Laboratory studies in tropical medicine / C.W. Daniels and H.B. Newham.

LABORATORY STUDIES IN TROPICAL MEDICINE

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IN

TROPICAL MEDICINE

BY

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THIRD EDITION

Thoroughly revised, with many new and additional illustrations

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PREFACE TO THIRD EDITION.

SINCE the appearance of the last edition of this book many advances have been made in the study of tropical diseases. Although no revolutionary discovery is to be recorded, still, new facts dealing with disease and its propagation have been made out and new details of technique have been elaborated.

The book is intended essentially to be a practical one, and although it is necessary to give brief descriptions of the more important protozoa, helminths, &c., in no sense is it to be taken as a complete account of parasites in general.

The classification of parasites, insects, &c., is constantly changing as new facts regarding them come to light, and no authoritative schemes of classification is given. Those inserted have been found by practice to be useful, and although differing in many details from those adopted by authorities at the moment, will, the authors believe, be found of utility to the average man.

New details and additions have been made, which it is trusted will serve to enhance the value of the book as an aid to the practical worker for whom it is intended.

Our best thanks are due to Professor Minchin and Messrs. Macmillan for kindly permitting us to make use of the illustrations showing the development of Gregarines and Rhinosporidium.

C. W. D.
H. B. N.

May, 1911.

PREFACE TO SECOND EDITION.

RAPID advance has been made in all branches of tropical medicine since the first edition was published. In this edition these advances are considered, and especially information as to the known carriers of disease has been added, including ticks, biting flies and fleas.

The subjects are now so large that a certain amount of systematic classification has become necessary, and tables are therefore included showing, in brief, the zoological relationship of parasites and of their carriers.

A sub-division of the chapters has been made, as it is hoped from time to time, by publication of the further advances made, to supplement the information given in each chapter.

The general idea remains the same, the book is intended for the lonely worker in his private laboratory.

We are indebted to many friends for advice and assistance, particularly in the revision of proofs, and especially to Dr. H. B. Newham, and A. W. Balch, Surgeon, U.S. Navy.

C. W. D.
A. T. S.

September, 1907.

PREFACE TO FIRST EDITION.

THE object of this work is to assist practitioners in the Tropics in the application of simple laboratory methods to the practice of medicine.

The writer has had personal experience in several countries of the peculiar difficulties that a student desirous of advancing the knowledge of tropical medicine, or of practising it conscientiously, will meet, and the plan of study advocated is the outcome of this experience.

The subjects include an outline of animal parasitology and the development of the best known of these parasites. The part played by insects in spreading disease is so important that it is necessary to have a sound working knowledge of the more important known carriers of disease.

Chapter vii. has been kindly written by Mr. F. V. Theobald for this book, so as to enable the student to differentiate the more important genera of the *Diptera*. No exhaustive study of any one subject has been made, but it is hoped that sufficient information is given to enable the practitioner to commence the effective study from the laboratory point of view of the more important problems. Simple methods are selected as far as possible, and those recommended are in the main those adopted by the writer for teaching purposes at the London School of Tropical Medicine, and can be relied on as applicable to the circumstances.

Few references are given, as the practitioner in the Tropics has rarely access to a library.

I am much indebted to Dr. G. C. Low, Medical Superintendent at the London School of Tropical Medicine, for valuable assistance, and the revision of the proofs has also been kindly undertaken by him.

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CHAPTER I.

THE LABORATORY.—In few places in the Tropics is there any institution that corresponds to the British idea of a laboratory. Tap-water, gas and electric light usually have to be dispensed with and substitutes employed. The isolated worker has to arrange and make his own laboratory, either in the house or attached to a hospital. A separate building will rarely be available.

The first essential is a good light, and if, as is usual, work is done by daylight, the light must come neither from east nor west. A north or south aspect should be chosen, according to whether the worker is north or south of the line, so as to avoid direct sunlight.

A corner of a verandah can be made into a good laboratory by placing blinds or jalousies on two sides, and leaving only the one side, that facing north or south, open. The side from which the light is received should be closed in with a window if possible, to prevent the entrance of rain and dust.

Another important consideration is wind, and with the wind the amount of dust. If there is a glass window this is of less importance, but if working on an open verandah, a portion of the verandah sheltered from the prevailing wind must be selected, even if this choice involves the sacrifice of the most favourable light.

If a room with a north or south aspect is not available

any other aspect will suffice, provided that there is a deep, low verandah outside the window.

On the wall of the laboratory should be fixed a number of plain wooden shelves. One of the lower of these, at a convenient height, should be strong and broad enough to receive heavy weights. On this shelf may be kept mosquito cages, maturing larvæ and other objects awaiting immediate examination or requiring constant attention.

It is convenient to have two tables—one on which to work with the microscope, and also for papers, notebooks and any book actually in use, another on which staining processes, dissections and the rougher and more messy work can be done. Individual habits of neatness and arrangement make a difference. Though much excellent work has been done by untidy workers, there is no doubt that in the limited space available on the narrow verandahs usual in many parts of the Tropics work is easier and more comfortable if the habit of tidiness be cultivated. Persons who are exceptionally neat and methodical in their habits will probably find one long table more convenient than the two recommended here.

For work with the microscope a firm steady table is required, and this should be placed a few feet from the window. The second table, which should be also strong, must be placed in a good light, and it is better to have the light falling from the left-hand side of the table.

For the other side of the laboratory jalousies are most convenient, as they let in plenty of air and can be turned so as to regulate the amount of air and to stop the entrance of rain. A cheaper arrangement is to use reeds, as natives in the most parts of the world are good workers with reeds. Sufficient air will pass through to keep the room cool. Native mats can be used, but must be nailed on to a framework, otherwise they will be blown about by the wind.

Water must be kept in bulk, as tap-water is rarely available. A small tank—an empty, thoroughly cleaned

kerosine tin will serve—should be kept filled with water. This should be filtered, and a glass syphon tube with a rubber tube and clamp attached can be used to draw

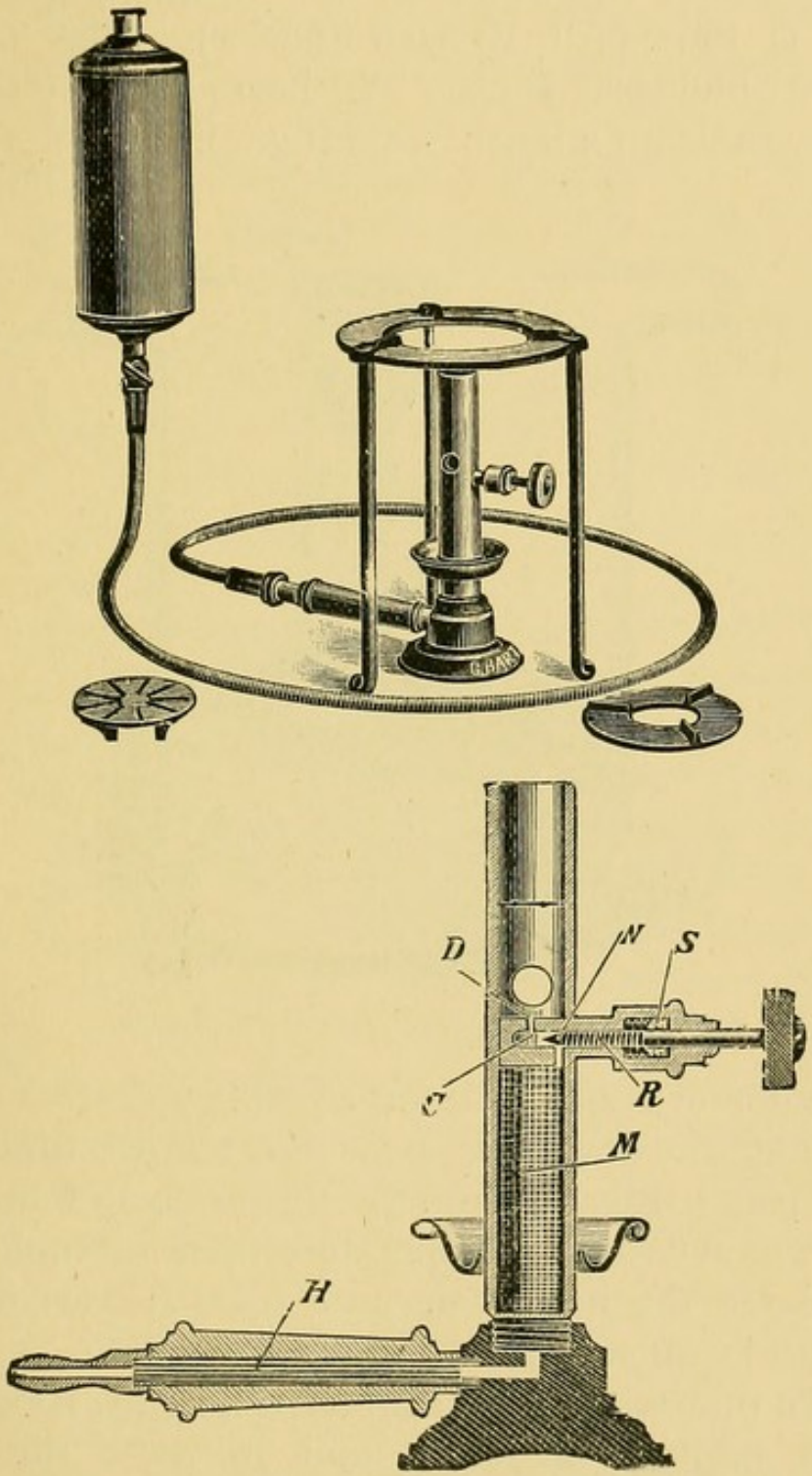

FIG. 1.—AUTOMATIC BUNSEN BURNER FOR METHYLATED SPIRIT.

it off. The tank must be kept covered with a well-fitting lid, and a basin or other receptacle should be placed underneath to receive the waste and washings of stains, &c.

Distilled water must be kept in bulk in a well-stoppered bottle, from which a sufficient amount is taken as required into a wash bottle for immediate use.

An excellent substitute for the ordinary gas Bunsen burner is the spirit Bunsen (fig. 1). The "Primus" Kerosine Smokeless Burner will be found very useful for heating vessels on a larger scale (fig. 2).

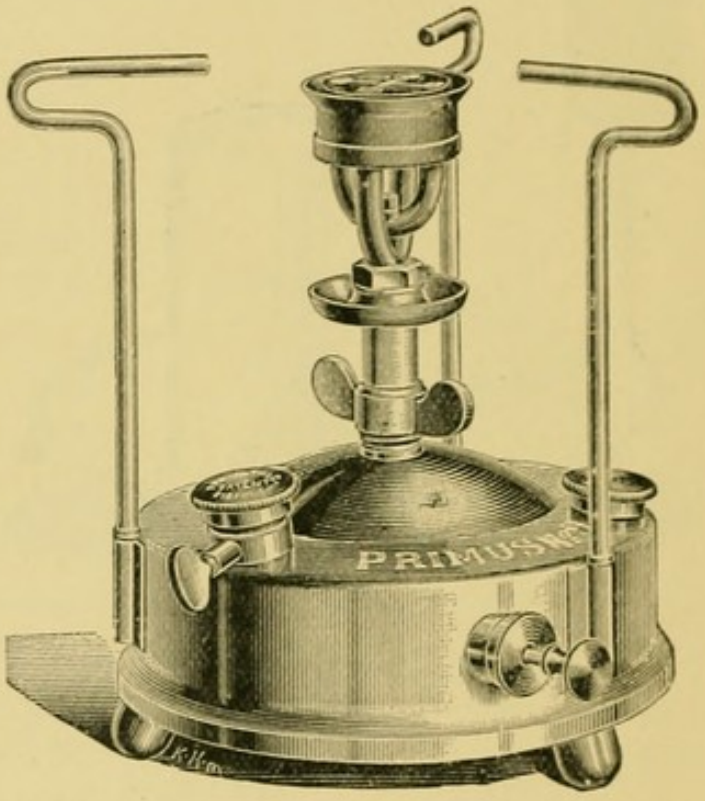

FIG. 2.—"PRIMUS" PARAFFIN LAMP.

An incubator is an enormous advantage and for accurate bacteriological work is essential. The temperature in most tropical places ranges from 75° upwards, and organisms grow better at "room" temperature than in England. In many places the nocturnal and diurnal variations are small, and in such the need for an incubator is not so great. In others there is a great difference between the day and night temperature, and in these the need is great.* A cold incubator is useless unless ice can be obtained.

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* With practice and the exercise of some ingenuity a workable incubator can be made by placing one tin inside a larger one (or a chemist's water-oven may be employed). The space between

Cultures should be kept in a dark cupboard, which must be as dry as possible.

Above the long broad shelf running along the wall two or three rows of narrow shelves should be fitted up on which stains in use can be kept. These are better kept exposed than in cupboards. The main stock can, of course, be kept out of sight.

For night work a good lamp is required. The lamp must be low, and the flame not more than six inches from the table.†

Equipment: A good microscope with a sub-stage condenser, iris diaphragm and mechanical stage is essential. An oil immersion $\frac{1}{12}$-inch objective, a low power, say $\frac{2}{3}$-inch, and a fairly high power, say $\frac{1}{6}$-inch, will be required. For many purposes a $\frac{1}{2}$-inch is a very useful lens. It is well to have two eye-pieces.

The choice of suitable microscopes is a large one, and the differences between those of different makers are not very great, the points of difference being such that it is difficult to say which is the best. In the choice much depends on the conditions under which the work has to be conducted. If much travelling has to be done it is advisable to have a microscope that is easily carried and can be set up for use at a moment's notice. Of these travelling or portable microscopes there are several different forms all fulfilling the main requirements—lightness, compactness, and usefulness. The folding microscopes made by some makers, though compact and easily packed, are heavy and therefore inconvenient to carry.

If most of the work can be done at a fixed station

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the two is filled with water. A small kerosine lamp placed beneath the tins will heat the water, and by varying the height of the lamp a sufficiently equable temperature can be maintained.

† For the best definition the narrow edge of the flame should be used as the source of the illumination and focussed accurately on the object.

one of the ordinary forms of microscope is the most convenient to work with. If the expense is no object it is well to have two stands, one portable and one for

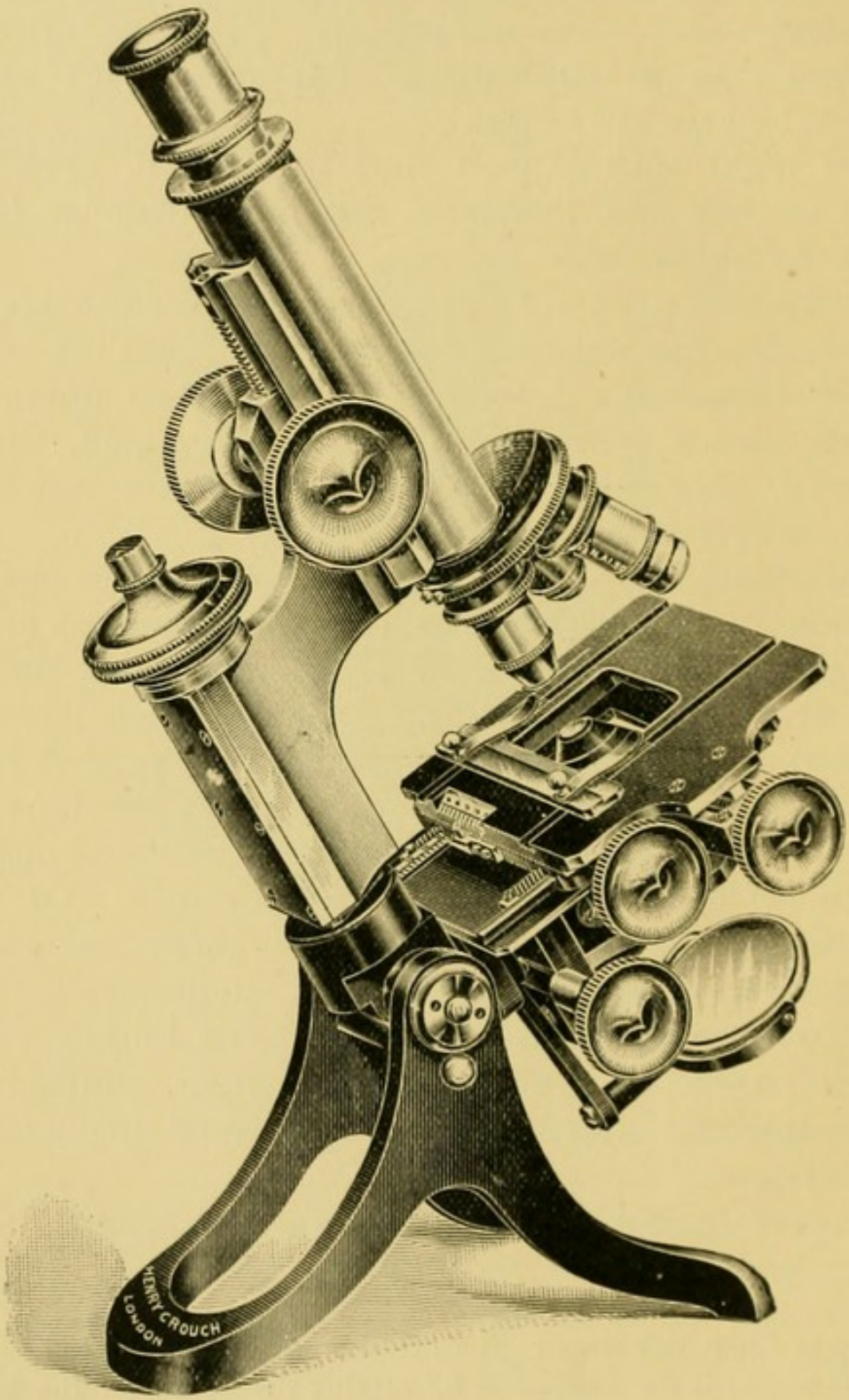

FIG. 3.

stationary work. The objectives and eye-pieces can be used for either, and therefore the additional expense is not very great.

PARTS OF A MICROSCOPE.—The base or stand is a stage fixed either to a tripod or to a vertical column rigidly attached to a solid and heavy footplate. The tripod is to be preferred, as from the wide spread of the legs greater stability is secured, and the level is less affected by irregularities in the table on which the microscope is placed.

In the folding and portable microscope the legs of the tripod are jointed at or near their junction with the stage, and can be folded back so as to economize space in packing.

The stage itself is a fixed plate firmly attached to the upright carrying the optical parts of the instrument, viz., the mirror and sub-stage condenser below the stage, and the tube, eye-piece and objective above.

To this solid plate is fixed the mechanical stage, of which there are two main types :—

(1) Those in which a lighter stage carrying the object to be examined is attached above the fixed stage. This can be moved by a rack and pinion in two directions at right angles to each other.

(2) Those in which the slide is seized by catches and moved over the solid stage.

Some mechanical stages have in addition to the rectangular motions a circular one in the same plane. This motion is not required.

Of the two types, preference should be given to the first, as it can be used for objects of all sizes and shapes, not simply, as with the second, for objects mounted on the regulation slides. With care it does not get out of order any more readily than that of the second type.

The microscope tube is attached to the upright in such a manner that it can be moved up and down parallel to the upright, but allows no lateral movement in any direction. The length of the tube is important, as with the higher objectives the best definition is obtained with a certain known length of tube. This distance varies with the objectives of different makers. To provide for

this variation there is a second or draw-tube inside the outer tube, which can be drawn out so as to lengthen the tube to the required extent. The length of tube required for an objective should be ascertained, and the draw-tube should be, and usually is, marked so that the corresponding length can be obtained.

In the portable and folding microscope the outer tube is so short that it is always necessary to use the draw tube.

The adjustments by which the object is focussed are of two kinds :—

(1) The coarse adjustment, by which the tube is moved by a rack and pinion and brought approximately into focus. The range of the coarse adjustment is great, but the movement is too coarse to focus easily and correctly with higher powers.

(2) The fine adjustment, which may be a differential screw or of the lever pattern. The range of this adjustment is small, but very delicate movement is obtained.

ILLUMINATING APPARATUS.—Good illumination is absolutely necessary for useful work with higher powers. The parts of the microscope providing for this illumination and modifying it are the mirror, the sub-stage condenser and the iris diaphragm, where, as is most usually the case, the object is to be examined by transmitted light. For opaque objects which can only be usefully examined with low powers illumination comes from above the stage.

The Mirror is attached below the condenser. It has two surfaces, one concave and the other plane. The plane mirror is that employed for work with higher powers. Too small a mirror should not be used.

The Sub-stage Condenser.—This is placed between the mirror and the stage, and collects the rays of light received from the mirror into a cone of large aperture, which can be focussed on to the plane of the object.

It must be centred so that the optical axis corresponds with that of the objective, and must be movable so that

it can be moved up or down in this axis. The movement is better performed by a rack and pinion, but in most of the portable microscopes this has to be done by hand.

THE LENSES.—To the tube are fixed at each end the two systems of lenses used for the magnification of the object. The lower system of lenses, which is screwed on to the lower end of the tube, is the objective, and forms a real image of the object, which is further magnified by the system of lenses at the upper end of the tube —the eye-piece.

To save time, annoyance, and wear of screws, a nose-piece is fitted to the lower end of the tube, to which can be screwed the three objectives in use instead of screwing them directly to the lower end of the tube.

These are the essentials of a microscope for the work here contemplated. It can be purchased complete for about £20 from several well-known makers. The price varies a little, but the reader is strongly advised to pay little attention to slight differences of price in the selection of an instrument that suits him. Much more expensive instruments can be purchased, but at about the above-mentioned price an instrument can be obtained suitable for the work contemplated. The portable microscopes with the same objectives are about £3 or £4 less.

No microscope should be bought without spending some time in careful examination and testing of the lenses and adjustments. The points to which special attention should be paid are: (1) The rigidity of the stand. This rigidity must be constant both with the tube vertical and inclined. (2) All the adjustments and screw movements must be tested to see that they work smoothly and evenly, and that every movement of the milled head results in effective movement of the screw and of the part of the instrument which it is intended to move.

With the mechanical stage it is further necessary to satisfy one's self that the movement imparted to the stage is all in one plane, otherwise as the object is moved it will also move out of focus. This can be ascertained by examining an object, such as a uniform blood-film, under various powers, and determining how far the object remains in focus. When using a $\frac{1}{12}$-inch objective, even with the best stages, some focussing will be necessary, but it should be slight, and the object should be very little out of focus with considerable movements of the mechanical stage. A slide and film of uniform thickness must be used for this test, and the result of the examination should be confirmed by using a series of slides.

The nose-piece should centralize the objective correctly, otherwise an object that is in the centre of the field with a low power may not be in the field with a higher power. This is tested by centralizing with the highest power some object that is visible with the lowest power, and seeing how near the centre of the field this object is when viewed with the other objectives. The order should also be reversed if the test appears to be satisfactory.

In testing the objectives, the points to be most closely investigated are :—

(1) Definition. Unless the object is sharply and clearly defined, the magnification is wasted.

(2) Flatness of field. Many lenses give good and sharp definition at the centre of the field, whilst objects a little removed from the centre are blurred, and those at the periphery are out of focus. In using such lenses, if any other part of the field is brought into focus, the objects in the centre of the field will be out of focus. With such a lens the field is not flat. It is perhaps too much to hope that the periphery of the field will be in sharp focus at the same time as the centre, but at any rate for blood work the greater part of the field must be flat, otherwise objects such as malaria parasites may easily be overlooked.

(3) Chromatic aberration must be entirely corrected and no particoloured fringe seen round the edge of the field.

(4) Magnification. As a test object a well-stained, evenly spread blood-film is as good an object as any, and as the object is a familiar one the degree of magnification can be readily estimated. Both eye-pieces should be used in turn.

In the use of the microscope great attention must be paid to the illumination. The light in the Tropics is not good, as it so often has to be derived from blue sky. The mirror should be turned so as to receive the light from a white cloud when possible.

When very sharp definition is required the narrow edge of a flat flame should be used as the source of illumination. In using the low power this should be focussed on the object, and by means of the centring screws of the condenser brought right across the field of vision. A higher power, say $\frac{1}{6}$, should then be used, and again the condenser should be centred so that the image of the flame stretches across the field. Finally, this process is repeated with the oil immersion lens.

The condenser must be raised until the image of the flame is as sharply defined as the object under examination. In this way very sharp definition is obtained, but the greater part of the field is not illuminated.

For the finer work the condenser as well as the objectives must be apochromatic.

In using a low power the condenser should be low so as to be out of focus, or if the stand permits it, swung out so as not to be between the mirror and the object. With a $\frac{1}{6}$-inch objective it should be higher, and with the $\frac{1}{12}$-inch oil immersion objective close to the under-surface of the slide.

The brightest and most uniform light that can be obtained with the iris diaphragm open is the best. If it is desired to reduce the light, it should be done by closing the diaphragm, not by altering the position of the condenser or of the mirror.

Both the mirror and condenser should be kept clean.

It is well to have a spare mirror, as these silvered mirrors sometimes deteriorate rapidly in the Tropics.

In focussing it is well to bring the objective nearer to the object than is necessary, and then, using the coarse adjustment, whilst looking down the microscope to withdraw the objective from the object till it is seen more or less distinctly. For exact focussing the fine adjustment should be used, but not till the object is nearly in focus. The range of the fine adjustment is small, and if used over too extensive a range there is risk of straining it.

When working with the oil immersion lens it is well to place the oil on the object and screw down the tube till the objective touches the oil. In doing this the drop of oil should be viewed from the side, and it will then be easy to see when the objective touches the oil. Afterwards very slowly focus on the object. Before using an oil immersion lens the field should be examined with a low power to make certain that there is something visible in the field. In a fresh blood-film, for instance, if a part be selected in which there are no corpuscles there may be nothing to focus on, and in such a case there is risk of screwing the objective down on the cover-glass.

If black specks are visible in the field it is well to rotate the eye-piece; if these rotate with the eye-piece there are particles of dirt in some part of the eye-piece. Dirt on the objective shows as a general haziness; such haziness may also be due to a cloudy or dirty cover-glass or a badly-prepared specimen.

All glass, and particularly the softer and more highly refractile glass of which lenses are made, is liable in a hot, moist climate to deteriorate and become cloudy or white, resembling very fine ground glass. When lenses become affected in this way they require regrinding. Some lenses spoil more quickly than others, and in purchasing objectives it should be stated that they are required for work

in the Tropics. Various less serious conditions are sometimes mistaken for this change in the glass. The cement may run so that it partly covers the inner aspect of the objective. In other cases water condenses between two lenses and causes a want of definition similar to that due to frosting of the glass. Either of these conditions may be detected by unscrewing the lenses and examining the surface with a watchmaker's glass or hand-lens. These conditions, when discovered, are easily remedied. It is well to use only lenses that can be unscrewed, and from time to time to unscrew and clean the surface of the lenses carefully. They will keep longer if this is done, but must not be expected to last as long as they do in England. Lenses not in use are best kept in a perfectly dry stoppered bottle. There is no objection to having some dehydrating agent, such as well-dried calcium chloride, in a separate compartment in the same bottle.

Lenses after use are best cleaned with a soft rag dipped in alcohol or xylol. If these are not at hand a soft handkerchief moistened with saliva forms an excellent substitute.

A camera lucida or drawing camera is a great convenience, and so useful for measurements that some form of this instrument should be obtained. That of Leitz is a cheap and simple form, the use of which it is easy to learn.

For measurements a micrometer slide ruled to $\frac{1}{100}$ of a millimetre is a useful accessory ; failing it, any of the standard ruled scales, such as the counting chamber of a Thoma-Zeiss' or Gowers' hæmocytometer, can be used as a substitute.

A micrometer scale (fig. 4), to be placed in the eye-piece in focus with the front lens, is useful for some measurements, but can be dispensed with if measurements are made with a camera lucida. A more useful form of eye-piece micrometer is ruled in squares (fig. 6.) Once they are standardized these can be used for blood counts, and the ruled scales used for the counting

chamber of a hæmocytometer, &c., dispensed with. For many purposes it is convenient to subdivide the field,

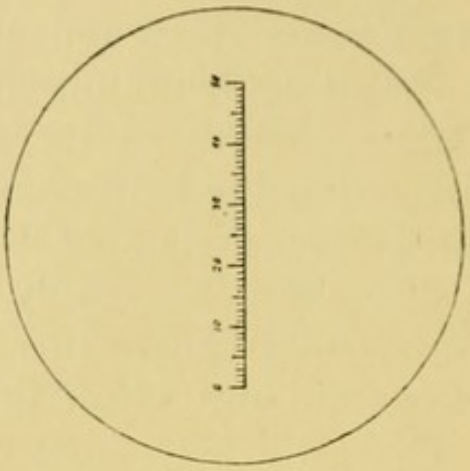

FIG. 4.

