means is not clear.*

* KOLLE. *Zeitschrift fur Hygiene*. 1898.

8. *H. metchnikowi.*—Found in the blood of *Trionyx indica*, or *Chitra indica*, a large fresh-water tortoise, in many Indian rivers. All adult specimens of this tortoise from the Jumna were infected.

The parasite resembles *H. danilewskyi* (halteridium), in that two forms are easily distinguished in the blood—(1) a hyaline form with large pigment grains, staining very slightly with methylene blue; (2) a granular form, with fine pigment, staining deeply with methylene blue. These forms correspond to the male and female gametes respectively. In one of SIMOND's figures it is interesting to observe a male and female gamete in the *same* red cell, which, so far as we know, has never been observed in the case of *H. danilewskyi*. But besides these pigmented forms there are also found unpigmented forms,

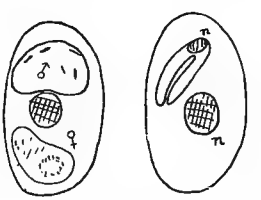

Fig. 69. *H. metchnikowi, Gametes and Vermicule*

which have the typical gregarine look, that is to say, curved, worm-like bodies. The exact relationship of the haemogregarine to the haemaebae forms is not understood. SIMOND, however, points out that halteridium has a vermicule stage, and there is the possibility of the relationship being similar in this case (Fig. 69).

Genus Haemogregarina*

The haemogregarines are unpigmented unicellular organisms, which, at one stage of their development, have a worm-like form. They occur as endoglobular parasites, and also as free forms in the plasma. The vermicule stage may be both endoglobular and free. The sexual and asexual cycles occur, as far as is known, in the same host. They occur in fish, amphibians, and reptiles, but not in mammals, and, unlike the gregarines, not in invertebrates. They are, so far as is known, non-pathogenic, and they cannot be transmitted by inoculation from one animal to another. The cycle of development, as far as it is known, will be described under the various species.

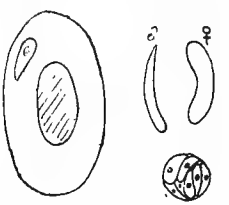

Fig. 70. H. ranarum (or Drepanidium ranarum) young form, Gametes free in the Plasma, and Fission forms in Spleen. (Partly after Minchin)

1. *H. ranarum (= Lankesterella ranarum).* Found in the blood of *Rana esculenta* (edible frog). This species includes, according to Laveran, two species, *H. princeps* and *H. monilis*, described by Labbe. Here, as in other species of

Note.—Ample material for the study of these parasites is readily obtainable in the tropics. Frogs, toads, lizards, snakes, tortoises, etc., are commonly infected. Examine especially the liver and internal organs for developmental forms. Examine carefully for ticks, lice, etc., and possible cycles in these. Preserve all ecto parasites in spirit for subsequent identification.

haemogregarines, the sexual and asexual cycles occur in the same animal. The cycle of development is as follows :—

(i) *Sexuals Form or Schizonts.*—These are endoglobular, four to eight μ in length. Increase in size takes place, and eventually they become spherical and divide into a number of segments (schizonts). According to some observers segmenting forms are only found in the spleen.

(ii) *Sexual Forms.*—Free in the plasma, twelve to fifteen μ long. These are male and female, and are characterized by the same general differences as other gametes; the male mikrogametocyte is slender and finely granular; the female makrogametocyte is fat and coarsely granular.

(iii) A mikrogamete in the form of a small mass of chromatin separates off and fertilizes the (now) makrogamete.

(iv) A zygote results, which is at first motile. This becomes encysted as the

(v) Oocyst, which is found in the *epithelial cells* of the intestine. This passes out eventually in the faeces of the frog. Sporoblasts are formed as in the malarial cycle, and from these result

(vi) *Sporozoits.*—These would gain access to a fresh frog which had swallowed an oocyst. Hintze has shewn that frogs confined in pools are especially liable to infection.

2. *H. splendens* (= *Dactylosoma splendens*). —Found in the blood of *R. esculenta.*

The following forms are figured by Labbe (Fig. 71) :—

(i) Amoeboid forms.

(ii) Forms resembling in shape a finger-glove.

(iii) Segmenting forms as in *Haemamoeba relicta* (Proteosoma).

The protoplasm contains no pigment but refractile granules.

This differs from the typical development of haemogregarines, and it is probable that its position requires revision. According to HINTZE, it is a variety of *H. ranarum*.

Fig. 71. H. splendens.—Adult form with Refractile Granules

3. *H. magna.*—Described by GRASSI and FELETTI in *R. esculenta*. MINCHIN thinks it may be the makrogamete of *H. ranarum* or *H. monilis*.

In frogs not uncommonly curious rod-shaped bodies are found lying in a vacuole in the red cell. When these occur further search will show cysts filled with these rod-like bodies. Originally described as protozoan parasites *Cytamoeba*, they are considered by LAVERAN to be bacterial in nature.

4. *H. riedyi.*—Occurs in a Californian Salamander, *Batracheseps attenuatus*.

5. *H. stepanowi.*—It is found in the tortoise, *Cistudo europaea*. This may be taken as the type haemogregarine. It presents the following forms (Fig. 72):—

(i) Reniform parasites, ten to fourteen μ long. Curved and thickened at each end,

granular, non-pigmented. Intermediate forms occur between this and the next developmental stage.

(ii) Vermicule forms, also endoglobular, but after examining a fresh specimen of blood for some time, free forms are seen thirty to forty μ long and three to four μ broad. These are actively motile, and constrictions can be seen travelling down their length during the motion. Young forms and reproductive forms are not seen in the circulation. These are found in the liver. The reproductive forms are at first endoglobular, but later free. They occur as

(iii) Ovoid forms, ten to sixteen μ long by four to six μ broad, shewing as many as six nuclei (chromatin masses). The protoplasm finally segments and there is formed

(iv) An actively amoeboid young form.

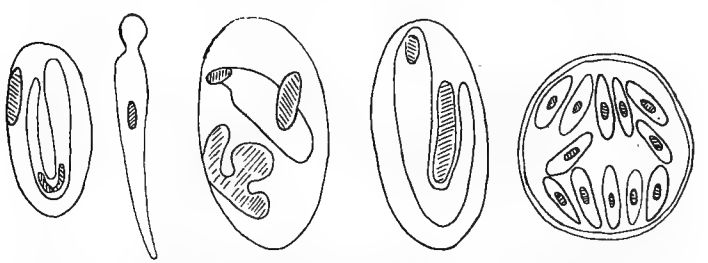

Fig. 72. H. stepanowi, Endoglobular and free Vermicules; H. lacertarum, shewing disintegration of Nucleus of Red Cell; H. lacazei, H. lacertarum, Cyst with Makromerozoits

The spores that are found in the kidneys of tortoises belong, according to LAVERAN, not to the haemogregarine at all, but are those of a *Myxosporidium (M. danilewskyi).*

6. *H. lacertarum (=Karyolysus lacertarum)* (Fig. 72).—Found in the blood of *Lacerta agilis*, *L. muralis*, and *L. ocellata*.

The parasite has a more compact form than some of the other haemogregarines. They exert a marked action on the red cells, which become much enlarged and anaemic, and, as the name of the species implies, a disintegrating action on the nucleus is one of its effects. The nucleus is either pressed to the side or broken up into fragments.

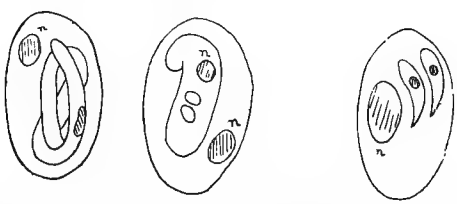

Fig. 73. (1) *H. mesnili, shewing characteristic looped Vermicule ;* (2) *H. laverani, shewing characteristic hooked Vermicule and two bright Granules (after* SIMOND) *;* 3 *H. bigemina in Blood of Blennies (after* MINCHIN)

The parasite in its endoglobular stage becomes encysted, and is then called a *cytocyst*. Further, it appears as if at this stage sexual differences appeared, for some of these cysts divide up into about a dozen *macromerozoits* (Fig. 72), while others divide up into twice as many or more *micromerozoits*. Corresponding to these we have free forms in the liver, twelve by three μ and eight by two μ respectively. According to SCHAUDINN it is transmitted by an Acarine.

7. *H. lacazei* (= *Haemocytozoon claratum*).

In the blood of lizards. The vermicules have a peculiar shape (Fig. 72). Here also cyst formation has been described in the spleen by LABBE.

8. *H. mesnili.*—In the blood of a tortoise, *Emys tectum* (Fig. 73).

Amoeboid forms, reniform, and vermicule forms occur. Besides these, free merozoits, but their origin is obscure. The form of the vermicule is characteristic at one stage of its development.

9. *H. laverani.*—In the blood of Indian tortoises, *Cryptopus granosus.* Similar forms occur to those of the last species. The vermicule is characterized by a blunt hook-like appendage, and the presence of two bright granules.

The parasite is endoglobular in all its stages.

10. *H. bigemina.*—Discovered by Laveran in the blood of blennies. A vermicule form occurs free in the plasma.

The endoglobular parasite divides by simple binary fission. In fishes we also have *H. delagei* in two species of ray and *H. simondi* in the sole.

11. *H. mauritanica.*—In *Testudo mauritanica.* Resembles *H. stepanowi* (Fig. 72). Two forms occur: (i) very granular, smaller forms, with two large refractile granules at each end; (ii) larger, uniformly pale forms. In stained specimens the smaller forms appear oval or reniform, with a nucleus transversely placed. The nucleus of the red cell is displaced. The larger forms have at one of the poles a pale blue mass, staining with difficulty. Division forms are found in the liver.

12. *H. tunisiensis.*—In *Bufo mauritanicus.* (i) Vermicule form. The two limbs of the vermicule are equal; the vermicule is encysted;

at one end of the cyst is a deep staining mass. According to BILLET there is a second cyst included in the first. This second cyst shows stippling with ROMANOWSKY, but the red cell is not hypertrophied. (ii) Elongated halter-form, 18 μ by 4-5 μ. Parasites especially abundant in the liver.

13. *H. viperini.*—Or *Karyolysus viperini.* The parasite bores into the nucleus, and, eventually, encysts there.

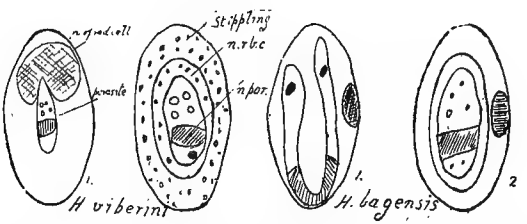

Fig. 73A. *H. viperini.* (1) *young parasite;* (2) *the parasite is encysted in the nucleus, the cyst and the red cell show stippling* (ROMANOWSKY). *H. bagensis.* (1) *vermicule;* (2) *encysted stage.*

14. *H. bagensis.*—In a tortoise (*Emys leprosa*) in Tunis. (i) Young stage does not exceed three-quarters the length of red cell. (ii) Vermicule stage encysted. The cyst does not stain. Nucleus variable in position, always central according to BILLET. (iii) Large oval forms. (iv) Division forms in liver.

15. *H. sergentium.*—In a lizard, *Gongylus ocellatus.* Has a destructive action on the nuclei of the red cells. They flatten and elongate, and

also fragment. Parasite is reniform, 15-18 μ by 5-6 μ. The protoplasm has numerous granules.

16. *H. curvirostris.*—In *Lacerta ocellata* var. *pater.* Differs from the previous one. (1) The vermicule form destroys the nucleus; (2) The encysted form becomes embedded in the nucleus.

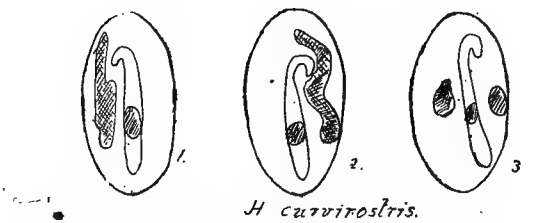

Fig. 73B. *Shewing disintegrating action on nucleus.*

Genus Piroplasma

SPECIES	HOST
P. bovis	Cattle (Texas fever organism)
P. canis	Dogs (Italy, Senegal)
P. ovis	Sheep (Italy, Roumania)
P. equi	Horse (S. Africa)
P. hominis	Man (producing 'spotted fever')

1. *P. bovis.*—'The parasite of Texas fever of cattle clearly does not belong to the proper group of malaria parasites, although it occurs in the red cell. It forms no pigment, and differs in its development from the malaria parasite' (Koch).

Technique.—Examine the blood of young calves, especially those showing some emaciation. The blood is most readily got from the ear. Wash the ear with a wet cloth, dry, and rub until the veins become prominent. Wash stained films momentarily in acetic acid, 1 in 400 H_2O, otherwise the deep blue of the red cells somewhat obscures the parasites. Examine very carefully, as parasites may be scanty in chronic cases.

The parasites are two to four μ in length, one to two μ in width. Various forms occur in the circulation—(i) a spherical or ovoid form, (ii) a piriform parasite in pairs or fours. These are characteristic, and give the name. Intermediate stages between (i) and (ii) occur. The spherical forms show a chromatic particle, and closely resemble 'young rings.' The chromatic body (nucleus) divides into two portions, one going to each end; the parasite elongates, and by this means the piriform body is got. The piriform parasites are two to three μ long and about one μ in diameter.

(iii) Bacillary forms showing, however, a red chromatic spot and blue protoplasm (Fig. 75).

(iv) Large double forms having a curious resistent appearance (*Vide* Plate) probably gamete forms of *P. bovis*.

The number of parasites in the peripheral circulation is proportionate to the severity of the disease—one to two per cent. of corpuscles are infected, at the end of the disease five to ten per cent. (or even twenty-five to thirty per cent.) The number in the blood is not so great as the number in the spleen (ten per cent.), liver (thirty per cent.), and especially kidneys (eighty per cent.)

Free parasites are found in the blood in the

later stages of the disease, but especially in the kidneys.

Post-mortem.—Haemorrhagic oedema about the stomach, kidneys, and retroperitoneal tissue. Intense hyperaemia of the spleen and kidneys, the latter are nearly black. Haemorrhagic erosions. Ulcers in various portions of the alimentary canal. Ecchymoses in pelvis of kidney.

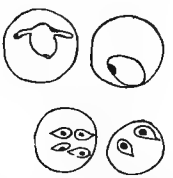

Fig. 74. Piroplasma canis (left), typical Piriform Parasites; (right) Amoeboid forms

Transmission by Ticks.—According to MOTAS (Bucharest), *Piroplasma ovis* can be transmitted by transferring *adult* ticks from an infected to a non-infected animal. He did not succeed in transmitting the disease by larvae or nymphae developed from ticks taken from infected animals. This is in direct opposition to the classical researches of SMITH and KILBORNE on *Piroplasma bovis*, corroborated by KOCH. SMITH and KILBORNE hatched young ticks from the eggs of ticks that dropped off infected cattle. It is these *young* ticks that communicate the disease.

2. *Piroplasma canis.*—The parasite is morphologically identical with *Piroplasma bovis*. It is a strictly specific parasite, and has not been transferred to any other animal than the dog. Native dogs in the tropics may harbour the parasite without shewing any symptoms.

PLATE III

Piroplasma bovis

(As seen in calves in Madras)

Fig. 1-3.—Amoeboid forms.

Fig. 4.—Dividing forms.

Fig. 5-8.—Typical piriform parasites.

Fig. 9.—Free forms.

Fig. 10.—Gamete form (?)

Fig. 11.—Bacillary form, fresh film.

Fig. 12-15.—Bacillary forms (Romanowsky).

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Technique.—Examine a number of pariah dogs, especially puppies and sickly dogs. With a scissors trim the hair off the tip of the ear and then snip the skin.

The disease exists in France, Italy, India, Africa, etc. In the chronic forms the parasite is rare in the circulation, but in the acute form with high fever, icterus, and haemoglobinuria, the parasite (typical pirosoma form) is found with great ease in the blood. Four to six parasites often occur in each cell. Kidney blood post-mortem is extremely rich in parasites. Young dogs, two to twelve weeks old, are the most easily infected, by intravenous injection. The tick, *Demacentor reticulatus*, is supposed to convey infection in Europe, and *Haemaphysalis laechi* in South Africa. Both these ticks appear to pass their larval stages on other hosts than the dog.

3. *Piroplasma ovis.*—The disease in Hungary is known as càrceag. BABES considers that the organism forms a connecting link between the bacteria and protozoa. Sheep that have recovered have a marked immunity.*

4. *P.* [*kochi*].—'African Coast Fever,' described by KOCH, is an exceedingly virulent form of piroplasma infection in Rhodesia and South Africa, eighty to ninety per cent. of infected cattle die. A peculiarity of the disease is that the anaemia is slight and, correspondingly, haemoglobinuria rare. The parasite is smaller than that of the piroplasma of Texas fever. The parasites are disc-shaped or leaf-shaped, and as the disease progresses may be found in almost every cell. Pear-shaped organisms are rare. It is suspected that *Rhipicephalus decoloratus*, or the

*Motas. *Soc. de Biol.*, 1903, p. 1,523.

blue tick, transmits the disease, for this tick transmits also Texas fever in South Africa.

It is in this form of bovine piroplasma especially that atypical forms have been described by THEILER, and subsequently more fully by LAVERAN.

(i) Forms resembling straight or curved bacilli, one to three μ long. They are thicker at one end, which contains a chromatin particle. One to four may occur in the same cell.

(ii) Forms resembling cocci, singly or in pairs. Two to four may occur in the same cell.

Fig. 75. *Atypical forms of Piroplasma* (*After* LAVERAN)

It is important to note that together with the atypical forms typical forms are always found, though these latter may be rare.

These atypical forms occur in the severe cases. Similar cocci-like forms, described by SMITH and KILBORNE in Texas fever, occurred in the *slight* cases. The post-mortem lesions, characteristic of this form of pirosomal disease, are local infarcts in various organs.

5. *Piroplasma hominis.*—This species of piroplasma is responsible for the disease known as 'spotted fever,' occurring in Montana and Idaho, U.S.A., and possibly in Egypt. As the name implies, there accompanies the fever an eruption of spots. The mortality in the United States is as high as seventy to eighty per cent. It is much

less in the cases described in Alexandria, Egypt. It is possible that they are not the same diseases, and the subject requires elucidation. In the Montana disease, piriform, ring-shaped, and cocci-like forms occur.

6. *Piroplasma equi* (LAVERAN).—Found in horses in South Africa.

TICKS

Life History.—The female, after satiating herself with blood, falls to the ground and, in a few days or weeks, lays eggs.

Eggs.—The eggs are laid in masses of several thousands (*Ixodidae*), of some hundreds (*Argasidae*). The process lasts about a week. They are small, oval, opaque bodies. They may take weeks or months to hatch out. From the eggs is developed—

The Larva.—These are hexapod. They cling to blades of grass, etc., and may do so for several months before attacking a host. The larval stage lasts six to ten days. The moult then takes place, and there emerges from the skin—

The Nymph.—These are octopod. They resemble adult females. They have respiratory stigmata, but no sexual organs. The nymphal stage lasts seven to ten days. The nymph moults, and there emerges—

The Adult.—These again attach themselves to the host, and in a few days copulation takes place. The female gradually distends and remains attached for about nine to eleven days. The female then drops off. What the male does is uncertain. The female's whole cycle *on the host* is thus twenty-two to thirty-one days. The *entire* life cycle takes, probably, about two to three months.

The dimensions of the various stages of *I. ricinus* are :--

Eggs	...	0·4 by 0·3 millimetres.
Larva	..	0·6 by 0·4 millimetres.
Nymph	...	1-3 by 0·6-2 millimetres.
Adult...	...	2·5 by 1·5 (male) millimetres.
Adult...	...	10-11 by 6-7 (female full grown) millimetres.

Ticks have a predilection for certain hosts, but the same tick may be found on different animals, and, further, the larval and adult stages may be passed on different animals.

External Anatomy

Technique.—Collect ticks from the ears of dogs, bellies of cattle, sheep, etc. Preserve in spirit. Make out in fresh specimens the following points, dissecting, if necessary :—

1. *The Rostrum*, capitulum or head, is the small anterior projecting portion, it is joined on by a short neck to the scutum; on its ventral surface is seen—

2. *The Labium* or hypostome, a bi-laterally symmetrical structure, furnished with a number of teeth directed backwards. The number of rows of teeth and their disposition are very important in classification.

3. *The Mandibles* lie dorsally to the labium. The terminal portion (digit) terminates in two or three processes, apophyses, which bear hooked teeth directed backwards. They are important in classification.

4. *The Mandibular Sheath* lies above the mandibles. The anterior extremity is notched corresponding to the two halves of the sheath.

(2), (3), and (4) form the piercing organ or *haustellum*.

5. *The Palpi* are four-jointed and form a kind of sheath for the haustellum. The shape of the palpi, their spines and processes are of the greatest importance in classification.

Fig. 76. *Eggs, Larva, and Adult Tick.* (*After* MAASEN)

6. *The Scutum* is a dorsal structure, situated behind the base of of the rostrum. It is a hard leathery plate. In the male it practically covers *the whole* of the dorsum. In the female it is confined to a roughly triangular anterior portion of the dorsum. The males are thus readily distinguished from the females. It is absent in the *Argasidae*.

7. *The Porose areas* are dorsal structures forming two oval depressions one on each side of the middle line at the base of the rostrum. They are most conspicuous in the female, but exist in both sexes.

8. *The Eyes*, not always present, are small, almost globular structures, situated laterally at the margin of the scutum in the *Ixodidae*, or as punctiform structures on the supracoxal fold of the first leg in the *Argasidae*.

9. *The Stigmata.*—Situated ventra[illegible] and laterally behind the level of the fourth le[illegible] n the

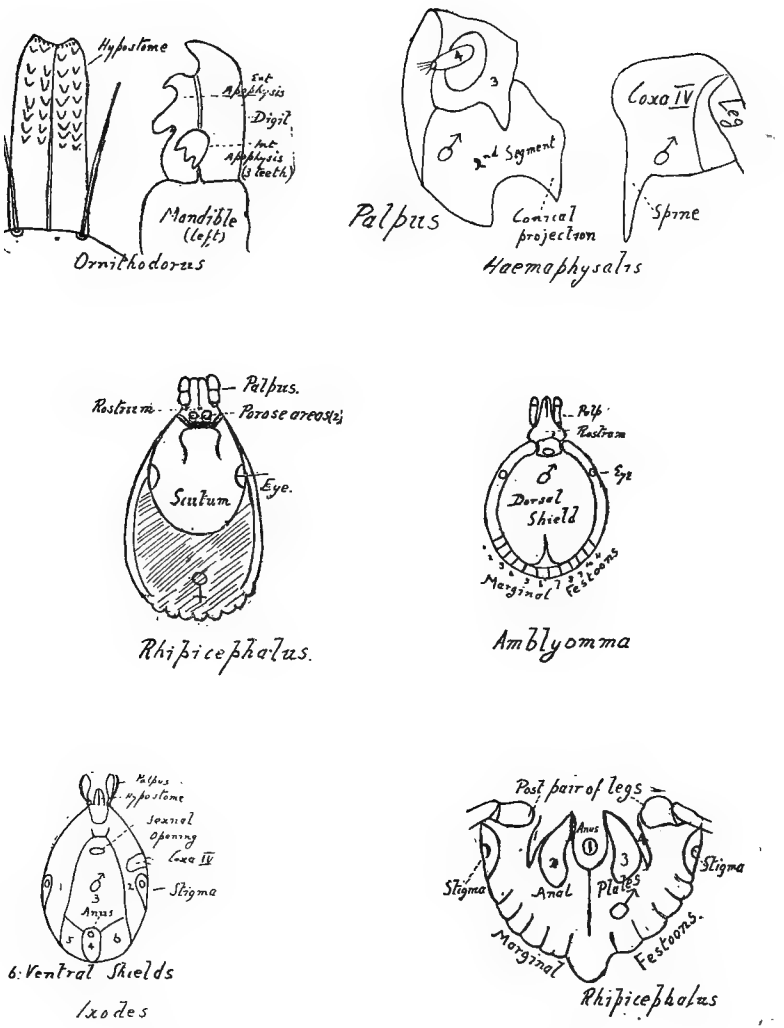

Fig. 76A. *Illustrating external anatomy of ticks.*
(Adapted from NEUMANN)

Ixodidae open into a stigmal plate or peritreme. In the *Argasidae* they lie between the third and fourth legs. The shape of the plates are important in classification.

10. *The Anus* is a little way in front of the posterior ventral margin. It has a valve.

11. *The Anal plates* or *clypei*, four in number, on either side of the anus, in the male. They are used for classifying. Not always present.

12. *The genital orifice* is in the middle line, a little way behind the rostrum.

13. *The Legs* are six in the larva, eight in the nymphs and adult. The claws have ventrally a well-marked pad or *pulvillum*. The coxa (with which the trochanter articulates) may have spines or teeth or larger 'shields.' These are used in classification.

Internal Anatomy and Dissection of Ticks

1. Take a large gravid female which has been kept a few days until it shews signs of shrinkage.

2. Taking care not to compress the tick, snip tangentially round the body.

3. Place the tick in normal saline, and now separate the dorsal from the ventral surface, and pull it gently over the head with a forceps.

4. The various viscera may now be fairly easily displayed. Observe—

(*a*) The very complicated diverticula containing blood (*d*).

(*b*) The alimentary canal similar in appearance passing direct from the head to the anus (*a*, *c*).

(*c*) The rectum. A terminal portion white in appearance (rect).

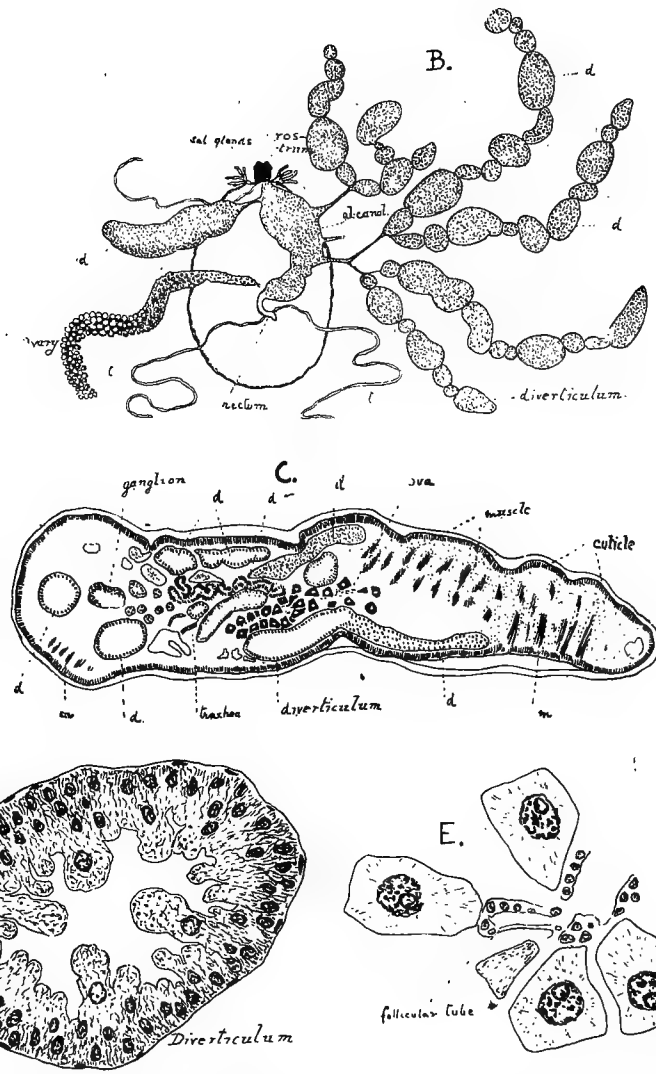

Fig. 77. Illustrating internal anatomy of ticks. B the viscera; C. a longitudinal section; D. transverse section of diverticulum; E. nearly mature ova.

(*d*) The salivary glands. A number of small tubules near the rostrum on either side.

(*e*) The ovary. A brownish elongated organ (*o*). Examine with a lens and note the large ova.

(*f*) The malpighian tubules ? (*t*).

5. *To cut sections.* Snip off portions of the chitin before placing in alcohol. Do not overharden. Use a sharp razor. Fix the sections to the slide by the hot water method. Stain with haematein. Observe the following tissues :—

(*a*) An external chitinous layer and a single row of columnar cells forming the cuticle.

(*b*) The alimentary canal and its appendages cut in various planes.

In the diverticula, distended with blood, the epithelium is flattened ; in the empty diverticula the epithelium is columnar, and the cells often have processes projecting into the lumen. Note the peculiar crystalline contents of the diverticula (altered blood).

(*c*) Large cells with large nuclei (ova). These are arranged around branches of the follicular tubes as in the mosquito.

(*d*) Undeveloped follicular tubes containing large cells.

(*e*) Tissue, rich in nuclei, especially in the posterior portion of the body (= Fat body of insects).

(*f*) A large nerve ganglion anteriorly.

(*g*) Muscle fibres passing chiefly ventrad and dorsad, tracheae supplying the viscera.

Classification of Ticks

Ticks are divided into two families—

1. *Argasidae*, scutum absent.
2. *Ixodidae*, scutum present.

The *Ixodidae* are divided into two sub-families—

1. *Rhipicephalinae.*—Palpi not longer than broad, rostrum short. Anterior portion of body emarginate to receive the rostrum.
2. *Ixodinae.*—Palpi longer than broad, rostrum long. Anterior portion of body straight or emarginate.

The *Rhipicephalinae* are the most important from our standpoint, as to the genus *Rhipicephalus* belong most of the ticks that are known to transmit parasites. The various genera are *Rhipicephalus, [Boophilus], Haemaphysalis,* and *Dermacentor.*

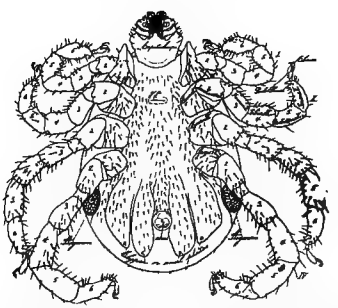

Fig. 78. Tick under surface, shewing Anatomy and parts used for classification. (*After* SALMON *and* STILES)

GENUS RHIPICEPHALUS

Eyes present. Base of rostrum hexagonal (dorsally), forming on each side a projecting angle. Palpi short and broad. Stigmata, comma-shaped; clypei, two pairs in the male. Coxae i, two large teeth. The genus includes nearly thirty species. Some of these, including the carriers of piroplasma, we may classify in the following way :—

Classification of part of Genus Rhipicephalus

Species	Furrows of Scutum	Labium	Mandibles	Scutum in Male	'Tail' in Male	Remarks
R. annulatus	Extend to post lat. margin	Eight rows of teeth	Int. apophysis bicuspid	Extends to post. margin	Absent	= *B. annulatus* = *B. bovis* Transmits American Texas Fever
R. caudatus	ditto	Ten rows	—	ditto	Distinct	
R. evertsi	ditto	Six rows	Tricuspid	Not extending to post.margin	Small	= Red Tick, South Africa
*R. decoloratus**	ditto	Six rows	Bicuspid and a rounded process	Extending to post. margin	Distinct	Transmits Texas Fever in South Africa. = Black Tick of S. Africa and Rhodesia
R. australis	ditto	Eight rows	Tricuspid and a rounded process	ditto	—	Transmits Texas Fever in Australia
R. sanguineus	ditto	Six rows	Tricuspid	Not extending	—	Europe, Africa, America, commonest tick in Rhodesia
R. pulchellus	Scutum with unequal punctations. Become obsolete in middle. Scutum white ♀ Black and white ♂	—	—	—	—	Zanzibar

* Koch describes a tick closely resembling this, but with eight rows of teeth on the labium along the East *Coast* of Africa. This species and *R. decoloratus* are possibly the transmitters of 'African Coast Fever' of Cattle

Genus Boophilus

Not admitted by Neumann as a genus.

Genus Haemaphysalis

Eyes wanting. Base of rostrum rectangular, twice as long as broad, palpi conical. Second segment of palpi has a well-marked lateral conical projection. Stigmata comma-shaped or circular. Anal shields absent in male. Coxa i not bifid. Coxa iv in male, a well-marked spur. There are about twenty-six species.

H. leachi (South Africa).—The dog is the usual host. Possibly transmits *Piroplasma canis*, occurs also on cattle.

Labium.—Four rows of teeth in ♀, five rows in ♂.

Palpi.—Dorsal surface as broad as long. Second palpal segment has a sharp lateral spine.

Coxa iv.—Has a tuberosity.

Genus Dermacentor

Eyes present. Base of rostrum broader than long. Palpi short and thick. Stigmata, comma-shaped. Anal shields absent in male. Coxa i bidentate in ♂ and ♀. Scutum ornamented. About twenty-four species.

D. Electus is the American dog tick.

The subfamily *Ixodinae* consists of five genera, *Ixodes*, *Haemalastor*, *Aponomma*, *Amblyomma* and *Hyalomma*.

Genus Ixodes

Eyes absent. Palpi long. Tarsi without terminal spurs. Anal groove surrounds anus anteriorly and opens posteriorly. Scutum in male does not cover the body laterally and posteriorly. Stigmata oval in ♂, circular in ♀. Male ventrally covered with six shields; two lateral, embracing the origins of the legs and the stigmata; one median, between the genital opening and the anus; two on each side of the anus (perianal); and one triangular posterior shield, carrying the anal orifice at its anterior corner. Female has dorsally three longitudinal grooves on the abdomen, ventrally two bell-shaped grooves, the first has its apex at the vulva, the second at the anus. There are a large number of species.

1. *I. ricinus.*—The castor-bean tick is common on sheep, goats, cattle: Europe.

2. *I. hexagonus.*—The European dog tick.

Genus Haemalastor

Eyes wanting. Rostrum long. Palpi piriform ♂, claviform ♀. Anal grooves as in *Ixodes.* Stigmata circular in both sexes. Legs very long. Dorsal and ventral chitinous thickenings in the male; fine grooves in the female. There are seven species.

Genus Aponomma

Eyes wanting. Anal groove surrounds anus posteriorly, and opens anteriorly. Anal plates absent. Base of rostrum pentagonal. Scutum

covers the dorsum entirely; usually marked with green spots. Stigmata, comma-shaped. Female, scutum shorter than broad, three green spots. The species are parasitic on reptiles.

Genus Amblyomma

Eyes present, conspicuous. Anal groove as in *Aponomma*, anal plates absent. Rostrum long. Scutum often has coloured designs. Stigmata usually triangular, nearly always eleven posterior marginal festoons in the male. There are over eighty species.

A. variegatum.—Is frequent on cattle in Rhodesia.

Genus Hyalomma

Eyes present, conspicuous. Rostrum long. Anal groove opens anteriorly. Body elongate oval. Colour deep-brown. Male, two pairs of ventral shields, two perianal, large, triangular, and two small external. Scutum covering nearly the whole of the dorsum. Crenellated or festooned posteriorly. Male, stigmata comma-shaped, with a long tail; female, stigmata with a short tail. Three species only.

H. aegyptium.—Attacks cattle especially, also dogs and cats. Occurs in Egypt, North and South Africa.

Argasidae.—Scutum absent, rostrum inferior (except in larva). Stigmata between third and fourth legs. Pulvillum of tarsi wanting in adult. Palpi, free, short, filiform, four segments. Tegument leathery, without dorsal or ventral shields. Sexual dimorphism not marked.

Genus Argas

Eyes absent. Rostrum, which is concealed

by the cephalo-thorax, is situated at least its own length behind the anterior margin. No projecting hood. Body oval or orbicular. The species are nocturnal in their habits, infest birds mainly. There are eleven species. A species of Argas is the transmitter of 'spirillar fever' of poultry.

A. reflexus infests pigeons, European.

A. persicus = Garib-Guez of Persia. The bite is said to produce severe local and constitutional effects.

A. tholozani = Kéné of Persians, similar effects ascribed to it.

Genus Ornithodorus

Eyes present or absent. Rostrum hidden under a projecting beak (hood), close to the anterior margin of the body, so that the tips of the palpi are visible from above. Lateral borders of body generally straight, sometimes concave, tegument mammillated.

O. moubata = 'Garrapata,' tick of Tete on the Zambesi. The bite is said to occasion severe local and general disturbances. It is the cause of 'tick fever' in Uganda (Christy).

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Chapter XXVII

THE TRYPANOSOMIDAE

Trypanosomata and Trypanoplasmata

The *Trypanosomidae* comprise two genera—(1) *Trypanosoma*, (2) *Trypanoplasma*. The genus *Trypanosoma* is characterized by the possession of a longitudinal undulating membrane, the thickened border of which takes its origin posteriorly from a blepharoplast, and terminates anteriorly in a free flagellum. Division takes place longitudinally. The genus *Trypanoplasma* has two flagella, one anterior the other posterior. Both arise from one blepharoplast; the anterior forms the thickened border of the undulating membrane; the posterior flagellum curves around the posterior end of the parasite, and then is prolonged into a flagellum about equal in length to the anterior one.

The *Trypanosomidae* occur in fish, amphibia, reptiles, birds, and mammals. Most of these are very incompletely known, and it is only some species in mammals that have been at all closely studied.

Trypanosomata of fish.—These are common in tropical fish, but have not yet been accurately described. Those recorded below occur in fish of temperate climes. Among marine fish they occur chiefly in the cartilaginous fish. The sole is the only osseous marine fish so far found infected.

Here, as in the case of other trypanosomes, morphological appearances often make it a difficult matter to distinguish species. The fact, however, that the trypanosome of one species of fish cannot be transmitted to another species of fish is in favour of the specific nature of the trypanosomes of each species of fish.

Mode of infection.—This, probably, is effected by ecto-parasites of the fish, especially leeches. Experimentally, it has been shown that leeches can transmit the infection. Inoculation of fish is most successful when made intra-peritoneally.

1. *T. remaki.*—In the pike (*Esox lucius*). Never very numerous. Two varieties or species occur: (i) *T. remaki*, var. *parva*, 28-30 μ long, flagellum included, by 1·4 μ broad. The blepharoplast is rather small. (ii) *T. remaki*, var. *magna*, 4·5 μ long, by 2·5 μ broad. These two varieties resemble one another very closely, except in size.

2. *T. danilewskyi.*—In the carp (*Cyprinus carpio*). 35-45 μ long, by 3 μ broad. Blepharoblast large. The protoplasm in stained specimens has many chromatic granules.

3. *T. tincae.*—In the tench (*Tinca tinca*). Generally scanty in the blood. Motility very active, and curls on itself. 35 μ long, by 2·5-3 μ broad. Posterior end is blunt.

4. *T. abramis.*—In the bream (*Abramis brama*). Recorded by LAVERAN and MESNIL, but undescribed.

5. *T. granulosum.*—In the eel (*Anguilla vulgaris*). They vary in size from 44-80 μ in length, and 2·5-3 μ in width. Very active in their movements. Undulating membrane broad. Posterior end sharply pointed. Stained specimens show

large chromatic granules. They live in blood kept at a temperature of 10-19° c. for a week.

6. *T. soleae.*—In the sole (*Solea vulgaris*). 40 μ long. Flagellum very short, 8 μ (Fig. 82).