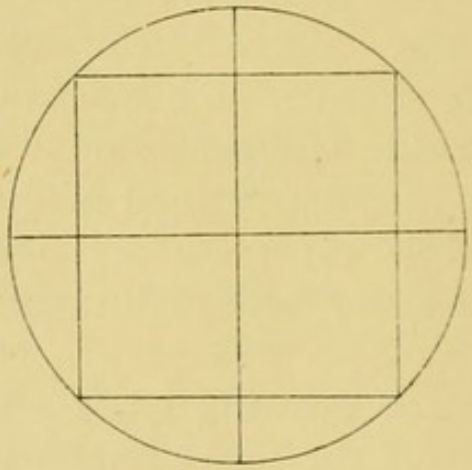

FIG. 5.

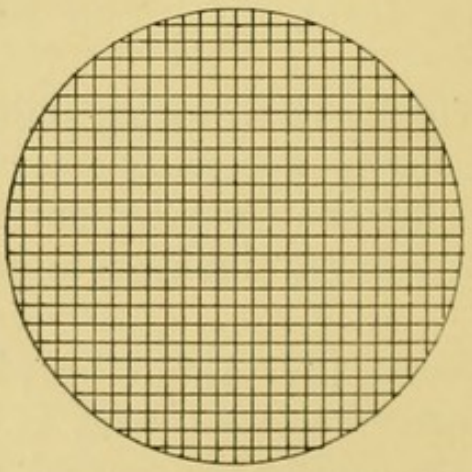

FIG. 6.

and this can be more readily done with a micrometer eye-piece ruled in squares than in any other way.

These eye-piece scales are simply placed in the eye-

piece and rest on the diaphragm between the two lenses. It will usually be necessary to move the diaphragm slightly in order to bring the scale sharply into focus, but this is easily accomplished.

These eye-pieces require standardization for the value of the squares or scale. The micro-millimetre scale is used as the object, and for each objective the number of micro-millimetres in a division of the scale noted. This can be done once for all and the records preserved. There is no object in having the divisions of a scale or the squares of an accurately known size. As seen in the eye-piece they are magnified.

A warm stage is not so much needed in the Tropics as in England, but is a convenience. The simplest form is a copper plate perforated with a hole the size of a shilling. From the plate a copper tongue extends in front for for about six inches. The under-surface of the plate is covered with cloth and is placed on the stage so that the aperture corresponds to the central aperture in the stage. The object is placed on the slide on the copper plate and examined, and by heating the tip of the tongue of copper projecting from the plate by means of a spirit lamp, the heat will be conducted to the plate and the slide kept warm. By heating the tongue nearer to the plate a higher temperature will be obtained, and by lowering the spirit lamp, or moving it further off, a lower temperature. With a little practice there is no difficulty in maintaining a fairly uniform temperature which can be estimated by touch. More elaborate warm stages are to be procured in which the temperature is kept steady by the circulation of hot water.

A dissecting microscope is useful but not essential; it consists of a single compound lens fixed on a vertical carrier which can be raised or lowered by a rack and pinion; the stage is of glass and there are wooden movable hand-rests at each side. For illumination there is a plane reflector, and as an alternative on the other side of the mirror a plaster of Paris disc.

For most of the purposes for which the dissecting microscope is used a watchmaker's glass does equally well, and for some purposes it is better, as both hands are free, and no stage is required.

A good large hand-lens on a handle is useful for observing the habits of mosquito larvæ.

Reagents, stains, slides and cover-glasses are required. The slides should not be of the best quality; the thin, white slides deteriorate in the Tropics more rapidly than the coarser glasses. No. 2 quality is to be preferred. They require thorough cleaning, and a stock cleaned and ready for use, requiring only to be wiped, should be kept in hand. They are best cleaned by placing in a saturated solution of carbonate of soda which is just brought to the boil. Afterwards they are well washed in running water, wiped with a soft linen rag, and kept in spirit in a stoppered or well-corked, wide-mouthed bottle. Before use the slide must be taken out of the spirit and well rubbed with a soft, clean linen rag.

Cover-glasses are best sent out in oil or covered with oil, as even in the course of a voyage lasting only two weeks they may become frosted. The whole mass of cover-glasses, say half an ounce, is placed in oil of cloves, and the cover-glasses are separated so that the oil penetrates between them. They are then taken out of the oil, wrapped in cotton-wool and can be replaced in their boxes. Treated in this way they will keep for months even in the worst climates. They also keep well if sent out in spirit, but this is not recommended, as if the spirit evaporates completely the glasses deteriorate very quickly.

Cover-glasses treated with oil are not very easy to clean, as the oil will have hardened and dried to a large extent. A good deal of the oil can be removed by placing the cover-glasses in 1 per cent. lysol solution or in xylol, and separating them or stirring them up. This saves the spirit which is necessary to more completely remove the oil. They must not be left more than an

hour in the lysol, and then should be placed and kept in spirit, which will gradually remove nearly all the oil.

A small stock should be further prepared so as to be ready for immediate use. This may be done by first just bringing them up to the boiling point in a saturated solution of carbonate of soda, washing well, preferably in running water, and transferring them to strong 50 per cent. sulphuric acid; in this they should be left over night, then again well washed in water and finally transferred to a wide-necked, well-stoppered bottle half filled with spirit. For use they should be taken out with forceps and well rubbed with a soft linen rag.

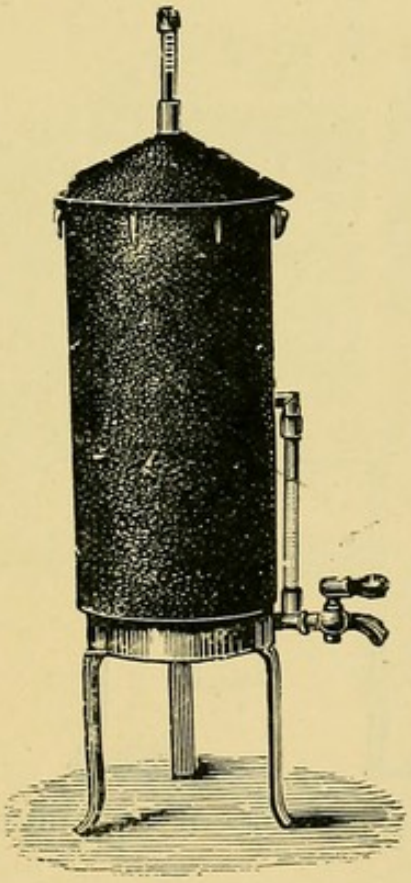

FIG. 7.

Such cover-glasses should be free from both grease and grit, and are then fit to use for making fresh fluid blood-films or for other preparations. As alternatives to the treatment with carbonate of soda and sulphuric acid, some prefer strong nitric acid and others bichromate of potash (2 parts), sulphuric acid (3 parts) and water (25

parts); others, again, sulphuric acid alone. In any of these solutions the cover-glass can be kept indefinitely and washed in water immediately before use.

Cover-glasses should be of the best quality, and for blood work the thinnest (No. 1) should be used. A smaller stock of thicker cover-glasses should be kept for the examination of fæces and making "squash" preparations. These thicker cover-glasses do not deteriorate so rapidly.

For bacteriological work some form of steam sterilizer such as Koch's (fig. 7) is necessary to sterilize vessels, media, &c. With this all requisite sterilization for ordinary

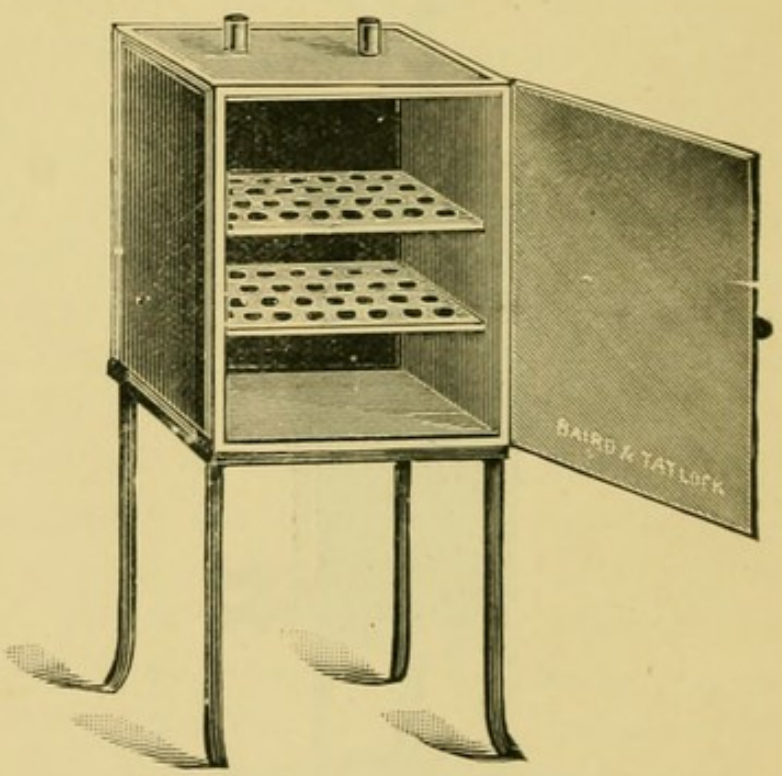

FIG. 8.—HOT-AIR STERILIZER.

work can be done, but a hot-air sterilizer is an advantage for the quicker and easier sterilization of vessels, Petri dishes and some instruments.

A steam sterilizer (fig. 7) is simply a tall metal vessel covered with a lid with a vent for the escape of steam, and containing water at the bottom. As it is not well to immerse the objects to be sterilized in the water, there is a perforated false bottom above the level of the water

on which such objects rest. The whole vessel to prevent loss of heat is covered with some non-conducting material. There is no great difficulty in improvising such a sterilizer, but those sold are more sightly and convenient.

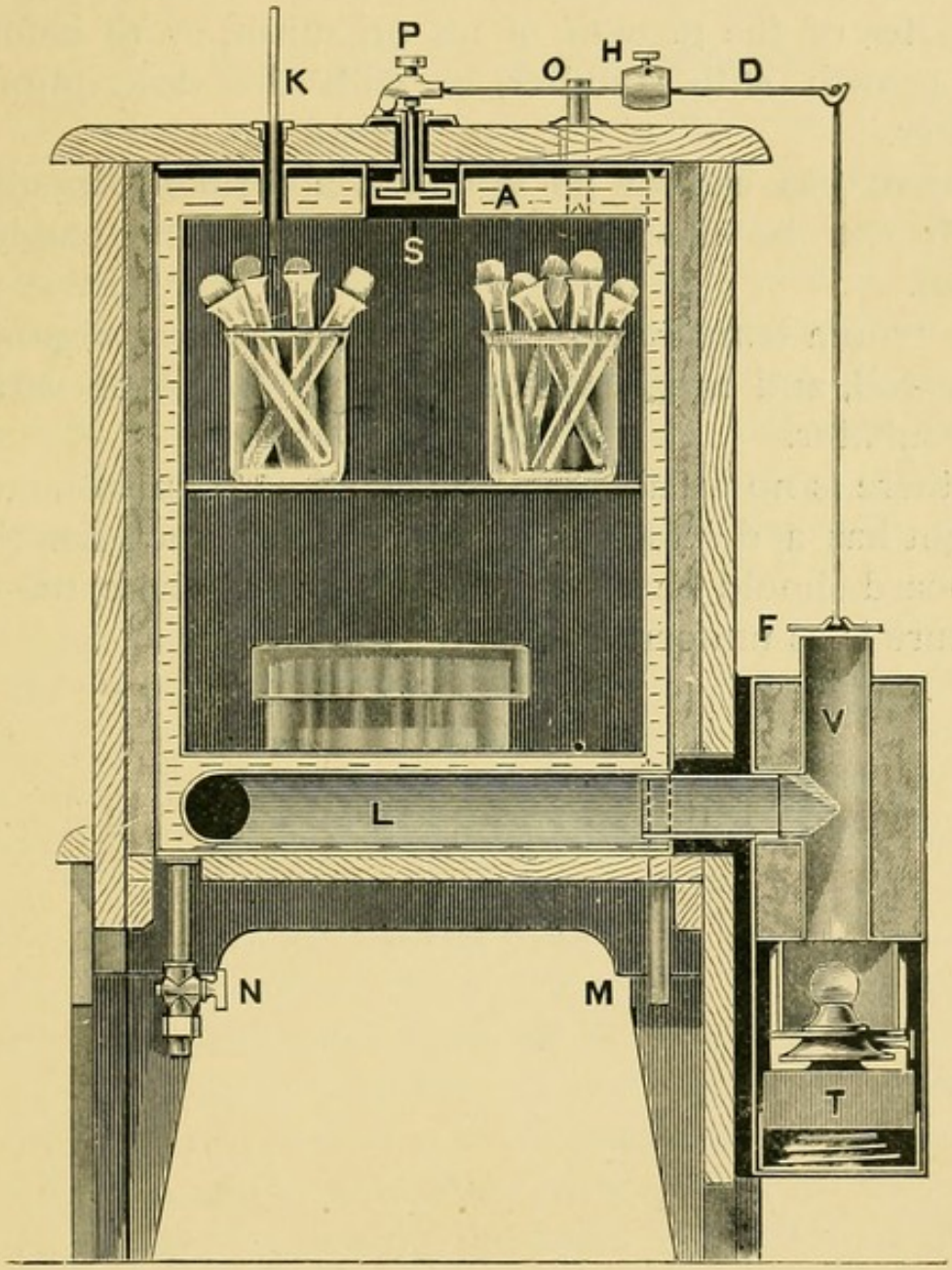

FIG. 9.—HEARSON'S INCUBATOR, WORKING WITH PETROLEUM LAMP.

They can be heated by a paraffin lamp, and the "Primus" is one of the best. At a pinch a wood or other fire may be used.

The hot-air sterilizer (fig. 8) is a metal case enclosed in a second larger one, the two being separated by an

air-space. The double case can be dispensed with, but the heating is then less uniform. A temperature of about 160° C. is required.

Incubators are needed where it is important that growth should take place at a uniform temperature, and are essential when it is desired to describe accurately the character of the growth of an organism, or to compare one growth with another, or with the description of another.

Where gas cannot be obtained a form of incubator which can be used with kerosine must be employed (fig. 9).

At "room temperature" in the Tropics most organisms grow well, and much useful work can be done without an incubator.

If there is no incubator a dark cupboard must be used, as light has a deleterious effect on most bacteria. This cupboard should be fixed in a dry place, where the temperature is as uniform as possible.

CHAPTER II.

Post-mortem Examinations.

Post-mortem examinations in the Tropics present certain differences from these examinations in temperate climates. *Post-mortem* changes are more rapid, so that it is essential that the examination should be made as soon as possible after death. This is not only on account of the rapidity with which putrefactive changes occur, but also because many of the animal parasites die, and some, such as the sporozoa, disintegrate and cease to stain well even before putrefactive changes set in.

A large proportion of tropical diseases are those affecting the abdominal viscera, and to study the exact relationship of the parts it is often advisable to remove the thoracic and abdominal viscera *en masse*. The abdomen should be freely opened and room gained by subcutaneous division of the muscles attached to the pubes below, and sternum and ribs above.

The attachments of the diaphragm to the sternum and costal margins must next be divided with the knife close to the chest wall, and the parietal peritoneum stripped off the abdominal wall with the hand as far as possible. The trachea and vessels going to the neck are then to be freely divided by passing the hand and knife in front of the lungs and cutting transversely above the root of the lungs, whilst with the hand the thoracic viscera are grasped firmly at the root of the lungs and steady traction exercised. When the division is complete, the lungs and heart will be easily pulled downwards through the lower opening of the thorax. With the knife the posterior attachments of the diaphragm are divided from above,

and steady traction, aided by a few touches with the knife, will strip the peritoneum off the remainder of the wall of the abdomen, and all the abdominal viscera with the aorta and kidneys will be completely separated except at their pelvic attachments. These can be divided, or better, the peritoneum stripped off the pelvis at each side, and the urethra and rectum divided as near the perineum as possible.

The mass of organs can be now examined from every aspect and the relations of the different parts readily observed.

By this method the root of the mesentery, the posterior mediastinum, and other parts which are usually overlooked can be displayed, and in these regions parasites are sometimes found.

Certain special observations are worthy of attention :—

(1) The weights and relative weights of the organs vary considerably from European standards, both in health and disease. With the lungs, in recording the weight, it is essential to note also the time that has elapsed between death and the examination, as the weights of these organs increase a few hours after death, probably by aspiration of fluid. If the examination is made two or three hours after death the lungs will be barely half the weight taken as the standard in Europe, whereas if the examination is made later the weights may be much the same.*

(2) Variation in weight of the organs with age differs in different races, and the curves obtained for the organs are in many cases different from those recorded in Europe. For example, the brain weight in Europe attains its maximum between 45 and 50, whilst in the negro the maximum is reached between 20 and 25.*

(3) Abnormalities are common, and some of them occur with unusual frequency in certain races, such

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Vide tables in Appendix.

as Meckel's diverticulum in the Chinese, and deeply-fissured lungs in the negro races. Diseases also affect organs differently according to race, and of this the age incidence of splenic enlargement in negro and other races living under the same conditions is a striking example. While in childhood all are equally affected by malarial disease, in adult life the spleen in the negro tends to subside, but in other races, Indian, Chinese and aboriginals, it remains increased in size, and is commonly found *post mortem* to be three or four times the weight of the normal organs in Europeans.*

(4) Abnormal appearances, such as congestion, ecchymosis, &c., are more common in the Tropics, and are observed under different conditions. On the one hand, as the examinations are made much earlier the appearances resemble more closely those in the living subject; and on the other hand, as putrefactive changes occur so easily, particularly in the vicinity of the intestine, patchy, irregular *post-mortem* staining is common, and is frequently mistaken for disease.

(5) Certain special putrefactive changes may be a source of error. As a result of putrefaction some of the organs, and particularly the spleen, often appear of a slaty colour, which may be mistaken for malarial pigmentation. Section of the organ will show that the discoloration of early putrefaction only extends for a short distance into its substance. The substance of the spleen in section sometimes appears very dark, but this colour can be distinguished from that of acute malaria by noticing that the dark colour changes to bright red after exposure to air. The only satisfactory test of malarial pigmentation of an organ is by examination of a portion of the tissue with the microscope. It is

* *Vide* tables in Appendix.

not necessary to cut sections; a small portion of the organ can be forcibly compressed between two slides and examined at once for pigment.

A diffluent spleen is often described, but is not met with in *post-mortem* examinations made sufficiently early after death. The spleen, even in the most acute cases of malaria, though enlarged and black, is firm, and wedges with acute angles can be cut. These angles retain their sharpness even when exposed to a jet of water. Such a spleen is easily forcibly compressed, and if allowed to decompose speedily becomes "diffluent."

Organs, shortly after death, are firm and hard from *rigor mortis* of the tissue elements. When this passes off the organs become flaccid and softer.

Many of the putrefactive organisms form gas, and consequently emphysematous changes are produced; such emphysema of the liver and other organs is common. In the intestines small emphysematous patches form in the submucosa, and present a peculiar and rather deceptive appearance.

Gaseous distension of the whole intestine is very common, and the stretched walls appear unusually thin and are often described as atrophied.

EXAMINATION FOR ENTOZOA.—In the examination of intestines in the Tropics it is not advisable to wash out the intestine before opening it. The intestine should be opened and examined for entozoa first by passing the intestine slowly between the thumb and first finger so as to remove the intestinal contents, and subsequently washed to see the condition of the mucosa. If the intestines are washed out first, the entozoa will be carried away, and may escape notice. The washings should be collected and the deposit examined separately.

Worms and intestinal parasites die as a rule within some six to twelve hours after the death of the host, and some, such as the ankylostomes, lose their hold on the intestinal walls even earlier.

MUSEUM PREPARATIONS.—In the older methods for

the fixation and hardening of macroscopical specimens, alcohol, and, later, formalin were employed. These methods preserved the form and relations of organs well, but were practically valueless for preserving the natural colours.

Modern methods in use result in the preservation of the natural colours of the specimens and harden them at the same time. The basis of the methods is the use of a first bath of formalin, which has the power of changing oxyhæmoglobin into acid hæmatin; of a second bath of spirit, which converts the acid hæmatin into alkali hæmatin, which in colour is very similar to oxyhæmoglobin, so that the tissues regain their natural colours; finally the specimens are preserved in a solution containing potassium acetate, glycerine and water.

Kaiserling's original method, which gives as uniformly satisfactory results as any of its modifications, is as follows :—

(1) The organs are fixed by keeping in the formol mixture until they are just hardened—thirty-six to forty-eight hours or even longer. The best results are obtained by fixing in the dark.

Formalin...	200 c.c.
Water	1,000 c.c.
Nitrate of potassium ...	15 grm.
Acetate of potassium ...	30 grm.

As a substitute 10 per cent. formalin may be used. Weak formalin must not be used, as this dissolves out the hæmoglobin.

A section of a solid organ such as the spleen or liver can be safely placed in a vessel containing this fluid, but in dealing with softer tissues it is advisable to wrap the specimen loosely in cotton-wool before immersion, and to give it plenty of room. In dealing with an entire organ it is best to inject it with the fluid, as the organ then retains its shape better, and the hæmoglobin in its interior is more completely

converted, and does not afterwards leak out and colour the mounting medium.

(2) After fixation the specimen is placed in the second bath, which consists of spirit. It was originally recommended to employ increasing strengths of spirit, but equally good results are obtained by placing the specimen at once in 90 per cent. spirit. The length of time necessary for this bath must be determined by the appearance of the specimen; it should be removed when the original colour fully returns, which is usually in twenty-four to thirty-six hours.

(3) Mount in jars containing :—

Glycerine	400 c.c.
Acetate of potassium...	200 grm.
Water	2,000 c.c.

A few crystals of thymol or a trace of formalin may be added to prevent the growth of moulds.

Preparation of Tissues for Microscopic Examination.

Parts of an organ or tissues kept for microscopical examination may be examined fresh or preserved and hardened.

From the examination of the fresh specimen much information may be gained. Smears of the fluids that exude from the cut surface may be made, small portions of the tissue may be squashed between a slide and cover-glass, or the material may be frozen and sections made. In the last case treatment in a strong solution of gum arabic is desirable. The details of the methods useful for the determination of parasites is considered with the description of these parasites. It is from the fresh specimens that cultures must be made. For this purpose the spleen and lymphatic glands are the most suitable places. Blood should be withdrawn by a sterile syringe from the unopened heart after first searing the

surface with a hot iron for culture-making and for inoculation of animals.

Fixation and Hardening.—Although formalin is commonly recommended as a fixing fluid when ordinary tissue changes are to be demonstrated, it will be found in tropical practice that alcohol is the most generally useful reagent, as protozoal and bacterial organisms stain better after fixation by this method. It has the disadvantage of causing great shrinking of the tissues from rapid dehydration.

To fix, the tissue should be first placed in 80 per cent. alcohol and in two to four hours transferred to 95 per cent. alcohol. The tissue must be in small cubes not more than half an inch in their greatest length, and placed in at least ten times their volume of alcohol in a closed glass vessel.

If a piece of tissue is simply put into a bottle and spirit poured on it, the blood coagulates at the edges where it is in contact with the glass, and the fluid does not penetrate between the glass and tissue. This is avoided by placing some cotton-wool or small pieces of crumpled paper at the bottom of the bottle before introducing the piece of tissue.

In certain cases, especially where it is desirable that the blood should be retained in the vessels, a larger piece of tissue can be taken, and after partial fixation subdivided into pieces of the right size. This is particularly to be recommended when the object is the examination of the tissue for malaria parasites or filaria *in situ*. At the end of six hours the alcohol should be changed, and again changed in twelve hours. By this time in a warm climate the specimen will be sufficiently fixed, and longer immersion in strong alcohol will render the specimen too brittle. In colder weather, where the average temperature is under 70° F., it can be left for some hours longer in the alcohol.

The specimens when fixed can be kept in methylated spirit till required. If greater accuracy is required the

specimens can be kept in 60 per cent. absolute alcohol. This will keep the specimens, and stronger alcohol at tropical temperatures soon overhardens them.

FORMOL ALCOHOL.—For the more rapid fixation of tissues where examination for malaria parasites is not required, alcohol and formalin give excellent results. This solution is made by the addition of formalin in the proportion of 2 to 10 per cent. to the absolute alcohol. It penetrates rapidly and causes less shrinking than alcohol alone, but the tissues should not be left in this solution for more than twelve hours or they will be overhardened. They are then fit for further processes or can be kept in spirit.

MÜLLER'S FLUID.—Pot. bichromate 2·5 parts, sodium sulphate 1 part, and water 100 parts is very extensively used and gives good results, but is slow in its action. The pieces of tissues are placed in an abundance of the fluid, which should be changed in a few hours, and again daily for a week, after that once a week will be sufficient. Some tissues will be sufficiently fixed in two or three weeks, but others, as the parts of the central nervous system, may, even in a warm tropical climate, require many weeks. When fixation is complete the specimens should be washed for twenty-four hours in abundance of water, which is frequently changed, preferably in running water, and then kept in methylated spirit.

Animal parasites do not stain well in tissues which have been fixed in bichromate solutions.

ORTH'S FLUID.—Müller-formol is made by adding 10 per cent. of formalin to Müller's fluid. This must be added immediately before use. It is a rapid fixative, and at blood heat only three or four hours are required for thin pieces of tissue. Two days are usually sufficient at room temperature.

When hardened the tissues may be cut directly on the freezing microtome, or after thorough washing passed through increasing strengths of alcohol and then imbedded.

ZENKER'S FLUID.—For the examination of skin, which is readily overhardened, Zenker's fluid gives good results. This is composed of 5 parts of corrosive sublimate, 2·5 parts of potassium bichromate, 1 part of sodium sulphate, 5 parts glacial acetic acid, and 100 parts of water. The slices of tissue to be examined must be very thin, not more than a tenth of an inch in thickness. The time required for fixation is twelve to twenty-four hours, according to the thickness of the specimen and the temperature.

After the tissues are fixed they must be thoroughly washed in water, which is frequently changed, for at least twelve hours, and should then be placed in spirit to which a little tincture of iodine, or a few drops of Gram's iodine solution, has been added, to remove any mercury deposited in the tissues. If the colour of iodine disappears from the fluid more iodine is to be added until the colour no longer disappears. The specimen can then be kept in spirit till required for use. Or the specimens can be kept in spirit and the cleaning with iodine done after the sections are cut.

OSMIC ACID MIXTURES.—Two other useful fixative agents are Flemming's solution :—

Chromic acid 1 per cent., aqueous solution 15 c.c.
Osmic acid 2 per cent., aqueous solution... 4 c.c.
Glacial acetic acid ... ... 1 c.c.

and Hermann's solution, in which 1 per cent. solution of platinum chloride is substituted for the 1 per cent. solution of chromic acid in Flemming's solution.

These solutions must be freshly made up before use, and as the penetrating power of the fixative is low, the specimens must be very thin, not more than one-twelfth of an inch in thickness.

Fixation takes from one to two days, and the specimens require to be thoroughly washed, preferably in running water, for one day, and then placed in 80 per cent. alcohol.

BOILING METHOD.—For the rapid fixation of tissues

they may be placed in water that has been heated just to the boiling point. If small pieces of tissue are used a few minutes will suffice to coagulate the albumins. This method is also useful for the examination of renal casts and for the contents of cysts.

Imbedding. — The tissues having now been fixed, hardened, and completely dehydrated, are assumed to be in absolute alcohol. Sections of hardened tissues can be cut with a razor by hand, or with a microtome knife after fastening the specimen in the microtome clamp. Fair sections of firm tissues can sometimes be obtained in this way or by means of the freezing microtome. With these methods, however, portions of the tissue are likely to fall out of the sections, and it is desirable to have the tissues imbedded in some material with which they become permeated and which preserves the component parts in their relative positions and surrounds them with a protective coating. They can then be cut into thin sections on a microtome.

The two substances in common use for imbedding purposes are paraffin and celloidin. Paraffin imbedding is the most useful if very thin sections are desired. For certain purposes celloidin is indispensable, as when it is desired to keep any loose bodies *in situ* in a tissue, there being no necessity to remove the celloidin before mounting in Canada balsam.

Hard tissues, and tissues which easily become brittle, such as muscle and skin, are cut with considerable difficulty by the paraffin method.

(1) PARAFFIN IMBEDDING.—The general principle is to pass the specimen through alcohol till it is thoroughly dehydrated, then to place it in a fluid in which paraffin is soluble, which will dissolve out the alcohol, and then to replace this fluid by first a weak solution of paraffin, then a strong solution of paraffin, and finally melted paraffin wax. Excess of paraffin is poured round the tissue, and it is allowed to cool ; when the paraffin solidifies not only is the piece of tissue enclosed in a solid block of

paraffin wax but the tissues will be permeated with the wax.

There are many modifications, some of which are rendered necessary for special tissues.