7. *T. scyllii.*—In dog-fish (*Scyllium canicula*, etc). The trypanosome is curved on itself. 70-75 μ long, by 5-6 μ broad. Posterior end conical. Undulating membrane much folded.

8. *T. rajae.*—In rays (*Raja punctata*, etc.) Like the last it is curved on itself. 75-80 μ long, by 6 μ broad. Posterior end much drawn out in some forms, blunter in others.

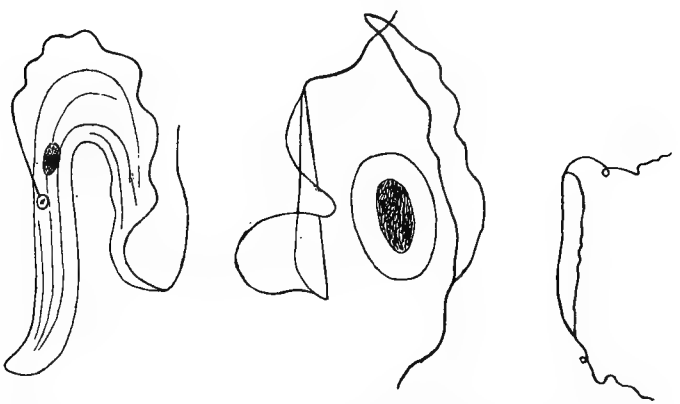


Fig. 79. *T. rotatorium, T. cobitis, T. carassii, Tp. danilewski. Left to right.* (*After* LAVERAN *and* MESNIL, MITRAPHANOW *and* DANILEWSKI)

9. *T. carassii* (MITRAPHANOW).—Besides the forms with undulating membrane and flagellum, disk-like forms are described. In the blood of fish (*Carassius vulgaris*) ; in the tench (*Tinca vulgaris*). Also described in the blood of the stickleback, pike, etc. (Fig. 79).

PLATE IV

Senegambian Trypanosomes

Birds

Fig. 1.—*T. johnstoni*, n. sp. From the blood of *Estrelda estrelda*. x 2,000.

Fig. 2.—Trypanosoma, sp. incert. x 2,000.

Frogs

Fig. 3.—*T. rotatorium*. x 2,000.

Fig. 4.—*T. mega*. n. sp. x 2,000.

Fig. 5.—*T. karyozeukton*. x 2,000. Showing chromatic granules extending in a chain from the centrosome to the nucleus.

PLATE IV

Fig. 1.

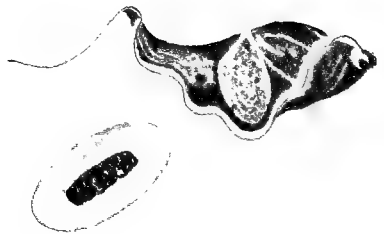


Fig. 2.

10. *T. cobitis* (MITRAPHANOW).—In the blood of the mud-fish (*Cobitis fossilis*). Thirty to forty μ long, one to two μ broad. Flagellum ten to fifteen μ. It is long and thin. Forms without an undulating membrane are described, and also without a flagellum (Fig. 79).

TRYPANOSOMATA OF BATRACHIANS

1. *T. rotatorium.*—Occurs in various species of frogs: *Rana esculenta*, *R. temporaria*, *Hyla arborea*, etc. It is characterised by extreme variation both in dimensions and in appearance. Thus we have (i) forms with striation (Fig. 79); (ii) forms without striation; (iii) spherical forms in which the undulating membrane is retracted.

The position of the blepharoplast or centrosome is also variable. Generally, it lies close to the nucleus, and, consequently, the membrane does not extend further back than this point; at other times it lies near to posterior end, with a consequent more extensive development of the membrane.

Dimensions: 40-60 μ long, by 5-40 μ broad.

It is doubtful whether the trypanosome occurring in these frogs belongs to one or to several species.

2. *T. mega.*—In a Gambian frog. (*Vide* Plate IV, Fig. 4).

3. *T. karyozeukton.*—In a Gambian frog. (*Vide* Plate IV, Fig. 5).

LAVERAN and MESNIL think that these may only be varieties of *T. rotatorium.*

4. *T. inopinatum.*—Occurs in *Rana esculenta* in Algeria. 25-30 μ long (including flagellum),

3 μ broad. Resembles *T. lewisi* and *T. rematki*. BILLET thinks it may be a developmental form of a haemogregarine occurring with it in the blood.

5. *T. nelspruitense*.—In frogs in the Transvaal, characterized by its long flagellum, 20-35 μ, giving a total length of 40-70 μ.

TRYPANOSOMATA OF BIRDS

Though first described in 1888 by DANILEWSKY, our more precise knowledge is due to LAVERAN.

1. *T. avium*.—Occurs in the owl (*Syrnium aluco*). 33-45 μ long (flagellum included). The undulating membrane is well-developed, and has several folds. The posterior extremity is pointed.

2. *T. johnstoni*.—In the blood of *Estrelda estrelda* in Gambia. It resembles a spirochaete in appearance. There is no free flagellum. (*Vide* Plate II, Fig. 1). 36-38 μ long, by 1·4-1·6 μ broad.

3. *T. paddae*.—In the blood of *Padda oryzivora*. 30-40 μ long, by 5-7 μ broad. Posterior end very pointed. Undulating membrane narrow and folded, but difficult to stain. Division takes place longitudinally. Pathogenic (?).

Other trypanosomes have been described by various observers in many different birds. The trypanosomes are, generally, scarce. DANILEWSKY states that while rare in the blood, trypanosomes may be abundant in the bone-marrow.

4. *T. noctuae*.—In the blood of an owl (*Athene noctua*) occur halteridium-like bodies. They are male and female (and hermaphrodite), and have the appearances in fresh and stained preparations which characterize other gametes. According to the remarkable work of SCHAUDINN, these so-called

halteridia are simply stages in the life history of a trypanosome. The 'halteridia' undergo their development in *Culex pipiens*. (*Vide* note, p. 368). The following stages occur :—

(1) The blood is sucked into the stomach, the gametes become spherical, and the mikrogametes (flagella), (which themselves are built on the same fundamental plan as trypanosomes) fertilise the makrogamete (female).

(2) The result, as in the case of the malaria parasite, is a zygote or copula which in eight to thirty-six hours becomes a vermicule or ookinet.

(3) These ookinets are of three kinds—hermaphrodite, male, and female.

A. Development of the ookinet into an indifferent trypanosome.—

The ookinet now develops into a typical trypanosome with nucleus, blepharoplast, undulating membrane, and flagellum.

[The locomotor apparatus of a trypanosome arises, according to SCHAUDINN, from the mitotic processes which take place in the nucleus of the ookinet. Three nuclear spindles are formed by successive divisions. The third of these becomes the locomotor apparatus. The excentric spindle fibres become the thickened edge of the undulating membrane, the central fibres form the free flagellum, while the contractile mantle fibres, eight in number, which direct the movements of the nuclear chromosomes on the spindle, become 'myonemes,' four of which run on either side in the (Ektoplasmic) undulating membrane].

We thus get an indifferent trypanosome.

B. Development of the indifferent trypanosome in the stomach of Culex pipiens.

(1) Multiplication by longitudinal division takes place ; a fresh locomotor apparatus is developed, the old one remaining unchanged. The resulting trypanosomes are small in size.

(2) The trypanosome has a resting or 'gregarine' stage. It bores its way into and even through the epithelial wall of the stomach, contracts, and the flagellum becomes a short rod-like process of attachment. It may divide in this stage, forming extensive clusters. This stage is adopted when the blood supply is scanty in the stomach. If blood is imbibed the parasites again become actively motile.

C. *Development of the indifferent trypanosomes in the blood.*

1. Some of the larger forms sub-divide in the blood.

2. The smaller forms apply themselves closely to the surface of the red cells and gradually penetrate, they lose their flagellum and now resemble young *halteridia.*

3. Pigment appears in twenty-four hours.

4. The parasites, however, again leave the red cell, *chiefly at night time*, and again become trypanosomes though now increased in size. These changes also occur especially in the internal organs, bone marrow, spleen, kidney, and liver. This process is repeated several times till after six days the fully-developed trypanosome and halterida stages are reached.

5. The fully-developed trypanosome divides rapidly till a large number of young flagellates are formed, which go through the same process until a massive infection of the red cells is produced. The developmental cycle in the blood thus takes six days. Whether in other species a formation of spores as in malaria, and as originally described by DANILEWSKY, takes place remains to be seen.

A. *The development of the ookinet* (♀) *into the female trypanosome.*

1. By a similar process the female trypanosome results. It is larger and stains more deeply than the indifferent form. The membrane is less well-developed, the blepharoplast is smaller, and the flagellum is shorter. It moves more slowly and soon takes on the gregarine form in the stomach.

B. *The development in the mosquito.*

1. They are the most resistent forms and can be found in the stomach two to three weeks after feeding, when all the other forms have died.

2. In this fasting condition they have penetrated the epithelium, and resemble malarial zygotes. It is these forms also by which the infection is transmitted to the egg as they remain alive in the ovary, and these forms can also, by parthenogenesis, give rise to all three forms, either in the stomach or in its halteridium form in the blood.

C. *Development of* ♀ *trypanosome in the blood.*

1. They enter the blood cells and grow slowly.

2. They leave the red cell not as a trypanosome but in a worm-like gregarine form.

3. They eventually form the characteristic makrogamete. At the end of the acute infection they are the only forms

remaining in the blood, but they may again, by parthenogenesis, produce all the other forms and so a relapse of the disease (Cf., relapses in malaria by parthenogenesis of the makrogamete).

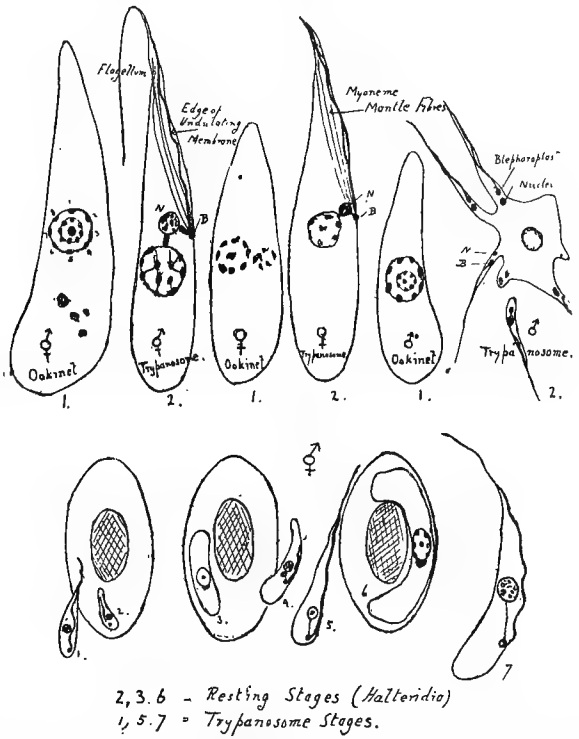


Fig. 79A. *Upper figure: shewing the three kinds of ookinet and the three trypanosomes developed from them in the mosquito's stomach. Lower figure: shewing the development of the indifferent trypanosomes into 'halteridia' in the blood (after* SCHAUDINN).

A: Development of the male trypanosome from the ookinet.

The male ookinet is hyaline without reserve stuffs, coarsely vacuolar and refractile. It is smaller than the two other ookinets, but has a relatively larger nucleus rich in chromatin. By similar nuclear changes trypanosomes are developed.

The male trypanosome is much smaller than the other two forms, but has a more fully-developed locomotor apparatus. The blepharoplast is relatively much larger, and the flagellum is longer, they are consequently very actively motile.

B. Development in the mosquito.

They do not develop further. They only become functional when they leave the blood and fertilize the female in the mosquito's stomach.

C. Development in the blood.

As the males rapidly die out in the stomach it is not often that they reach the blood. If they do they probably die quickly. The mikrogametocytes of the blood are developed from the indifferent trypanosomes.

The fertilization of the makrogamete (female trypanosome) by the mikrogamete (male trypanosome) takes place when blood is sucked into the mosquito's stomach, and thus the cycle is completed.

The circulation of the trypanosomes in the mosquito.

1. The gametes in the blood of the owl are sucked in. The formation of the ookinet, and development into trypanosomes takes place in the mid-gut in about twenty-four hours.

2. If no more blood is given the parasites (female) are now found in the resting (gregarine) stage attached to the mid-gut, and they may occur in such quantity as to kill the mosquito.

3. If a second meal of blood is given development into the motile stage occurs, followed by the resting stage. The parasites are now collected in enormous masses (thousands) around the proventriculus and commencement of the mid-gut.

4. If a third meal of blood is given these masses are washed onwards by the entering blood, eventually reach the ileum and the great upward curvature of the colon.

5. Here they penetrate the epithelium, reach the blood stream, passing some backwards to the ovaries, some forwards until they reach the blood spaces surrounding the pharyngeal pump. They accumulate here in such masses that they eventually pass through the epithelium of the pharynx and so reach the lumen.

6. They are then washed out when the contents of the diverticula, carbonic acid gas, bacteria, etc., are ejected prior to the sucking act.

7. The parasites that reach the ovaries develop there, and so can be transmitted through the larval stages to the newly-hatched mosquito.

TRYPANOSOMATA OF MAMMALS

Examine the blood or the oedema fluid, if present, of camels, cattle, horses, dogs, etc, especially those showing emaciation, oedema of any part, watery discharge from the eye, skin eruptions, etc. Apparently healthy animals may not infrequently harbour trypanosomes, *e.g.*, calves in India (Madras), the big game in Africa, etc. Compare carefully the trypanosomes found.

Fig. 80. *T. lewisi.*—(1) *Dividing form with two blepharoplasts;* (2) *Adult form;* (3) *Young forms resulting from division* (*fresh preparation*)

Trypanosomes are bodies easily detected in fresh blood with a one-sixth or one-seventh lens. They are actively motile, and may be seen displacing the red cells by their motions. As they come to rest the undulating membrane and flagellum are visible. They are bodies about twenty μ long. In stained specimens (ROMANOWSKY) an oval nucleus lies about the middle of its length, and near the blunt posterior end a small stained particle is clearly seen, the centrosome, or rather blepharoplast. From this, the flagellum starts, and can be seen as a distinct wavy thick red line extending the whole length of the organism and continued beyond as the long (anterior) free flagellum. The portion (unstained) between this external wavy margin and the blue stained body of the organism is the undulating membrane (Fig. 80).

1. *T. lewisi.*—Is found in a small percentage of ordinary sewer rats in England. It is non-pathogenic. It is common in rats of tropical countries. White rats are easily infected, but never spontaneously show infection. Possibly there are various species of this trypanosome. It is slenderer than *T. brucei.* Its undulating membrane is less wide. It is stated that infection can be transmitted by a rat flea.

2. *T. brucei.*—This is the highly pathogenic trypanosome of Ngana, or tsetse fly disease. Length 27 μ by 2 μ. *T. brucei* is fatal to nearly all, if not all, mammals. In the horse there occurs watery discharge from the eyes and nose; puffy swelling under the belly; sheath of penis, etc., marked anaemia, wasting.

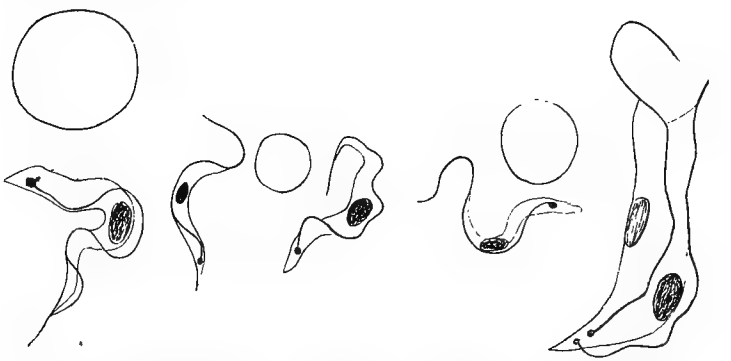


Fig. 81. *T. gambiense, T. lewisi, T. brucei, T. equiperdum, and dividing form of T. brucei, shewing two nuclei, two blepharoplasts*

The disease was shown by BRUCE to be conveyed from infected animals to healthy ones by means of *Glossina morsitans.* The fly after biting remains infective from twelve to forty-eight hours.

3. *T. evansi*.—The trypanosome of Surra. A common disease in many parts of India, *e.g.*, Bombay, at certain seasons especially, though probably always in a latent condition. It is possible that its increase at a particular time is associated with the prevalence of a biting fly. Surra is characterized by a similar train of symptoms to those of Ngana. ROGERS states that the disease in India is conveyed by *Tabanidae* (horse flies). This statement has not yet been confirmed.

Whether the 'surra' of camels in India is produced by the same trypanosome there is no evidence to shew.

LAVERAN and MESNIL, who have recently been able to make a comparison of *T. brucei* and *T. evansi*, state that *T. brucei* is shorter and more compact than *T. evansi*. The movements of *T. brucei* are also less extensive. The posterior end of *T. brucei* is also blunter than that of *T. evansi*. The free portion of the flagellum is shorter in *T. brucei* than *T. evansi*, and the protoplasm of *T. brucei* has more numerous and larger granules than that of *T. evansi*. The nuclei and the blepharoplasts are morphologically indistinguishable. Further, the mean length of *T. brucei* is less than that of *T. evansi*, and the width of *T. brucei* is greater. The distinction between Surra and Ngana is, however, best proved by the fact that an animal immunized against Ngana is yet susceptible to inoculation with Surra.'

4. *T. equinum* (Mal de Caderas).—In Central and South America. A disease affecting horses.

The symptoms—remittent fever, oedema, wasting—resemble those of Ngana and Surra.

Most characteristic is the paralysis of the hind legs, from which the disease takes its name.

It runs a chronic course, two to six months. In donkeys six to twelve months. There is occasionally haemoglobinuria.

Mice, rats, rabbits, dogs, guinea pigs, etc., are susceptible. Incubation period, five to eight days. Horned cattle are the most refractory.

It is thought that the infection is transmitted by a biting fly *(Stomoxys calcitrans)*. There appears to be, however, some connection between Mal de caderas and a disease affecting a rodent (*Hydrochaerus capybara*), allied to the guinea-pig. When an epidemic occurs among these, then Mal de caderas breaks out among the horses (LAVERAN). The parasite morphologically resembles *T. brucei*. In the latter the centrosome is, however, larger. In *T. equinum* it is so small that its existence has been denied. Moreover, an animal immunized against *T. equinum* is still susceptible to *T. brucei*.

5. *T. equiperdum* (Fig. 81).—This trypanosome is the cause of the disease among horses in Algeria and India, known as *Dourine*. In asses the symptoms are slight. In horses, and especially stallions, the symptoms are much more marked. It is conveyed, as far as is known, under natural conditions by 'coitus' only, and not by means of flies.

In eleven to twenty days after coitus, oedematous swellings of the genitalia appear.

In forty to fifty days characteristic 'plaques' on the skin. These are very occasionally absent, as in asses, but when present are pathognomonic. These 'plaques' last only one to eight days. Around these there is oedema. The animals

PLATE V

T. gambiense

Fig. 1.—*T. gambiense* in Gambian native. x 2,000.

Fig. 2.—*T. gambiense* in tame rat, 'long form.' x 2,000.

Fig. 3.—*T. gambiense* in tame rat, showing longitudinal division. x 2,000.

Fig. 4.—*T. gambiense* in tame rat, from a specimen taken one week before death, 'stumpy form,' showing chromatin granules. x 2,000.

Fig. 5.—*T. gambiense* in tame rat, from a specimen taken one week before death, 'round form,' showing granules. x 2,000.

T. dimorphum

Fig. 6.—*T. dimorphum*. The small 'tadpole-shaped' parasite in the early stage of the disease. x 2,000.

Fig. 7.—*T. dimorphum*, 'stumpy form,' in tame rat. x 2,000.

Fig. 8.—*T. dimorphum*, showing longitudinal division of 'tadpole-shaped' parasite. x 2,000.

Fig. 9.—*T. dimorphum*, 'long form,' showing longitudinal division. x 2,000.

Fig. 10.—*T. dimorphum*, 'long form.'

PLATE V

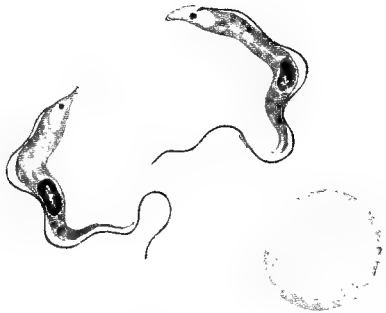


Fig.I.

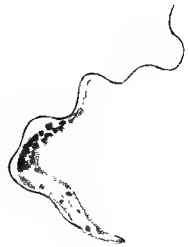


Fig.II.

Fig.IV.

become anaemic, complete paraplegia sets in, and death in two to ten months.

Trypanosomes are most easily found in the 'plaques,' with difficulty in the blood.

Post-Mortem.—There is inflammation of the urogenital mucosa, and in two cases areas of softening have been found in the spinal cord. Ruminants are refractory (to *T. brucei* they are very susceptible). Dogs which have been immunized against *T. equiperdum* yet succumb to *T. brucei*, so that Dourine and Ngana are distinct.

6. *T. gambiense* (DUTTON).—This, the first human trypanosome to be described, was discovered by DUTTON in the blood of a European in the Gambia. The clinical symptoms of the case were :—

(1) Irregular relapsing fever.
(2) Oedema, especially about the eyes.
(3) Congestion of the skin.
(4) Erythematous patches, associated with thickening of the skin.
(5) Increased pulse and respirations. Loss of flesh.

The trypanosomes are generally scanty in the blood, and it may be necessary to centrifugalize and examine the leucocytic layer.

N.B.—Europeans in an area where trypanosomiasis (or sleeping sickness) is endemic in the native population, may be infected with trypanosomes and show none of these signs, at least at first, except, perhaps, a daily rise of temperature in the evening. Examine repeatedly and carefully Europeans with an irregular intermittent temperature on which quinine has no effect.

TRYPANOSOMIASIS (SLEEPING SICKNESS)

In 1902 CASTELLANI found a trypanosome in the cerebro-spinal fluid of a case of sleeping

sickness. BRUCE furnished the proof of the causal connection of this trypanosome with the disease. BRUCE further showed that the disease was transmitted by a particular 'tsetse' fly, *Glossina palpalis*. It has since been shown that the trypanosome in this disease is identical, morphologically and in its pathogenic properties, with *T. gambiense*. In fatal cases a streptococcus can often be isolated from the organs, but death may occur with all the typical symptoms and yet the organs be completely sterile; so that the streptococcus occurs only as a terminal infection.

1. *Blood examination.*—Parasites may be absent from the peripheral blood for a month or more at a time, and even if abundant, seventy to a cover-slip, may again completely disappear. The number of parasites bears no relation to the severity of the symptoms.

2. *Cerebro-spinal fluid.*—Obtained by lumbar puncture. Seldom more than one to five trypanosomes occur in the centrifugalized sediment.

3. *Cervical lymphatic glands.*—GREIG and GRAY state that trypanosomes can be most certainly found by puncturing the cervical glands.

4. *Post-mortem.*—Parasites are often found in the pericardial, pleural, and peritoneal fluids even without centrifugalizing.

T. gambiense is from 18-25 μ in length, by 2-2·8 μ broad. It occurs in two main forms—(1) a 'long form' with a pointed posterior end, and (2) a short stumpy form with many chromatic granules (*Vide* Plate V).

Pathogenic action.—It is pathogenic for many mammals, *e.g.*, rats, guinea-pigs, rabbits, monkeys (except *Cynocephalus*), etc. On the whole, the

disease is a chronic one in animals, and recovery may take place. White rats are easily infected, best by intraperitoneal inoculation; while guinea-pigs are most convenient for experimental work. About half the inoculations into animals fail, and *post-mortem* material never gives a positive result.

Morbid anatomy.—The changes in the central nervous system are those of a chronic meningo-encephalitis and myelitis (Mott). The pia-arachnoid is sometimes opaque, and the vessels are considerably congested. Purulent meningitis is the most frequent complication. The brain in these cases is covered with lymph, and the vessels of the pia-arachnoid much injected.

Microscopically.—The pia-arachnoid shows a mononuclear infiltration most marked over the cerebellum and medulla. The blood in the vessels also shows the mononuclear character.

7. *T. theileri.*—This is found in the blood of cattle in South Africa, subject to a disease known as 'gal ziekte,' *i.e.*, gall sickness. Length, thirty to sixty-five μ; width, two to four μ.

Theiler states that a biting fly, *Hippobosca rufipes*, transmits the disease.

T transvaliense.—Found in the blood of oxen, eighteen to fifty μ long by four to six μ broad. The blepharoplast of this trypanosome almost touches the nucleus. The undulating membrane is consequently little developed.

Theiler considers this to be only a variety of *T. theileri*. *T. theileri* is infective for cattle only.

8. *T. dimorphum.*—In the horse in Gambia. The disease is characterized by progressive weakness and emaciation. There are no oedematous swellings as in Ngana. Various animals are susceptible. It occurs in three forms (Pl. V, Figs. 6-10).

Various other trypanosomes have been described in cattle, horses, and camels in Uganda, Algeria, Soudan, Somaliland, Togoland, German East Africa, etc. Their relationships are at present doubtful.

Trypanosomes have also been recorded from time to time in many other mammals, *e.g.*, rabbit, hamster, dormouse, bat, squirrel, lemming, souslik, guinea-pig, Gambian mouse, field mouse, and mole. In the Gambian mouse the trypanosome is said to possess no undulating membrane. The trypanosomes in these various animals have a general resemblance to *T. lewisi*, but most are insufficiently described.

DIMENSIONS OF TRYPANOSOMATA FOUND IN MAMMALS

1.	*T. lewisii*	24-25 μ by 1-4 μ
2.	*T. brucei*	25-30 μ by 1·5-2·5 μ
3.	*T. equiperdum*	18-26 μ by 2-2·5 μ
4.	*T. evansi*	20-30 μ by 1-2 μ
5.	*T. equinum*	20-25 μ by 2-3 μ
6.	*T. gambiense*	18-25 μ by 2-2·8 μ
7.	*T. theileri*	30-65 μ by 2-4 μ
8.	*T. transvaliense*	18-50 μ by 4-6 μ

Considerable variation exists between the data of observers, and though these figures can be considered as approximately correct, they do not suffice for distinguishing the various species.

Whether it will be possible to distinguish nearly allied species morphologically, *e.g.*, *T. brucei* and *T. evansi*, remains to be seen. Differences in the position of the blepharoplast and differences in staining properties hardly suffice in similar species that resemble one another closely, and at present the only certain method is their pathogenic properties.

Inoculation.—The most certain and rapid method is intraperitoneal, *e.g.*, in the case of

T. lewisii, but subcutaneous is almost equally certain, and in *T. brucei* scratch inoculations nearly always succeed.

Blood Examination.—If present in fair quantity there is no difficulty in detecting them fresh with a low power. If very scanty, it may be necessary to centrifugalize the blood. The most delicate test of a successful infection which may have resulted, even though no parasites be found, is a subinoculation into a highly susceptible animal.

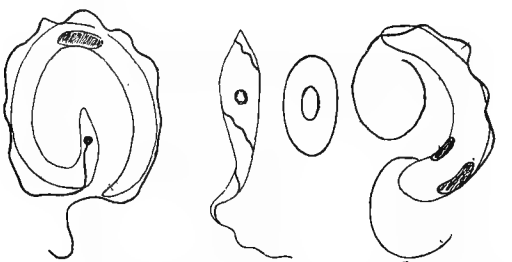


Fig. 82. *T. soleae*, *T. avium* (*after* DANILEWSKY) *Tp. borreli* (*after* LAVERAN)

Where parasites cannot be found by an ordinary examination in the blood, they may, however, be readily discovered in the oedematous swellings so often found in trypanosomiasis. Thus it is often extremely difficult, if not impossible, to detect trypanosomes in the blood of a rabbit infected with *T. Brucei*, yet they are easily found in the oedematous fluid about the ears, muzzle, etc.

Cultivation of trypanosomes.—NOVY and MCNEAL have succeeded in growing trypanosomes *in vitro*. Of those yet tried *T. lewisi* is the most readily cultivated. The medium consists of a mixture of nutrient agar and defibrinated blood. The proportion of blood to agar is 2 : 1, though *T. lewisi* will also grow

when the proportion is 1 : 2, or even 1 : 10. *T. brucei* is much more difficult to grow, the best proportions of blood to agar are 2 : 1 or 3 : 1.

The culture medium is best poured into flasks so as to get a large but shallow layer of condensation water. After inoculation the cultures are kept at a temperature of 25° C. As soon as a good growth is obtained subcultures should be made. Bacterial contamination must be scrupulously avoided.

In cultures of *T. lewisi* forms 1-2 μ (excluding flagellum) in length are found, and filtrates that have passed through a Berkefeld filter are infective. The virulence of cultures may be completely lost, though the trypanosomes are still active and growing in subcultures.

Genus Trypanoplasma

1. *Trypanoplasma borreli.*—Twenty μ long, three to four μ broad. Each flagellum, fifteen μ long. It is curved in shape. The undulating membrane on the convexity. The anterior end is more pointed than the posterior. Found in the blood of the red eye (*Leuciscus erythrophthalmus*). Also a similar, if not identical, species in minnows (*Phoxinus laevis*). They may cause anaemia, wasting, and death of the fish (Fig. 82).

2. *T. cyprini.*—In the carp (*Cyprinus carpio*). Ten to twenty to thirty μ long. The flagella are of unequal length. The anterior flagellum is twice as long as the posterior. Pathogenic (?).

Addendum.

T. noctuae (*Vide* p. 345).—Drs. Ed. and Et. Sergent have confirmed Schaudinn's results. *Culex pipiens* were fed on an owl containing 'halteridia.' A month later they were allowed to bite on four occasions a non-infected owl. Two days after the last meal, the owl showed 'halteridia' in its blood.

LITERATURE

Trypanosomes et Trypanosomiasis. Laveran and Mesnil. 1904.

Chapter XXVIII

The Leishman-Donovan Bodies

Leishmania donovani

1. The parasites known by this name were discovered in the spleen in the disease so common in India, known as chronic 'malarial' cachexia, Dam-dam fever, Kala-azar, tropical spleno-megaly, etc. They have since been found by splenic puncture in a few cases of chronic fever from Africa.

2. The same parasites also have been found in the granulation tissue of Tropical ulcer, Delhi boil, Aleppo button, Scinde sore, Oriental sore, etc.

Clinical Characters of the Fever

1. *Great enlargement of the spleen*, which frequently reaches the umbilicus and even the pubis. This is the most distinctive character of the disease.

2. *Emaciation.*—Usually present in advanced cases, and in fatal cases it is extreme.

3. *Irregular pyrexia.* — Uninfluenced by quinine, the accompanying chart indicates its character in a well-marked case.

4.—*Abdominal symptoms.*—Dysenteric ulceration with blood and mucus in the stools in advanced cases. Death from peritonitis following perforation is not uncommon.

5. *Ulcerations.*—Cancrum oris, noma vulvae, or other phagedaenic processes are common. Small ulcers occur about the knees and elbows, or

larger ulcers on the leg. The occurrence of these ulcers should arouse suspicion of a systemic infection with the parasite, for in Madras all cases affected with noma or cancrum oris yielded parasites on splenic puncture.

6. *Skin lesions.*—Epecially in advanced cases, papular eruptions occur about the thighs and scrotum.

7. *Haemorrhages, epistaxis, petechiae, purpura,* etc.

8. *Oedema of the feet.*—Occasionally but not constantly present.

9. *Pigmentation of the skin.*—Not usually in excess of the normal.

Technique.—(1) For puncturing the spleen use a hypodermic needle. Boil it previously in normal saline, or in normal saline containing 0·1 per cent. ammonium oxalate. Puncture between the ribs if the splenic enlargement is not great, otherwise where it is most prominent. Make a number of dry and wet films. (2) To examine the granulation tissue of 'Tropical ulcer' snip off with a curved scissors pieces of tissue from papules or ulcers. Crush a fragment on a slide by means of another slide and make *thin* smears. Imbed other pieces for section cutting.

Examine films made by splenic puncture and in stained specimens (ROMANOWSKY); observe the following characters of the parasite (Pl. VI):—

1. The presence of small round or oval bodies containing *two* chromatin masses—a large and a small. These are so distinctive that they cannot be mistaken, and could not possibly be confused with platelets (Figs. 1-6).

2. Observe that some of these bodies are free but that the majority occur in leucocytes, and in fragments of the cytoplasm of splenic cells (matrix of Ross, zooglea of MANSON), which have a close resemblance to unaltered red cells (Figs. 12-14).

PLATE VI

THE LEISHMAN-DONOVAN BODIES

Fig. 1.—A rare form of the parasite without vacuole or secondary chromatin body.

Fig. 2.—Small form of parasite.

Fig. 3.—Parasite with 'tail' joining the chromatin masses.

Fig. 4.—Pear-shaped form.

Fig. 5.—A large form with very large chromatin mass.

Fig. 6.—Tail-like structures distinct from that of Fig. 3.

Fig. 7-11.—Dividing forms.

Fig. 12.—Parasites in apparently altered blood cells.

Fig. 13.—Parasite in matrix, the remains of protoplasm of a leucocyte.

Fig. 14.—Parasites in bodies after treatment with hypotonic ammonium oxalate solution.

Fig. 15.—Parasites and pigment in apparently an altered red cell.

Fig. 16.—Parasites in a polynuclear leucocyte.

Fig. 17.—Parasites in a large mononuclear leucocyte.

Fig. 18.—Endothelial cell containing parasites, from the femoral vein.

Fig. 19.—A macrophage containing parasites.

Fig. 20.—Endothelial cell from testis with parasites.

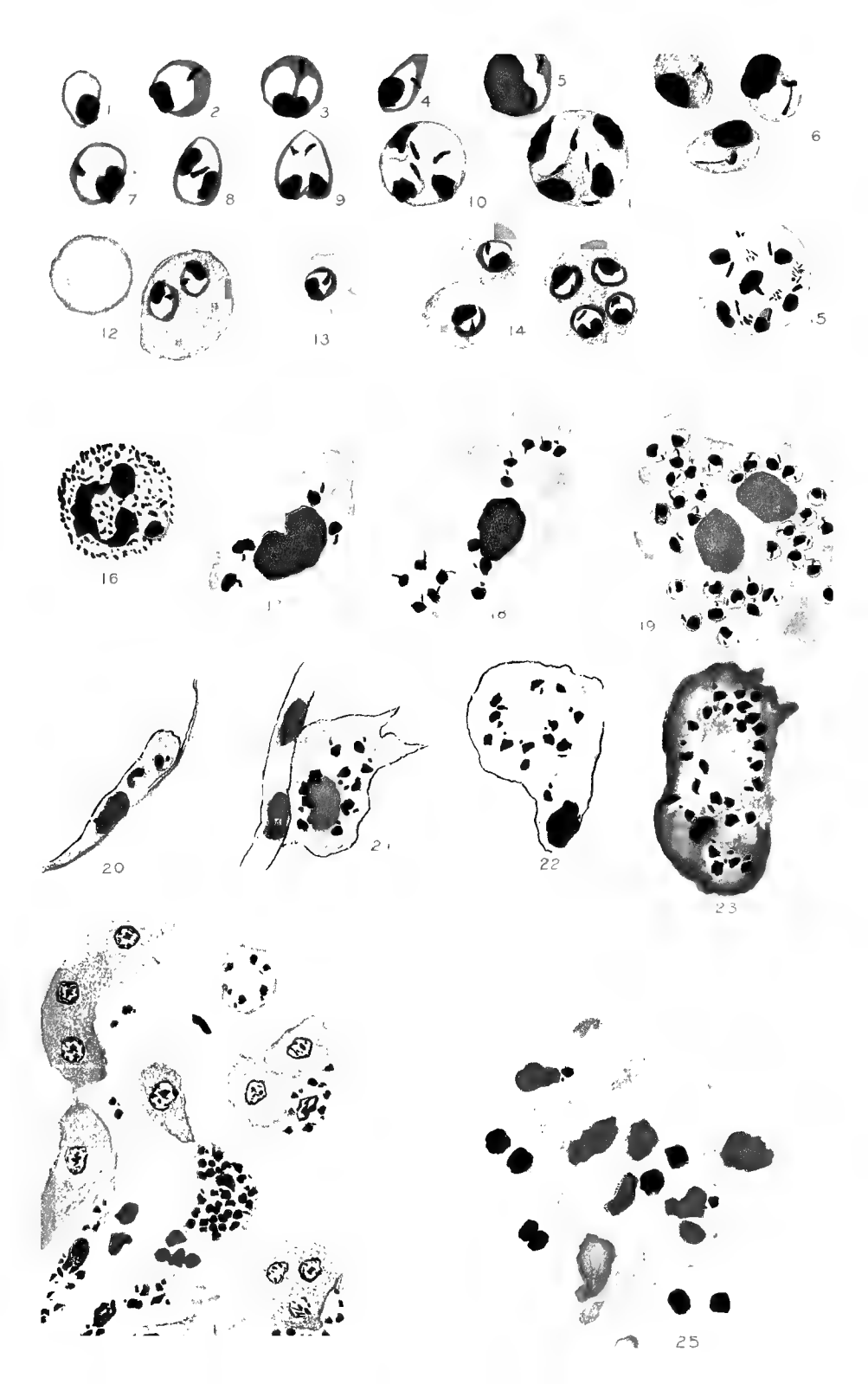
Fig. 21.—Swollen endothelial cell from granulation tissue with parasites.

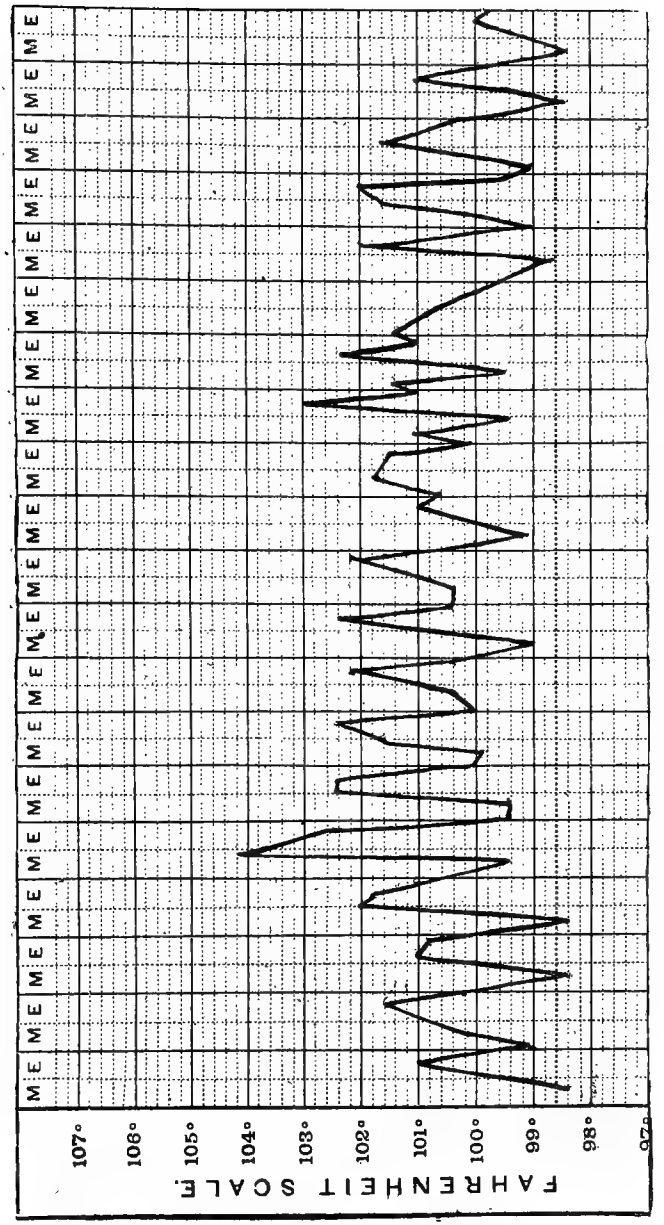
Fig. 22.—Necrotic macrophage from spleen with parasites.