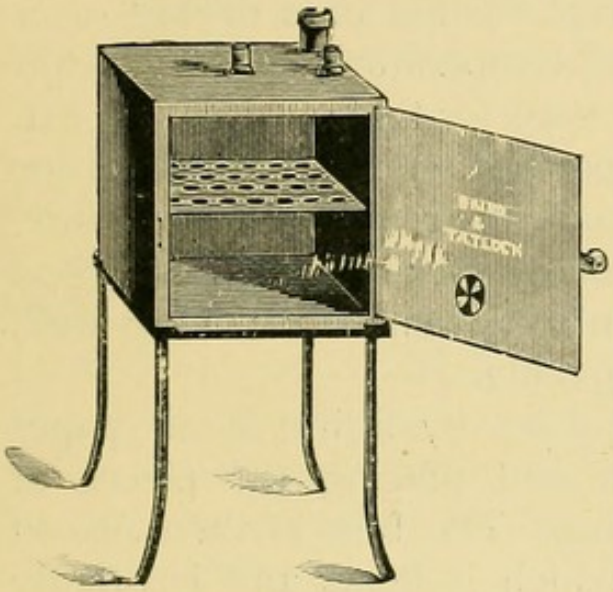

FIG. 10.

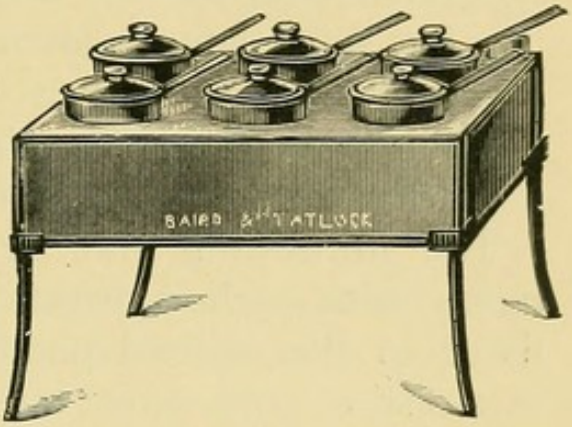

FIG. 11.

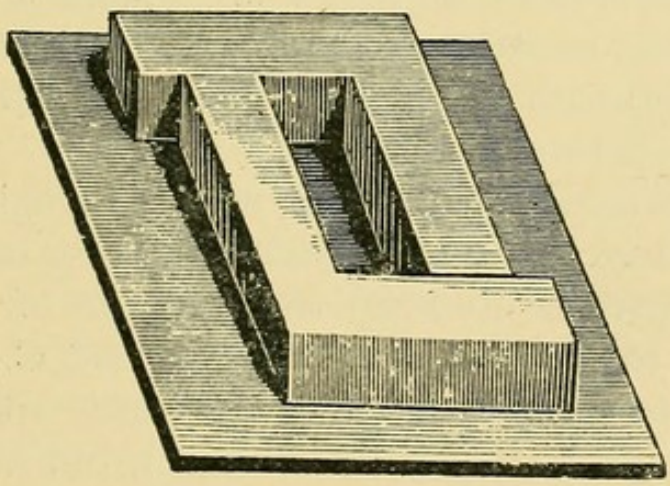

FIG. 12.

For general work with specimens taken from strong spirit:—

(1) Place the specimen in absolute alcohol for twenty-four hours. If the specimen has been removed from weaker spirit or from water, before placing in the absolute alcohol it should be placed in methylated spirit for forty-eight hours.

(2) Remove from spirit, drain off excess of spirit

for a few minutes and place in aniline oil. One day.

(3) Place in xylol. One day.

(4) Place in paraffin and xylol, equal parts. One day.

(5) Place in melted paraffin wax for one day. The paraffin wax can be kept melted in a drying oven (fig. 10) at the required temperature, or a paraffin embedding bath can be used for this purpose (fig. 11). As a considerable amount of spirit is required for the spirit lamp to maintain the required temperature, it is well to imbed as many specimens as possible at the same time. The imbedded specimens keep well.

(6) Imbed and cool quickly.

The imbedding may be done by filling small paper boxes with melted paraffin and placing the pieces of tissue in this melted paraffin. The box is then placed in a dish of cold water on which it floats and is rapidly cooled, so that the paraffin sets without crystallizing. Or L-shaped pieces of metal are placed in contact on a smooth slab, as in the diagram (fig. 12), and the space between filled with the melted paraffin and the specimens placed in as before.

Modifications.—The paraffin used in England melts at too low a temperature for satisfactory work in the Tropics. It is well therefore to keep two varieties of paraffin, one melting at 48° C. and the other at 60° C., and to use a mixture of them. Such a mixture with a melting point about 54° C. is usually sufficient, but in the warmest weather either a larger admixture of the paraffin at the higher melting point will be required, or the pure paraffin melting at 60° C.

RAPID PARAFFIN IMBEDDING METHOD.—For the rapid examination of small objects the process of imbedding may be shortened by the use of acetone, which hardens and dehydrates the tissue and at the same time prepares it for immersion in paraffin.

(1) Fix small pieces of tissue in 10 per cent. formalin for one-half to four hours.

(2) Place in pure acetone for one-half to one and a half hours.

(3) Transfer directly to fluid paraffin (52° to 56° C.), and place in the oven for one-half to one and a half hours. The acetone evaporates and the paraffin permeates the tissues.

(4) Prepare the paraffin block. Cut and stain as usual.

The acetone may be used again by placing fired copper sulphate at the bottom of the vessel.

(2) CELLOIDIN IMBEDDING. — Commercial celloidin (Schering) is a purified gun-cotton. It is sold in granules, in shavings, or in flat slabs. The latter require to be cut into small squares before using.

To imbed in celloidin, the general principle is the same as that for paraffin, but the agents employed and the methods differ.

(1) The specimen is kept in absolute alcohol, after being in weaker spirit, for twenty-four hours.

(2) It is then soaked in a mixture of equal parts of ether and absolute alcohol for twenty-four hours.

(3) Place in a weak solution of celloidin (3 per cent.) in alcohol and ether for twenty-four hours or more; two days is usually ample.

(4) It is then to be transferred to a thicker celloidin solution, 6 per cent. celloidin dissolved in alcohol and ether, and kept in this for at least one day, and better for several days.

(5) The specimen is then placed on a small block of wood, on which a few drops of the thick celloidin have been placed. Leave exposed to the air for a few minutes, and pour a little thick celloidin solution over the specimen. Expose to air for a few minutes until a whitish film appears on the surface, and place in 60 per cent. alcohol, which will harden the celloidin. In cutting celloidin specimens, the knife must be oblique, and must be kept constantly moistened with spirit.

Section-Cutting. — For this purpose a microtome is required, and the greater number of those available may be used for either celloidin or paraffin. For cutting frozen sections a special instrument is necessary.

Cathcart's microtome (fig. 13) is the simplest efficient freezing microtome, but in the Tropics its use is necessarily limited, as freezing with ether is not practicable in most tropical countries.

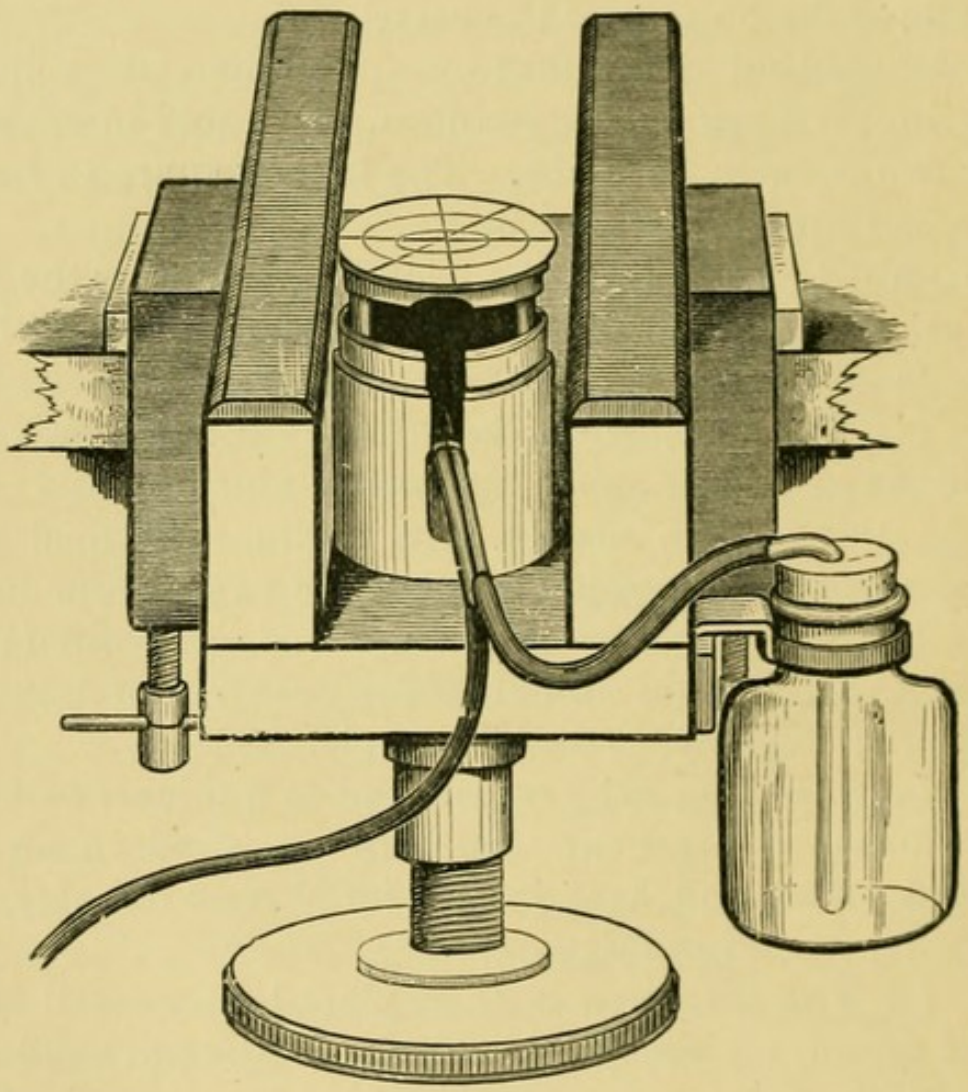

FIG. 13.—MICROTOME, CATHCART'S, WITH SPRAY BELLOWS.

Imbedded sections can be cut with this microtome, but must not be frozen. The carrier is heated and the paraffin block pressed against it. The paraffin will be melted, and will then adhere to the zinc plate; but better sections can be obtained with other microtomes.

For freezing in the Tropics a mixture such as ice and salt is the best.

Swift's microtome consists of a circular wooden box (figs. 14, 15) (A) from the centre of which rises a metal tube surmounted by a horizontal zinc plate raised above the level of the top of the box. The box is covered with a glass plate perforated in the centre with a hole large enough to allow the tube and zinc plate to pass

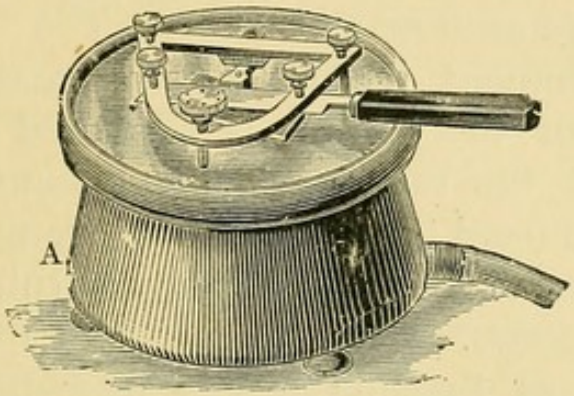

FIG. 14.

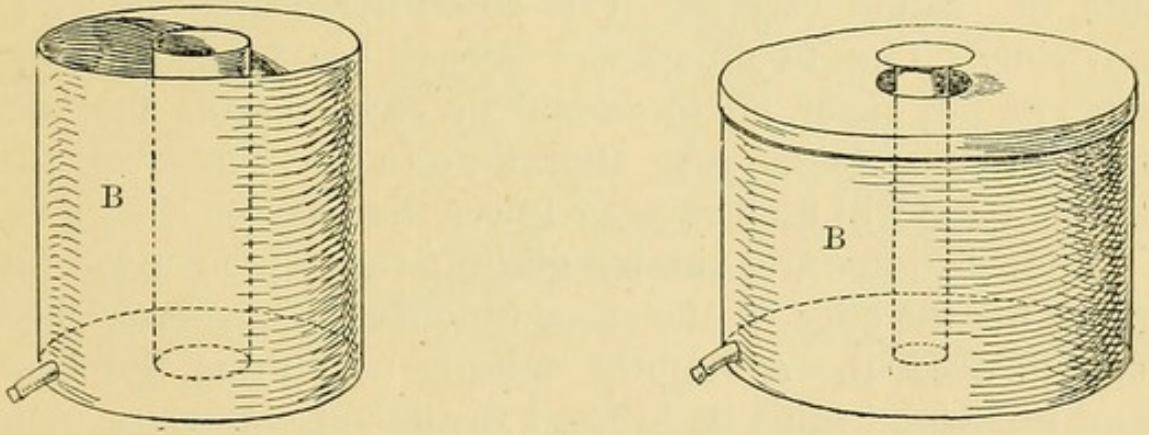

FIG. 15.

through. When arranged for use, the box is filled with a mixture of well-crushed ice and salt. The lid is placed on and the zinc plate projects above its level. The substance to be frozen is placed on the zinc plate and well-covered with a strong solution of gum. If the air temperature is not too high, the specimen with the surrounding gum freezes.

In many tropical countries this does not suffice unless the specimen is also surrounded by a cold atmosphere.

This is done by placing a second metal box (fig. 15, B) on the top of the glass plate. This metal box has a central tube rising from the bottom and open below, and this tube must be wide enough to enclose the zinc plate and specimen. If the metal box is also filled with the freezing mixture, the air in the central tube will be cold, and the specimen surrounded by this cold air freezes readily.

When the specimen is frozen, the upper metal box can be removed, and sections cut.

In this instrument the specimen remains fixed, and the thickness of the section is regulated by alterations in the level of the razor. This is arranged by having the razor blade fixed on a tripod; the length of the legs of this tripod can be regulated by turning the milled heads of the screws. The feet of the tripod are tipped with bone so as to slide evenly over the glass. For use the blade of the razor must be wetted with water, and the tripod carrying it is so arranged that one leg is anterior. The two posterior screws are turned till the edge of the razor is horizontal or parallel with the surface of the glass. Any alteration in the screw of the anterior leg will then raise or lower the edge of the blade.

The sections are cut by gliding the tripod over the plate till the edge of the razor touches the specimen, and cuts through it. A slightly oblique motion is the best, and the tripod must be pressed firmly on the glass plate and the movements must be rapid, more like a thrust. The knack of making the correct movement is soon acquired.

When a section is cut, the tripod is drawn back, slightly tilting it to avoid touching the specimen, the anterior screw turned to an amount regulated by the thickness of the desired section, and again thrust forward. This process should be repeated until there are several sections on the upper surface of the blade of the razor, and these can be removed with a camel's hair brush to a vessel containing water which has been recently boiled, so as to be

free from air. The sections will float, and can be floated on to a slide, and either examined directly or stained.

Sections of fresh, unfixed tissues can be cut and examined unstained, or, to show structure better, they can be stained. They should be soaked in gum before freezing.

Well-fixed specimens can also be frozen and cut. It is necessary before freezing to thoroughly remove the last traces of alcohol by washing in water, and to soak in an aqueous solution of gum arabic for some hours. Where ice cannot be obtained further hardening and imbedding is necessary.

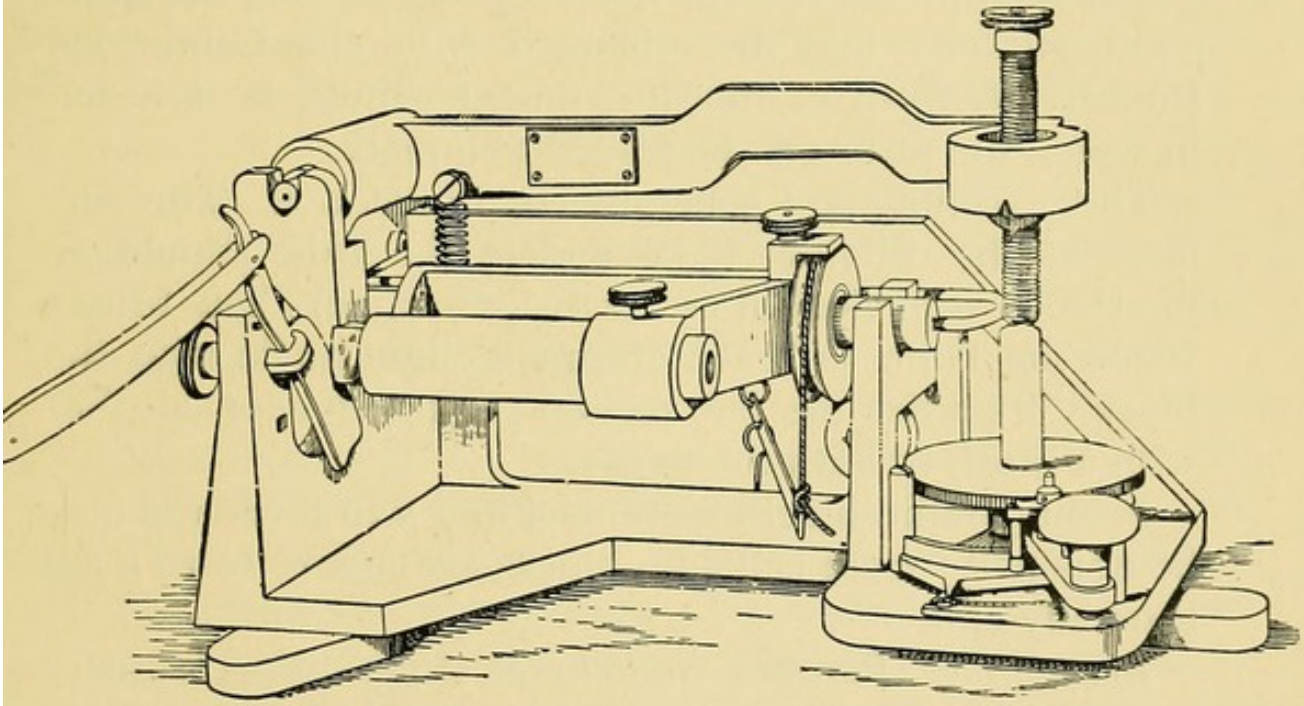

FIG. 16.—CAMBRIDGE ROCKING MICROTOME, NEW PATTERN FOR CUTTING FLAT SECTIONS, WITH LARGE ARTICULATING APPARATUS AND ONE RAZOR.

PARAFFIN SECTIONS.—Of the simpler and cheaper forms of microtome suitable for cutting paraffin sections, the Cambridge Rocker is the most convenient.

Full directions for the use of this instrument are sent with the microtome, but the chief points to observe are :—

(1) That the razor must be rigidly clamped.

(2) That the paraffin block must be firmly fixed on the metal carrier. This is done by heating the

carrier and applying the paraffin block firmly to it, and keeping it in position till the carrier is cold.

(3) That the thickness of section must be graduated in accordance with the nature of the tissue, its brittleness, and object of the section. If the specimen is too hard or brittle it is useless to expect thin sections.

Sections showing large parasites, such as filaria, in tissues like the lung, should not be too thin. For the details of nerve structure, and for sections showing bacilli, or the parasites of malaria, the thinnest possible sections are the best.

CELLOIDIN SECTIONS.—For cutting celloidin sections a slide microtome, such as that of Jung, is best, but quite good sections may be obtained with the Cambridge Rocker. A form of this instrument should be selected in which the razor can be placed obliquely.

These sections are generally cut in alcohol. The surfaces of the celloidin block and of the knife should be moistened with alcohol by means of a camel's hair brush. Place the knife so that it forms a slight angle with the block. Transfer the cut section to 70 per cent. alcohol with a brush or with the finger.

From paraffin blocks sections 5 to 7 μ in thickness may be obtained; from celloidin blocks sections 10 to 15 μ are generally cut.

Fixation of Paraffin Sections on Slide.—For ordinary work the sections, when cut, are placed on the surface of some warm water at about 44° C., or rather more if paraffin of a higher melting point is used, in order to straighten them out, and can then be floated on to a slide. The water is allowed to drain off, and the slide is then placed in the hot incubator for twelve to fifteen hours. The section will then be fixed to the slide.

Occasionally it will be found that the sections after removal of the paraffin fall off. In such a case the other sections may be very gently warmed over a flame till the paraffin *begins* to appear more translucent and then allowed to cool.

Another method is to employ a mixture of albumin and glycerine to fix the section. This mixture is prepared by beating up the white of an egg, filtering, and adding to it one third the quantity of glycerine. A crystal of carbolic acid or thymol may be added to prevent the growth of moulds. A thin film of the mixture is placed on a slide, and on to this the paraffin section previously straightened out on the surface of warm water is floated. As before, the slides are placed in the hot incubator after draining off the superfluous water.

Paraffin sections can be rapidly fixed by floating them out on a drop of water placed on the slide. The slide is warmed till the section flattens out, and the water is then drained off and the section firmly pressed to the slide with a piece of clean blotting paper. This should leave the dried section closely applied to the dried slide. The slide is now again heated until the paraffin begins to appear translucent, and again firmly pressed to the slide. This method should not be used for delicate tissues such as the brain, as the firm pressure required is harmful.

Treatment of the Sections.—To remove paraffin from the sections so that aqueous and other stains can be used, the slide carrying the section should be placed in xylol and agitated in it for two or three minutes. This dissolves the paraffin. To remove the xylol place in strong spirit or absolute alcohol and again agitate, so that fresh surfaces of spirit are brought in contact with the section. As a precaution it is well to rinse in fresh spirit. The slide can then be placed in water to remove the spirit, and stained as is considered advisable. If the tissue has been hardened in corrosive sublimate it should be rinsed in iodine solution and again washed before staining.

After staining, dehydrate in alcohol, clear in oil of cloves, wash with xylol if aniline stains are used, and mount in xylol Canada balsam. If the alcohol used is strong enough the oil of cloves need not be used.

CHAPTER III.

Blood.

Examination of the blood is of such importance in tropical work that in all cases of difficulty and doubt recourse to this method of diagnosis is essential. A thorough knowledge of the constituents of normal blood is a necessary preliminary. The abnormal forms of cells met with in various diseases must be readily recognized. Last, but not least, the various methods used for the finding and recognition of parasites must be known. Many methods of examining blood have been employed and most of them are good. Fallacies and mistakes have occurred with all, and the sources and causes of these errors and the recognition of them have to be studied. These are dealt with under each method described.

Blood is composed of a nearly colourless fluid, the plasma, in which are floating cellular elements, the red and white blood corpuscles and blood-plates. The more solid elements, the blood corpuscles, will be considered first. They vary in number, in their relative proportions and in their characters.

Other cells, not normally present in the blood, are found under certain conditions in that fluid. Most of these cells are normally present in the tissues of the healthy body, though not normally in the blood.

Parasites occur in the red corpuscles and in the plasma, and are sometimes found, in a more or less disorganized condition, in white corpuscles or phagocytes, which have devoured them. None have been observed in the blood-plates.

The two main methods employed for the examination of blood are :—

(1) In the fresh and fluid condition.

(2) As films which are allowed to dry, and fixed and stained in various ways.

These two methods are of general application. For special purposes, so as to reveal abnormal bodies scantily present in the blood, thick films can be made and the hæmoglobin removed. In this way a quantity of blood that would not be sufficiently transparent if treated by the ordinary methods can be rapidly examined.

EXAMINATION OF FRESH BLOOD.—This method is the only one by which vital changes can be observed. Of the normal blood elements, the amœboid and phagocytic properties of the leucocytes can thus be observed. Living organisms abnormally present, such as filariæ, trypanosomes, and the parasites of malaria, can be watched, and such developmental and degenerative changes as occur in shed blood observed.

No description or observation of new parasites is complete without an examination of these parasites in the living conditions. It is noteworthy that most of the important mistakes made even by experienced observers in the description of bodies met with in blood have been due to neglect of the examination of fresh fluid blood.

The essential point in the preparation of fresh fluid blood films is that a great part of the film should be so thin that the blood corpuscles are lying flat and separate from each other.

(1) The simplest method of making such a film is to take a small drop of blood on the centre of a cover-glass and drop it on the slide (fig. 17). In a well-made film by this method three zones are apparent. The edge of the film is thick and irregular. Here the corpuscles are in rolls or masses, and it is too thick for the examination of the individual red corpuscles (fig. 17, a).

Internally to this is an area with a slightly opaque or ground-glass appearance. Here the red corpuscles will

be found lying flat and not to any great extent overlapping each other. This is the part of the film best suited for examination of the red corpuscles and the parasites contained in them (fig. 17, c).

The centre of the film is clear and transparent, and here few corpuscles are found, as this part is composed almost entirely of the plasma (fig. 17, b).

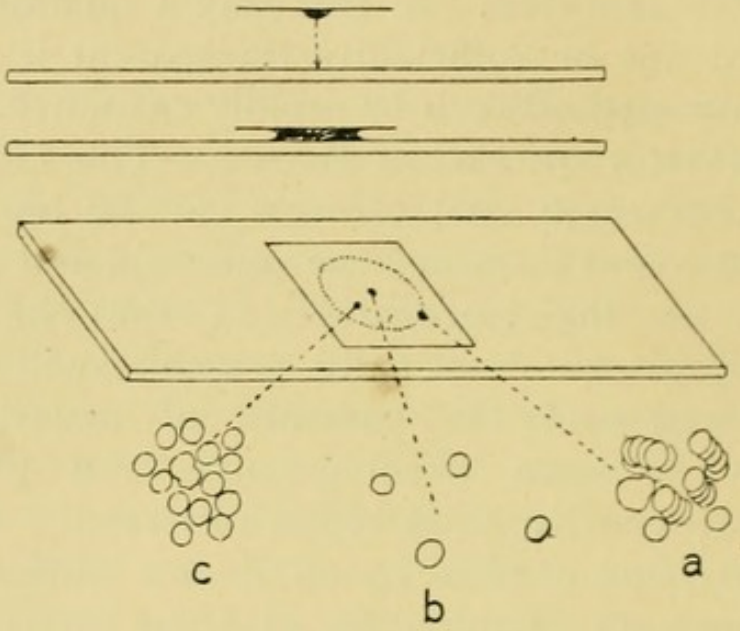

FIG. 17.

To get good films by this method there are certain points to be observed :—

(1) The slide and cover-glass must be free from grease, otherwise the blood will not spread out (*vide* cleaning slides and cover-glasses).

(2) They must be freed from grit; this is best done by rubbing well, immediately before use, with a soft linen rag.

(3) The drop of blood must be so small that, when spread out, it does not extend to the edges of the cover-glass. If the blood is too abundant it floats up the cover-glass, and sufficient space is left between the slide and cover-glass to allow of the formation of rouleaux.

(2) Another method is that of Braddon. Here the

square or oblong cover-glass, freed from grease and grit, is placed on a slide similarly cleaned. The cover-glass is so placed that its edge corresponds with one edge of the slide. Pressure is exercised on the centre of the cover-glass, or it can be fixed with Cornet forceps.

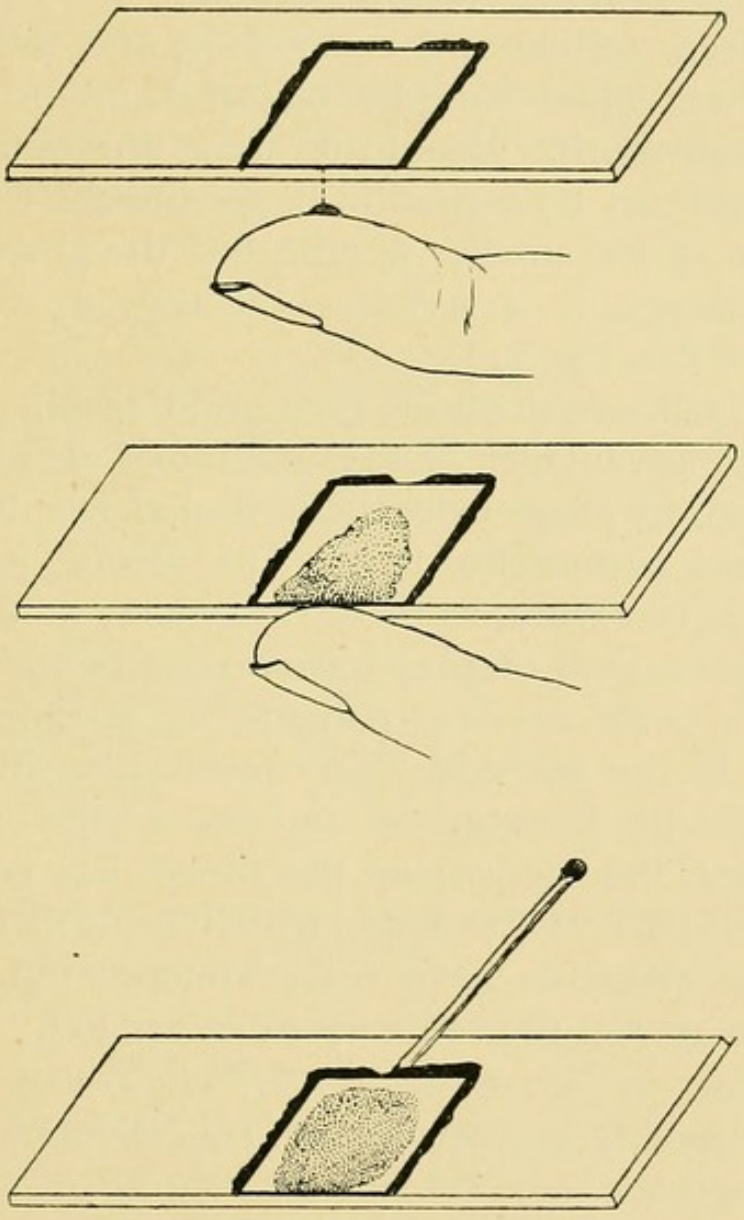

FIG. 18.