Fig. 23.—A similar cell reduced to a mere pellicle with parasites.

Fig. 24.—Section of liver shewing macrophages in the capillaries with parasites.

Fig. 25.—Young granulation tissue from an ulcer shewing two parasites.





Advanced case of tropical splenomegaly. Spleen reaching to umbilicus.

3. Further observe that polynuclear leucocytes contain only one or two of these bodies (Fig. 16); large mononuclear leucocytes one to six (Fig. 17); cells of an endothelial type one to twelve (Fig. 18); large cells with a hyaline or finely granular or vacuolated cytoplasm (macrophages) up to several hundreds (Fig. 19).

4. The parasites are approximately circular or oval, 2·5-3·5 μ in size, clearly outlined, and appear to possess a distinct cuticle, as they retain their shape and are rarely seen distorted in films.

5. The two chromatin masses are characteristic, the large one staining lightly and the small one intensely with ROMANOWSKY. The masses are usually situate opposite each other in the short axis of the parasite. The larger chromatin mass always forms part of the periphery of the parasite.

6. Most of the parasites contain one or two vacuoles which may displace the cytoplasm of the parasites to the periphery.

7. Developmental forms. Division commences at the thick end of the parasite, and the large chromatin masses may be widely separated before the small chromatin mass has begun to divide. As many as three to six bodies are formed in this way, the large nuclei being arranged peripherally, and the smaller centrally (Figs. 7-11).

OCCURRENCE IN PERIPHERAL BLOOD

In two cases only, approaching a fatal termination, have we found parasites in the *peripheral blood*. The parasites were of the typical structure, but were all included in leucocytes. During a count of five hundred leucocytes we counted altogether thirty-seven parasites.

Leucocytic Changes

Leucopenia is the most marked change. So much is this so, that it is necessary to take several large films in order to make accurate leucocytic counts. Two thousand leucocytes per mm^3 is a common value and still smaller numbers are not uncommon. The *relative* leucocytic values, however, do not vary much from the normal.

	Case I	Case II	Case III	Case IV
Large mononuclear	11·4	8·6	10·6	16·0
Small mononuclear	24·0	20·4	29·2	7·0
Transitional	1·8	0·6	—	—
Intermediate	·6	0·0	—	—
Myelocytes	1·2	0·2	0·6	—
Polynuclear	60·0	69·8	56·0	73·0
Eosinophil	1·0	0·4	3·6	4·0

POST-MORTEM CHANGES

Spleen.—The appearance of the spleen and the liver are almost pathognomonic. The spleen retains its shape when removed from the body as if hardened in situ. It is firm but friable, not tough like a fibroid spleen.

Liver.—Firm but friable, retaining its shape like the spleen on removal. On cutting into it

an arborescent appearance is noticed, due to the deposit of a white tissue (macrophages containing parasites) in the centre of the lobules.

Large intestine.—Extensive multiple ulceration is almost constantly present. Fungating granulation tissue occurs in association with the ulcers. Purulent peritonitis, broncho-pneumonia, septic infarcts, are commonly met with. The other organs show no particular change to the naked eye.

MICROSCOPICAL CHANGES

1. Make *thin* smears of spleen pulp, liver, bone marrow, lung, kidney, testis, lymphatic gland, suprarenal. Stain with ROMANOWSKY. Parasites occur in immense numbers in the spleen, liver, and bone marrow. To a less extent in the lungs and testis. They are present also in the suprarenals and lymphatic glands.

2. Make thin smears from granulation tissue of ulcers of the skin and intestine. Parasites are present in both situations; in the skin they are scanty, in the intestine they may be very numerous.

3. Place small pieces of these tissues on cover glasses. Harden in alcohol or corrosive sublimate. Embed in paraffin. Cut sections. Stain by the modified ROMANOWSKY method (p. 51), or with haematein. The study of sections is essential for a clear understanding of the relation of the parasite to the tissues. Observe the following conditions:—

Liver.—In the lumen of the capillaries of the lobule, often applied closely to the capillary wall, occur numerous large cells crowded with parasites. These cells are sometimes retracted and globular, but more usually they are characteristically extended, and suggest the idea that they are actually

moving inside the capillaries. These cells are of doubtful nature but resemble the macrophages seen in the organs in malaria. In some cases these cells contain melanin. The parasites in these cells have the characteristic structure. They appear to lie in vacuoles, but these are undoubtedly the body of the parasites (Fig. 24).

Spleen.—The parasites occur in similar cells. They are very conspicuous in sections. Large mononuclear cells containing parasites are more abundant than in the liver. Neither do the red cells contain parasites, nor do free forms occur.

In contrast to what is seen in blood films made by spleen or liver puncture where most of the parasites are either free or contained in a matrix, in sections no such relation exists; the parasites lie in cells. These cells are of various types.

(*a*) But slightly modified endothelial cells. These have an oval nucleus and extensive protoplasm showing vacuolization (Fig. 20). The protoplasm may show buds or protrusions. These cells contain six to twelve parasites. Identical cells are seen in the capillaries of the testis and of granulation tissue.

(*b*) Large round cells with a large nucleus. The protoplasm has a ground glass appearance and is vacuolated. In the testis and in granulation tissue these cells are attached at one point to the capillary wall, the rest of the cell projecting freely. They also occur in the blood taken *post-mortem* from the large veins. They contain twenty or more parasites (Fig. 21).

(*c*) Very large cells with one or two vesicular nuclei. They occur in the liver and spleen in immense numbers. They occur either extended along the capillary wall or in a retracted form. In the spleen their processes extend among the smaller cells of the pulp. They contain numerous parasites.

(*d*) Large cells staining more intensely than the last and sometimes showing signs of necrosis. The nucleus is pushed to the side The centre of the cell is occupied by a large vacuolated space, around which are arranged numerous parasites. The cells, in fact, contain so many parasites that they appear to be on the point of rupture, and such cells are rarely seen whole in films unless fixed extremely carefully with osmic acid vapour. They contain as many as two hundred and fifty bodies (Figs. 22, 23).

Bone marrow.—In films the parasites occur in macrophages in immense quantity. To some extent also in large mononuclear cells, and a few in polynuclear cells, and in myelocytes.

Large intestine.—Parasites occur in large numbers in the granulations, and in the mucous membrane in the early stages of infiltration. They occur in similar cells to those found in other situations.

Granulation tissue.—Sections of papules or ulcers of the skin show a few parasites in what are apparently endothelial cells of the fine capillaries. In larger capillaries cells may contain three or four parasites, while in small vessels large cells similar to those in the liver and spleen are found crowded with parasites. These cells are attached at one point to the capillary wall.

Lymphatic glands.—In those draining the area of a skin lesion parasites are found. They occur in large cells in the lymph sinuses and in cells of the reticulum.

We have thus in an infection caused by these parasites two processes—(1) 'Tropical ulcer,' a local invasion of the nature of a granuloma; (2) a systemic infection of the nature of a septicaemia, involving chiefly the visceral endothelia. The endothelial cells increase in size, and become so distended with parasites as eventually to undergo necrosis. Such cells would appear eventually to rupture, and the parasites set free to be again taken up by other cells.

Development of the parasites.—ROGERS states that he has observed further development of these bodies *in vitro* To splenic blood from a case of Kala-azar he added some citrate of soda to prevent coagulation, and then incubated the solution at about 20° C. He then observed:—

(1) Multiplication of the parasites so that they became far more numerous.

(2) In a few days flagellates made their appearance. These closely resembled trypanosomes except that they had no undulating membrane.

One of us, S.R.C., confirmed the observation that flagellates are formed, but these flagellates are certainly not trypanosomes.

Chapter XXIX

SPIRILLAR FEVER

It seems highly probable that the 'spirochaetes' found in the blood are protozoa and not bacteria. Firstly, they will not grow on any medium; secondly, the character of the temperature chart is unlike that associated with any bacterium; thirdly, the spirillar disease of fowls is conveyed by ticks; and fourthly, SCHAUDINN'S remarkable work on *Spirochaete ziemanni*, if we can extend it to others, seems to complete the proof of their protozoan nature.

1. *Spirochaete obermeieri.*—In man occurs as a fine spiral thread-like body ten to forty μ in length, by, at most, one μ broad. The number of spirals is on an average about ten. Examined with high powers they appear to be uniform in structure, or at most present minute unstained spots. They have been said to possess flagella but the observation has not been confirmed. The formation of tangles or rosettes is an uncommon phenomenon. The presence of spirochaetes in the blood can be detected with comparatively low powers by the disturbance among the red cells. The blood should be examined during the pyretic attack; they disappear entirely during the apyrexia. Stain with ROMANOWSKY. Outside the body the spirilla, at a temperature of 20° C., will survive as long as a fortnight. The only susceptible animals are monkeys.

2. *Spirochaete anserina.*—Highly pathogenic to geese, death occurring in about a week. The spirochaete closely resembles the former morphologically. The blood shows immense quantities of the spirochaetes, and tangles are common. They appear with the pyretic attack and disappear as the temperature falls. Besides geese, ducks are susceptible to inoculation, and, to some extent, hens.

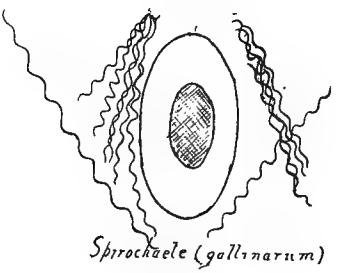


Fig. 84

3. *Spirochaete (gallinarum).*—Affects fowls in Rio de Janeiro. The spirochaetes are found during the pyrexia. Tangles are numerous. Death takes place in four to five days. The spleen and liver are much enlarged. The disease is conveyed by ticks of the genus *Argas.* When subcutaneous inoculations are made spirochaetes do not appear in the blood until the second day. They then increase until the fifth or sixth day, when they *suddenly* disappear.

4. *Spirochaete theileri.*—In cattle in Transvaal and Cameroons. The spirochaetes are actively motile, twenty to thirty μ long. Small forms only eight μ also occur. In some of the infected animals bacilliform *Piroplasma* were present. In others,

Piroplasma, *T. theileri*, and spirochaetes. The spirochaetes are probably the cause of death.

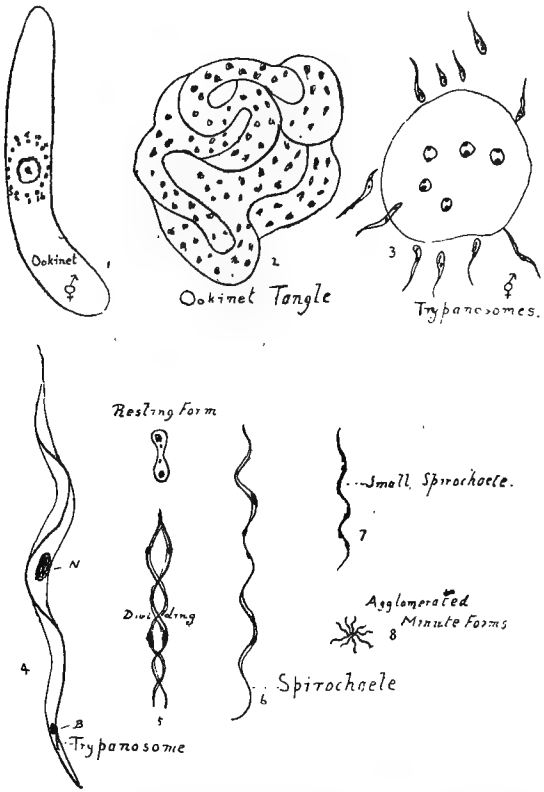


Fig. 85. *Shewing development of the ookinet of Sp. zicmanni into trypanosomes and then spirochaetes, in the mosquito (after* SCHAUDINN)

5. *Spirochaete ziemanni.*—In the blood of *Athene noctua* besides the halteridia described in the previous chapter, ZEIMANN has described peculiar large fusiform parasites. Male and female forms occur and flagellation has been observed.

SCHAUDINN has traced their further development in *Culex pipiens*. The general development is similar to that of *T. noctuae*, for these also have a trypanosome stage.

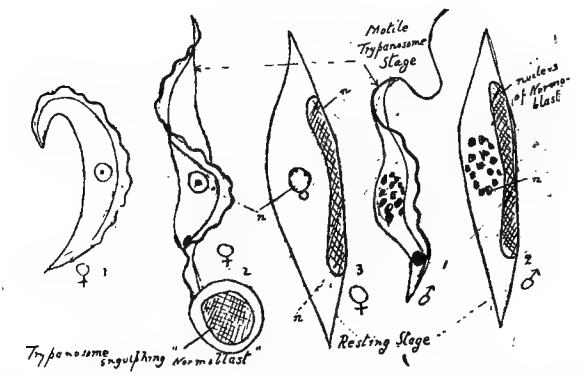


Fig. 86. *Development in the blood of Sp. ziemanni and change to resting forms* (*after* SCHAUDINN)

Development in mosquito.

1. Ookinets of three kinds are developed in the stomach.

2. From these are developed trypanosomes which, in this case, are minute, and the males so much so that they are very difficult to see except when agglomerated in rosettes.

3. The backward and forward movement of spirochaetes results from the fact that two of these trypanosomes, after division, remain attached.

4. Multiplication goes on in the stomach, and the resulting forms are so extraordinarily minute that they are invisible except in agglomerated masses.

5. These trypanosomes now penetrate the epithelium of the malpighian tubes, multiply here, and come to rest. They eventually pass out with the malpighian secretion, reach the great curvature of the colon, and then follow the same course as *T. noctuae*. The mosquitoes can infect a fresh owl after their third meal of blood.

Development in the blood.

1. A large development of the indifferent trypanosome takes place, causing an acute infection.

2. The sexual forms appear. These parasites infect leucocytes or rather young Hgb-free erythroblasts, so that development goes on mainly in the internal organs. The sexual forms are distinguished from the indifferent forms by their much greater size They are much larger than the cells they attack, and, in fact, take up the cell into their protoplasm They are found in the blood as in *T. noctuae*, both in the resting stage and in the motile trypanosome stage When the blood enters the mosquito's stomach flagellation takes place, the makrogamete is fertilized, and the ookinet results.

Trypanosomes and spirochaetes have been found together with piroplasmata in cases of red water in cattle, and it is suggested that possibly piroplasmata develop in ticks by means of a trypanosome stage.

6. *Tick Fever.*—Ross and MILNE have found spirilla (scanty) in all cases examined by them (*vide* p. 349).

Chapter XXX

FILARIA

The *Filariidae* form one of the families into which the *Nematodes* are divided. Other families in this sub-order are the *Ascaridae*, *Strongylidae*, *Anguillulidae*, etc. The *Filariidae* are divided into several genera, only one of which concerns us immediately, viz., the *Filaria*, and first, we shall consider those species of filaria which have their embryos in human blood. They are the following :

1. *F. bancrofti*, syn, *F. nocturna* (MANSON).
2. *F. diurna.*
3. *F. perstans.*
4. *F. ozzardi.*
5. *F. demarquai.*
6. *F. loa.*
7. *F. megalhaesi* (adults).
8. *F. gigas* (PROUT).

The following are the characters of the embryos of each species :—

F. bancrofti.—Occurs at night in the peripheral blood, found in the internal organs by day, especially in the vessels of the lungs. *Ce. argyrotarsis* and *Ce. albipes* are efficient hosts.

1. Length about three hundred μ fresh (one hundred and eighty μ, stained specimens) by seven to eleven μ wide.

2. Enclosed in a sheath considerably longer than body of embryo.

3. If placed rapidly beneath microscope shews at first active progressive movement (ANNETT and DUTTON), later the anterior tip of the sheath appears to become attached to the glass, and movements of the embryo, though active, are not progressive.

4. The embryo shews an anterior abruptly rounded off end and a posterior, tapering for two-fifths the length. There is a six-tipped prepuce and a short very fine fang.

5. The stained specimen shews (i) an irregular transverse break about twenty-one per cent. of the length.

(ii) A V-shaped spot or transverse irregular break at a distance of about thirty per cent. of the length from anterior end. Nearly always present.

(iii) An area of varying length with cells loosely arranged, sixty-three per cent. length. This is constant and represents the central aggregation of fresh specimens.

(iv) An irregular, sometimes oval spot, often present, eighty-five per cent. length.

(v) A small central bright spot occasionally, ninety-one per cent. length.

F. diurna.—No differences are distinguishable between the embryos of *F. diurna* and *F. nocturna* either in the fresh or stained specimen (ANNETT and DUTTON). DUTTON and ANNETT have found the embryos taken from the adult female *F. loa* to be practically identical with those of *F. diurna*, and describe a case in which infection with *F. loa* was associated with embryos present in the blood during the day and not to the same extent at night.

Embryos of *F. loa*, taken from the female, are described by ANNETT and DUTTON.

1. Length, 208 μ.
2. Possess a sheath.
3. Spots as follows : —

(i) An oval or diamond-shaped spot, twenty-four per cent. length from anterior end.

(ii) An indistinct lateral area containing scattered nuclei, thirty-seven per cent. length.

(iii) A longer portion of worm which stains badly, sometimes divided into anterior and posterior portions.

(iv) A small lateral bay, eighty-six per cent. length.

F. perstans.—Embryos present in peripheral blood day and night.

1. Length, two hundred μ by four μ to five μ breadth in fresh, and about ninety μ in stained preparations.

2. Do not possess a sheath.

3. Movements extremely active, and progressive movement continues for many hours. They possess the power of considerable elongation and shortening.

4. No hooked prepuce, a fang is generally observed protruded and retracted. The body tapers gradually for two-thirds of length, and is abruptly truncated at the tail and slightly bulbous.

5. In stained specimens the following spots are made out :—

(i) A narrow irregular transverse band at distance of 26·4 per cent. length, nearly always present.

(ii) A wider irregular transverse spot, at thirty-six per cent. length. Occasionally.

(iii) The largest of the spots an irregular transverse area, sixty-three per cent. length. Not always present.

(iv) A very inconstant central bright speck at eighty-three per cent. length.

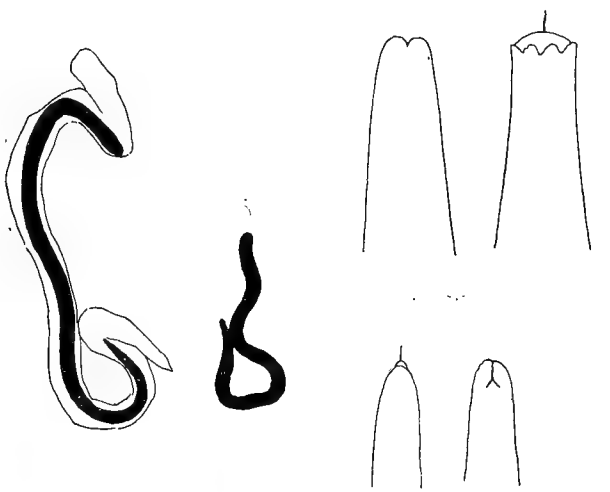


Fig. 87. *F bancrofti, Embryo shewing sheath; F. perstans, Embryo (sheathless); F. bancrofti (Embryo), prepuce and fang (above); F. perstans (Embryo), fang (below)*

6. They are readily distinguished from the embryos of *F. ozzardi* by their blunt tails.

7. The observations of FIRKET and CHRISTY point to the fact that there is more than one species of *F. perstans.*

F. ozzardi:—

1. Length, one hundred and seventy-three to two hundred and forty μ by four to five μ.

2. They are *sharp-tailed* and sheathless.
3. They have no periodicity,

F. demarquaii :—

1. Length, two hundred and five μ by five μ.
2. Tail sharp and sheathless.
3. Cephalic armature, ill-developed prepuce and spine.
4. A V spot exists fifty-two μ from the head (seen in wet films).
5. There is no periodicity.
6. *Ce. argyrotarsis* and *Ce. albipes* are inefficient hosts.

F. loa.—Two cases of infection with the adult *F. loa* have been described in which *F. diurna* occurred in the blood. It is possible then that *F. diurna* is the embryonic form of *F. loa* (*vide F. diurna* antea). On the other hand, *F. diurna* embryos are indistinguishable from those of *F. bancrofti*, the adult forms of which are well known.

F. megalhaesi.—Adults only known.

F. gigas :—

1. Blunt tailed.
2. Has no sheath.

The Characters of the Genus Filaria

They are long slender worms of almost uniform breadth throughout their length. The anterior extremity is rounded, and the mouth often has no lips. The males are distinctly smaller than the females. They have an incurved or spiral tail, the latter sometimes having lateral membranous outgrowths. They usually have

four pre-anal and a variable number of post-anal papillae and spicules, which vary in size and appearance. In the females the vulva opens in the neighbourhood of the mouth. The host in which the filaria reaches full maturity, giving rise to embryos, is the definitive host, the other host is the intermediary or secondary host. Thus *F. bancrofti* has for its definitive host, man, for its intermediary host, certain species of *Culicidae*.

F. recondita.—Definitive host, dogs. Intermediary host, *Ct. canis* (dog-flea).

Adult Filariae

1. *F. bancrofti*.—The adult male and females are found together, sometimes in the lymphatics or in cyst-like dilatations of these. The embryos gain access to the circulation by the thoracic duct.

2. *F. diurna*.—Adult form doubtful. According to Annett and Dutton it is *F. loa*.

3. *F. perstans*.—The adults were found by Daniels at the root of the mesentery, behind the abdominal aorta, and beneath the pericardium.

4. *F. ozzardi*.—Adults found by Daniels in the sub-peritoneal tissue.

5. *F. demarquaii*.—Adults doubtful. A female form has been described differing slightly from that of *F. ozzardi*.

6. *F. loa*.—Adults found in the subcutaneous areolar tissue, also in the eyelids, and beneath the conjunctiva.

7. *F. megalhaesi*.—Adults only known, found in the left ventricle of the heart by Figueira de Saboia.

8. *F. gigas*.—Adults unknown.

The following are the characters of the respective species :—

F. bancrofti.—Males and females found together in lymphatics.

♀ 1. Length, eighty-five to one hundred and fifty mm.

2. Distinct neck; one-third width of body.

3. Body plain, tapering somewhat abruptly to neck, and tapering towards tail.

4. Cuticle with striations.

5. Tail ends bluntly, and has a small depression, surrounded by two small lips.

6. Mouth simple, minute, terminal.

7. Ova twenty-five to thirty-eight μ by fifteen μ.

8. Anus ventral opening on summit of a bilobed papilla.

♂ 1. Length, eighty mm.

2. Body cylindrical, tapering to tail. No neck.

3. Mouth circular, simple, terminal.

4. Cloaca ventral, four pairs pre-anal, four post-anal, papillae (MANSON doubts the presence of these). Two unequal spicules.

5. Genital tube simple. Oesophagus thick-walled.

6. Tail vine-tendril like, with one or two spirals.

F. perstans :

♀ 1. Length, seventy to eighty mm.

2. Neck longer than *F. bancrofti.*

3. Body without markings.

4. Tail incurvated. Tip of tail mitred. This is *characteristic* of this species.

5. Mouth minute, simple.

6. Embryos in utero, blunt tailed, not sheathed.

♂ 1. Length, forty-five mm.

2. Head end as in female.

3. Two caudal ends much coiled.

4. One spicule and two papillae.

5. Low describes four pairs of pre-anal and one pair of post-anal, minute papillae.

F. ozzardi.—Adult.

1. Dimensions much the same as those of *F. bancrofti.*

2. Distinguished by the *bulbous* tail; in *F. bancrofti* it is not bulbous but circular.

F. loa.—Adult forms travel about in connective tissue.

♀ 1. Length, thirty to forty mm. (average). Varies from sixteen to seventy mm. Breadth, 0·57 mm.

2. No neck. Head cone shaped.

3. Body cylindrical, tapers sharply towards head and tail.

4. Cuticle with bosses, except over the head.

5. Tail terminates in a short incurved portion, and has two small tubercles at its extremity.

6. Mouth simple.

7. Ova, containing embryos, thirty-five μ by twenty-five μ.

8. Anal orifice on low, broad papillae, 0·3 mm. from the tip.

♂ 1. Length, twenty-five to thirty mm.

2. Uniform thickness except at head and tail.

3. Cuticle with bosses, but not so numerous as in the female.

4. Tail not spirally twisted, merely incurved. It possesses well-marked lateral alae.

5. Three well-marked pre-anal papillae and two unequal post-anal papillae. Two slender unequal spicules.

F. megalhaesi.—Adult males and females in left ventricle.

♀ 1. Length, one hundred and fifty-five mm. by 0·7 mm.

2. Club-shaped oral end.

3. Swollen oesophagus well marked.

4. Mouth simple.

5. Cuticle, fine striations.

♂ 1. Length, eighty-three mm. by 0·4 mm.

2. Four pairs pre-anal, four post-anal papillae, and two spicules.

To Examine Blood for Filaria Embryos

The technique varies somewhat according to what end the observer has in view.

1. To facilitate detection, it is well, as Manson advises, to make thick films of blood. Dry. Then wash out the haemoglobin with water or one-third per cent. acetic acid, and stain with haematin, or gentian violet, or fuchsin.

For the latter stains, a few drops of a saturated alcoholic solution of the dye are added to half a watch-glassful of water.

Search the slides stained (or fresh) with a half-inch lens.

2. (For studying the minute structure of the embryos, the above method is not advisable). Make a film in the ordinary way. Fix in alcohol and stain with haematein. The 'spots' and granules of the embryo are most beautifully shewn.

Filaria in Mammals

F. immitis.—Adults found in right ventricle of dog, fox, and wolf. Embryos in blood. Embryos develop in the malpighian tubes of *Anophelines*. Afterwards they enter the general body cavity and pass towards the labium.

Dogs in the tropics commonly harbour this filaria. The filariae are most numerous at night.

F. recondita.—A single female adult has been found in kidney of dog.

Embryos in Blood.—Embryos develop in *Ct. canis* (dog-flea), and *P. irritans* (man and dog); also in a dog tick. They are found in the intestine and body cavity. The filaria has, however, not been transmitted from infected fleas to healthy dogs.

F. equina.—Serous cavities, intestines, and liver of horses, donkeys, and mules.

Embryos in Blood.—Appear like *F. bancrofti*, but smaller.

F. haemorrhagica.—Male and female live together in tissues of horse and donkey. They form hemispherical tumours the size of a nut beneath the skin. These burst and discharge blood. Fresh tumours appear in from one to two days.

F. irritans.—Found in 'summer sores' of horses and donkeys.

F. evansi.—Lung and mesentery of camel. Embryos in blood.

F. lacrymalis and *F. palpebralis.*—About the eyes of horses and cattle.

F. osleri.—The adults cause broncho-pneumónia in dogs.

Avian Filariae

Filarial embryos are very common in the blood of birds, and the adult forms are found in the most diverse positions, notably in the subcutaneous tissues. In some form the embryos appear to be confined to the lymph.

In the description of *Avian filariae* the following should be noted:—

1. The species of bird concerned.
2. The site of the adult filariae.
3. The description of the adult filariae, female and male; the use of Cobb's formula gives uniformity to descriptions. The measurements are taken with the animal in profile from the anterior end.

(i) To the base of the oesophagus.
(ii) To the nerve ring.
(iii) To the cardiac constriction.
(iv) To the vulva in the female, or to the middle in male.
(v) To the anus, noting when this is terminal.

At each of these points transverse measurements are taken and noted below the above, so:—

Longitudinal.
Transverse.

The unit of measurement is one-hundreth part of the length of the worm.

This formula should be used with caution, since it rests on the assumption that the proportions of the various parts of the body are constant in different individuals (Shipley).

Drawings should be made of the head and tail, and the mouth, anus, and vaginal orifice carefully described.

4. The description of the embryo. Where found, blood or lymph. Presence of a sheath. Length and breadth of embryo and sheath. The exact description of spots and the distance of these from the anterior extremity. The following spots and markings may be seen :—

(i) A transverse slit, about twenty-five per cent. length. Sometimes not seen.

(ii) A clear, sometimes lateral, sometimes transverse spot, about thirty to forty per cent. length.

(iii) A long space in which the nuclei are loosely arranged, often ending anteriorly and posteriorly in clear space. About sixty-five per cent. length.

(iv) A small spot, about seventy-six per cent. length.

(v) Very small lateral spot or slit, ninety per cent. length.

Development of Filaria in the Mosquito

Experiments made so far have been chiefly with *F. nocturna* (*F. bancrofti*).

Both *Anopheline* and *Culicine* mosquitoes may act as the hosts of *F. bancrofti*. Certain species of both genera, however, do not act as hosts. The following have been shewn to act as hosts :—

C. pipiens	*P. costalis*
C. ciliaris	*Mym. rossii*
C. fatigans	*Myz. sinensis*

The following have been shewn by BANCROFT not to allow full development to take place. In some species partial development occurs, the larva, however, eventually disappearing :—

C. notoscriptus (SKUSE)
C. annulirostris „
C. hispidosus „
C. vigilax „
C. nigrothorax (MACQUART)
C. procax (SKUSE)
[*A.*] *musivus* „

GRASSI and NOE's experiments shew that *F. immitis* is capable of developing in *A. claviger*. As regards the re-infection of a healthy dog, the experiments are somewhat inconclusive, for, in the dog used, a single immature worm only was found, about sixteen days after the period of 'biting.' They state, however, that of a batch of *Anophelines* dissected before the 'biting,' many of the labia contained filaria, whereas, of a batch dissected after the 'biting,' none contained filaria, the conclusion being that the filaria had escaped through the labia into the blood of the dog during the 'biting.'

Seven stages of development of the embryo are usually described. The following is a resumé of the changes undergone in *Culex pipiens* :—

First Stage.—One hour after removal of blood by mosquito, the sheath is cast and the embryos exhibit active locomotive movements. In twelve to eighteen hours many have bored through the stomach wall, and have reached the

muscles. Some die within the stomach. In the muscles the cuticular striation disappears, movement ceases, and the body becomes thicker.

Second Stage.—For two to three days the embryo becomes much thicker, and the mouth begins to be faintly indicated.

Third Stage.—An anus appears in front of the tail, and a mouth is very distinct with four fleshy lips. Cells are seen in the body, and these form an alimentary and tegumentary layer. The embryo is now about 0·3 mm. long.

Fourth Stage.—Rapid growth takes place, and the tail becomes relatively smaller.

Fifth Stage.—Lengthening takes place. The whole worm becomes fibrous and transparent in appearance. It has cast the cuticle. Some large cells at the end of the tail form papillae which are characteristic of this stage of the larva. The parasite is now about 1·5 mm. (one-sixteenth inch). Time, about seventh day.

Sixth and Seventh Stages.—Movements become more active and, when the filariae have reached their highest stage of development in the thoracic muscles, they leave that tissue and travel forward in the direction of the head of the mosquito (Low and JAMES). They reach the loose tissue about the salivary glands and pass into the neck. Some are found in the abdomen. Numbers of the filaria larvae enter the lower part of the head, lying beneath the large head ganglia. Eventually one or more worms pass into the substance of the labium, where they are readily found by dissection. The larva at this stage measures about one-sixteenth inch in length.

The Transmission to Man

According to Dutton, who has very minutely described the structure of the proboscis, the worms can only leave the labium at one point, *i.e.*, by perforating an extremely delicate membrane, which closes in the extreme end of the labium (see p. 167). If they escape elsewhere, they must penetrate the dense and hard chitinous envelope of the labium—a very improbable occurrence.

Development of Filaria in the Louse

In the lymph, *e.g.*, of the subcutaneous tissue, of the swift (*Cypselus affinis*) occur the embryos of *F. cypseli*. Dutton has traced the development of these, up to an almost mature stage, in a louse, *Leiothina sp.*, which infests these birds. The mode of escape of the filariae from the louse and infection of a fresh bird is uncertain.

Filariasis and Eosinophilia

Wurtz and Clerc found in a case of *F. loa* (no embryos present in the blood) a marked eosinophilia. A similar increase has been found by us in a case of 'tropical swellings' of doubtful nature, but resembling somewhat Calabar swellings. The leucocytic counts were :—

	F. loa	Tropical swellings
Large mononuclear	5	1
Small mononuclear	13	23
Polynuclear	29	26
Eosinophil	53	50

LITERATURE

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APPENDIX

BLOOD-SUCKING FLIES

The Diptera or flies are two-winged insects (the posterior pair of wings are transformed into *halteres*), and are so distinguished for example from the Hemiptera or bugs which generally have four wings. In the Diptera the metamorphosis is complete, eggs, larva, pupa, insect; in the Hemiptera it is not so. The following have blood-sucking habits:—

The *Nematocera* (*νημα*, thread; *κέρας*, antenna).

1.—*Blepharoceridae.*

Wings irridescent, ample, bare, with creases, no 'discal' cell on wing (the discal cell lies between the second posterior cell and the second basal cell). Posterior tibiae with stout spines, anterior tibiae unarmed. The fourth vein is the one immediately preceding the large posterior fork, the incomplete vein not being counted. They resemble midges. The larvae have suckers, and are found attached to stones in the water.

Genus *Curupira* (? blood-sucking).

No incomplete vein A long vein between the first and fourth. Eyes contiguous. *C. torrentium*, Brazil.

Genus *Snowia* (? blood-sucking).

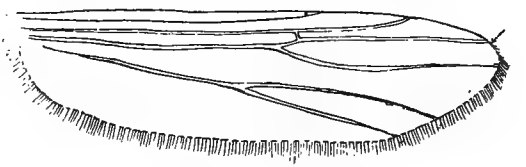
Eyes separated by a broad frons. Palpi four-jointed, well developed.

2.—*Culicidae.* Mosquitoes or gnats.

3.—*Chironomidae.* (Midges).

Head small, often retracted under thorax, which has no transverse suture. Simple eyes (ocelli) absent or rudimentary. Antennae up to fifteen segments, densely pectinate in ♂, often simple in ♀ and smaller Legs long and slender Tibiae and tarsi nearly cylindrical. Costal vein ends at apex of wing.

[Genus *Chironomus*] not blood-sucking. Larvae are 'blood worms.' 'Vers de Vase.'



Wing of Chironomus plumosus.

Fig. 88. *The arrow indicates the point at which the costal vein ends.*

Genus *Ceratopogon.*

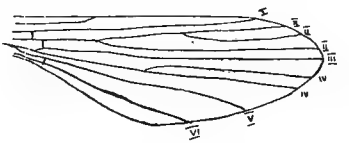
Very minute midges. Wings generally spotted. Head depressed in front, produced into a short rostrum. Antennae thirteen segments, the first eight bead-like, the rest elliptical. Sub-costal vein ends beyond half the length of the wing. Second long vein ends near the tip, third long vein at the tip. Femora armed beneath with spines. Larvae mostly non-aquatic. *C. varius.* A pest in Scotland.

Genus *Tersesthes.* New Mexico.

4.—*Psychodidae* (Moth flies).

Very small. Antennae very hairy. Wings very hairy (*Vide* Fig. 12). Larvae of some genera amphibious. The larvae and pupae resemble those of *Ceratopogon.* The eggs are laid in a cluster on the water.

Genus *Phlebotomus.* Europe and tropics.



Wing of Phlebotomus sp.

Fig. 89

5.—*Simulidae* (Sandflies, Buffalo-gnats).

Small hump-backed flies. Antennae destitute of hairs. Wings relatively large. Proboscis short, thick, consisting of

epipharynx and hypopharynx. Antenna eleven segments. Palpi four segments.

Simple eyes (ocelli) absent; thus distinguished from *Bibionidae*. Eyes in ♂ joined together (holoptic). The facets on the upper part of the eye are the larger.

Egg.—Deposited in a compact layer on stones and grass. Egg measures 0·40-0·18 mm.

Larva.—Twelve segments. On under side of anterior portion is a subconical retractile process crowned with bristles. Anal extremity bristly, with three short retractile tentacles.

Pupa.—Has a respiratory tuft on each side of thorax. Pupa has spines by which it anchors itself to the cocoon. These can be found under small stones. Pupal stage about five days.

Genus *Simulium*.

Body small, hump-backed, with a hairy felt-work (tomentum). Head small. Palpi four segments, the fourth composed of numerous annuli; larger in ♀ than in ♂ Antennae eleven segments, narrowing to the tip, a little longer than the head. Wings large. First, second, and third veins dark, remainder pale. ♂ generally black, ♀ cinereous. Eyes contiguous in ♂ (holoptic) remote in ♀ (dichoptic).

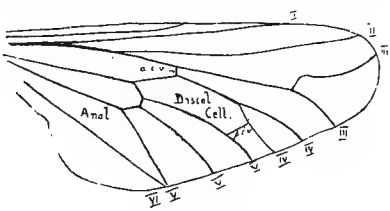


Fig. 90. *Wing of Lepidoselaga* (*Hadrus*), *a Tabanid.* *a.c.v.* = *anterior cross vein*; *p.c.v.* = *posterior cross vein*

The *Brachycera* (βραχύς short, κέρας antenna) include the following:—

1.—*Tabanidae* (Horse-flies or gad (=sting) flies).

Large flies. Antenna three-jointed, not terminating in a style or arista (the arista (when bristle-like) or style (when thick) being an appendage of the terminal portion (flagellum) of the antenna). Third segment of antenna annulated.

Labium enclosing four stylets in ♂, six in ♀. The terminal joint of the palpi is inflated, and the palpi hang down in front of the proboscis. Eyes in ♂ holoptic (contiguous), occupying most of head area. In ♀ dichoptic (separate). The male fly does not bite.

Egg.—Spindle-shaped. They are laid in spherical or flat groups on the stems of grass, etc.

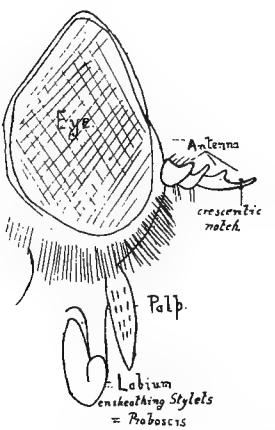
Larva.—Are aquatic or live in damp earth. They are carnivorous. They are about an inch long.

Pupa.—Aquatic or terrestrial. Over an inch long.

The *Tabanidae* are divided into two divisions, comprising more than thirty genera and over thirteen hundred species. It is only possible to mention here the commonest genera.