Vaseline is then applied to the slide at the edges of the cover-glass, leaving the side applied to the edge of the slide and a small space at the edge opposite to this free (fig. 18). The Cornet forceps are then removed.

These slides can be prepared in the house or laboratory and are then ready for use. If the edge of the slide be

applied to a drop of blood, the blood will run up by capillary attraction and spread itself out in the space between the cover-glass and slide in a film thin enough for examination.

(3) A third method is to make the film between two cover-glasses, the lower one being much the longer. The blood spreads more readily between two cover-glasses than between a slide and cover-glass, and the free edge of the lower glass can be clamped on to the slide (Horder's method). Beautiful films are obtained, and cover-glasses can be carried in larger numbers than slides on account of the smaller weight, but the greater fragility of cover-glasses is a serious objection to the general adoption of this method.

All of the above methods give good results. The first has decided advantages in that the blood elements are all present and, to some extent, distributed evenly throughout the best part of the film. The second is very convenient for class work, as there is no delay at the bedside, and a large number of preparations can be made quickly. It is useful with nervous patients, as no preparation is necessary at the bedside. The more adhesive elements of the blood, the blood-plates and leucocytes, are crowded together near the edge where the blood has entered. In the thinner part of the field, which is farther from the edge, these elements have been "filtered out" and few solid elements but the red corpuscles are left.

In the freshly drawn blood the elements normally present are :—

(1) Red corpuscles or erythrocytes.

(2) White corpuscles or leucocytes.

(3) Blood-plates or platelets, Hayem's hæmatoblasts.

(4) Plasma.

In the red corpuscles the points to note are the colour, the size and the shape, and variations in these. In many of the corpuscles, particularly if pressure has been

applied to the cover-glass, clear, transparent spaces, vacuoles, which may be either circular, oval, or even slit-shaped, will be found (fig. 19 c). These must be recognized for what they are, and not confounded with unpigmented parasites nor with the natural deficiency in colour seen towards the centre of the corpuscles. From both of these the vacuoles can be distinguished by the sharpness of their outline. An oscillatory or vibratory motion of the hæmoglobin edge of the vacuole is highly characteristic, but must not be mistaken for amœboid movement.

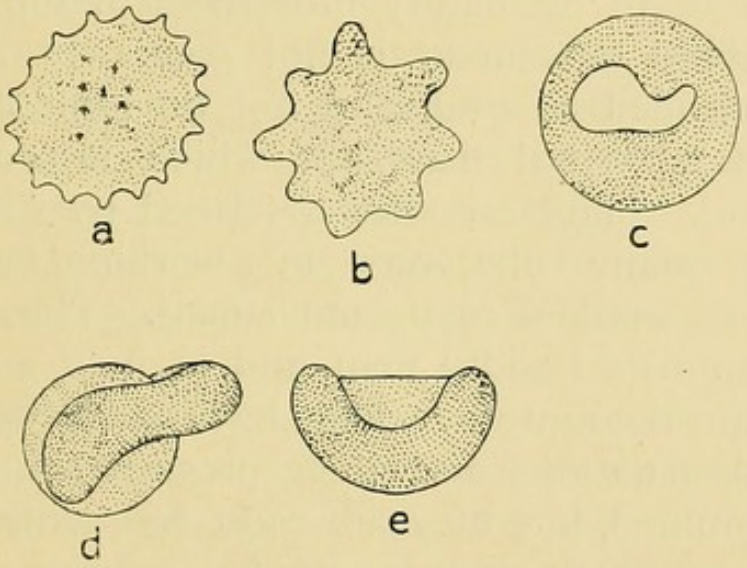

FIG. 19.—a, b, Crenated corpuscles; c, vacuolated corpuscle; d, e, buckled corpuscles.

CRENATION.—If the blood corpuscles be watched for some time they will be seen to become distorted and projections are thrown out, either as a few blunt processes or as sharper projections (fig. 19 a, b). This change is known as crenation, and the projection may be feebly motile and portions may break off and be discharged into the plasma. These crenations are readily recognized when they occur at the edge of the corpuscles. When they occur on the flat surface they produce an irregularity in the colouring and, as they are not flat, cause refraction, and so produce an appearance of dark spots surrounded

by a lighter ring, or a light spot surrounded by a dark ring, according to the focussing.

Some of the red corpuscles, particularly if pressure has been used in making the film, are bent on themselves or "buckled" (fig. 19, d, e). Such corpuscles may assume very varied shapes, and, as the hæmoglobin is readily expressed from any part of the corpuscle irregularities in colour are usual.

All these appearances can be easily distinguished from parasites by careful focussing. Occasionally, however, one meets with red blood cells within which are bodies closely simulating ring forms. These may be motionless, or have a slight oscillatory movement at the edge, but true amœboid movement is not seen. In shape they may be rounded or oval, and not uncommonly appear to possess a central dark dot, which is probably due to refraction. Similar bodies described by Cropper and noted by many observers in working with fresh blood have a marked rotary movement. They are found in other animals besides man, and in man and animals in non-tropical countries. They have never been stained in dried preparations and their precise nature has not been determined, but they are most frequently found in cases where there is evidence of degenerative changes in the blood.

LEUCOCYTES are distinguished by their size and the absence of colour. As seen in the fresh blood they are usually granular, the granules being best seen on closing the iris diaphragm of the microscope. Variation in the granules will be noted, and the coarse, highly refracting granules of the eosinophile leucocytes are quite characteristic. *These granules are often mistaken for pigment by beginners.* Letting in more light, which brings out pigment granules more strongly and shows these normal granules to be translucent, will remove this difficulty. The characters of the nuclei and of the granules are best studied in stained specimens. The amœboid movements and phagocytic properties are best seen in these fresh living fluid films.

THE BLOOD PLATELETS are the most difficult objects to see, as they are colourless, non-granular and differ little in refractive index from the plasma.

The size and arrangement in groups, points that vary in different specimens of blood, should be noted. The irregular serrated margins they acquire in a short time, from the formation of filaments of fibrin, are characteristic of these bodies. These elements are more readily seen in stained or over-stained specimens.

STAINING OF FRESH FILMS.—Many methods of staining blood, whilst still in a fluid condition, by admixture with stains have been employed.

The usual practice is to place a drop of sufficiently dilute stain on the slide, then take a minute drop of blood on the cover-glass and drop this on the drop of stain, so that the blood and stain spread out together. A certain admixture takes place at the edge of the drop of blood, and in a little time the stain diffuses further into the blood.

Various solutions of stain have been used. Braddon's is perhaps as good as any.* In this, as well as in other aqueous stains, the water causes a liberation of the hæmoglobin, and the dissolved hæmoglobin precipitates the stain, or *débris* is stained by the stain.

If this process takes place in the serum little confusion is caused, but if, as frequently happens, it takes place as the stain penetrates the red corpuscles it causes the formation of a complicated arrangement of stain in the interior of the red corpuscles, which has been mistaken for parasitic growth. To avoid this error, a strong salt solution is used by some, so that the hæmoglobin is not discharged from the red corpuscles. Others, for the same reason, use ascitic fluid.

Malaria parasites are well stained by this method, and

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* Braddon's solution is composed of 1 per cent. pot. citrate, $\frac{1}{2}$ to 2 per cent. methylene blue : water to 100 parts.

it has the advantage of requiring no fixation, and consequently is rapid.

DRIED FILMS.—These can be made in many ways, most of which after a little practice give excellent results. In all methods it is important that only the top of the drop of blood and not the skin should be touched by the slide, cover-glass or paper. Neglect of this precaution will result in the admixture of epithelial scales, oil globules and micrococci with the film.

(1) In this method a drop of blood is taken on the surface of a slide near one end. The edge of another slide is brought into contact with this drop, which then spreads out so as to fill the angle between the two slides along the whole extent of the line of contact. On pushing the upper slide towards the other end of the lower slide a film of blood will be left behind. The thickness of this film is easily regulated, as if the angle between the two slides is acute the film left behind will be very thin ; if the angle be nearly a right angle a thick film will be left. An angle of about 45° gives the desired thickness, but it is well to slightly vary the thickness of the film by alternately slightly increasing and diminishing the angle made between the two slides as the upper one is pushed along, so that different parts of the film will be suitable for examination for different purposes.

A slight modification of the method is to take up the drop of blood on the edge of the upper slide and bring the drop of blood and the edge of this slide into contact with the upper surface of the lower slide and proceed as above (fig. 20).

(2) A drop of blood is taken on a slide rather nearer one end than the other, and the larger the drop the farther from the middle. Another slide, a glass rod, or, perhaps best, the shaft of a needle, is then applied to this drop so that the blood spreads along the whole of the line of contact. The upper slide, glass rod, or needle is then drawn across the lower slide and an excellent film will be left (fig. 21).

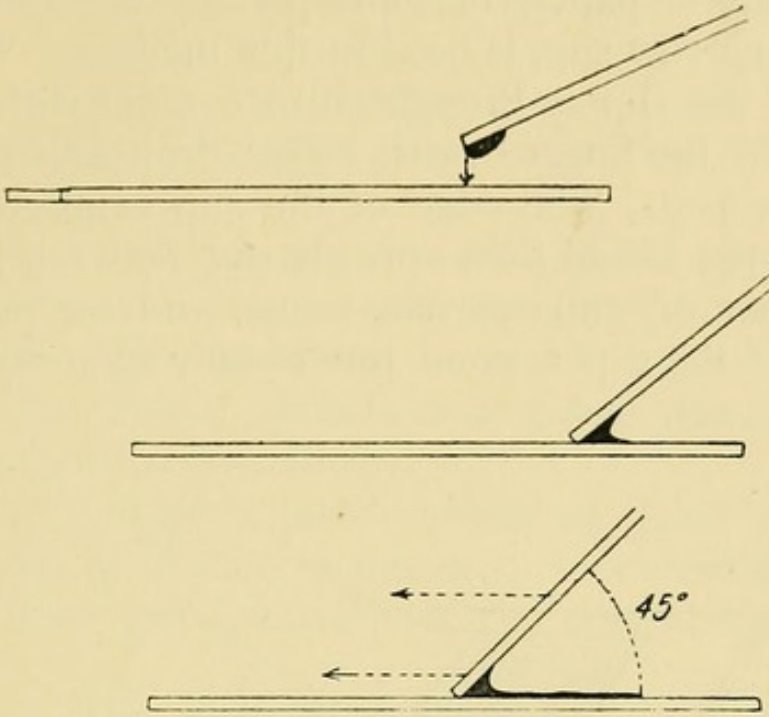


FIG. 20.

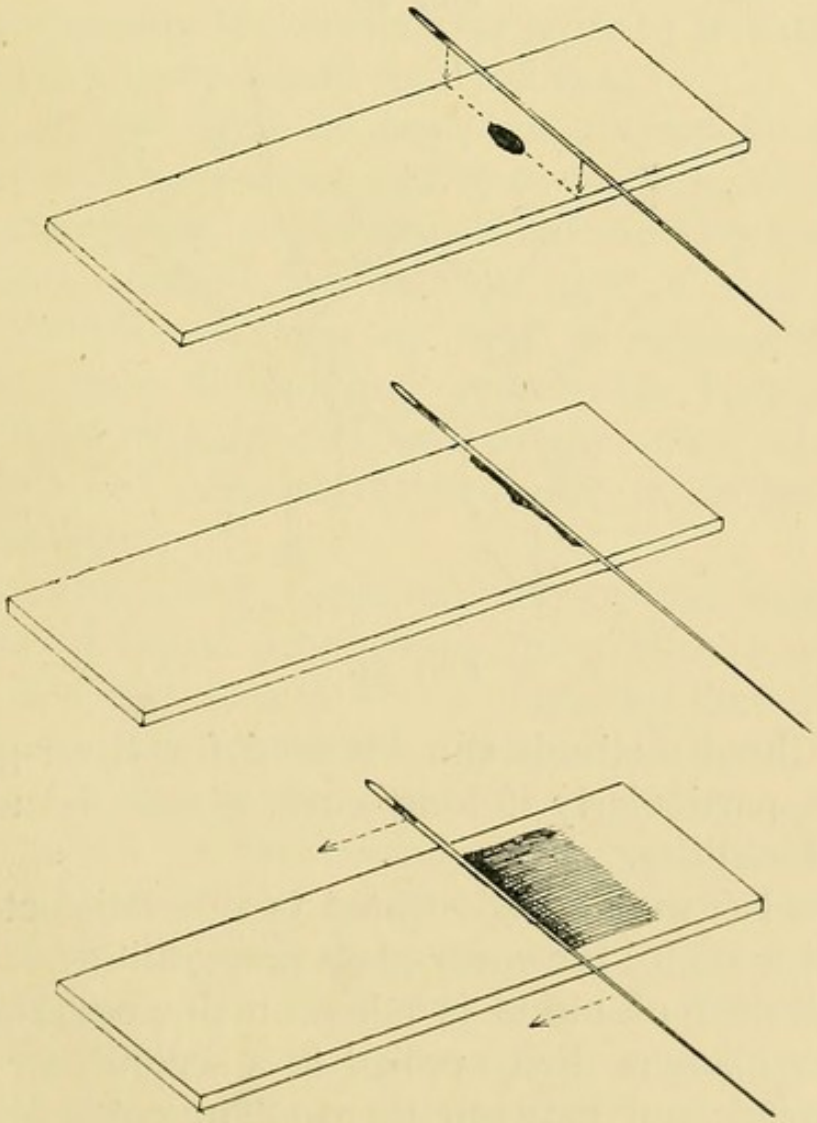


FIG. 21.

(3) Cigarette paper, or gutta-percha tissue cut in the form of a narrow slip, is used in this method. The lower surface of the slip is brought into contact with the drop of blood on the finger or ear. This drop adheres to the slip on removal. The edge of the slip is placed on the slide and the blood then spreads out between it and the tissue paper or gutta-percha tissue, and on pulling the free end of the slip a good but usually scratchy film will be left (fig. 22).

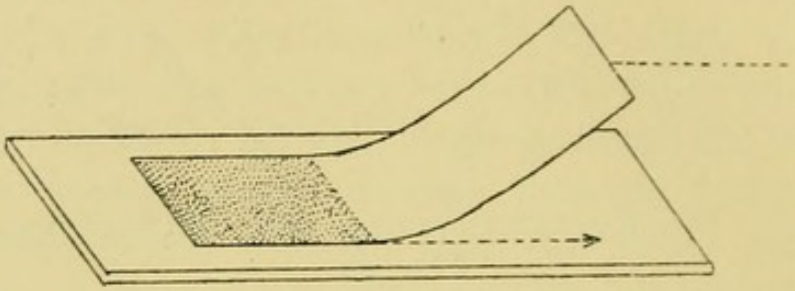


FIG. 22.

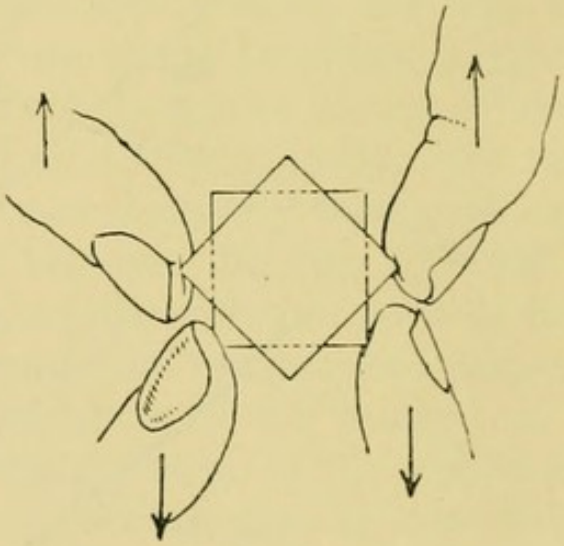


FIG. 23.

These three methods can be used for cover-glass preparations, particularly if long cover-glasses be used, but are best for slides.

(4) The last method advocated is not satisfactory with slides but is useful for cover-glass preparations.

A small drop of blood is taken on one cover-glass and this cover-glass is then applied to a second, so that the blood spreads out between them. The cover-glasses are

arranged diagonally, so that the corners of each cover-glass can be taken hold of. The upper cover-glass is then drawn or slid over the lower, care being taken that it is not *lifted off*. A good film should be left on each cover-glass (fig. 23).

In case of emergency any piece of flat glass, broken window-pane, &c., can be used and good films obtained, but the best films are those in which the slides are of good quality, even in thickness and free from scratches, dirt, grease, or irregularities.

The films, however made, should be dried rapidly by waving them to and fro in the air, but not heated; otherwise crenation and distortion of the corpuscles will take place.

Fixation. — If such films were placed in water or aqueous solutions of stains the hæmoglobin would be dissolved out, and the corpuscles more or less destroyed; it is therefore necessary to fix the films.

Films can be fixed by heat, but a temperature above a certain point vacuolates and distorts the red corpuscles.

As a general rule in blood work, fixation by heat should be avoided, though for one method of staining—the Ehrlich-Biondi—fixation by heat is necessary. Good results are more difficult to obtain in the Tropics by this method than in England, and, as the same information can be obtained by easier methods, it is not recommended for tropical work.

Fixation by absolute alcohol, or by absolute alcohol and ether in equal proportions, gives good and reliable results. Fix for ten minutes or more and then dry in air.

There are other methods of fixation, and of these exposure of the film to the vapour of 40 per cent. formaldehyde (formalin) for two minutes is perhaps the best.

Saturated solution of perchloride of mercury does not give good results with films of malarial blood, as the parasites do not stain well after the use of this reagent, but for other blood work the results are fairly satisfactory.

Staining of Dried Films.—When fixed and dried the film

can be stained, and the number of stains that have been employed is very large. Of the methods most generally applicable, the following have the special advantages and drawbacks indicated. Often two or more methods can be employed with advantage on different slides, in order to bring out special features in the blood.

HÆMATOXYLIN.—Any good hæmatoxylin stain will stain most of the basic elements in the blood and most of the parasites. The number of preparations used is large. The formula recommended is composed of a mixture of—

Hæmatin...	2·5 grm.
Absolute alcohol	50 c.c.
Alum	50 grm. or to saturation.
Water	1,000 c.c.

The hæmatin is dissolved in the alcohol and added to the solution of alum in water and the vessel containing the mixture is left loosely corked and exposed to the light in order to hasten the maturing of the stain.

In warm weather this stain matures rapidly, two or three weeks being sufficient. When mature the sides of the vessel containing the mixture are deeply stained. Like all other hæmatoxylin stains, it must be tested before use to find the time required for staining. When properly mature this preparation requires about seven to ten minutes to stain blood well. It need not be filtered immediately before use. The stain may be placed over the film, or the slide with the film on it may be immersed in a pot of the stain, which should be well shaken before use.

If the stain is placed on the slide do not pour off the stain, but *flush it off*. If well flushed, even when a dirty stain is used, little deposit will be left on the film. If the stain be poured off, however much the slide is then flushed or washed, dirt from the stain will adhere to the film. After flushing off the stain leave in ordinary tap water for five minutes. The effect of the tap water is to

change the dirty purple colour of the film to a clear blue. The process is commonly spoken of as "blueing" the film. Drain and allow to dry.

As a counter-stain eosin is useful. An aqueous 1 per cent. solution of yellow eosin (soluble in alcohol) is used. It will stain in twenty to thirty seconds; then wash and allow to dry. A film so prepared is in a fit condition for examination with an oil immersion lens. The oil can be placed directly on the film, but if it is intended to keep the film, it is simpler to mount in xylol balsam and then examine. Plate IV. shows the appearances of the blood-cells stained in this manner.

RED CORPUSCLES.—The red corpuscles are stained by the eosin, the depth of the colour varying according to the richness of the corpuscles in hæmoglobin. As in fresh blood, the size, depth of colour and shape of the red corpuscle should be observed. Among the rarer forms in normal blood are red corpuscles which hardly stain with eosin, the shadow or ghost corpuscles; polychromatic corpuscles, which are faintly stained with both stains, so as to have a purplish colour, and red corpuscles containing granules which stain deeply with the basic stain used, hæmatoxylin. The last-named cells are described as containing basophilic granules (Plate IV., 9). Nucleated red corpuscles are very rarely present in the blood of healthy individuals, but are found not only in blood of patients markedly anæmic but also in some cases of malaria, &c. The nucleated red corpuscles have a nucleus frequently fragmented and staining deeply, but not evenly, with hæmatoxylin (Plate IV., 3 and 4). They have a sharply defined margin. Not unfrequently the nucleated red corpuscle itself is polychromatic, or contains basophilic granules. Such nucleated red corpuscles may be larger (megaloblasts), smaller (microblasts), or the same size (normoblasts), as the normal.

THE BLOOD PLATELETS are stained feebly with both stains and have a uniform faint purple colour. In an

overstained specimen the network of fibrin filaments starting from either a single plate or a group, is plainly brought out, but in a normally stained specimen only the platelets and the bases of these filaments are revealed (Plate IV., 5).

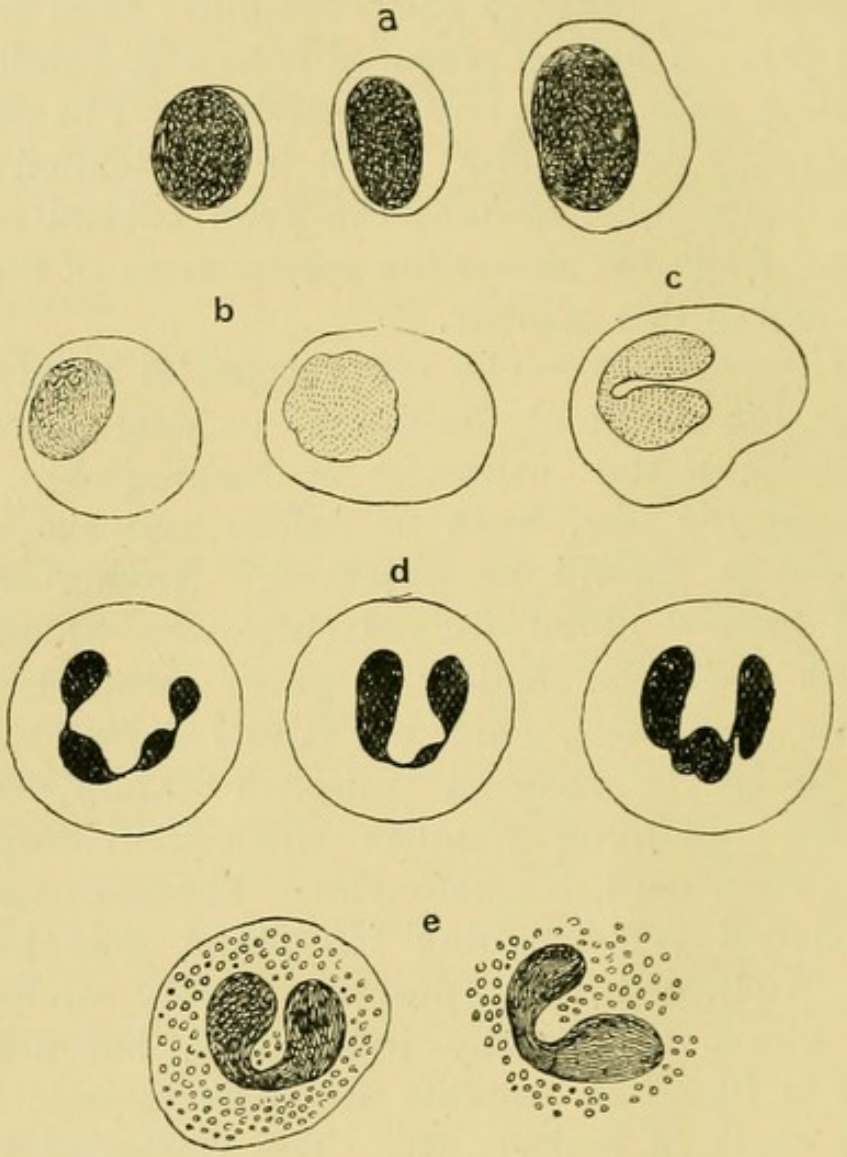


FIG. 24.—*a*, Lymphocytes ; *b*, large mononuclear leucocytes ; *c*, transitional leucocyte ; *d*, polymorphonuclear leucocytes ; *e*, eosinophile leucocytes.

WHITE CORPUSCLES.—The leucocytes have their nuclei stained deep blue. The protoplasm is stained differently in the different varieties of leucocytes, but granules, with the exception of those staining deeply with eosin, are not brought out by this method. They are, however, visible in the unstained leucocytes and can be demonstrated by other stains.

In normal blood four varieties of white corpuscles can be differentiated. Of these two have a single, more or less rounded, nucleus. These mononuclear leucocytes are of two classes, though it is not always easy to say to which class a given mononuclear leucocyte belongs. Still, with practice the number of doubtful instances greatly diminishes. The points to be considered in the differentiation are the size of the corpuscle, the shape and staining reactions of the nucleus, the stain taken up by the protoplasm, and the relative amount of protoplasm as compared with the nucleus.

(1) The small mononuclear leucocyte, or LYMPHOCYTE, is usually not much larger than a red corpuscle and varies from 7 μ to 12 μ in diameter. The nucleus stains deeply and forms the greater part of the corpuscle. The protoplasm is often reduced to a mere rim, and in any case is relatively scanty in proportion to the size of the nucleus (fig. 24, *a*). The protoplasm is stained faintly pink, much the same as the protoplasm in the polymorphonuclear leucocyte.

(2) THE LARGE MONONUCLEAR LEUCOCYTES (fig. 24, *b*), sometimes called the hyaline cells, are variable in size, but some of them form the largest white elements in normal blood. The nucleus is not so deeply stained as in the lymphocyte. The protoplasm is relatively abundant and stains slightly with basic stains. It may be unstained or faintly blue, or, if pink, is less so than the polymorphonuclear leucocyte.

All these points have to be taken into account in the separation of these leucocytes.

Some corpuscles are found with the nuclei deeply indented, or horse-shoe shaped. In staining reactions they resemble the large mononuclear and are probably advanced forms of these, and not, as usually described, transitional forms between these and the polymorphonuclear leucocytes (fig. 24, *c*).

The other two classes of leucocytes are much easier to distinguish.

(3) THE POLYMORPHONUCLEAR LEUCOCYTES (fig. 24, *d*), sometimes incorrectly called polynuclear, form the greater number of the leucocytes. They are rounded cells, which are granular in the fresh blood, but the granules are not stained by the method we are now discussing. The characteristic of these cells is the variety in form of the nucleus. The nucleus stains deeply with the hæmatoxylin, and at first sight appears to be multiple. Closer examination shows that the different parts of the nucleus are really connected together, though often by a mere string or filament.

The form in dried uncompressed specimens is round, the size fairly uniform, and the protoplasm stains a faint pink.

(4) THE EOSINOPHILE LEUCOCYTES. — The fourth variety has a deep indented nucleus, sometimes divided into three. The nucleus does not stain so deeply with hæmatoxylin as in the polymorphonuclear leucocytes, but the characteristic of this leucocyte is the presence of a large number of coarse granules which stain deeply with eosin. Hence these leucocytes are called *eosinophile* This leucocyte is more loosely held together than any other, and it is no uncommon event for one to be ruptured in making the film, so that the nucleus is seen surrounded by a cloud of granules stained with eosin (fig. 24, *e*).

Relative Proportions.—These four varieties of leucocytes are all present in normal blood, but in relative numbers varying within comparatively small limits. The variations in appearance are shown in fig. 24.

The normal proportions are given variously as :—

Lymphocytes	10-25 per cent.
Large mononuclear	5-10 „
Polymorphonuclear	65-75 „
Eosinophiles	2-4 „

It will be seen that the lymphocytes are the most variable elements and, in an individual, may vary during

the same day from hour to hour, according to the stage of digestion.