1. Hind tibiae with spurs at the tip; ocelli in most cases present. *Pangoninae.*

2. Hind tibiae without spurs at the tip; no ocelli (simple eyes). - - *Tabaninae.*



Head of Tabanus sp. (after Delofond)

Fig. 91

1. *Pangoninae*

Genus *Pangonia.*

Face and front in ♀ *without* tubercles or callosities. Proboscis often long, thin, horizontal. In some, three to four times length of body, piercing, even when the fly is on the wing. Third segment of antenna, eight rings. Species about two hundred and fifty.

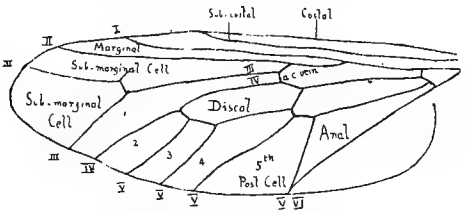
Genus *Chrysops*.

Front *with* a tubercle or callosity. Three ocelli. Second segment of antenna almost as long or as long as first. Eyes golden green. Flight silent. Wings widely separated; spotted. Hind tibiae spurred. Species about a hundred and fifty.

Ch. caecutiens attacks the eyes especially.

Genus *Silvius*.

Second antennal segment very much shorter than first. Wings without any spots. Third antennal segment five rings as in *Chrysops*. Species about twenty-six.



Wing of Haemolopola pluvialis.

Fig. 92

2. *Tabaninae*

The two most numerous genera are Tabanus and Haematapota.

(*a*) Front much longer than broad. Frontal tubercle when present not transverse.

Genus *Tabanus*.

Proboscis short and thick; vertical in the female, oblique in the male. Antennae scarcely longer than head. Third segment five rings. First ring is characteristically notched in shape of a crescent with a basal process (*Vide* Fig. 91). Eyes bare. Large flies with humming flight. Species about a thousand.

(*b*) Front as broad as it is long or broader. Frontal tubercle transverse, about four times as broad as long.

Genus *Haematapota*.

Terminal segment of antennae not crescentic. Third segment has four rings. Wings adjacent like the side of a roof. They have transparent markings. No ocelli. Flight silent. About fifty species.

H. pluvialis. Common in woody lanes in England in the summer.

The third group (*Cyclorrhapha Schizophora*) include the muscinae, sarcophagidae, and oestridae. The last two groups are not blood-suckers, but are included here for their pathological interest; also only some of the first group are blood-sucking.

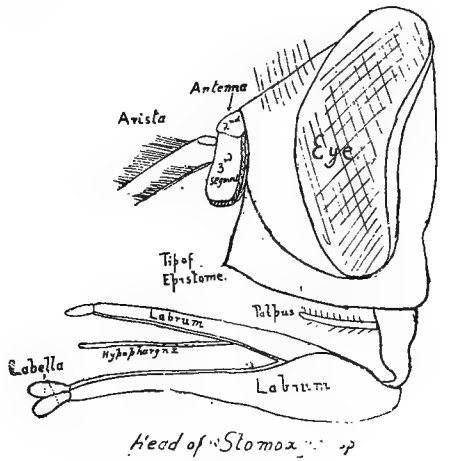


Fig. 93

1.—*Muscinae=Muscidae* (restricted).

Antennae *dependent* in front of head. They have three segments. The third segment is flattened and pod-like in shape, with an arista plumose generally to the tip. Hind body devoid of stiff bristles.

(*a*) Genus *Musca.*
House flies (not blood-suckers).

(*b*) Genus *Calliphora.*
Blow flies or blue bottles (not blood-sucking).

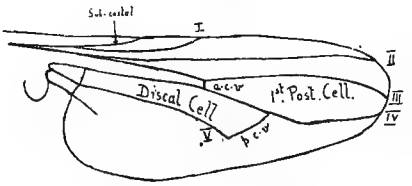
(*c*) Genus *Lucilia.*
Green bottles (not blood-sucking).
L. macellaria. The larva of this fly is the American 'screw worm,' infesting the nasal fossae and frontal sinuses of man.

(*d*) Genus *Auchmeromyia.*
A. luteola. The larva is the blood-sucking floor maggot of the Congo, etc.

Blood-Sucking Genera

Genus *Haematobia.*

(*a*) Palpi shorter than proboscis, partly ensheathing it. (*b*) Labellae fleshy, easily visible. (*c*) Aristà plumose dorsally; three to four hairs ventrally. (*d*) Third and fourth long veins reach the *apex* of wing. Small mottled flies.



Wing of *Stomoxys. sp.*

Fig. 94. *Shewing the first posterior cell open at the margin of the wing.*

Genus *Lyperosia.*

(*a*) Palpi long and flattened, ensheathing the proboscis. (*b*) Arista plumose dorsally. (*c*) Wing as in *Stomoxys.* Differs thus from *Glossina.* These flies are common on camels. *L. irritans* is the 'horn-fly' termed *H. serrata* in U.S.A.

Genus *Beccarimyia.*

(*a*) Palpi shorter than proboscis. (*b*) The first post-cell of the wing is *closed* before the margin.

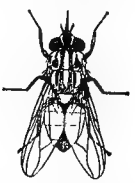


Fig. 95. *Stomoxys, shewing resting position of Wings,* × 2. (*After* Austen)

Genus *Stomoxys.*

(*a*) Palpi very small, bearing some hairs; not projecting beyond the epistome. (*b*) Proboscis is bent at its base like an elbow joint. (*c*) Arista plumose dorsally, distally forms a fine hair. (*d*) Third and fourth long veins reach the apex. The fourth is bent beyond the posterior cross vein. Wings diverge widely. *S. calcitrans*, the 'stable' fly, is common about farm-yards.

Genus Glossina

TSETSE FLIES*

Abdomen generally, but not always, has pale but well-marked dark-brown bands interrupted in the middle.

1. Dull-coloured, brownish flies, seven to twelve mm. long (excluding proboscis and wings).

2. Wings in resting position, closed flat, one over the other, scissors-like, projecting beyond the abdomen.

3. Proboscis ensheathed in palpi, projecting horizontally in front.

4. Base of proboscis suddenly expanded into a large onion-shaped bulb.

5. Arista feathered on upper side only.

6. Male genitalia (hypopygium) highly characteristic, oval and tumid, with a vulviform median groove (anus) running from anterior margin to beyond the middle. Sex easily distinguished by this mark.

7. Wings absolutely characteristic, especially in the course of the *fourth longitudinal vein* (*vide* Fig. 96, iv). The anterior transverse vein is very oblique. The bend in the course of the fourth vein, before it meets the anterior transverse vein, is absolutely diagnostic.

* The data of this section are compiled from *A Monograph of the Tsetse Flies*, by E. E. Austen, and from an article in the *British Medical Journal*, September 17, 1904, by E. E. Austen.

Larva and Pupa.—According to BRUCE, tsetse flies (or at least one species) do not lay eggs, but extrude a yellow-coloured larva. After a few hours this changes into a pupa. The pupa is six mm. long and three mm. broad. It consists of twelve segments. The twelfth segment is produced into two large lips, enclosing a pit, the site of the respiratory stigmata in the larva. At the anterior end is a longitudinal groove, through which the fly eventually emerges.

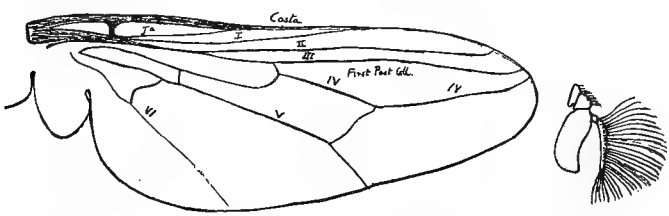


Fig. 96. *Wing Venation of Glossina, and antenna with Feathered Arista.* (*After* AUSTEN)

CLASSIFICATION OF SPECIES

(1) Hind tarsi entirely dark.

(*a*) Abdominal segments: sharply defined pale hind borders. Second segment: a conspicuous square or oblong pale area in the centre.

1. *Gl. tachinoides.*—The smallest tsetse fly (8 mm,). ♂ smaller. In the ♀ the tarsi basally somewhat pale.

(*b*) Abdominal segments: hind borders, if lighter, extremely narrow. Second segment: pale area triangular. Larger species than (*a*).

2. *Gl. palpalis.*—Darkest of all species of *Glossina.* Third joint of antenna dusky-brown to cinereous black.

3. *Gl. pallicera.*—Third joint of antenna orange-buff. Front in both sexes *narrower* than in *Gl. palpalis*. In ♂ the arista is stouter and longer than that in *Gl. palpalis*.

(2) Hind tarsi not entirely dark.

Small species, length is rarely ten-and-a-half mm. (A) Last two joints of front and middle tarsi have sharply defined dark-brown tips.

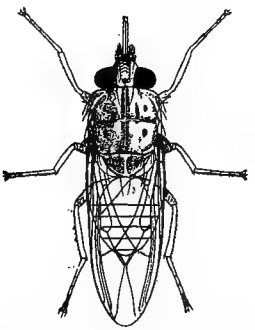


Fig. 97. Glossina, shewing scissors position of Wings when at rest, × 2. (*After* Austen)

4. *Gl. morsitans.*—
   1. Smaller than *G. longipalpis*.
   2. Head narrower.
   3. Front paler and wider.
   4. Eyes in ♂ and ♀ distinctly converging towards vertex.
   5. Abdominal bands less deep, pale hind margins of segments therefore deeper.
   6. Hypopygium in ♂ larger, paler, somewhat more oval in outline, and clothed with fewer hairs.
   7. Tip of ♂ abdomen less hairy laterally.

8. Bristles on sixth segment in ♂ stouter and more conspicuous than in *longipalpis*.

5. *Gl. longipalpis*.—

(B) Last two joints of front and middle tarsi entirely pale.

6. *Gl. pallidipes*.—

Large species. Length, at least ten-and-a-half mm. (in this respect they contrast markedly with the other small species).

7. *Gl. longipennis*.—

1. Thorax with four sharply defined dark-brown oval spots.
2. Ocellar spot, dark-brown, *very conspicuous* compared with the body.
3. Proboscis shorter than in *G. fusca*, and *relatively* shorter, compared with the body, than in any other species.
4. In both sexes the *front* is broader than in *Gl. fusca*.

8. *Gl. fusca*.—Thorax without spots.

2.—[*Sarcophagidae*].

Not blood-sucking. Arista feathery at the base, bare at the tip. Large flies, about 14 millimetres long.

Genus *Sarcophaga*. Elongated thorax, three black bands, abdomen spotted. Third segment of antenna three times the second segment.

S. carnaria, *S. magnifica, and S. ruficornis* (India), give rise to terrible forms of myiasis in man and animals.

3.—[*Oestridae*] (Bot. (=Larva) Flies).

Not blood-sucking. Large flies. Proboscis rudimentary. Antenna very short. Arista segmented. Flight humming.

(*a*) Genus *Gastrophilus*, *e.g.*, *G. equi*. The white eggs can be easily seen on the horse's hair. The larvae are swallowed and they attach themselves to the mucosa of the stomach.

(*b*) Genus *Hypoderma*, *e.g.*, *H. lineata*. Larvae produce ox warbles (=tumours) in the ox.

(*c*) Genus *Oestrus*, *e.g.*, *O. Ovis*. Larvae in the respiratory passages of the sheep.

(*d*) Genus *Cephalomyia, e.g., C. maculata.* In the camel.

(*e*) Genus *Cephenomyia, e.g., C. rufibarbis.* In red deer. Scotland,

(*f*) Genus *Dermatobia, e.g., D. cyaniventris.* Larva is the 'ver macaque' (America), producing myiasis in man and cattle.

(*g*) Genus *Ochromyia, e.g., O. anthropophaga.* Larva is the 'ver de Cayor' (Senegal), producing myiasis in man.

Myiasis is common in Africa and in the tropics, but the larvae have been identified in but few instances as yet.

The fourth group, the Pupipara (to which *Glossina* also belongs, from the point of view of its life history), comprises:

1.—*Hippoboscidae* (spider flies).

They run rapidly over the body, hiding in hair or feathers. Head circular. No distinct neck. Clypeus distinct, separated from the head by a curved suture. Antennae lie in cavities in its anterior angle. Antennae: one segment with or without a style (arista). Palpi absent. Abdomen leathery, capable of much distension in ♀. Tarsi: fifth segment longest, with two or three claws. Empodia (*between* the claws) distinct. Wings large, or mere strips, or absent.

Fig. 98. *Hippobosca Rufipes, left* × 2—*right, natural size* (*After* THEILER)

(*a*) Genus *Hippobosca.*

Wings large, obtuse. No ocelli; arista nude; legs long and extended. Claws bidentate.

H. equini. Runs rapidly over the body; is the [New] forest fly of England.

H. camelina. Attacks camels in Egypt.

H. rufipes transmits Trypanosoma theileri (?).

(*b*) Genus *Melophagus*.

Wings extremely minute. Eyes small. No arista on antennae. Claws bidentate.

M. ovi is the sheep 'tick.' Four millimetres long.

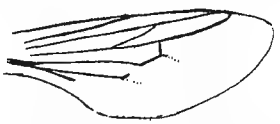
(*c*) Genus *Ornithomyia*.

Wings large. Four millimetres long.

O. avicularia. Occurs on birds.

(*d*) Genus *Lipoptena*, *e.g.*, *L. cervi* on the red deer.

(*e*) Genus *Stenopteryx*, *e.g.*, *S. hirundinis* of the swallow.



Wing of Hippobosca rufipes.

Fig. 99

2.—*Nycteribiidae*.

Found on bats. They have no wings.

FLEAS†

Fleas, or Siphonaptera, are considered to be aberrant forms of flies, and hence follow naturally after the division Pupipara, of the flies.

Life History

Eggs: About a dozen are laid, in floors, in cracks, etc.; sometimes in the hair or fur of animals. They are 0·7 by 0·4 millimetres (*P. irritans*). The eggs hatch in about a week or more.

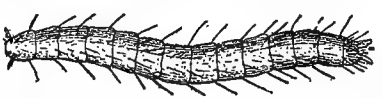


Fig. 100. *Larva of a Flea* x 20 (*after* Railliet)

† Rabinowitsch has obtained positive results in the transmission of the rat trypanosomes by fleas. In this case, and in the case of Glossina there is no evidence to show that the trypanosome undergoes any developmental change; nor, even in the case of Glossina, has it actually been shewn that trypanosomes occur in or on the proboscis during the biting of an uninfected animal.

Larvae: Are worm-like, whitish, consisting of fourteen segments. They are about one-and-a-half by one-tenth millimetres in size. They feed on organic refuse (?) and on blood. In about eleven days they are full grown.

Nymphs.—Have legs but are stationary. After the lapse of eleven days the flea emerges (the evolution thus taking about a month).

Anatomy

1. The head is small, not distinctly separated from the body.

2. The antennae are placed in fossae behind the eyes. They consist of two basal segments, and a third of diverse form irregularly segmented. The maxillary palpi must not be mistaken for them.

3. The mouth consists of (*a*) hypopharynx (the central stylet) serrated above, tubular below; (*b*) two serrated mandibles hollowed on their inner surfaces and forming with (*a*) a gutter, along which the blood flows; (*c*) a labium single for a short distance, then bifurcating and forming two labial palps, which form a sheath for the piercing organs (*a*) and (*b*); (*d*) two maxillae having the form of expanded plates, each bearing a four-jointed palpus.

4. The thoracic segments, three in number, are separate. The metanotum has a 'wing-like' flap or epiphysis especially well developed in the *Sarcopsyllidae.*

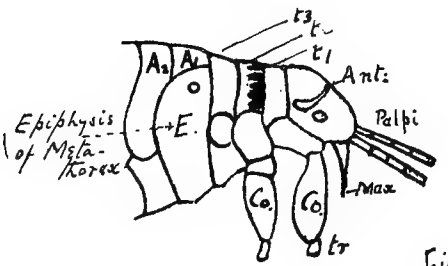
5. Abdomen consisting of ten segments, overlapping; the dorsal and ventral portions not being united, and so allowing of distension. The terminal segments are highly specialized forming the genital apparatus. The sexes are readily distinguished by this means (*Vide* Fig.)

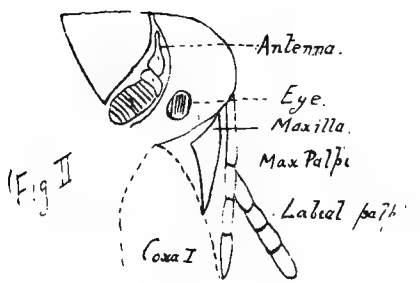
Capture of Fleas

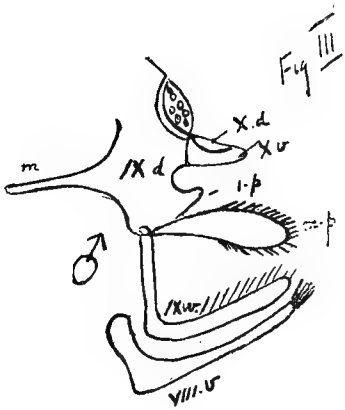
Small animals, such as rats, are best chloroformed in a box with a glass top. The fleas are subsequently carefully brushed out. The nests of small animals, such as mice, birds, etc., are often a rich source of fleas. The nest should be put in a bag for subsequent examination. For lifting the fleas use the tip of a stiff feather dipped in spirit. Very careful manipulation is necessary to avoid damaging bristles or spines.

Identification of Fleas

The number of described species already approaches two hundred. Consequently all that we can attempt here is to indicate some points in the structure of fleas. The external







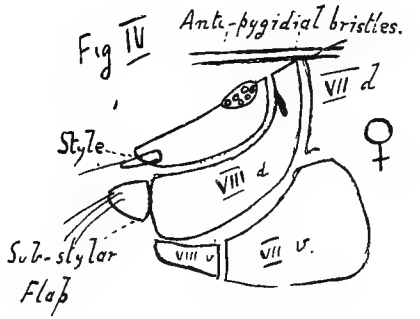


Fig. 101.

I.—*t*1-*t*3, *thoracic segments*; *A*1, *A*3 *first and second abdominal segments.*
II.—*Head of a flea shewing antenna* (*three segments*) *lying in antennal groove.*
III.—*Hind segments of male flea shewing claspers.* *d* = *dorsal segment*, *v* = *ventral segment*; *i.p*, *immovable process*; *m.p*, *moveable process*; *m* = *manubrium* (*diagrammatic*).
IV.—*Hind segment of female flea* (*diagrammatic*).

copulatory organs of the male are of great importance in classification. A complete description of a flea, however, involves a description of practically every bristle or series of bristles on the body. The following classification is taken from Baker :—

1. Thoracic segments short and narrow. Labial palpi without pseudo-joints, third antennal segment without clearly separated pseudo-joints. 2.

Thoracic segments not short and narrow, labial palpi with three or more pseudo-joints. Third antennal segment with nine or more fairly distinct pseudo-joints. Maxillary palpi almost always shorter than anterior coxa. Epiphyses (flaps) of meso- and metathorax extending over only one abdominal segment. 3.

2. Maxillae without, or with, very short and broad projecting laminae. Maxillary palpi extending beyond anterior coxae. Head produced into a sharp point in front in ♀ and ♂ Metathoracic epiphysis extending over two to three abdominal segments. *Sarcopsyllidae.*

Maxillae with a narrow long curved lamina. Maxillary palpi as long as anterior coxae. Head evenly round. Metathoracic epiphysis extends to one abdominal segment. *Hectopsyllidae.*

3. Labial palpi largely developed with eleven to thirteen pseudo-joints, abdomen of gravid ♀ much swollen, antepygidial bristles absent *Vermipsyllidae.*

Labial palpi three to five pseudo-joints; antepygidial bristles present. 4.