In many diseases, and for some time after these diseases, there is a marked variation in the relative proportions of these blood elements. A most important variation is that which occurs during, and still more markedly after, a malarial attack. The leucocytic variation, which occurs in malaria, is a relative increase in the number of large mononuclear elements, so that they may constitute 20 per cent. or more of the leucocytes found. The increase appears to be constant and it is rarely less than 15 per cent. It may be much greater, so that they may constitute 40 per cent. or even more of the total leucocytes.

It occurs in all forms of malaria and persists after all other signs or symptoms of malaria have disappeared. It is found sometimes three months or more after an attack and rarely disappears, or even diminishes, in a month. It is not affected by quinine.

A similar leucocytic variation is occasionally, but rarely, found in typhoid and Malta fever and in other conditions, but in these diseases it is not persistent, and in these cases the difficulty of distinguishing the true large mononuclear cells from the numerous large lymphocytes is great. In trypanosomiasis and kala-azar it appears to be constant, but is less marked than in malaria and is associated with an increase in the lymphocytes.

The total number of leucocytes varies during an attack of malaria. During the pyrexial period there is leucopenia sometimes as low as 2—3,000 per c.mm., in the apyrexial periods there is an increase to, or even decidedly above, the normal. According to Vincent there is a period immediately after the paroxysm begins during which there is an increase in the number of leucocytes, whilst according to Ross the total mononuclear cells increase rapidly after a paroxysm, so that about seven days later the total leucocytes are above the normal.

A relative increase in the polymorphonuclear elements occurs in pneumonia and in many septic conditions, particularly when deep-seated abscesses form, such as in perityphlitic or hepatic abscess. In such cases there is also an absolute increase in the number of leucocytes. A differential leucocyte count is therefore an important aid in distinguishing these diseases from malaria, which they may resemble clinically.

Increase in the relative proportion of eosinophiles occurs from many causes, some of which are unknown. It is marked in most cases of anæmia from ankylostomiasis, and occurs also in many cases of filariasis and in some cases of bilharzia infection, and is said to be well marked and constant in trichinosis. The blood examination may often give a hint as to the presence of some of these parasites. As the increase occurs also from unknown causes, and in some skin diseases, and is often associated with chronic bronchitis and asthma, in itself it is of no certain diagnostic value.

The proportion of lymphocytes is so variable that only an enormous increase is of importance. This occurs in some cases of scurvy and is associated with an increase in the other mononuclear elements.

A *differential* leucocyte count must not be confused with an *actual enumeration* of the number of leucocytes present in a given volume of blood. That has to be determined separately, as we shall see subsequently. To make a differential count of the leucocytes a dried film of a small but uncertain volume of blood is prepared and stained. All the leucocytes found in a systematic examination of a part of this film are counted and the percentage of each different variety met with is thus ascertained. For accurate work not less than 500 should be counted, but for clinical purposes 200 will often suffice. The edges of the film where leucocytes are most numerous should not be included in the enumeration.

Variations in the total number of leucocytes and in the relative proportion of the different kinds of leucocytes

occur in healthy persons to a moderate extent. The number of leucocytes in healthy adults is rarely under 6,000 per c.mm. or over 12,000. In new-born children the number is much greater—up to 20,000, and in pregnancy is increased up to 15,000. During active digestion there is an increase in the number of leucocytes.

Variations in the relative proportion of the different forms of leucocytes readily occur in healthy children: but in adults, with the exception of the lymphocytes, such variations are comparatively small.

The following table gives examples of the relative proportions of the different leucocytes in certain diseases, as well as the number of leucocytes per c.mm. usually met with in such diseases :—

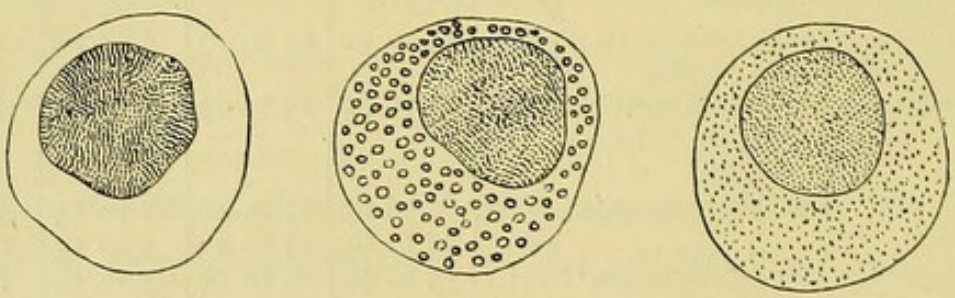
	No. per c.mm.	Polymorphonuclear Per cent.	Lymphocytes Per cent.	Large mononuclear Per cent.	Eosinophiles Per cent.
Pneumonia ...	Great increase up to 60,000	85 to 95	15	5	1
Sepsis... ...	Increase up to 30,000 or 40,000	75 to 90	15 to 25	5 to 10	1 to 8
Liver abscess...	Increase varies, often slight, 12,000 to 20,000	75 to 85	15 to 25	5 to 10	2 to 4
Typhoid ...	Slight increase at most	50 to 65	25 to 40	5 to 15	1 to 3
Malta fever ...	,, ,, ,,	50 to 65	25 to 40	5 to 15	1 to 3
Relapsing fever	Great increase up to 50,000	75 to 90	10 to 20	5 to 10	1 to 2
Malaria ...	No increase; decrease during pyrexia	45 to 65	15 to 25	15 to 30	1
Trypanosomiasis	No increase	50 to 65	20 to 30	15 to 20	2 to 4
Kala azar ...	Marked decrease — 1,000 to 3,000	50 to 60	25 to 35	15 to 20	2 to 4
Ankylostomiasis	Usually increased, especially in early cases	66 to 70	10 to 20	5 to 10	8 to 50
Beri-beri ...	Slight increase — 11,000 to 14,000	24 to 49	30 to 68	1 to 12	1 to 5
Pellagra ...	7,000 to 9,000 ...	50 to 60	32 to 42	3 to 6	1 to 4

It must be remembered that multiple infections are common. In relapsing fever lung complications are so common that possibly the leucocytosis is due to this.

The tendency to leucocytosis and an increase in the polymorphonuclear leucocytes due to any disease will mask the mononuclear increase due to malaria.

ABNORMAL CELLS. — Abnormal elements resembling leucocytes are present in certain diseases, particularly in leucocythæmia. These abnormal elements are known as MYELOCYTES (fig. 25) from their similarity to cells found normally in the bone-marrow. They are of three kinds, all mononuclear.

(1) The first form is variable in size, the greater number of them being much larger than the large mononuclear leucocytes. With eosin and hæmatoxylin, as the granules which they contain are not stained, it is sometimes difficult to distinguish the smaller ones from the larger of the mononuclear leucocytes. For practical purposes the difficulty is unimportant, as when myelocytes occur they are common and most of them are readily distinguished from the leucocytes.



1. 2. 3.

FIG. 25.

In these myelocytes the nucleus stains less readily and is therefore paler than that of the large mononuclear leucocytes. The edge of the nucleus is frequently ragged. The protoplasm is abundant and stains in many cases more deeply with eosin than the large mononuclear leucocytes do. Mitotic figures are often met with.

(2 and 3) The other two forms of myelocytes contain granules which stain deeply with eosin. They are subdivided according to the size of these granules, which may be coarse, as in the eosinophile leucocytes, or fine.

The distinction is probably unimportant. These myelocytes are distinguished at once from eosinophile leucocytes by the single nucleus, and from each other by the size of the eosinophile granules.

These cells are abnormal cellular constituents that may be met with in blood specimens stained with eosin and hæmatoxylin, and they must be clearly recognized before any satisfactory examination for parasites can be made. In themselves they are of considerable importance in the recognition of various diseases and for prognosis.

Cells known as "mast-cells" are occasionally found in normal blood and more abundantly in some of the blood-diseases, *e.g.*, leucocythæmia, &c. They are probably degenerated leucocytes, usually of the polymorphonuclear type. The protoplasm of the cell contains numerous coarse metachromatic granules and the nucleus is often obscured. These cells are best shown when stained by the Romanowsky method or one of its modifications.

Their significance is unknown.

In pernicious anæmia and in chlorosis the changes in the red corpuscles, the irregularity in their size, shape, and colouring are of clinical value, and in most tropical anæmias, including that occurring in malaria, the changes are similar to those in a mild case of pernicious anæmia.*

Leucocythæmia is readily recognized by the enormous increase in the number of the white elements which, as we have seen, take on basic stains. This increase is so great that the appearance of a dried film indicates it

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* Between these two forms of anæmia the main difference observed in blood examination is that in chlorosis the number of corpuscles is not diminished, but the hæmoglobin is, so that each corpuscle is poor in hæmoglobin. In pernicious anæmia there is a great diminution in the number of corpuscles, but the hæmoglobin value of the corpuscles averages much the same as normal blood. Mixed or intermediate cases occur.

unmistakably, and it is not, for diagnostic purposes, necessary to make any count. The presence in numbers of the eosinophile myelocytes is conclusive proof of the implication of the bone-marrow, whilst the absence of this form of abnormal cell indicates more probably a lymphatic leucocythæmia.

In all forms of leucocythæmia decided changes are also found in the red corpuscles. Irregularities in size, shape and depth of colour are common, and nucleated red blood corpuscles occur, often in large numbers. Polychromatic red corpuscles and red corpuscles showing basophilic granules are also common.

If no abnormal cells are present the relative proportions of the normal cells may be so changed that we can diagnose with some degree of probability septic processes, recent malaria, or helminthiasis.

Eosin and hæmatoxylin can be used for staining any of the parasites found in blood. The stains are easily prepared, keep well, and their use is not dependent on distilled water or appliances which are not obtainable everywhere.

It is not a very brilliant stain, and therefore other stains giving more marked contrast are for some purposes preferable. It does not stain the granules present in many of the white elements of the blood, and though of general application, other stains are of greater value for special purposes and have special advantages.

DOUBLE STAINS.—Combinations of methylene blue and eosin dissolved in methyl alcohol are much used. The first is the LOUIS JENNER stain. It is made by adding an aqueous eosin solution to one of methylene blue. The stains combine and form a precipitate which is collected in a filter, dried and dissolved in methylic alcohol.

This stain can only be used with films that have *not* been fixed. The methylic alcohol does all the fixing required. Distilled water is an essential.

The stain may be placed on the film, slide or cover-glass for three and a half to four minutes, or, and this is

better, the slide or cover-glass can be placed in the stain in a well-stoppered bottle for the same length of time. The time must be kept accurately, carelessness in this respect leading to poor results. The stain must be flushed off with distilled water, and it is better to allow the distilled water to stand on the film for half a minute after washing. The water can then be drained or blotted off, the film allowed to dry, and the specimen examined directly with the oil immersion. When it is considered desirable to keep the specimen a drop of xylol balsam should be placed on the film and covered with a cover-glass.

With this stain the red blood corpuscles are stained pink, the depth of colour varying with the amount of hæmoglobin which the corpuscles contain.

The nuclei of the leucocytes are stained a clear blue, the eosinophile granules are stained deep red, and the granules in the polymorphonuclear leucocytes, which it will be remembered are not stained with eosin and hæmatoxylin, are brought out as fine dull-reddish granules.

Basophilic granules contained in cells are stained blue; this occurs both in white cells and in some red corpuscles. This stain is a good stain for many parasites, particularly those of malaria. Bacilli and cocci are also stained blue.

Some specimens of the Louis Jenner stain bring out clearly the important constituent of nuclei known as *chromatin*. A modification of the method and of the methylene blue is required to bring out the chromatin with certainty. Many methods have been employed for this purpose, most of them modifications of Romanowsky's method.

LEISHMAN'S STAIN.—The simplest, the most rapid, and on the whole the most satisfactory of these methods is that introduced by Leishman. A saturated aqueous solution of methylene blue, preferably "Hochst's pure medicinal," is made. This solution has to be rendered polychrome, so that in addition to the pure blue colour

of the ordinary methylene blue it is in part changed into a red stain. The change is indicated by a change in colour of the solution, so that in thin layers it has a reddish tinge. The solution of methylene blue becomes to some extent polychrome when exposed to air for some months, but for practical purposes a quicker transformation is required. There are many methods. Repeated heating in a sterilizer accelerates the change. Leishman uses a 1 per cent. solution of methylene blue (Grübler's), and adds 5 per cent. sodium carbonate to it. This solution he keeps at a temperature of 65° C. for twelve hours, and then exposes to air for a week or more.

J. H. Wright adds to a ½ per cent. solution of sodium bicarbonate 1 per cent. of methylene blue (Grübler's B. X., Koch's or Ehrlich's rectified). This solution is steamed in a steam sterilizer for one hour to effect the required transformation, and the solution may be used as soon as it is cold without filtering.

A convenient method, and more suitable where steam sterilizers are not available, is to treat the saturated solution of methylene blue with freshly precipitated oxide of silver. A solution of sodium hydrate is added to a solution of nitrate of silver till no more precipitate forms. The precipitate is washed till the washings are neutral to litmus paper. The precipitate, oxide of silver, is added to the saturated solution of methylene blue, and it is allowed to stand for twenty-four hours or more. A considerable proportion of the methylene blue in solution will be converted into polychrome methylene blue. The superjacent solution should be decanted off from the precipitated silver salts and filtered before use. It improves with keeping.

Whatever method be adopted for rendering the methylene blue polychrome the subsequent proceedings are the same.

One hundred cubic centigrammes of this solution of polychrome methylene blue are placed in a large shallow vessel (a half-plate photographic tray is a suit-

able one), and then a 1 in 1,000 aqueous eosin solution is added till a thick film forms on the surface and the fluid just shows the colour of the eosin. About 400 c.c. or a little more will be required, but the change in colour is the guide. The mixture should be well stirred and then allowed to stand exposed freely to air for some hours, stirring occasionally, and afterwards filtered. The residue in the filter is composed of the stain. It should be well washed with distilled water till the washing has only a faint bluish tinge, and then thoroughly dried, preferably in an incubator at blood heat. The stain must be finely powdered before use.

Two centigrammes of the powder can then be dissolved in 100 c.c. of pure methyl alcohol, and the stain is ready for use. It is, perhaps, more convenient to make a saturated solution of the stain in methyl alcohol, filter in the cold, and dilute with one-tenth of its bulk of methyl alcohol, so that a solution is made which is not quite saturated. The " tabloids " of the stain (Leishman's stain) keep well, and give excellent results. It is cleaner than the home-made stain, but the preparations do not keep so well.

To Use the Stain.—With a pipette two or three drops of the stain are placed on the dried *unfixed* blood-film on slide or cover-glass, and allowed to stand on it for half to one minute. If it shows any tendency to dry over any parts of the film in this period fresh stain must be added. To the fluid stain on the slide at the expiration of this half or one minute distilled water must be added drop by drop, and by oscillating the slide the stain and water are mixed as rapidly as possible. The amount of water required should be about double that of stain, but a better guide is to add the water in such an amount that when mixed with the stain the dark blue colour of the latter is replaced by a pinkish colour in the mixture, whilst the precipitated stain can be seen floating in the fluid. With a little practice the right amount of water required in each case is easily

found, and slight variations from exactitude are not of great importance. The water mixed with the stain should be allowed to remain on the film for five minutes, or with old or thick films for a longer period. It is quite easy to watch the staining under a iow power on the microscope, and the staining of the leucocytes is the best guide.

The stain is then flushed off with distilled water, and a drop of distilled water is ailowed to remain on the film for about one minute. A certain amount of the blue is dissolved, and the red corpuscles acquire a clearer red colour. This clearing with distilled water is essential to obtain good results. The more deeply the specimen is stained the longer will be the time required for clearing. This stage of the process is watched under the microscope and stopped when the clearing is sufficient. The water is then washed off rapidly with distilled water, the specimen drained or blotted and allowed to dry. Mount in xylol balsam and examine.

The principle of all modifications of the Romanowsky stain for chromatin is that the staining takes place during the precipitation of the stain, in the original processes during the precipitation of the mixture of aqueous solutions of the stains, and in Leishman's method during the precipitation by water of the combined stains dissolved in methyl alcohol.

Absolute alcohol with 2 per cent. aniline oil can be used as the solvent instead of methyl alcohol, and the solution treated as Leishman treats the methyl alcohol solution. The results are not so good as with methyl alcohol for a solvent, but are very fair. It is not to be recommended except where methyl alcohol cannot be obtained: the time for all the stages of the process should be doubled if this solvent be employed.

Other modifications can be used for films previously fixed in alcohol and ether.

In the first of these staining takes place during the admixture and mutual precipitation of the eosin and polychrome methylene blue. A 1 per cent. solution of

pure medicinal methylene blue (Grübler's) is made in distilled water and ½ per cent. sodium carbonate added. This solution keeps well and is fit for use when a reddish tinge appears. This change is expedited by keeping in an incubator. A second solution is a 1 in 1,000 aqueous solution of eosin extra B.A. (Grübler). This is fit for use at once, and keeps well if not exposed to light.

These are stock solutions, and should be diluted with twenty-four parts of pure water before use. The solutions are rapidly mixed and stirred, and the slides or cover-glasses are placed with the film side downward in the mixture. The dish should be rocked from time to time, and the films left in the stains for half an hour or more till well soaked. This is tested by examination of the slide whilst still wet under a low power.

The specimen should then be washed in distilled water, rapidly dried, and examined again under a low power. If too deeply stained a little distilled water may be left on the slide for a minute or more to clear it. Blot off the water, dry in the air and examine directly, or after mounting in Canada balsam.

GIEMSA'S METHOD.—This is another modification useful for fixed films where intense basic staining is required, and also for the restoration of faded preparations.

The staining mixture consists of :—

Azur ii., eosin	·3 grm.
Azur ii.	·8 ,,
Glycerine (Merck, chemically pure)	250 grm.
Methyl alcohol (Kahlbaum i.) ...	250 ,,

Immediately before use the stain is diluted with distilled water, 1 of stain to 15 of distilled water.

The films are fixed in methylic alcohol for two to three minutes, or in absolute alcohol for five minutes and then dried.

The diluted stain is poured on the films, and left from fifteen to thirty minutes, or for deep staining up to twenty-four hours.

The films are then flushed with a strong jet of tap water, dried in the air, and mounted in Canada balsam.

EOSIN AZUR METHOD.—This method is a modification of Giemsa's method, and has the advantage over the latter of being more rapidly carried out. The stain is supplied in "tabloid" form.

To make the solution one "tabloid" is dissolved in 10 c.c. of pure methyl alcohol, and the mixture allowed to stand for twenty-four hours. Only unfixed films must be used. The details of the process are the same as laid down for Leishman staining, with the exception that the dilution of the staining solution with distilled water must be greater: one part of stain to three parts of distilled water gives the best results. Staining takes seven to fifteen minutes. This method is especially of advantage in staining those parasites in which a difficulty is experienced in bringing out the chromatin clearly with other stains. It is particularly useful for staining trypanosomes and halteridium.

In specimens of blood stained by Romanowsky's method and its modifications, there are several distinct colours to be observed (Plates V. and VI.).

CHROMATIN is stained red. Other elements taking basic stains are mostly stained blue in various shades, and the red corpuscles are stained a peculiar pale pink with the eosin. Some granules, as those in the so-called "mast cells," are said to be metachromatic, as, though they stain deeply, the colour is different to that of any of the component colours of the stain used (Plate V., 7).

Polychromatic red corpuscles are stained purple and basophilic granules are well brought out as blue dots. The nuclei of nucleated red blood corpuscles are found to be rich in chromatin, and consequently the nuclei are stained a deep violet-purple.

In corpuscles invaded by certain parasites, viz., those of human benign tertian malaria, granules or dots staining red are found. In amphibian blood corpuscles invaded by one species of hæmogregarine, similar granules occur.

These granules are known as Schüffner's dots, and indicate a peculiar form of degeneration (Plate V., 21, and Plate VI., 7, 8, 9).

Granules staining with the basic stain, blue, are sometimes seen in corpuscles invaded with subtertian parasites. They resemble Plehn's bodies but are somewhat larger. They have been called Maurer's bodies. Plehn's bodies or these bodies are in some cases of malaria very common in the uninfected corpuscles (Plate VI., 20).

THE BLOOD PLATELETS are stained faint blue, with numerous red particles which sometimes form a meshwork. These particles are deeply stained and render the platelets very conspicuous (Plate V., 2).

THE LEUCOCYTES, with the exception of the eosinophile, are well stained, but in well-stained specimens the eosinophile granules do not show a clear red, as the protoplasm in which they are embedded stains a deep blue. The large size of the granules is shown, and there is no real difficulty even in a badly stained specimen in recognizing these elements. They do not, however, form such conspicuous objects as in specimens stained by Louis Jenner's stain.

The nuclei of the polymorphonuclear leucocytes stain purple. The staining is not regular but in patches. The protoplasm contains minute granules, usually in very large numbers, staining brownish-red. The protoplasm itself is very faintly stained.

THE LARGE MONONUCLEAR LEUCOCYTES.—The nuclei stain faintly purple. The staining is not uniform, but usually presents a faint mottled appearance. The protoplasm stains a faint blue, and imbedded in it are granules, which may be coarse or fine, and stain a deep clear red. These are known as chromidia (Plate V., 4).

THE LYMPHOCYTES.—The nuclei stain a deep purple from the large amount of chromatin contained. The staining is more uniform than in most of the leucocytes. The protoplasm is stained deep blue, is nearly uniform, and has no granules staining a different colour (Plate V., 3).

MAST CELLS.—The nuclei are stained very faintly, and

when the basophilic granules are numerous are difficult to make out. The granules in the protoplasm form large and irregular masses, and stain a deep purple-brown (metachromatic) (Plate V., 7).

MYELOCYTES have in most cases a rather feebly staining nucleus, poor in chromatin. The nuclei are large, but the relative amount of protoplasm varies greatly; in many cases a mere rim only of protoplasm is found (Plate V., 10, 11, 12, 13).

Granules taking either the acid or basic stain, or both, are present in most of the cells, sometimes in small, but more commonly in large numbers. Sometimes two, or even three, classes of granules are present in the one cell (Plates III., V., 10 to 13).

A detailed classification of these abnormal cells would be very difficult, as intermediate forms abound.

Amongst these cells are a small number with a large nucleus richer in chromatin than most of the myelocytes, and a rim of protoplasm staining a deep blue. These are not unlike the large cells found in cases of trypanosomiasis and in other ill-determined blood conditions, but in those the protoplasm is, relatively to the nucleus, in larger amount (Plate V., 9).

The true myelocytes include the eosinophile myelocytes, but in these there is, in most cases, some admixture of neutrophile or basophile granules, as shown by this stain.

The main classes of granules revealed by Leishman's stain are pure oxyphile or eosinphile staining pink, basophile staining blue, and neutrophile, which take up both acid and basic stains, including in some instances the red modification of the methylene blue, and metachromatic granules. According to the relative proportions of the three stains, these granules may present a range of colours from blue to red, or to a purple-brown, and also differ in the intensity of the staining and in the size of the granules. Too little is known of the micro-chemistry of such cells, or of their origin in detail, for the meaning and value of the different granules found to be of much practical importance at present.

CHAPTER IV.

ANIMAL PARASITES FOUND IN BLOOD.—Of the four great divisions of the protozoa, representatives of the sporozoa and mastigophora only are found in human blood.

To the SPOROZOA belong the parasites which cause malaria in man. These are found in the red blood corpuscles.

The MASTIGOPHORA (flagellate organisms) are represented by trypanosomes, and spirochætæ, which are found in the blood plasma, and also by Leishman-Donovan bodies found in the leucocytes.

Belonging to the higher animal kingdom are TREMATODA, of which the *Schistosomum hæmatobium* and *S. japonicum* are found in certain blood-vessels, and NEMATODA, represented by the filaria and filarial embryos, or micro-filaria. Sexually mature forms are found in the blood in lower animals, and one only, *F. magalhãesi*, once in man.

EXAMINATION OF THE BLOOD FOR PROTOZOA.—An essential feature of the examination consists in the examination of the fluid blood as soon as possible after its removal from the body. Many of the parasites exist in the red blood corpuscles, so that the film must be so thin that in a great part of it the red corpuscles are all lying flat and separate from each other. The methods of making such thin fluid films, already described, must be strictly adhered to. It is often urged that examinations of stained films are more convenient and better, but it cannot be insisted upon too strongly that most of the important errors which have occurred have been due to the exclusive use of stained specimens, and also that the phenomena of life can only be satis-

factorily observed in the fluid blood. These include some points of diagnostic value, namely, the character and movements of pigment, the activity of amœboid movement and the formation of flagella.

Stained films have their value, and show more clearly some points in the structure of the parasites. In busy practice it is often more convenient to defer for some hours the examination of the films, and in such cases stained specimens are more useful. In any case of difficulty, or when dealing with a parasite believed to be new, both methods should be employed.

Dried films can be made by any of the methods already described, and the parasites stained by the methods recommended. The films deteriorate when kept, and should therefore be examined as early as convenient, though a delay of a few days is not of much importance.

Other methods can be adopted if only the presence or absence of parasites has to be determined.

The methods generally used can be divided into three groups :—

A.—Those in which preliminary fixation is required before staining.

B.—Those in which fixation and staining are effected together.

C.—Those in which preliminary fixation is avoided.

A.—Films are fixed by immersion in absolute alcohol or in absolute alcohol and ether for ten minutes or more.

(1) HÆMATOXYLIN alone, or HÆMATOXYLIN and EOSIN. These stains can be used as already described, but better results are obtained by doubling the time for staining with hæmatoxylin.

(2) BORAX METHYLENE BLUE.—This stain is composed of methylene blue 2 grm., borax 5 grm., and water 100 c.c.

Place a few drops of the stain on the dried and fixed film and leave it for thirty seconds. Wash well with water, allow to dry, and examine directly, or mount in xylol balsam.

It is very easy to overstain by this method, and in such a case the red corpuscles will also be stained a deep blue and the parasites will not stand out clearly.

This stain is most rapid in its action, and on account of the risk of over-staining some authorities dilute it with one, two, or three times the volume of water. The stain keeps well.

(3) CARBOL THIONIN.—A stock solution of thionin 1½ grm., alcohol 10 c.c., and 1 in 20 aqueous carbolic acid solution to 100 c.c. is made. This stock solution keeps well, but is too strong to use for films. Before use it should be diluted with three parts of water and filtered. This diluted solution does not keep for more than a few days. For use, cover the film and leave the stain on for five minutes or more. It does not easily over-stain, so that only the minimum time need be remembered ; still, to get good results, half an hour is about the limit. Flush off the stain, allow to dry, and examine directly, or mount in Canada balsam. Old films stain with this method much more rapidly than ones recently made.

It is a good, clear, transparent basic stain and gives a very fair contrast. Bacteria, as well as animal parasites, are well stained, and it is one of the best stains for the demonstration of parasites in tissues.

(4) TOLUIDIN BLUE is a stain which has some points of resemblance to thionin. The stain is best kept as a saturated alcoholic solution, and diluted for use with twenty parts of 1 in 80 aqueous solution of carbolic acid. The fixed film should be covered with the stain and left for ten minutes or more. It is difficult to over-stain, and good results are obtained even if the film be left in the stain for twenty-four hours. This is an advantage, as the specimens can be left to stain whilst other occupations are pursued. The main advantage of the stain is that the pigment is less obscured than in specimens stained by carbol thionin.

If blood examinations are frequently required, it is well to keep the stains in a wide-necked stoppered bottle, and

simply place the slide in the stain for the time required instead of putting the stain on the slide. Many of the stains form films on the surface, and if this film of stain comes into contact with the blood-film it will adhere to it. The bottle of stain should be shaken vigorously before use. The fixing agent can be kept in the same way (fig. 26).

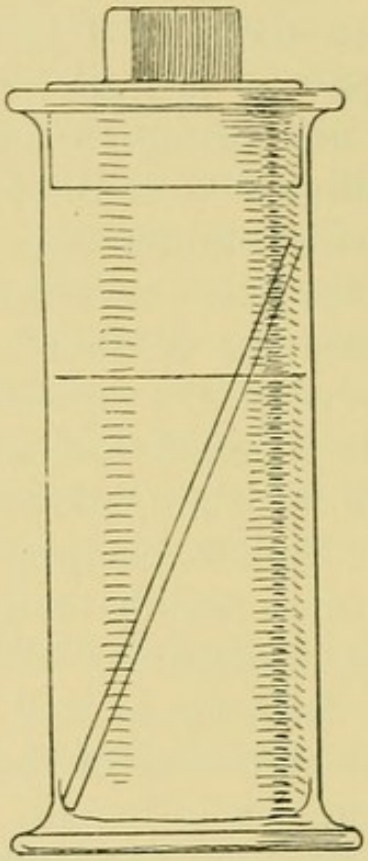


FIG. 26.

Giemsa's stain also gives good results with films fixed in absolute alcohol, but better results are obtained if methylic alcohol is used for fixation.

B.—*Staining solution also fixes.* These methods include the use of Louis Jenner's stain and the stain used in Leishman's modification of the Romanowsky method, as in both of these the methyl alcohol fixes the film. The method of using these stains for the examination of normal blood has been already described. It suffices for the demonstration of all protozoa. The stains, particularly Leishman's, give most brilliant results, and show more points in the structure of parasites than

any other method. The disadvantages are: (1) The necessity of having distilled water, though where the rainfall is heavy and away from the sea rain-water can often be used; (2) methyl alcohol is very volatile; (3) the stains, under circumstances not thoroughly understood, seem to lose their strength in the Tropics, and consequently are not so universally reliable as the simpler stains first described.

Louis Jenner's stain in particular is unreliable, and seems to deteriorate either when kept in the solid condition or when dissolved. The usual failing is in the basic portion of the stain, and unless the nuclei of the leucocytes are stained a brilliant blue the stain is worthless for the demonstration of parasites. Leishman's stain also deteriorates, but can be made satisfactorily in the manner described. Both these stains must be rejected, however, if the normal constituents of the blood are not satisfactorily stained by them.

The results obtained by the use of these stains are so clear and good that it is a pity to discard them, and a film can be much more rapidly examined when stained by Leishman's method than when stained by any other. The worker must, however, be prepared to make up his own stain, and, if need be, to distil water before he is justified in trusting to these stains alone.

C.—*No preliminary fixation.* When parasites are scanty they may be easily overlooked if thin films only are examined. Thick films, if fixed, are too opaque for examination after staining.

A useful method with the larger parasites, is to make a very thick film and allow it to dry. When dry place in water, the hæmoglobin will be dissolved out and only parasites, leucocytes, blood platelets and fibrin, with the decolorized remnants of the blood corpuscles, will be left. Such decolorized films, when dry, can be stained with any of the basic stains. There is usually considerable distortion of the parasites, but many of them, particularly crescents, are quite recognizable.

Trypanosomes can be more readily found by this method than in thin films, but are so much distorted that results are often unreliable. A good stain to use with these decolorized films is carbol fuchsin, diluted with two parts of water. Ross prefers to decolorize the film with a weak aqueous solution of eosin, and counter-stain with a weak solution of polychrome blue. It is a useful diagnostic method, but not suitable for obtaining good specimens of the more delicate parasites, and without considerable practice mistakes are frequently made.

General Summary of the Development of the Hæmosporidia.

The Hæmosporidia are parasitic in their entire existence, and require for complete development two hosts: the one a warm-blooded animal, and the other usually an insect. In the warm-blooded host reproduction takes place asexually, by the breaking up of each organism into a number of young forms or spores.

Each of these spores enters a red corpuscle, and when it has reached its full development it, in turn, breaks up into spores. This is the ENDOGENOUS or ASEXUAL CYCLE OF DEVELOPMENT—SCHIZOGONY. The host, during this cycle, is the INTERMEDIATE HOST. The parasites which develop in this manner are known as SCHIZONTS, and the individual spores as MEROZOITES.

Some of the merozoites, however, instead of becoming schizonts, develop into the sexual or GAMETOCYTE form. These do not reproduce, or undergo any further change, whilst in the intermediate host. If they are taken up by the definitive host they become sexually active, conjugation takes place, and further development follows. The product of the conjugation, the fertilized female or zygote, increases in size and forms a cyst. The contents of this cyst divide into several masses, SPOROBLASTS, from which small, thread-like bodies, SPOROZOITES, are formed. These bodies, when introduced into a suitable animal—the intermediate host—become schizonts.

This cycle is a sexual one and is known as SPOROGONY, and the host during this period is therefore the DEFINITIVE HOST. Mosquitoes belonging to several genera of the *Anophelinæ* are therefore the definitive hosts of the parasites of malaria, whilst man is the only known intermediate host.

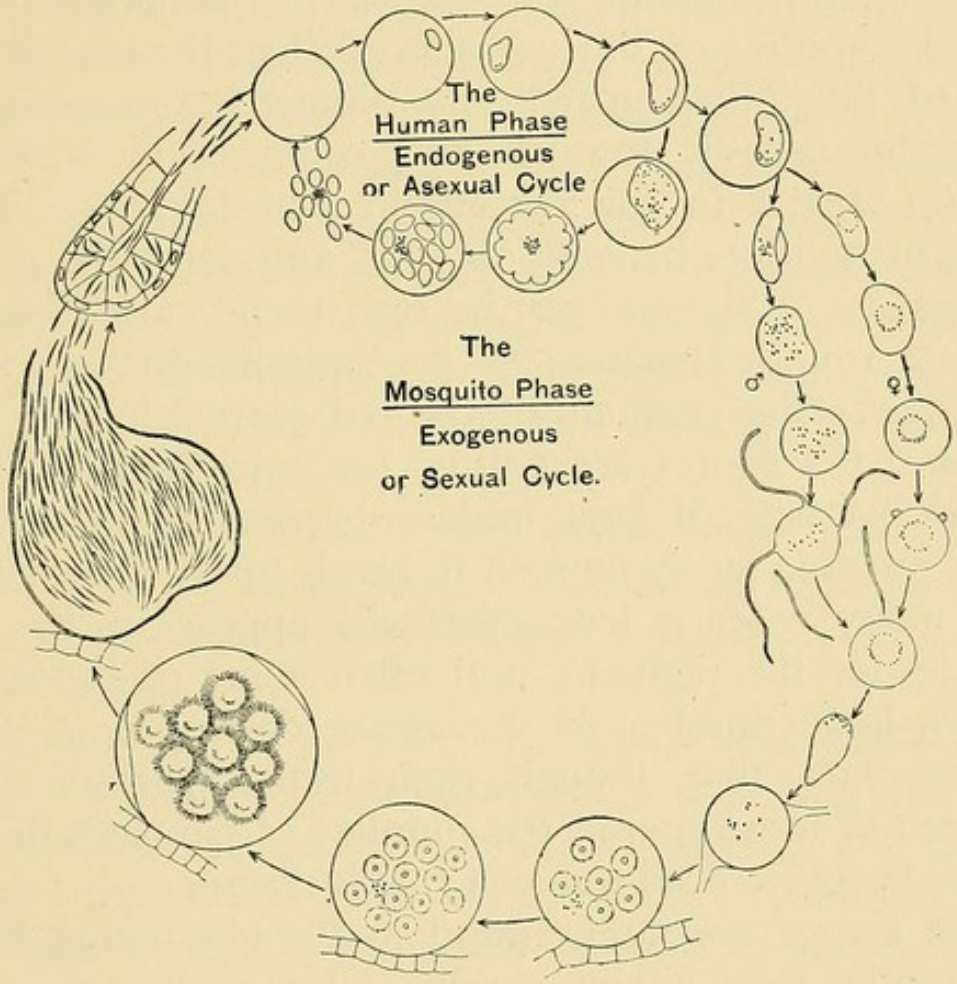


FIG. 27.

The diagram (fig. 27) represents in a graphic form the two methods of reproduction. The smaller circle represents the asexual cycle of reproduction, and the larger the conjugation of the male and female sexual forms, with the further development of the fertilized female.

Parasites of Malaria.—As seen in fresh living blood the youngest form of malaria parasite is a small, white, rounded body in or on a red corpuscle. A clearer portion can sometimes be made out inside it. At this stage it varies, according to the species of the parasite, from

one-eighth to one-quarter the diameter of the red blood corpuscle. Even the youngest forms of the parasite often show at the edge some sign of amœboid movement. The parasite increases in size, and the amœboid movements become pronounced. A parasite seen a few hours later will be observed to be not only larger, but to have a few grains of pigment scattered about inside it. The colour of the pigment and the size of the grains varies with the species of the parasite. When the parasite has reached the fullest growth its pigment commences to aggregate, usually towards the centre, and traces of division appear in the surrounding protoplasm. These traces of division become obvious, and soon the central aggregation of pigment can be seen to be surrounded by separate rounded masses of protoplasm—the spores or *merozoites*. The remnant of the red corpuscle then gives way and the spores are poured out into the plasma. A mature parasite, if kept under observation on a warm stage, can frequently be seen to break up in this manner.

In many cases a leucocyte will appear in the field and devour the pigment, and often some of the spores. That a large number of spores are destroyed is shown by the fact that though each tertian parasite forms eighteen or more spores, the number of parasites in each successive cycle does not as a rule increase.

Free spores are rarely found in the circulating blood. Apparently they either rapidly take shelter in a red blood corpuscle, or are destroyed by leucocytes or in some other manner.

Stained specimens demonstrate some further points in the structure of the parasites. The youngest form, the amœbula, is shown to consist of a ring of protoplasm staining with basic stains, a clear unstained space, the vesicular nucleus, and a deeply stained spot, or nucleolus, usually in contact with the ring of protoplasm.

This is the type of the young form of all the hæmosporidia. They are all composed of a nucleolus staining deeply, a "vesicular" nucleus, which does not stain

with either acid or basic stains, and a surrounding protoplasm which stains, but much less intensely than the nucleolus, with basic stains. It is only this surrounding protoplasm which is amœboid, and consequently in the very young forms the range of amœboid movement is not very great, as this protoplasm is so scanty.

The increased growth of the parasite is mainly due to the increased growth of the ring of protoplasm, though the vesicular nucleus also enlarges. Pigment, the residue of the digested hæmoglobin, is deposited in this protoplasm only. With further growth the vesicular nucleus breaks up and disappears, and all that is seen is an irregularly stained parasite with pigment scattered through it. Later the pigment becomes pushed into one block, and the surrounding protoplasm is seen to be divided into masses, each with a deeply stained spot, the nucleolus of the young spore.

Beyond showing the "ring form," none of the simple stains, such as hæmatoxylin, thionin, methyl blue, &c., disclose any structural changes beyond those seen in fresh blood.

Romanowsky's method, or, better, Leishman's modification of this method, shows more markedly the structural changes, and, in particular, the varying arrangement of the chromatin. With this stain the youngest form, amœbula or ring form, is shown to have the chromatin arranged as a solid block — the nucleolus — which is stained deep ruby red. The ring of protoplasm stains blue, whilst the vesicular nucleus is unstained. At a later stage the chromatin, instead of being in a solid mass, is seen to be composed of scattered points, arranged at part of the periphery of the vesicular nucleus. Still later, when the vesicular nucleus disappears, points of chromatin are found diffused through the protoplasm. This is called "fragmentation" of the nucleolus. Still later the chromatin aggregates into small masses towards the periphery, and a secondary division of the masses takes place, resulting in the formation of a number of

small chromatin nodules, the nucleoli of the young spores.

When sporulation is complete each of these chromatin nodules is situated in the interior of a portion of the protoplasm of the parasite, and so forms the spore or merozoite. The pigment takes no part in the process, and with a small residual portion of the protoplasm of the parasite is pushed into a mass, usually towards the centre of the group of spores. When the corpuscle bursts and the spores are liberated, the pigment is devoured by leucocytes, usually the large mononuclear leucocytes. These thus become "pigmented leucocytes."

The chromatin in the parasites destined to become *gametocytes* undergoes different changes. The first stage is the same as in the young parasites, which will ultimately divide asexually into spores—the schizont. The chromatin in the young or "ring" form of the gametocyte, as of the schizont, is arranged in a solid block. This chromatin subsequently divides into separate granules, but does not become diffused throughout the protoplasm as it does in the schizont. In the full-grown gametocyte the chromatin, composed of numerous particles packed together, forms one mass in the interior of the parasite, surrounded by a zone free from pigment and staining feebly. The changes in the arrangement of the chromatin after the blood is shed and the gametocytes become sexually active, will be considered with the sexual or mosquito phase of the existence of the malaria parasite.

All the human malaria parasites, the similar parasites in other mammalia and birds, as far as is known, conform to this general type.

The distinctive points on which the division of the human parasites into distinct species is made are as follows :—

(1) Duration of the asexual cycle.
(2) Number of spores formed at each sporulation.
(3) Activity of movement.
(4) Preferential sites for sporulation.

(5) Differences in digestive processes in different parasites as indicated by the differences in pigment.

(6) Effect of the parasite on the corpuscle which contains it.

(7) Shape and appearance of the gametocyte.

The methods of examination described are ample for determining these points.

(1) *The length of cycle* can be readily ascertained in the case of parasites which sporulate in the peripheral blood. The blood is examined at intervals, so as to determine the length of time between the sporulation of a group of the parasites and the steady growth of this group up to the next period of sporulation. In benign tertian and quartan this is readily done, and it will be found that the period or length of cycle is approximately forty-eight and seventy-two hours respectively. It is difficult to determine in malignant tertian (æstivo-autumnal or sub-tertian) malaria, as only the young schizonts and mature gametocytes are common in the peripheral blood. The period for this species is certainly variable, and the parasites are commonly in several stages of growth, so that periodicity is not so clearly defined as in the other species of parasites of human malaria.

(2) *The number of spores* can be counted in the fresh or stained blood when the parasites are fully mature. If stained for chromatin, the number of spores can be counted earlier. It will be found that in benign tertian the spores are usually about 20, but may be as low as 15 or as high as 25, or even more. In benign quartan 12 is a maximum rarely exceeded, whilst 8, 9, or 10 are the common numbers. The number in sub-tertian is more variable—7 to 30.

(3) *The activity* of the amœboid movement can only be determined with certainty in the living blood. Internal movement in the parasite itself is also shown in the fresh fluid blood by movement of the pigment in the parasite.

Amœboid movements can be inferred in stained specimens, as the parasites present great varieties in shape, and frequently where amœboid movements have been active when the film is dried, the pseudopodia can still be seen.

(4) *The selective site* for sporulation is of great importance, as one species, the malignant tertian (sub-tertian) sporulates almost exclusively in the internal organs, and the occasional malignant clinical course of the disease caused by this parasite is due to the selection of the brain or other important organ as a site for sporulation.

The absence of full-grown forms and the determination of the absence of sporulating forms indicate that the parasites are sporulating elsewhere, *i.e.*, in the internal organs. *Post-mortem* examination of fatal cases shows in which organs the sporulating parasites are, but the clinical symptoms often give a clue.

Benign tertian parasites sporulate to a considerable extent in the circulating blood, though the splenic sinuses are their preferential resort at this period. Quartan parasites sporulate freely in the circulating blood, whilst sub-tertian (malignant tertian) is hardly ever found sporulating except in the visceral capillaries.

All the phases of benign tertian and quartan can be observed in the blood obtained by pricking the finger or ear, and therefore the determination of the length of the cycle with these parasites is easy. With malignant tertian, on the other hand, the stages of sporulation, or even the full-grown schizonts, are rarely to be observed in the peripheral blood. The full-grown gametocytes are common in the blood, but the intermediate stages of growth cannot be found except in the visceral capillaries. Puncture of the spleen in the living subject may show these forms. If undertaken aseptically the operation is considered to be practically free from risk to the patient; but as accidents have occurred this method should not be employed except in cases where certainty of diagnosis is absolutely necessary.

In fatal cases with cerebral symptoms, the sporulating and full-grown forms can be observed in enormous numbers in the brain and often in other organs—lungs, suprarenals, liver, &c. In other fatal cases they may be found in greatest numbers in the intestinal mucosa, pancreas, and rarely in the kidneys.

The organ in which the parasites are most commonly found *post mortem* is the brain, and cerebral symptoms are common in so many cases that recover that it seems probable that this is a favourite site. It must be remembered, however, that, as the blocking of the cerebral capillaries is the most common cause of death in acute malaria, the proportion of fatal cases with this complication gives an exaggerated idea of the frequency with which this site is selected by the parasites.

For diagnostic purposes it suffices to take a small portion of the fresh brain substance and squash it between the slide and cover-glass. The capillaries in a case of cerebral malaria will then be seen to be filled with grains of black pigment. Though the parasites themselves cannot be seen, these grains of pigment are diagnostic, as they are contained in the full-grown or sporulating parasite.

It is not absolutely necessary to open the skull, though it is better to do so. The needle of a large exploring syringe can be forced through the orbital plate of the frontal bone and the brain stirred up a little; suction with the syringe will then usually bring away sufficient brain matter for examination. As the puncture is made through the conjunctiva no disfigurement results, and the site of puncture will be covered by the eyelid.

The vessels on the *pia mater*, particularly at the base of the brain, are frequently pigmented. This pigmentation must not be confused with malarial pigmentation. The pigment is not contained, as it is in malaria, in the capillaries, but in their walls, and is insoluble in alkalies which readily dissolve melanin. The finely granular or streaky arrangement of this natural pigmentation differs from

the coarser arrangements of the melanin particles, and the colour is brown, not black. This pigmentation, non-malarial, occurs in all races, but is commoner in the coloured races. It is found in new- or still-born children whose organs are free from malarial pigmentation.

To demonstrate the arrangement of the pigment granules of malaria in a hardened brain, thick sections should be cut. These can be quite easily cut by hand, and without any staining passed through absolute alcohol and then oil of cloves to dissolve the fatty brain constituents and render the section transparent. The section can then be mounted in balsam, and in a malarial case every capillary will then be seen to be mapped out by the contained pigment granules almost as if it had been injected.

These methods, though useful for rapid diagnosis, do not show the parasite. With the fresh brain specimens, whether a squashed fragment or a fragment drawn out with the exploring springe be examined, parasites will often be seen in corpuscles which have escaped from the capillaries.

To show the parasites well it is necessary to stain them. With the fresh brain it is not necessary to cut sections nor is it advisable. A smear should be made of the brain substance, and this should be dried rapidly by waving it in the air—not by the application of heat. The smear need not be very thin, as the greater part of the brain matter is subsequently dissolved. The smear can be stained by Leishman's method, but must then be thoroughly dried to dehydrate, and mounted in xylol balsam. This method shows the chromatin in the parasites, but the drying causes much distortion of the surrounding tissues.

If this method be not adopted, hæmatoxylin gives good and permanent results, and carbol thionin also gives very good results. The procedure is as follows : Fix the smear in absolute alcohol or alcohol and ether for ten minutes and allow to dry.

To stain with hæmatoxylin, cover the smear with a hæmatin solution and leave for ten minutes. Flush off the stain and place the slide in water for five minutes. Dehydrate with spirit and oil of cloves. Mount in xylol balsam.

With carbol thionin the procedure is rather more complicated and requires more care. It is, however, a general method, and is a suitable one also for the demonstration of vegetable micro-organisms in tissues.

Fix the smear in absolute alcohol as before, and cover it with the strong carbol thionin solution. Leave for ten to fifteen minutes. It is essential that at this stage the specimen should be very much over-stained, as much stain is lost in the subsequent processes. Flush off the stain with water. Pass rapidly through methylated spirit, *not* absolute alcohol. Much stain will come away, and care must be exercised that the specimen is still over-stained when removed from the spirit. The time the specimen is left in the spirit is determined entirely by the colour. It cannot be completely dehydrated at this stage, or too much colour would be removed. Drain off and *gently* blot off excess of spirit. Cover with oil of cloves and place under the microscope. The oil of cloves will dissolve out the brain fatty matter, complete the dehydration, and slowly remove the excess of stain. When it is observed that nearly enough stain is removed, a cover-glass can be placed over the specimen and an examination made with an oil immersion lens. If the specimen is well stained the cover-glass can be removed, and the specimen placed in xylol to remove the oil of cloves, which would otherwise ultimately decolorize the specimen. Finally it is mounted in xylol Canada balsam.

Specimens of brain hardened in absolute alcohol can be used. Thin sections are required, embedded in paraffin for choice. The processes are the same as for brain smears after the paraffin has been removed from the specimen by xylol, the xylol by alcohol, and the

alcohol by water; but the section must not be allowed to dry at any stage.

Van Gieson's method is also useful for staining parasites in tissues. Van Gieson's solution is composed of 1½ per cent. acid fuchsin dissolved in a saturated aqueous solution of picric acid.

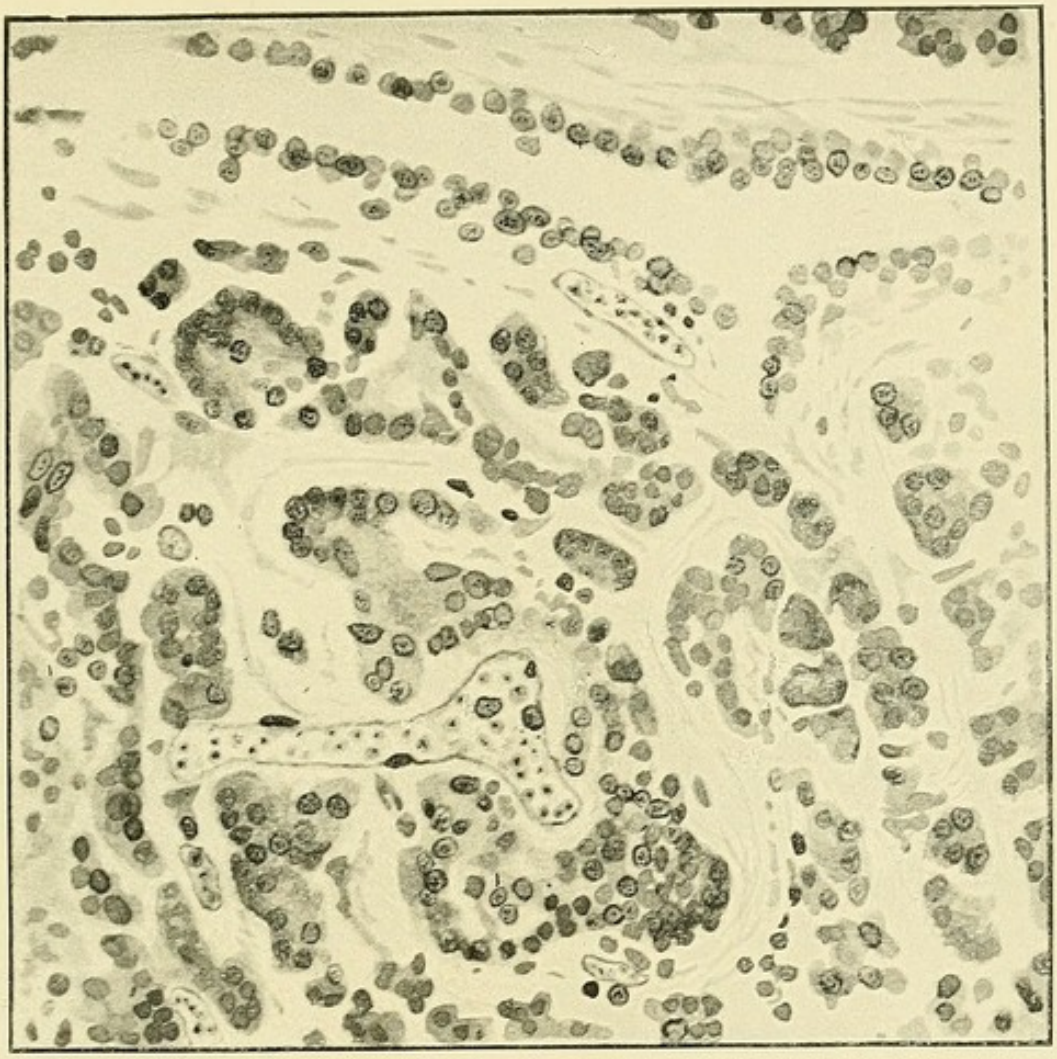
The method is as follows: Remove the paraffin with xylol, the xylol with spirit, and the spirit with water in the usual way. Stain with hæmatoxylin or hæmalum for ten to fifteen minutes and flush off the stain with tap water. Leave the specimen in tap water for five minutes to "blue." Then treat with van Gieson's solution for half to one minute, not longer. Wash this solution off with *spirit*, not with water, clear with oil of cloves, and after removing the oil of cloves with xylol, mount in xylol balsam.

The parasites and the nuclei of cells are stained with the hæmatoxylin, the protoplasm of the tissue cells with the picric acid, whilst fibrous tissue is stained red with the acid fuchsin.

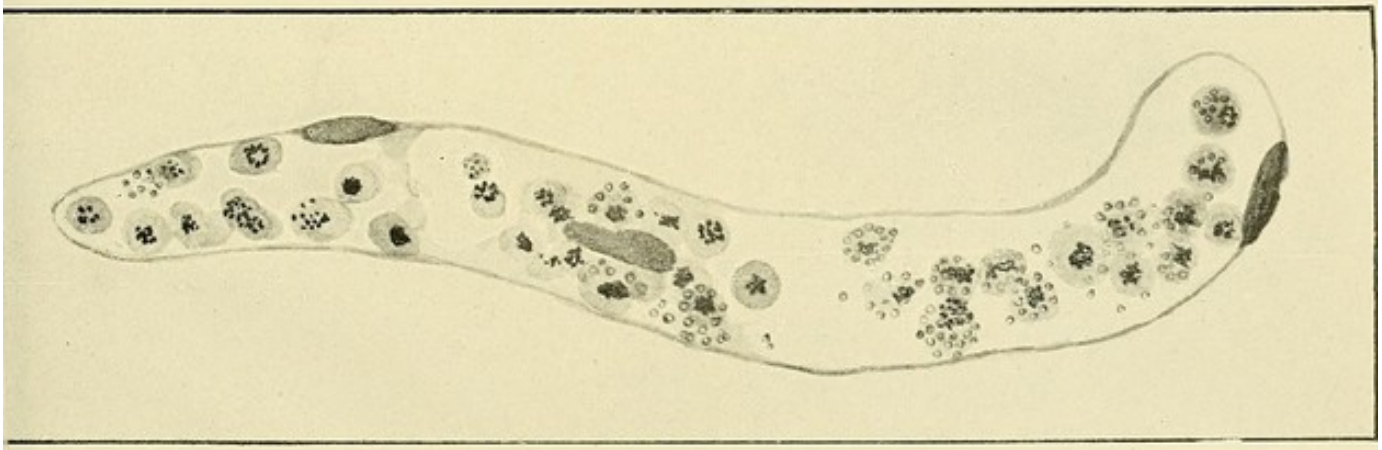
The parasites in sections show well, but are smaller, only about half the size of those in the smears made from the fresh brain, as the fixative agent causes much shrinking (Plate IV., 3*a*, 4*a*). As this parasite is the smallest of the human malaria parasites, and when full-grown often little more than half the diameter of the red blood corpuscles, there is in these shrunken specimens considerable difficulty in making out the spores into which the parasites are broken up.

In these specimens the corpuscle containing the parasite is not lying singly or flat, as it is in the blood-film, or smear, but is one of the many corpuscles packed into the capillary, so that it is exceptional for the outline of the corpuscle containing the parasite to be made out (fig. 28).

These methods are *general* methods for the demonstration of protozoa in tissues. With carbol thionin bacteria are also shown.



a.—Pancreas.



b.—From Intestine.

FIG. 28.

e p. 86.

In the large vessels parasites are not so common. In the small vessels the corpuscles containing the parasites are often found only in contact with the wall of the vessel, and no parasites are contained in the corpuscles towards the centre of the vessel. The largest number of corpuscles containing parasites are in the capillaries.

This occurrence in the minute capillaries results in a blood stasis more or less complete. Such a stasis involving a large part of the brain results in headache, drowsiness, and coma in adults, rarely delirium, and in convulsions and coma in young children, and is the most common cause of death in acute malaria. The process is often spoken of as *thrombosis*. This is incorrect; there is no coagulum formed; no fibrin, and the leucocytes are not aggregated in the capillaries and take no part in the process. Clinically, where active treatment is adopted we have abundant evidence that the condition is a transient one. Speedy and complete recovery from the condition of complete coma frequently takes place under energetic treatment with quinine.

The parasites themselves are usually at different stages. Quite young parasites, hardly larger than the spores, may be found. More commonly the great majority of the parasites contain centralized pigment and have lost their vesicular nucleus. In some specimens a large proportion, in others a small proportion, will be found sporulating.

The number of spores varies greatly, and in some specimens only seven or eight spores will be found to each parasite. In other cases the number will be twenty or more.

This variation in the number of spores is one of the distinctions on which reliance has been placed for the subdivision of this species into three.

In most parts of the body a temporary partial stasis of the blood in the capillaries leads to no sudden fatal changes or symptoms, and consequently, unless the brain is also involved, a fatal result is not common. There are, however, peculiar risks attending another region—

the intestines. Stasis occurring in the capillaries of the mucosa impairs the vitality of the cells and renders them liable to be invaded by some of the bacterial contents of the alimentary tract. Secondary inflammation and superficial necrosis may thus result, and so indirectly, by lowering the nutrition of the mucosa, a fatal enteritis may be set up. There is some reason for believing that sufficient attention has not been paid to the indirect results of repeated blood stasis in the various viscera, consequent on malarial infection.