4. Fore tibiae: posterior border has a few black teeth. Fifth tarsal segment greatly enlarged, those on forelegs as long as rest of tarsus; claws of all legs nearly as long as fifth joint. Fore coxae with but few long spines. Body of gravid female swollen. *Megapsyllidae.*

Fore tibiae: post border has slender spines. Fifth tarsal segment not greatly enlarged, never as long as rest of tarsus. Fore coxae with several or numerous rows of bristles. Gravid female not swollen so as to expose membrane between sclerites. *Pulicidae.*

Sarcopsillidae ;—

1. Genus *Sarcopsylla.*

Maxilla without a projecting lamina, angle of head produced, metathoracic epiphysis extending to three abdominal segments. Fifth tarsal segment without lateral heavy spines and legs almost spineless, *e.g.*, *S. penetrans*, the Chigoe or Jigger, etc.

2. Genus *Xestopsylla.*

Maxilla with a short projecting lamina, angle of head not produced, metathoracic epiphysis of medium size extending to hardly two abdominal segments. Fifth tarsal joint with the usual spines. Legs with spines, *e g.*, *X. gallinacea*, infests hens U.S.A.

Pulicidae.

1. Maxillae long triangular, acute at apex. Abdominal segments with no ctenidia (combs). Post tibial spines in pairs not in a very close set row. Last tarsal segment on all the tarsi with a marginal row of four stout spines. Eyes large. ♀ one antepygidial bristle on each side.

(*a*) Head without ctenidia. Genus *Pulex*, *e.g.*, *Pulex cheopis*, associated with the transmission of plague. It is distinct from *P. pallidus.*

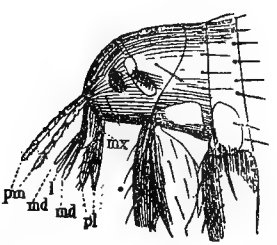


Fig. 102. *P. irritans*, x 30. (*After* RAILLIET)

(*b*) Head with ctenidia. Genus *Ctenocephalus*, *e.g.*, *Ctenocephalus canis* on dogs.

Fleas for identification should be sent to the Hon. N. Charles Rothschild, Tring Park, Tring, Herts.

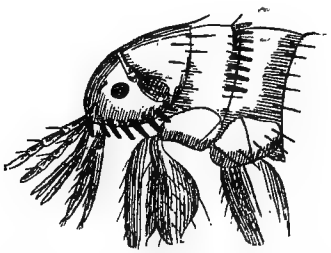


Fig. 103. *Ctenocephalus canis*, x 30. (*After* RAILLIET)

LITERATURE

Taschenberg. Die Flöhe.

C. F. Baker. A revision of American Siphonaptera or fleas, together with a complete list and bibliography of the group. *Proceedings of the United States National Museum*, Vol. XXVIII, pp. 365-469, with Plates X-XXVI (No. 1361).

STAINS, NUMERICAL DATA, ETC.

FIXING AND HARDENING SOLUTIONS

Alcohol is a fixative and dehydrating medium, and for ordinary work is the most convenient. The tissues, in small pieces, may be placed directly in methylated spirit (ninety per cent. alcohol), or absolute alcohol (ninety-eight per cent. alcohol). Change the alcohol a few times. Or pass through fifty, seventy-five, ninety-five, one hundred per cent. alcohols, leaving a few hours in each. After hardening, if the specimens are not to be imbedded immediately, transfer to alcohol of

about eighty per cent. for preserving. After the use of other fixatives, specimens should be washed and transferred to eighty per cent. of alcohol for preservation.

Rectified spirit of the British Pharmacopaeia, is equal to eighty-four per cent. alcohol.

Methylated spirit, containing *wood* naptha, is equal to ninety per cent. alcohol.

Ordinary methylated spirit contains mineral naphtha, and should not be used.

Absolute alcohol is equal to ninety-eight per cent. alcohol. For practical purposes the dilution of alcohols is sufficiently accurately made by means of the diluting formula (p. xvii).

Zenker's Fluid :—

Potassium Bichromate	2·5 grammes
Sodium Sulphate	1·0 grammes
Corrosive Sublimate	5·0 grammes
Water	100·0 grammes

Add glacial acetic acid to this stock solution, in the proportion of five grammes to one hundred c.c., before use. Fixation is complete in one to twenty-four hours. Wash thoroughly in alcohol to which enough iodine has been added to give a dark-brown solution. Or, if alcohol is undesirable, use tincture of iodine, two parts, potassium iodide, one part, glycerine, fifty parts, water, fifty parts. Renew until no further decolouration takes place.

Haematin or eosin and methylene blue give good results for malarial tissues.

Orth's Fluid.—This is Muller's fluid, to which formalin (*i.e.*, formaldehyde forty per cent. solution) is added in the proportion of ten c.c. to one hundred c.c. of Muller before use.

Fixation of small pieces takes place in two to three hours, if kept warm. Wash thoroughly. Pass through alcohol.

Muller's Fluid.—Potassium Bichromate, 2½ parts.
Sodium Sulphate 1 part.
Water 100 parts.

Add a little camphor or naphthalin to prevent the growth of moulds. Change the fluid after twenty-four hours, and then every few days for the first week. Tissues are ready in a fortnight or three weeks. They may be left much longer. Fix

in the dark. Wash thoroughly in water till colourless. Transfer to alcohol, seventy to eighty per cent., for preservation.

Flemming's Solution.—Chromic acid, 1 per cent., 15 vols.
Osmic acid, 2 per cent., 4 vols.
Glacial acetic acid 1 vol.

Mix in the above proportions before use. Use very small pieces. Fixation is complete in about twenty-four hours. Blackening due to the osmic may be removed by hydrogen peroxide. Blood films may be fixed in this solution.

Tissues thus fixed may be preserved in equal parts of alcohol and glycerine.

Formalin (forty per cent. solution of formaldehyde).—Use two to five per cent. solution in water. Small pieces are fixed in twelve to twenty-four hours. They may be left in solution or transferred to alcohol.

Corrosive Sublimate.—Best used as a concentrated alcoholic solution (or aqueous may be used). Fixation takes place in a few hours. Wash thoroughly in water and transfer to iodine solution (*vide* Zenker's fluid) till iodine no longer decolourized.

The concentrated alcoholic solution is a most rapid fixing and hardening reagent, and sections can be cut in a very short time, if small pieces are used.

Decalcifying Solution.—Tissues require fixing before and after these solutions—

(i) Phloroglucin, one gramme, nitric acid, ten c.c., water, one hundred c.c.; *or*

(ii) One to five per cent. solution of nitric acid in water or alcohol. Change the fluid daily. Decalcification takes place in two to three days.

(iii) Picric acid, a saturated solution (= about 0·75 per cent.) containing crystals. Decalcification may take weeks or months. Wash in alcohol.

Eau de Javelle (dissociating and decolourizing solution).—Add to a concentrated aqueous solution of chloride of lime a solution of potassium oxalate as long as a precipitate is formed. Filter and dilute if necessary. This may be used for softening the chitinous skeleton of mosquitoes and for decolourizing Madura fungus, etc.

For Fixing Paraffin Sections to the Slide

1. Celloidin, one part, oil of cloves, two parts; *or*
2. Thin solution of white shellac in creosote; *or*

3. Thoroughly mix equal parts of white of egg and glycerine ; filter. This is one of the simplest and best means ; *or*

4. Filtered white of egg, 50 c.c.
Glycerine 50 c.c.
Sodium salicylate 1 gramme.
Shake well and filter (this takes about a week). The solution keeps well for six months or more ; *or*

5. Simply use fresh white of egg ; smear thinly over ; dry.

Mounting Media, etc.

1. *Farrant's Solution.*—

i. Take equal parts of glycerine and a saturated solution of arsenious acid. Add powdered gum arabic till the solution is saturated ; *or*

ii. Pure gum arabic, 40 grammes.
Water 40 c.c.
Glycerine 20 c.c.
Carbolic acid 1 gramme.
[*or* Thymol 0·3 grammes.]
Powder the gum and dissolve in about one hundred and fifty c.c. of water by boiling, add the carbolic acid, dissolved in a a little water, filter through a hot filter, changing when clogged, evaporate until it is about eighty c.c., then add the glycerine.

2. *Acetate of Potassium.*—Saturated solution. Cement the cover-glass with gold size, dammar lac. This is used for osmic preparations, and for glycogen stained with iodine, etc.

Glycerine Jelly.—Glycerine 70 c.c.
Water 60 c.c.
Gelatine 10 grammes.
Thymol 0·5 grammes.
or one gramme of phenol to each one hundred grammes of the mixture.

Dissolve the gelatine at forty degrees in a water bath ; add the glycerine (warmed) ; powder the thymol and mix with a little water, and stir in ; add the beaten-up white of an egg ; stir continuously ; warm to eighty-five degrees ; filter through a hot filter.

To Mount Delicate Objects

Place in ten per cent. glycerine, allow this to concentrate in the air, and then transfer to the jelly.

To mount in Jelly.—Remove as much water as possible; place the slide, coverglass, and jelly in the incubator (if necessary). Before cementing see that the layer of jelly is not too thick. If too thick press some out and scrape away; then cool.

Dammar Lac.—Dissolve in equal parts of benzene and oil of turpentine. It does not render preparations as translucent as Canada balsam.

Fixing Blood

As we have already stated, for practical purposes alcohol is absolutely satisfactory. The following solutions have been used, and may prove useful occasionally:—

1. *Osmic acid.*—Osmic acid, 1·0; sodium chloride, 0·6; distilled water, 100·0.

A neat and practical method of using this is to moisten a camel's hair brush with the solution, then to touch the blood drop, and to immediately spread the blood out on the slide with the brush. Wash the brush, after use, in alcohol (Kornilowitch).

2. *Chloroform.*—Instead of heat in staining with Ehrlich's triacid. Fix for five minutes in chloroform (neutral to litmus paper). Stain for five minutes or more after fixing (Josué).

3. *Strong Flemming.* Especially for nuclear structures of parasites.

4. *Heat.*—Heat up from one hundred to one hundred and ten degrees C. in a hot oven, and then, when this temperature is reached, allow to cool again in the oven.

5. Osmic acid, two per cent., glacial acetic acid, equal parts. Expose to the vapour. For delicate work indispensable.

Staining Solutions for Blood, etc.

1. *Romanowsky stain* (p. 10).

2. *Haematin* (p. 50).—If the solution has become reddish on keeping neutralize with a little ammonia.

3. *Eosin and Methylene Blue* (consecutively).—(*a*) Stain for one to five minutes in a one-half per cent. solution of eosin in sixty per cent. alcohol, wash, dry with blotting paper, and stain (*b*) in a one per cent. solution of methylene blue for thirty seconds to one minute.

This is a useful and simple method for studying the acidophil and basophil reactions of granules.

4. *Safranin.*—Make a saturated solution in two per cent. anilin water. Heat to sixty per cent. C. Filter hot. Stain sections over night. Differentiate very carefully with one per cent. hydrochloric alcohol. Safranin is an excellent nuclear stain.

5. *Ehrlich's Triacid.* Stain for five minutes.

6. *Ehrlich's Haematoxylin-Eosin.*—Haematoxylin, five grammes ; acetic acid, twenty grammes; alcohol, one hundred grammes ; glycerine, one hundred grammes ; water, one hundred grammes. Allow this to ripen for a month in the sun, then add eosin to the extent of one per cent. ; stain for twenty-four hours. The solution is best got ready-made.

Solubility of Stains

10 c.c. of saturated alcoholic methylene blue contains 0·068 grammes. of the stain.

10 c.c. of saturated aqueous methylene blue contains 0·664 grammes of the stain.

10 c.c. of saturated alcoholic gentian violet contains 0·442 grammes of the stain.

10 c.c. of saturated aqueous gentian violet contains 0·175 grammes of the stain.

10 c.c. of saturated alcoholic fuchsin (basic) contains 0·292 grammes of the stain.

10 c.c. of saturated aqueous fuchsin (basic) contains 0·066 grammes of the stain. (Hewlett).

Loffler's Methylene Blue.—30 c.c. saturated alcoholic methylene blue.

100 c.c. of 0·1 per cent. caustic potash.

Ordinary Methylene Blue.—A saturated alcoholic solution is used as the stock, and a few drops added to a watch-glass-ful of water, according to strength required ; or ten per cent. solution is a convenient strength.

Borax Methylene Blue (Sahli's).—Saturated aqueous solution of methylene blue, twenty-four parts ; borax five per cent. solution, sixteen parts ; water, forty parts.

The times necessary for staining are best judged by the appearance of the films or tissues.

Tissue Stains

1. *Haematein* (p. 50).—Stain for about five minutes, according to the ripeness of the solution. Sections do not readily overstain. Decolourize, if necessary, with one per cent.

alum solution. Counterstain, if required, with a weak, watery solution of eosin, one-half to one per cent. ; or the sections may be stained first with a strong eosin solution, five to one hundred per cent., for five to twenty minutes. Combination of eosin and methylene blue can be used in a similar way.

2. *Alum Carmine.*—Carmine, two grammes ; alum, five grammes ; water, one hundred c.c. Boil together for one hour. Filter. Does not overstain.

3. *Ehrlich Biondi.*—Saturated aqueous solution of acid fuchsin, four parts ; orange g., seven parts ; methylene green, eight parts. Dilute fifty to one hundred times before using. Stain for twenty-four hours. Wash in alcohol. The sections may, before staining, be treated with acetic acid (two parts in one thousand of water) for a few hours.

Iron Reaction (Haemosiderin) in Malarial Tissues

1. Fix in alcohol.
2. Two per cent. aqueous solution of potassium ferrocyanide, five to twenty minutes.
3. Acid alcohol (HCl, one part, seventy per cent. alcohol, one hundred parts), five to ten minutes.
4. Wash in water.
5. Counterstain with alum carmine.

Staining of Amoeba Coli in Tissues (Mallory and Wright)

1. Fix in alcohol.
2. Saturated aqueous solutions of thionin, three to five minutes.
3. Two per cent. solution of oxalic acid, one-half to one minute.
4. Wash in water.
5. Dehydrate in alcohol.
6. Clean in oleum origanum cretici.
7. Wash in xyol.
8. Balsam.

Nuclei of the amoebae are brownish red, the nuclei of the mastzellen ' are blue.

Weights and Measures, etc.

1. Conversion from one temperature scale to another—

$$\frac{C}{5} = \frac{R}{4} = \frac{F-32}{9}$$

Thus to convert 100° F. to centigrade—

$$\frac{100 - 32}{9} = \frac{C}{5} \therefore C = 37.7^{\circ}$$

2. *Formula for dilution of Solutions.*—The number of parts required to dilute from one part of a solution of strength x per cent. to another strength y per cent. is $\frac{x}{y} - 1$.

Thus, to dilute a solution from 20 per cent. to 5 per cent., add to each volume of solution $\frac{20}{5} - 1 = 3$ of the diluting fluid.

3. *Approximate Values:*—

1 cubic centimetre = 17 minim. 1 minim = ·059 c.c.
100 ,, = 3 ounces, 4 drachms, 20 minims.
1 drachm = 3·55 c.c.
1000 cubic centimetres = 1·76 pints. 1 fluid ounce = 28·4 c.c.
4 litres = 7 pints. 1 pint = 568 c.c.
1 gramme = $15\frac{2}{5}$ths grains (avoirdupois). 1 grain (avoirdupois) = ·0648 grammes.
1 kilogramme = 2 pounds, 3 ounces, 119·8 grains (avoirdupois). 1 drachm = 1·77 grammes.
5 kilogrammes = 11 pounds. 1 oz. = 28·35 grammes.
1 grain apothecaries' = ·0648 grammes.
1 drachm apothecaries' ʒ = 3·88 grammes.
1 ounce apothecaries' ℥ = 31·1 grammes.
15·4 grains apothecaries' = 1 gramme.

4. 1 millimetre = ·039 ($\frac{1}{25}$) inches. 1 inch = 2·5 centimetres.
1 centimetre = ·39 ($\frac{2}{5}$) inches. $\frac{1}{1000}$ inch = ·0253 millimetres.
1 metre = 3·28 feet. $\frac{1}{100}$ inch = $\frac{1}{4}$ millimetre.
$\frac{1}{1000}$ millimetre (μ) = ·00004 inches.
$5\mu = \frac{1}{5000}$ inch. $\frac{1}{4}\mu = \frac{1}{10000}$ inches.

5. $\pi = 3{\cdot}14159$.
Circumference of a circle = $2\pi r = \pi d$.
Area of circle = πr^2.

LIST OF APPARATUS

		£	s.	d.
1.	A Beck microscope, including oil immersion, $\frac{1}{12}$	13	10	0
2.	Glass slides,† 3 x 1, ground edges, per gross	0	3	6

† Slides and coverglasses should be kept in spirit or alcohol to prevent their becoming opaque, as only too frequently occurs in the tropics.

		£	s.	d.
3.	Cover glasses, No. 1, $\frac{3}{4}$-in., $\frac{1}{2}$-oz. -	0	4	0
4.	Straight surgical needles, for making blood films and dissecting, $\frac{1}{2}$ doz. (Weiss & Co., Oxford Street)	0	1	6
5.	Stoppered jars (4), 13 x 7$\frac{1}{2}$ centimetres	0	3	6
6.	Porcelain dishes, square, flat, 1 doz.	0	1	0
7.	Specimen tubes, flat-bottomed, corked, 3 in. x $\frac{3}{4}$ in., 50 -	0	4	0
8.	Slide box to hold 25, sliding, cardboard	0	1	0
9.	Measures, 100 c.c. and 10 c.c.	0	1	9
10.	Drop-bottle for xylol (a toothpick inserted into the cork of a specimen tube serves the purpose) -	0	0	4
11.	Cedarwood oil bottle (a pin in the cork of a specimen tube makes a convenient dropper for the oil) -	0	0	9
12.	A 'Primus' paraffin burner, for boiling, etc.	0	12	6

STAINS, ETC.

1.	Romanowsky.—Methylene blue, pure medical, 10 grammes, or in 'soloids'	0	1	8
	Eosin, B.A., 10 grammes, or in 'soloids'	0	1	2
	Sodium carbonate, pure, in 'soloids'	0	0	2
2.	Leishmann's Stain—(*a*) In 'soloids' (Burroughs, Wellcome & Co.) = ·015 gramme. Dissolve in 10 c.c. of methyl alcohol (or methylated spirit).			
3.	Haematin, 5 grammes,	0	4	6
	Alum, 1 oz.	0	0	2
4.	Absolute alcohol, 1 lb. -	0	4	0
5.	Xylol, 1 lb. -	0	2	3
6.	Paraffin wax, melting points, 50°C. and 60°C. 1 lb. 2s. 6d. 1 lb. 3s.	0	5	6

ADDITIONAL APPARATUS

1.	A mechanical stage, fitting on to *the stage* (not the column) of the microscope -	3	5	0
2.	Browning's pocket spectroscope, indispensable for urine work in blackwater fever, etc.	1	0	0
3.	Haemacytometer. Thoma-Zeiss.	1	16	0
4.	Haemoglobinometer. Gower's	1	1	0

FOR MOSQUITO COLLECTION

		£	s.	d.
1.	A lens (the lens of an eye-piece of the microscope serves as well).	0	15	0
2.	Silver pins, No. 20, $\frac{1}{4}$ oz.	0	2	0
3.	Entomological pins, $1\frac{1}{2}$ in. long, 1 oz.	0	0	8
4.	Cardboard (fine Bristol board), 4 sheets	0	0	3
5.	Specimen tubes, flat bottom, each about	0	0	1
6.	Pill boxes.			
7.	A dissecting board, 12 x 3 in., half covered with black, half with white paper (made by self).			

Supplied by Messrs. C. Baker & Co., London.

LITERATURE

1. *Encyklopädie der Mikroskopischen Technik.* 2 vols. by Ehrlich and others.
2. *Methods and Theory of Physiological Histology.* Mann.
3. Merck's *Reagentien Verzeichniss.* A very useful compendium, giving the composition of all the best known stains and reagents.

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