The parasites in these and other situations are best demonstrated in sections stained with hæmatoxylin, carbol thionin, or Van Gieson, as already described for the brain. Carbol thionin has the additional advantage of staining the micro-organisms which have invaded the mucosa. These, however, can be shown in separate specimens somewhat better, particularly those micro-organisms which retain their stain when treated by Gram's method.

(5) *Melanin* malarial pigment, or simply "pigment," is the residue from the digestion of hæmoglobin, and contains the excess of iron over the minute amount required by the parasite.

In the different species it is deposited in different forms. In the quartan it is deposited as granules, which are coarse and black, and in the benign tertian the colour varies from a yellow-brown to a dark brown, while it is always in fine granules; in the sub-tertian the pigment is not commonly seen in the early forms present in the peripheral blood. When it is found it is in fine, black granules, which aggregate into a mass earlier than in the other forms of parasites.

(6) The parasite affects the corpuscle containing it in different ways.

In quartan fever, although the parasite is in the interior of the corpuscle, the bulk, or at any rate the diameter of a corpuscle containing the parasite, is slightly below the average in the majority of instances. The

colour of the red corpuscle is not lighter, and is frequently a trifle darker than the average of the red corpuscles.

In benign tertian there is a great difference, as the diameter of the corpuscle is decidedly above the average and the corpuscle is pale. This is well seen both in stained and unstained specimens. The corpuscle is easily distorted, and is consequently frequently compressed by

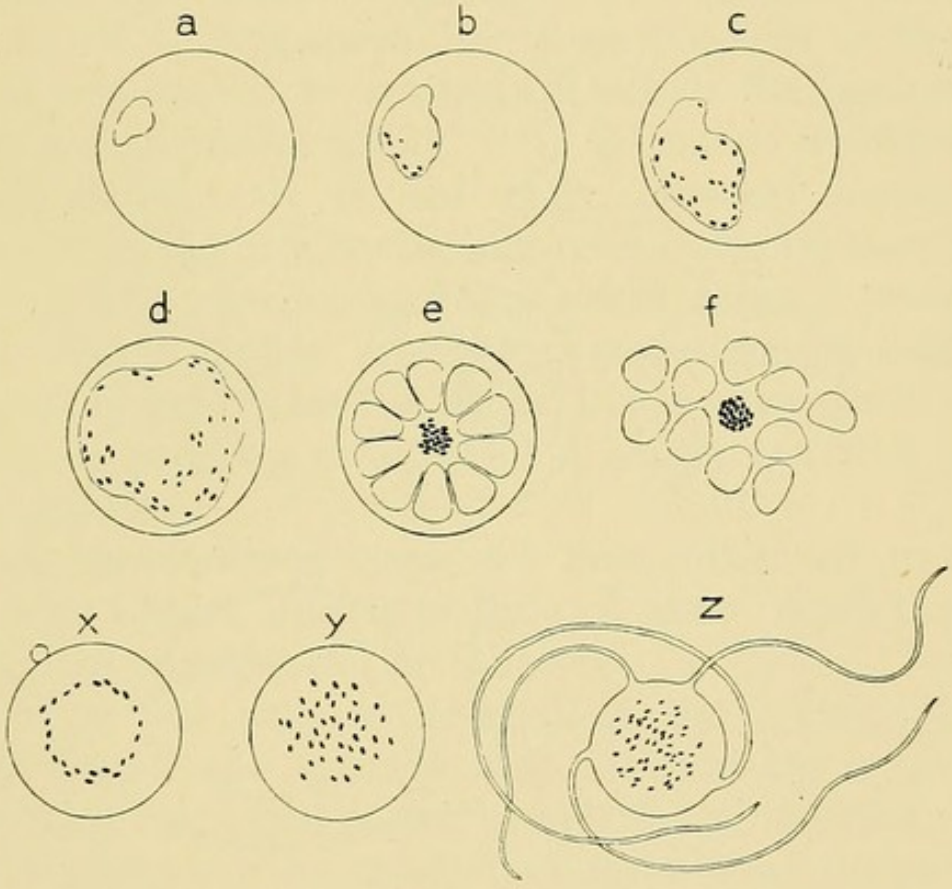


FIG. 29.—*a* to *f*, Phases in the asexual development of the quartan parasite; *x* to *z*, phases in the sexual development.

neighbouring blood-cells, when these are in contact with it. Leishman's and some other similar stains reveal still further changes, as granules staining deeply red are found throughout the corpuscle in parts not invaded by the parasites. These granules are small and not very distinct when the parasite is very young, but with the growth of the parasite become more numerous and more distinct (Plate VI., 7, 8, 9). They are not found with

the other human malaria parasites, nor with the hæmamœbidæ of mammals and birds, but are found in corpuscles infected by one species of hæmogregarine in the frog (Plate V., 21.)

To show them well, the mixed water and stain in Leishman's method must be left on double the normal time, and fifteen minutes is not too long with most specimens of the stain.

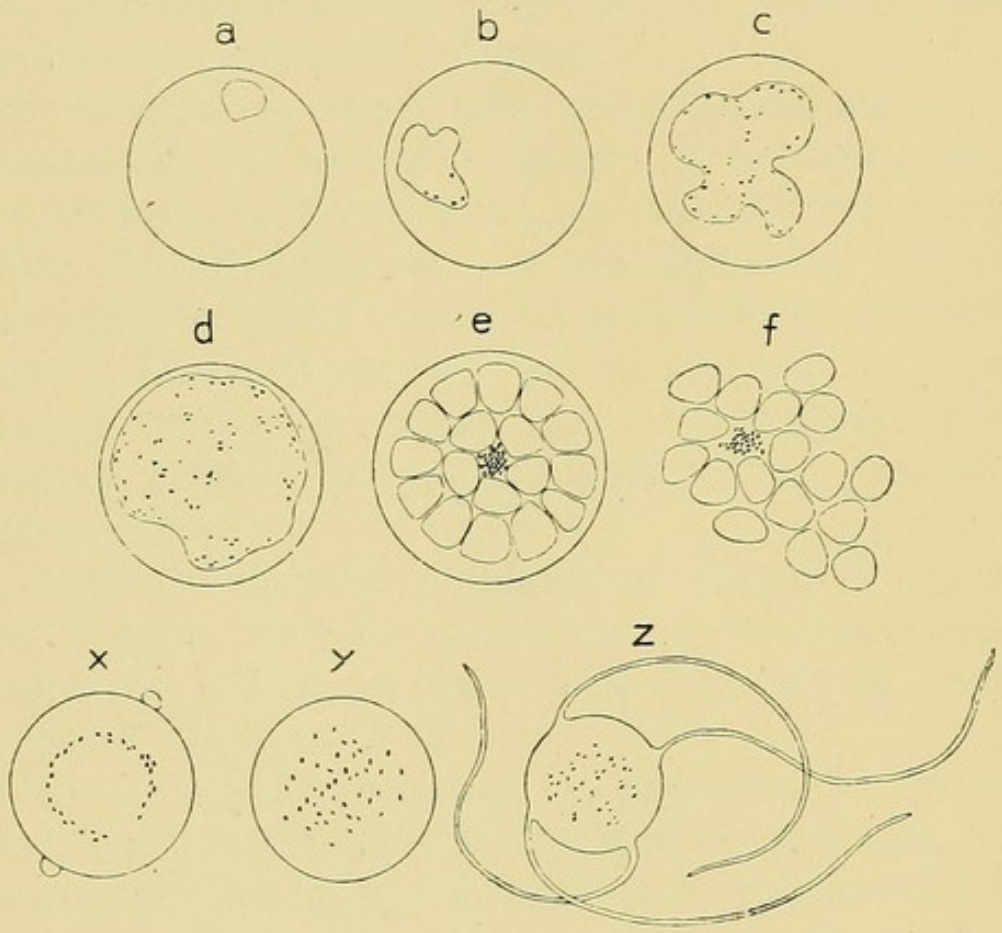


FIG. 30.—*a* to *f*, Phases in the asexual development of the benign tertian parasite ; *x* to *z*, phases in the sexual development.

These granules are known as Schüffner's granules or dots, and must not be confused with the basophilic granules, which stain blue by the same method, found in some instances abundantly in malarial blood, as well as in other diseases.

The effect of the malignant tertian parasite on the corpuscle is variable. The young forms met with in peripheral blood do not appreciably affect it, but occasionally the corpuscle is changed in colour to a yellowish

or brassy tinge. With the more advanced stages of the parasite the corpuscle, without increasing in size, becomes more or less decolorized. In the full-grown parasite, whether schizont or gametocyte, the remnant of the red corpuscle is a mere colourless shadow, which is not always easy to define (Plate IV., 10 to 19).

(7) The shape of the gametocyte in benign tertian and quartan is that of a rounded body indistinguishable by its shape from the full-grown schizont before sporulation has taken place. It can be distinguished by the presence of a clear space, free from pigment in the unstained specimen. In malignant tertian the gametocyte is the so-called "crescent." It is better described as sausage-shaped. It is not truly crescent-shaped, as, though often slightly curved, the two ends are broad and rounded, not tapering to sharp points as in a true crescent. These bodies always contain pigment, and this pigment is never in a solid block, but is always composed of discrete particles grouped in a cluster near the centre of the parasite.

These gametocyte forms are best observed in fresh fluid blood-films, and it is only in such films that the subsequent changes can be followed.

As it is not easy to distinguish the gametocyte of benign tertian and quartan malaria from the sporocyte, the subsequent changes are most readily observed in the case of malignant or sub-tertian malaria, as the peculiar "crescent" shape renders the identification of the gametocyte easy in this form of malaria.

These changes only take place when alterations in the blood occur, such as abstraction or addition of water. The crescents lose their peculiar shape, throw off the remains of the containing corpuscles, and become first oval and then spherical. Small portions, one or two, are extruded, the polar bodies, and remain usually adherent to the outer surface of the altered crescent.

Of these altered crescents, a proportion which varies in different specimens throw out long, filamentous flagella,

varying in number from two or three to six. These flagella are actively motile and lash about in the blood plasma, or over neighbouring red corpuscles for some

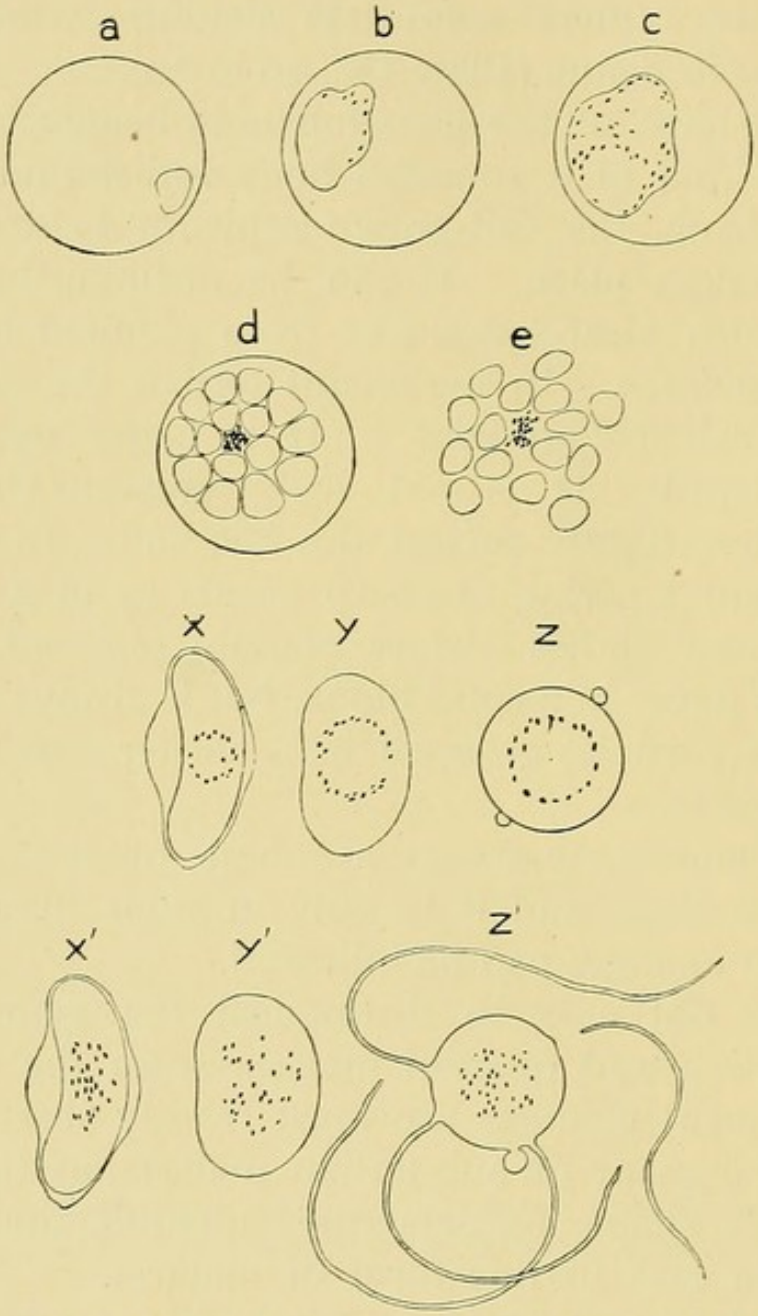


FIG. 31.—*a* to *e*, Phases in the asexual development of the malignant malarial parasite; *x* to *z*, phases in the sexual development—x *y z*, of the female: x^1 y^1 z^1, of the male.

time. Finally they break away, and can be seen moving rapidly through the plasma.

The crescents which undergo this change and flagellate are the males, and the flagella are equivalent to spermatozoa, and are known as MICROGAMETES. The residue of the crescent is a small protoplasmic mass containing

all the pigment. It soon dies, and is either broken up or devoured by a leucocyte.

The other altered crescents do not flagellate. After the extrusion of the polar bodies they retain their spherical shape, but the pigment in the interior is often in a state of violent agitation. They form the MACROGAMETES.

Very rarely a flagellum that has broken off the male crescent is seen to enter this rounded body, and is absorbed by it. These mature crescents which do not flagellate are the females, and the entrance of the flagellum is a process of fertilization. After fertilization further changes take place, the pigment is thrown into violent agitation, the whole body changes shape, one end becomes conical, and the body becomes actively motile, moving steadily through the blood serum.

This fertilized female, as it is the product of conjugation, is a zygote, but is known, whilst motile, as the "travelling vermicule" or OOKINET, and passes into the outer wall of the mosquito's stomach beneath the epithelial lining where it becomes encysted, and for this stage the general term ZYGOTE only is employed.

The male and female crescents can often be distinguished in the freshly-shed blood by the arrangement of the pigment. In the female there is usually a clear space in the middle surrounded by pigment, whilst in the male no such clear space is present and the pigment is less in a ring and more in a clump than in the female.

The young forms of crescents are sometimes found in the brain, spleen and elsewhere; they can be distinguished by their shape and the tendency of the pigment to be arranged in a central, irregular clump, and not in one mass.

The same series of changes occur in the gametocytes of benign tertian and quartan malaria after the blood is shed.

The gametocytes stain rather feebly with basic stains. The outlines of the red corpuscles which contain them can usually be made out, though, as nearly all the hæmoglobin is absorbed, the remnant does not stain, or only faintly, with eosin.

The gametocytes contain chromatin in considerable quantity, but this chromatin, though it stains with the red of the polychrome methylene blue, does not stain with hæmatoxylin or most basic stains.

Stained by Leishman's method, the gametocytes of benign tertian and quartan are easily recognized, as the chromatin granules are collected in a clump surrounded by an unstained area free from pigment (Plate VI., 6).

In the crescents the chromatin in the female is collected into a solid block in the centre, and round this the pigment is arranged. In the male there is no central block of chromatin, but numerous scattered particles are mixed up with the pigment. In the males the chromatin may be very abundant (Plate VI., 18, 19).

To make permanent preparations showing the changes that occur in shed blood, it is necessary to prevent the blood from drying and to examine at intervals of a few minutes. For this purpose the slides with the fluid film on them must be kept in a moist chamber. This is easily done by cutting windows in a folded piece of blotting paper and placing this blotting paper on a slab. The blotting paper should be moistened, and the windows each covered with a slide on which is the wet film face downwards. The evaporation from the damp blotting paper will render the air so moist that evaporation from the film will be very slow.

One of these slides can be taken off and allowed to dry every five minutes, and in this way we have a series of blood-films five, ten, fifteen, twenty minutes, or more, after the blood has been shed.

To obtain stained specimens of flagellating bodies thick films can be used, and when allowed to dry the hæmoglobin may be removed by placing the slide in water. After this the film is again allowed to dry, fixed in alcohol, and stained with a strong basic stain, such as carbol fuchsin.

To observe well the changes that occur in the arrangement of the chromatin these decolorized films cannot

be used, but the specimen with a moderately thick film must, after drying, be stained by Leishman's method. In both male and female a portion of the chromatin will be seen to be extruded in the polar body. The remainder increases in amount, and most of it in the male will be seen in the form of nodules at the periphery of the altered crescent. Finger-like processes of the protoplasm will be seen to project from the vicinity of these masses, at first without any chromatin, and these processes elongate and form long, slender flagella without chromatin. Ultimately, however, the chromatin enters the flagella as a long, thin filament, leaving a mere residuum in the remnant of the crescent at the base of the flagellum. When the flagellum breaks loose it has this chromatin filament running nearly its whole length. Even when all the flagella have broken away there are still remnants of the chromatin in the protoplasmic residual mass left behind.

In the female the chromatin forms a less compact mass after the extrusion of the polar bodies, and it is with this mass that the chromatin of the flagellum which fertilizes it probably fuses.

Minor differences in the crescents as regards shape, staining reaction, and colour of the pigment are described by those who subdivide the malignant or sub-tertian into two or more species.

As regards the genesis of the gametocytes, suggestions have been made from time to time that they may be formed by the union of two young parasites in one corpuscle. Two, or even three or four, parasites are not uncommonly found in one red corpuscle. These parasites may be in actual contact with each other, but there is no satisfactory evidence that conjugation or fusion of two such parasites ever takes place. On general grounds there is little or nothing to support this hypothesis of the formation of the male and female sexual forms. Schaudinn believed that the development of the gametocyte was slower than that of the sporocyte. As

occasionally we find a mature sporocyte and a full grown gametocyte in the same cell this is not probable.

The differences between the three main species of parasites are shown in tabular form on the next page (Table, p. 97.)

Mistakes can be made with every method of blood examination, but most of them after a little experience are easily avoided.

In fresh fluid blood-films the following are often mistaken for young non-pigmented parasites.

(1) The normal lighter colour of the central portion of the corpuscle, due to the bi-concave shape of the red corpuscle. The gradual shading and the absence of any definite edge to the lighter part is usually sufficient to prevent this error, and familiarity with this appearance in normal blood is of importance.

(2) Vacuoles or slits in a blood corpuscle are distinguished by the very sharp, abrupt edge of such a vacuole and by the oscillatory motion of the edge. It can be generally seen that whilst in a parasite there is a faint opalescence, in the vacuole the space is perfectly clear (*vide* fig 19, c).

(3) Blood plates resting on a blood corpuscle are in some cases difficult to distinguish. Round such blood plates there is usually a ring or "halo" where the hæmoglobin has been pressed away, and in some cases by focussing it can be determined that the body is one which is on and not a part of the red corpuscle.

(4) Small particles resting on a corpuscle will displace the hæmoglobin beneath them and cause a lighter-coloured patch in the corpuscle. Such particles, if dark, are often mistaken for pigment, and the pale area is taken for the parasite.

(5) Crenations, particularly when they occur as projections on the upper or lower surface of a corpuscle, are frequent sources of error. The effect of focussing, or alteration of the illumination, will show the true nature of these crenations (*vide* fig. 19, a, b).

	1 Length of Cycle	2 Number of Spores	3 Activity of Movement	4 Selective Sites for Sporulation	5 Character of Pigment	6 Effect on Red Corpuscles	7 Form of Gamete
TERTIAN (Benign tertian) *P. vivax*	48 hours ...	15 to 25 ...	Very active	Common in the circulating blood, but most abundant in spleen	Yellowish-brown fine granules	The corpuscle becomes swollen and pale. With special stains Schüffner's dots are found	Rounded body
QUARTAN (Benign quartan) *P. malariæ*	72 hours ...	5 to 12 ...	Usually sluggish	In circulating blood	Black, coarse granules	The corpuscle becomes smaller and darker	Rounded body
MALIGNANT TERTIAN (Sub-tertian) Æstivo-autumnal *P. falciparum*	Variable and difficult to determine; probably about 34 to 48 hours	Varies greatly in some cases; 7 or 8 are the common numbers, whilst in other cases 20 or more spores are common	Very active, but the processes are small	In internal organs, brain, lungs, intestines,&c. Very rarely found in circulating blood	At first fine and black, but aggregate into masses earlier than in the other parasites	At first little visible change, but later corpuscle is decolorized. In some cases the corpuscle changes in colour, becoming yellower "brassy bodies," and in others granules like large Plehn's bodies are present, Maurer's granules	"Crescent"- or sausage-shaped body

(6) Bent or twisted "buckled" corpuscles may cause confusion (fig. 19, *d* and *e*).

Many effects are mistaken for pigmented parasites. Some of these are due to insufficient illumination, as refraction effects with a dim light closely simulate grains of pigment. Crenated corpuscles, leucocytes, &c., are thus sometimes taken for pigmented parasites. Full illumination will dispel this illusion. Particles of dirt, or epithelial fragments with specks of dirt adhering, usually overlap at one edge or other the red corpuscle on which they lie. If they do not, by focussing it can often be determined that they lie on or beneath the red corpuscle. In most cases such fragments can be distinguished by their sharp angular outline, the irregularity in the size of the grains of dirt they contain, and by their high refractive index.

Flaws, specks of dirt, or grease on slides or cover-glasses may cause confusion, but may be distinguished in the same manner. In any case of doubt it is well to touch the edge of the cover-glass with a needle whilst observing the object, and in that way it will be seen that the movement of the object is independent of the corpuscle that was supposed to contain it.

In stained specimens there are similar fallacies, and, in addition, dirt from the stain, precipitated grains of stain, yeast cells, or other micro-organisms, may be present. It is well in any case of doubt to examine some part of the slide where the stain has extended beyond the blood film, and see if the same appearances are presented there.

In the great majority of cases, if the appearances met with in normal blood have been carefully studied, particularly the blood plates and the various forms of degeneration of blood cells and of stained precipitates, mistakes are rare. Very rarely we do get an appearance from stain precipitates deposited on a red corpuscle that is difficult to distinguish, and therefore we should avoid diagnosing malaria from a single body believed to be a

parasite. It is better in case of doubt to look carefully for a second parasite.

Crescents should never be diagnosed on the ground of the shape only. A crescent always contains pigment, and is longer than the diameter of a red blood corpuscle, and stains with basic stains. Three blood plates arranged in a row may be about the same size and shape as a crescent, but do not contain pigment or stain like a crescent. A transformed or altered crescent can be mistaken for a quartan parasite.

Groups of blood plates are sometimes taken for sporulating bodies, and if they surround, as they may, a mass of dirt, the mistake is easily made. Even in fresh fluid blood the peculiar appearance of the edges of blood plates should prevent this mistake, and in stained specimens the manner in which the blood plates stain will enable them to be recognized.

Imperfect fixation is a cause of some errors. In specimens fixed by heat, or fixed in alcohol that has absorbed water, small round bodies, artificial vacuoles, water or air, are often found in the red cells. They may be numerous in each corpuscle, or only one or two may be present. The sharp edge and high refractivity of these bodies, as well as the variation in size, distinguishes them from parasites.

Familiarity with the appearances of blood prepared in different ways is necessary, so that these appearances will cause no difficulty, and the recognition with certainty and rapidity of parasites will then be easy.

Leishman-Donovan bodies, which are described later, are found in kala-azar in the leucocytes of the peripheral blood, but usually they are very scanty. The large mononuclear leucocyte is the variety in which they are ordinarily found, but they also occur in the polymorphonuclear leucocytes. They can be distinguished by the double chromatin mass—a smaller deeply-staining mass, and a larger, more lightly-stained one (*vide* Parasites in tissues).

CHAPTER V.

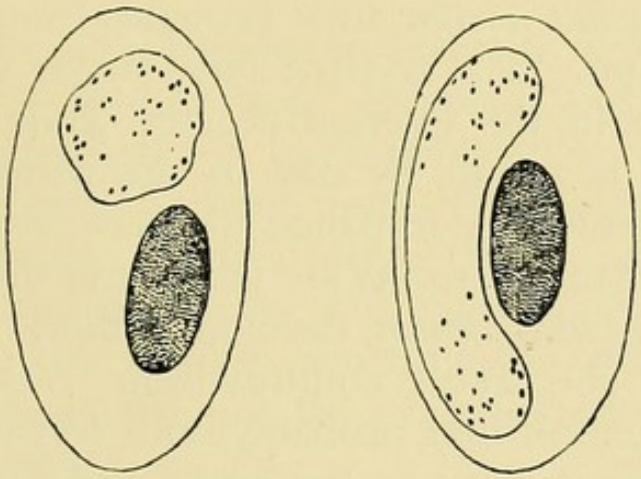
Parasites found in the Blood of Animals.

Birds harbour two well-known species of intracorpuscular parasites—proteosoma and halteridium, and others occur.

Proteosoma (*Proteosoma grassi*, fig. 32*a*) is found in comparatively few species of birds. Amongst these the Indian sparrow is the most common. Working with this parasite, Major Ross first demonstrated the sexual cycle of the hæmosporidia in a mosquito. Proteosoma in birds has many resemblances to the malaria parasite of man. In its earliest stage it is unpigmented; with continued growth pigment appears, and as the parasite grows it displaces the nucleus of the affected red blood corpuscle, and the corpuscle becomes paler. All stages of development, from ring forms to sporulating forms, are found in the blood at the same time. The gametocytes are very similar to those of benign tertian malaria. The infection is readily transferred from one bird to another by inoculation, and also to other birds, such as canaries. It is naturally transmitted by mosquitoes (*Culex fatigans*).

Halteridium (Plate V., 15) is a common parasite of many species of birds; it is found in pigeons, crows, jays, finches, parrots, &c.—often a high percentage of the birds of a susceptible species are found to be infected. The infection cannot be transferred from one bird to another by inoculation, and the method of its transmission in nature is doubtful. Recently it has been shown that infection may be transmitted from one bird to another by a fly belonging to the Hippoboscidæ and

known as *Lynchia maura*. It was with this parasite that MacCallum first demonstrated the fertilization of the macrogamete by the microgamete. This parasite is characterized by its peculiar curved shape surrounding the oval nucleus, but not displacing it as a rule. It does not cause any change in the red corpuscle which contains it. Halteridium resembles proteosoma in producing pigment, but is easily distinguished from it by its position in the cell (fig. 32*b*).



a *b*

FIG. 32.—*a*, Proteosoma; *b*, halteridium.

The forms of the parasite as seen in the peripheral blood are probably all sexual forms.

If fresh fluid films of blood containing this parasite be examined it will be seen that the halteridia leave the red cells and go through a similar series of changes like the "crescents" of sub-tertian malaria. The males proceed to flagellate, whilst the females are passive and receptive, and are fertilized by the microgametes with the production of an oökinet. In dry films stained by Leishman, or one of its closely-allied stains, the males can be easily distinguished from the females, the protoplasm of the latter staining a deep blue, whilst in the males the protoplasm is only very faintly stained.

The asexual phases of the parasite have not been clearly demonstrated.

Halteridium has attracted a good deal of attention in view of Schaudinn's remarkable observations on the life-cycle of *Trypanosoma noctuæ*, a trypanosome occurring in the blood of the little owl (*Athene noctuæ*). According to Schaudinn this flagellate attaches itself to the red blood corpuscle by means of its flagellum, and, gradually penetrating it, comes to lie by the side of the nucleus. In this process the trypanosome loses its flagellum and undulating membrane, and the blepharoplast becomes closely applied to the nucleus. The flagellate *Trypanosoma noctuæ* thus becomes transformed into the halteridium. The trypanosome stages are found at night chiefly in the internal organs, while the halteridium phase is found by day in the peripheral blood. When fully grown the trypanosome may divide asexually, or, if the gametocyte forms are taken into the stomach of the mosquito *Culex pipiens*, develop further. Schaudinn's observations still await confirmation. Some observers are of the opinion that probably both trypanosomes and halteridia were present in the birds, and that Schaudinn confused the changes occurring in these two classes of parasites. The frequency with which flagellates are found in insects adds to the doubt as to Schaudinn's hypothesis.

Various other parasites allied to those of human malaria have been found in animals. African monkeys harbour a parasite (*Plasmodium kochi*) closely resembling the sub-tertian parasite of man, and some species of Asiatic monkeys, bats, and flying foxes harbour parasites which are not unlike those of quartan malaria.

In snakes a pigmented parasite closely resembling a large halteridium has been described, and is known as *hæmocystidium*. Nothing is known of its life-history or mode of propagation.

PIROPLASMATA (BABESIA).—Another class of parasite, of which several representatives have been found in the blood of animals, is piroplasma. These parasites differ from the *Hæmamœbæ*, such as the parasites of malaria,

in that they form no pigment, that the nucleolus does not fragment, that division is into two or four only, and in the frequency with which extra-corpuscular forms are found. All the parasites of this class which have so far been investigated, have been shown to have as an intermediate host some species of tick of the sub-family *Ixodina*. The first of these to be discovered was *Piroplasma bigeminum* (Plate IV., 24), the cause of Texas fever in cattle. It is transmitted in America by *Rhipicephalus annulatus*, and in Africa and Queensland by *R. australis*.

P. parvum causes Rhodesian fever of cattle. It occurs in the blood in bacillary, spherical, and intermediate forms. It is transmitted by *Rhipicephalus appendiculatus*. An animal may harbour both *Piroplasma bigeminum* and *P. parvum* at the same time. *P. bigeminum* may be conveyed from one animal to another by inoculation, but similar experiments with *P. parvum* have been negative, and the offspring of infected ticks are not infective.

P. canis is the cause of epidemic jaundice of dogs. It is carried by *Hæmaphysalis leachi* in South Africa, and *Dermacentor reticulatus* in Europe, and *Rhipicephalus sanguineus* in India.

Other species of piroplasma described are: *Piroplasma ovis*, found in sheep and carried by *Rhipicephalus bursa*; *Piroplasma equi*, *P. muris*, and an unnamed species found in the monkey in Uganda has been described by P. H. Ross.

Experimental work has demonstrated that the parasites are peculiarly specific, the disease not being transmissible to other animals. Thus *P. canis* can only reproduce the disease in dogs and not in other animals. *P. equi* will produce the disease in horses and donkeys, but no other animals. Similarly with the other known piroplasmata. So far piroplasmata, though described in man on several occasions, have not been conclusively proved to occur. All attempts to find them in Blackwater Fever have failed.

An interesting fact is that ticks which feed on an infected animal are not in themselves infective, but hand down the infection to their offspring. These, sometimes as larvæ, in other cases not till they become nymphs or adults, are able to convey the infection to the animal on which they feed, provided that animal is susceptible to the particular piroplasma involved.

Development of Piroplasma.

Asexual Cycle.—This parasite has a free and an intracorpuscular stage in its asexual life-history. In the free stage they are pyriform in shape, and in the intracorpuscular at first rounded, afterwards dividing into two or more pyriform bodies by a process of budding. The newly-found parasites are at first connected together by a thin process, but finally become separated, and escaping

Fig. 33.

from the corpuscle invade other corpuscles. They may enter and leave two or three corpuscles before again becoming rounded and undergoing division; ultimately, however, the process of division is undergone, and so the cycle is repeated.

Sexual Stage.—Koch has described developmental forms of *P. bigeminum* in *Rhipicephalus australis*, *R. evertsi* and *Hyalomma ægypticum*, and Christophers has demonstrated similar forms of *Piroplasma canis* in *Rhipicephalus sanguineus*. From these observations it appears that in the stomach of the tick the parasite escapes from the red blood corpuscle and becomes elongated, one of its chromatin masses passing to the blunt (? anterior) end, forming a projection—the other chromatin mass remaining in the middle of the parasite. Next radial processes

arise near the projection, the other end of the parasite becomes pointed, and the general contour of the parasite becomes angular. After the second day couples of these forms are seen apparently connected together, a process of copulation. The male element, after giving up its chromatin, is thrown off or stretched out over the enlarged fertilized female. The fertilized element after becoming round, oval, or eventually club-shaped, leaves the gut and passes to the ovary. In the ova of infected ticks large pear-shaped bodies are described. Christophers also states that he has found further developmental forms in the cells of the gut of unfed nymphs reared from infected mothers, and has traced them to the salivary glands of nymphs of the second generation, which were about to become adults.

HÆMOGREGARINA.—The hæmogregarines are unpigmented parasites, occurring both as intracorpuscular forms, in which they are bent upon themselves so as to form a tail (Plate V., 19), and as free vermicular forms in the blood plasma (Plate V., 17 and 20). They are non-pigmented, and in stained specimens the nucleus stains well, and contains abundant chromatin as numerous granules sometimes arranged in radiating lines. Sporulation takes place not in the blood, but in certain cells of the viscera, usually of the liver.

The parasites are common in the blood of cold-blooded vertebrates. The effect on the red corpuscle varies. Some species displace the nucleus, some do not. At least one species causes the formation of granules which stain like the Schüffner's dots in the human corpuscle containing the parasite of benign tertian malaria (Plate V., 21). Several species have recently been found in the blood of mammals. None have been found in human blood.

The first of these mammalian hæmogregarines was discovered by Bentley in the leucocytes of dogs in Assam, and described by James as *Leucocytozoon canis* (correctly *H. canis*). Soon afterwards similar parasites were des-

cribed by Balfour as occurring in the red blood corpuscles of the jerboa, *Jaculus goudoni* (fig. 34*b*), and by Christophers in the Indian field-rat, *Gerbillus indicus*. Later, Patton described another hæmogregarine in the leucocytes of the Kathiawar squirrel, *Funambulus pennantii*, and Balfour a similar parasite in the leucocytes of the rat (*Mus decumanus*).

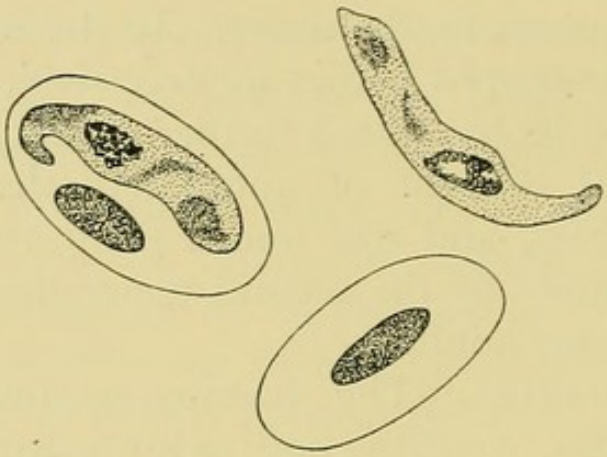


FIG. 34*a*.—Hæmogregarine in Reptilian blood.

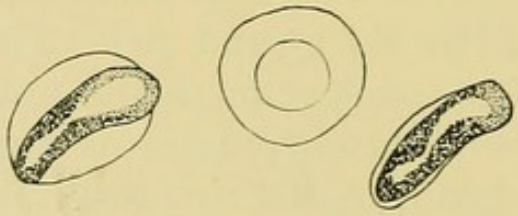


FIG. 34*b*.—Hæmogregarine in Mammalian blood.

H. canis occurs in the polymorphonuclear leucocytes only. The parasite is enclosed in a capsule which is not easily penetrated by the stain. It is an oblong body rounded at the ends. The contents are the hæmogregarine, which is sharply bent on itself, so that the nucleus is frequently horse-shoe shaped.

The development of this parasite has been studied by Christophers. It has been found to reproduce itself by encystment in the bone-marrow of the host, there forming merozoites (fig. 35). He also found that the sexual development of the parasite took place in the dog tick (*R. sanguineus*). When taken into the gut of ticks the parasite escapes from the capsule and shows active

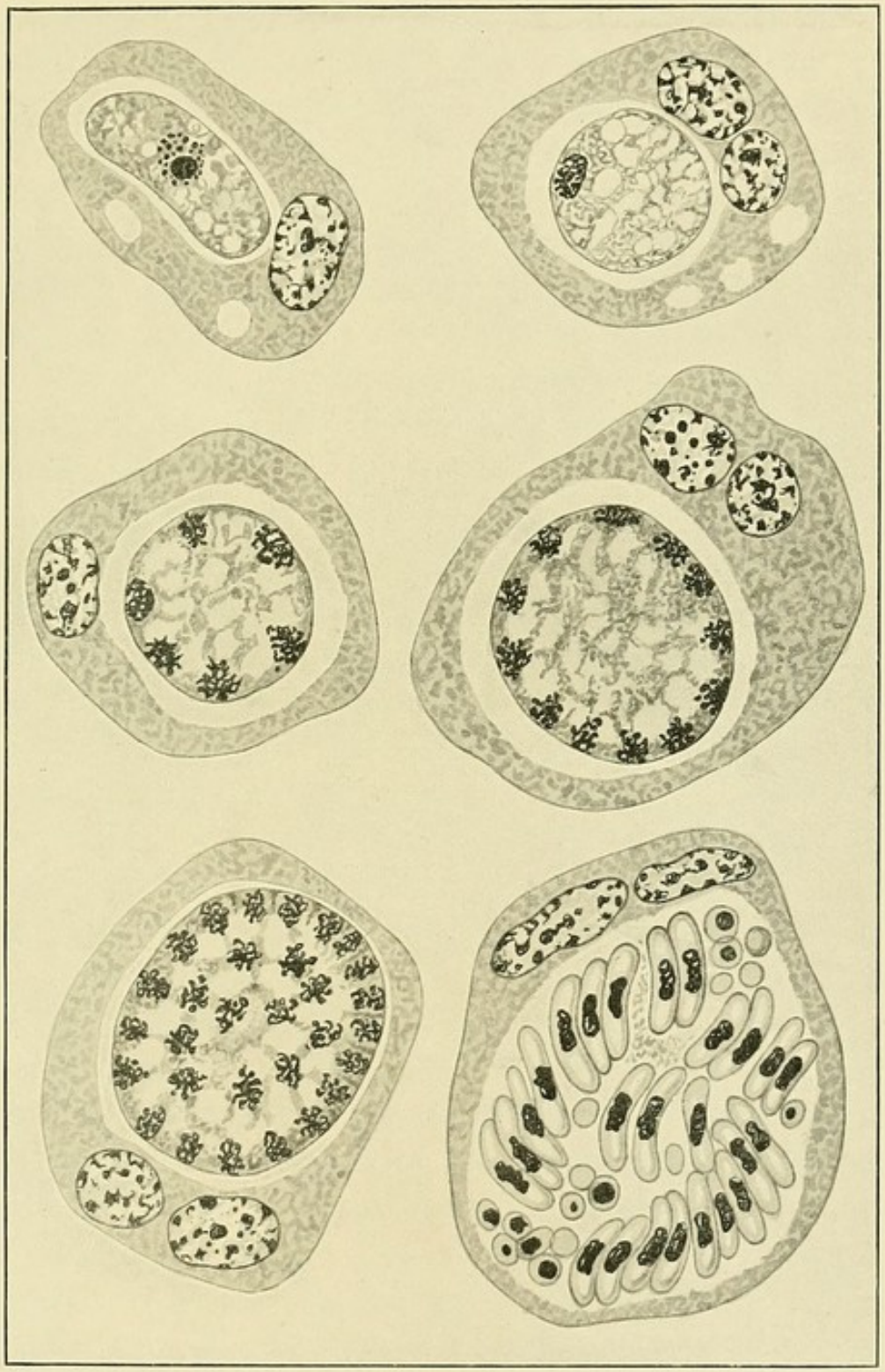


FIG. 35.

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vermicular movements. Within twenty-four hours these, associated in pairs, have lodged themselves in the large cells of the gut. On the second day conjugation between two similar individuals takes place, and a globular body is formed containing a single large homogeneous mass of chromatin. This chromatin mass spreads out to form a reticulum, and the protoplasm increases in amount. The chromatin collects at the periphery into irregular star-shaped masses, and on the third or fourth day the whole body splits up to form eleven to fourteen sporozoites. When set free the sporozoites are in the lumen of the gut. The method of re-entry in the dog has not been discovered.

H. balfouri occurs in the red blood corpuscles of the jerboa or desert-rat. As seen in stained specimens it is a slightly-curved body, with rounded ends lying either apparently free or in the remains of a red blood corpuscle. It is believed that the free forms owe their condition to the destruction of the red blood cell which contained them. A long oval nucleus, situated about the centre of the parasite, is present, and occasionally a few dots of chromatin in the pale polar areas.

The stage of schizogony has been observed by Balfour to take place in the liver cells of the host (fig. 35), and is similar to that described in *H. canis*.

H. gerbilli is a parasite similar to the preceding, occurring in the red blood corpuscles in the Indian field-rat.

The stage of sporogony occurs in the louse. The complete life-cycle has not been discovered.

H. funambuli is a parasite occurring in the large mononuclear leucocytes of the palm squirrel. It differs from *H. canis* in the absence of a capsule surrounding the parasite. A similar parasite has been found by Balfour in the Norway rat.

GREGARINES.—These protozoa are characterized by certain peculiarities in the reproduction. They are not known to occur in any true vertebrate, though they are found in amphioxus. They are pre-eminently parasites

of arthropods, but occur commonly in worms. They are very rare in molluscs.

The gregarine is primarily a parasite of the digestive tract, in which in the earliest stages it is attached to or contained in an epithelial cell of the gut. When the host cell is used up the parasite may remain free in the gut or may penetrate into the body cavity. When special portions of the cœlom are separated for a particular function, as in the case of the vesiculæ seminales of the earthworm, the parasites may be found in these organs.

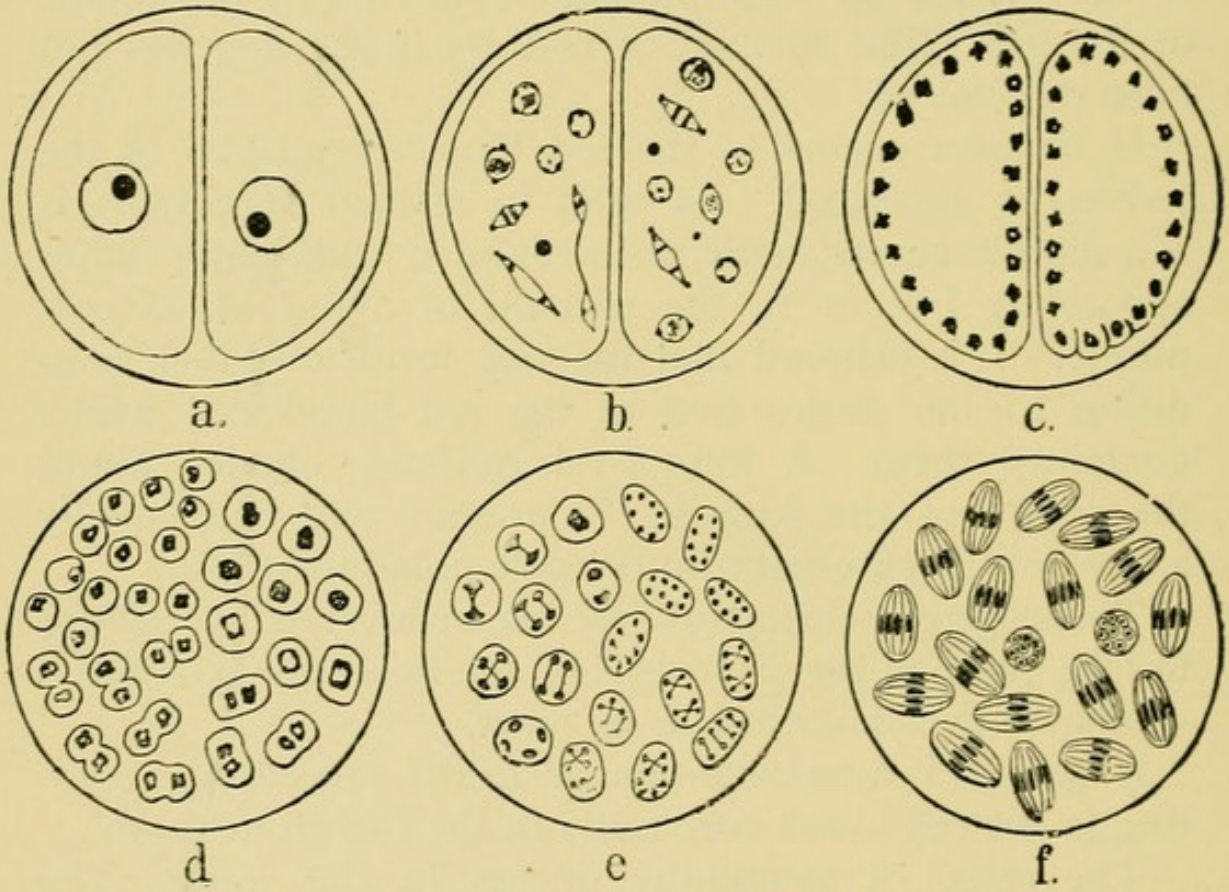


FIG. 35*a*.—Development of Gregarines. *a*, The two sporonts in a common cyst; *b*, various stages of nuclear division in the sporonts; *c*, commencing formation of gametes by a process of sporulation; *d*, union of gametes in pairs to form zygotes; *e*, stages in the division of the nuclei of the zygotes; *f*, cyst with ripe spores, each containing eight sporozoites.

Reproduction always takes place by sporogony, except in the small sub-order Schizogregarinæ. Hence Gregarinæ are divided up into two sub-orders—Schizogregarinæ and Eugregarinæ. The Eugregarinæ are further divided into Cephalina and Acephalina.

The Cephalina have a well-defined epimerite or fixative

organ, and the body is usually divided up by septa. An example is the gregarine found in the gut of the meal-worm.

The Acephalina are those which have no fixative organ or epimerite, and the body is not divided up by septa. The best-known examples are the *Monocystis magna* and *Monocystis agilis* found in the vesiculæ seminales of earth-worms.

The body form of gregarines is always well defined, never amœboid, and varies from a sphere or ovoid to an elongated wormlike shape. The body is composed of ectoplasm and endoplasm, in the latter of which is the large single ovoid nucleus.

Reproduction is by sporogony. Two full-grown gregarines come together, become rounded and develop a cyst wall around themselves. The nuclei become fragmented, and around these fragments of nuclei segmentation of the protoplasm takes place to form gametes.

Conjugation of gametes then takes place to form zygotes, each of which becomes encysted and develops within itself eight sporozoites (fig. 35*a*).

These sporozoites when introduced into a fresh host develop into adult gregarines.

ZOOLOGICAL POSITION OF THE MALARIA PARASITES.

Protozoa

Class *Sporozoa*

Sub-class *Telosporidia* — *Neosporidia*

Order (1) *Gregarinoidea* — *Hæmosporidia* — *Coccidiidea*

Genera :

(1) *Plasmodium* (*Hæmamœba*) — (2) *Hæmogregarina* — (3) *Piroplasma* — (4) *Halteridium* (?)

Species.

(1) *Plasmodium* (*Hæmamœba*)	(2) *Hæmogregarina*	(3) *Piroplasma*
P. vivax (Benign tertian parasite)	*H. ranarum*	*P. bigeminum*
P. malariæ (Quartan parasite)	*H. gerbilli*	*P. parvum*
P. falciparum (Sub-tertian parasite)	*H. balfouri*	*P. ovis*
P. præcox (Proteosoma)	*H. canis*	*P. equi*
P. kochi (found in monkeys)	*H. funambuli*	*P. canis*
&c.	&c.	&c.

CHAPTER VI.

Parasites found in Blood Plasma.

The most important animal parasites found in the plasma of the peripheral blood are trypanosomes and spirochætæ belonging to the class *Mastigophora*, and embryos of various filaria, nematode worms.

In some of the lower animals hæmogregarines are found during their motile stage, and the piroplasma pass a short period of their existence free and may be found in the plasma. The hæmamœbidæ are not found in the plasma, though for a brief period the "spores" must be free. As stated in the preceding chapter, according to Schaudinn, the halteridium has a free stage in the plasma.

In freshly-shed blood, trypanosomes are readily seen as actively motile, worm-like bodies darting about between the blood corpuscles. Their movements are so rapid and they are so transparent that it is difficult to make out their form clearly in the living condition.

The largest trypanosomes are found in fish, both fresh and salt water. They can readily be demonstrated in small fish by cutting off their heads and making a smear on the slide with the cut surface. These smears can be examined fresh by placing a cover-glass on the top of the exuded fluid, or the films can be dried and stained.

Trypanosomes are found in some birds. The method of examination of the blood for these is the same as that required for the hæmosporidia. If a small bird is to be examined it is held in the palm of the left hand and one leg is allowed to protrude between the fingers. A needle is then inserted deeply into the vascular pad surrounding

the root of the claw and left there for half a minute. On squeezing the leg so as to force the blood towards the claw the blood will exude in drops, and films can be made as with human blood. With larger birds an assistant is necessary to hold the bird, and the bird should be wrapped in a thick cloth for the protection of the assistant and operator.

The trypanosomes that have attracted most attention are those of the mammalia. Many species are known; they closely resemble each other in their appearance, but differ in size, shape, and motility to some extent; also in the positions they assume and the way in which they stain (Plate I., *a*, *b*, *c*, *d*, *e*). They may be differentiated by their pathogenic action. By inoculating a series of animals with the blood it will then be found which animals are immune and which are susceptible. For such inoculation the blood should be used at once, but if this is impossible then it may be mixed with some fluid that will prevent coagulation. Sodium citrate solution, 10 per cent., may be used, and the blood should be diluted with one-twelfth of this solution. Others use a weaker solution of citrate of soda, 1 per cent., and dilute the blood more freely. Injection of such diluted blood into the subcutaneous tissues will, in the majority of cases, lead to infection with trypanosomata of the animal which has been injected if it is susceptible.

Every mammalian trypanosome, so far as is known, belongs, with the exception of *T. cruzi*, to one of five types (Plate I.). These types are :—

(A) *T. lewisii*. The antiflagellar end is sharply pointed, the centrosome usually at some little distance from the end of the body, and the nucleus is always in the posterior half of the body. Size 25 to 30 μ (Plate I., *b*).

(B) *T. gambiense*. The antiflagellar end is usually bluntly rounded, the centrosome close to that end, and the nucleus at the middle of the body. Size 20 to 25 μ (Plate I., *d*).

(C) *T. nanum*. Characterized by its small size, being

only about 14 μ in length. The centrosome is small and the nucleus is round and in the centre of the body (Plate I., *c*).

(D) *T. theileri*. Characterized by its large size, being some 65 μ in length (Plate I., *e*).

(E) *T. dimorphon*. The important characteristics of this type are the broad body, the short free portion of the flagellum, the close application of the undulating membrane to the body and the presence of a vacuole between the centrosome and nucleus (Plate I., *a*).

The more important of the trypanosomata are :—

(A) (1) Those found in a large proportion of the rats in both tropical and temperate climates. These are non-pathogenic to full-grown rats, and all other animals experimented on are insusceptible to the infection (*T. lewisi* (Plate I., *b*).

(B) (1) NAGANA, or "TSETSE-FLY DISEASE." The trypanosoma of this disease (*T. brucei*) can be inoculated into a large number of wild and domesticated animals, but man is insusceptible. To cattle, horses, donkeys, dogs, guinea-pigs, rats, &c., this parasite is pathogenic, but the time required to cause death varies greatly in these animals. Wild game, and particularly the buffalo, harbour the parasite, which appears to be harmless to them. It is carried from animal to animal by biting flies belonging to the genus *Glossina*, usually by *G. morsitans*.

(2) SURRA (*T. evansi*). A disease fatal to horses ; cattle often recover. It occurs in India, Philippines, &c. It is carried by various biting flies, such as *Stomoxys calcitrans*, some of the tabanidæ, hæmatobia, &c.

In the living condition it can be distinguished from *T. brucei* by its greater activity, as it not only moves but actually progresses.

(3) DOURINE (*T. equiperdum*). A disease chiefly affecting mares and stallions and found in Southern Europe, Northern Africa and Chili. It is a disease transmitted by coitus and characterized chiefly by a purulent discharge from the genitals and great œdema of the abdomen. It

Plate I.

a.

b.

c.

d.

e.

Bale & Danielsson, Ltd del. et lith.

is accompanied by fever and great emaciation and usually proves fatal. The parasites are found abundantly in the discharges and scantily in the blood. Rats, dogs, rabbits, &c., are susceptible, whilst monkeys, cattle, sheep and goats are refractory.

(4) MAL DE CADERAS (*T. equinum*). A disease of horses in South America, notably in Venezuela. The parasite is characterized by having a very small centrosome. The natural host of the parasite is believed to be the capybara —a large water vole. The carrier is probably a stomoxys. The disease can be inoculated into most animals, but cattle are immune.

(5) T. GAMBIENSE (Plate I.) Man is insusceptible to all the above-mentioned trypanosomes, but in Africa, on the West Coast and throughout a large part of Central Africa, another species has been found in man. The parasites are found in small numbers in the blood in most cases, particularly in chronic cases, but can be found in larger numbers in fluid drawn with an aspirating needle from a lymphatic gland. The symptoms are constant irregular pyrexia, enlarged lymphatic glands, usually in the neck or above the clavicle, erythematous rash. Enlargement of spleen and liver have been noted. Monkeys are susceptible, and, with some strains dogs, white rats and guinea-pigs. After a longer or shorter period in man grave cerebral symptoms supervene, usually of the lethargic condition known as "sleeping sickness," and the patient dies. In this stage of the disease trypanosomes are found in the cerebro-spinal fluid. They are not numerous, and it is necessary to centrifugalize the fluid to demonstrate them.

(C) T. NANUM is found in cattle in the Soudan, causing in them fever, emaciation, &c. The carrier is believed to be stomoxys.

(D) T. THEILERI. South Africa. Cattle only are susceptible. No other animal has yet been inoculated successfully. It is one of the largest of the trypanosomes found in mammals. It is believed to be transmitted by *Hippobosca rufipes*.

(E) T. DIMORPHON occurs in horses in Africa, especially on the Gambia. The disease produced is like "Nagana" but more chronic. Various animals are susceptible. The parasite occurs in several forms.

T. CRUZI. This trypanosome, or rather schizotrypanum, was discovered in the blood of a child in Brazil who was suffering from irregular fever, progressive anæmia, and enlargement of lymphatic glands.

The carrier is believed to be a bug—*Conorhinus sanguisuga*—belonging to the Reduviidæ.

More recent work by Chagas on this parasite has shown that it differs in several respects from the ordinary trypanosomes. Instead of multiplying by longitudinal fission in the peripheral blood, it splits up into eight merozoites within its limiting membrane in the capillaries of the lung. In consequence of its mode of multiplication it has been named by Chagas—Schizotrypanum. The bug which is the carrier acts as a true host, for after feeding on an infected man or animal, it is not capable of infecting another till eight days have passed, but after that remains infective for an indefinite period.

For the examination of blood for trypanosomes, films prepared as for malaria are the best, as the parasite will then be seen undistorted. When the parasites are scanty and for purely diagnostic purposes, thicker films decolorized by the action of water may be used, but a good deal of distortion results. In some infections the parasites can only be found by injecting a highly-susceptible animal with the blood of a suspected case, as a large infection may then result in the animal which has been injected. This proceeding is necessary in many cases of dourine. In centrifugalized blood the parasites accumulate in the upper part of the mass of red corpuscles and can be found there more readily than by the ordinary method.

Typanosomes stain rather feebly with most basic stains, hæmatoxylin, methylene blue, &c. A stronger basic stain, such as carbol fuchsin, should therefore be

used. Clearer specimens are obtained by diluting the stain with three parts of water and leaving to stain for ten minutes.

Good results can also be obtained by overstaining with this stain and then decolorizing with ½ per cent. solution of glacial acetic acid in water, but the parasite is often swollen and distorted, though quite recognizable.

Leishman's stain, used as for other blood work, gives excellent results with fresh specimens and shows well the various points in the structure. The body is elongated and one extremity is bluntly truncated, whilst the other is prolonged into a long flagellum. In addition there is attached to the body of the parasite and running its whole length an undulating membrane frequently thrown into folds. The flagellum is continued throughout the body, running along the free edge of the undulating membrane and closely following its sinuosities. Slightly posterior to the termination of the flagellum is a deeply-staining nodule—the centrosome. About the middle of the body is a rounded mass, larger but less defined—the nucleus. In fission forms the centrosome first divides, then, successively, undulating membrane, nucleus and protoplasm (fig. 36). The flagellum does not divide but remains attached to one of the resulting individuals, whilst the other develops a new flagellum. The protoplasm with Leishman's stain is blue. The centrosome, nucleus and flagellum are red.

Multiplication is by fission. These fission forms are rarely found in the peripheral blood in man. Occasionally there are two flagella, with no signs of fission in centrosome or nucleus.

An interesting phenomenon frequently associated with trypanosome infections is that known as auto-agglutination. When a fresh living film of the infected blood is examined it will frequently be found that the red blood cells tend to run together into clumps, and as this phenomenon has only rarely been observed with other infections it appears to have some value as a diagnostic sign in trypanosomiasis.

The human trypanosome is carried by *G. palpalis*, but other species of glossina are also suspected of acting as carriers.

The mode of transmission may be direct, the trypanosomes being taken from any infected animal, and without any further development in the fly enter the next animal bitten. This only occurs if the fly after feeding on an infected animal proceeds to feed again almost immediately on another animal.

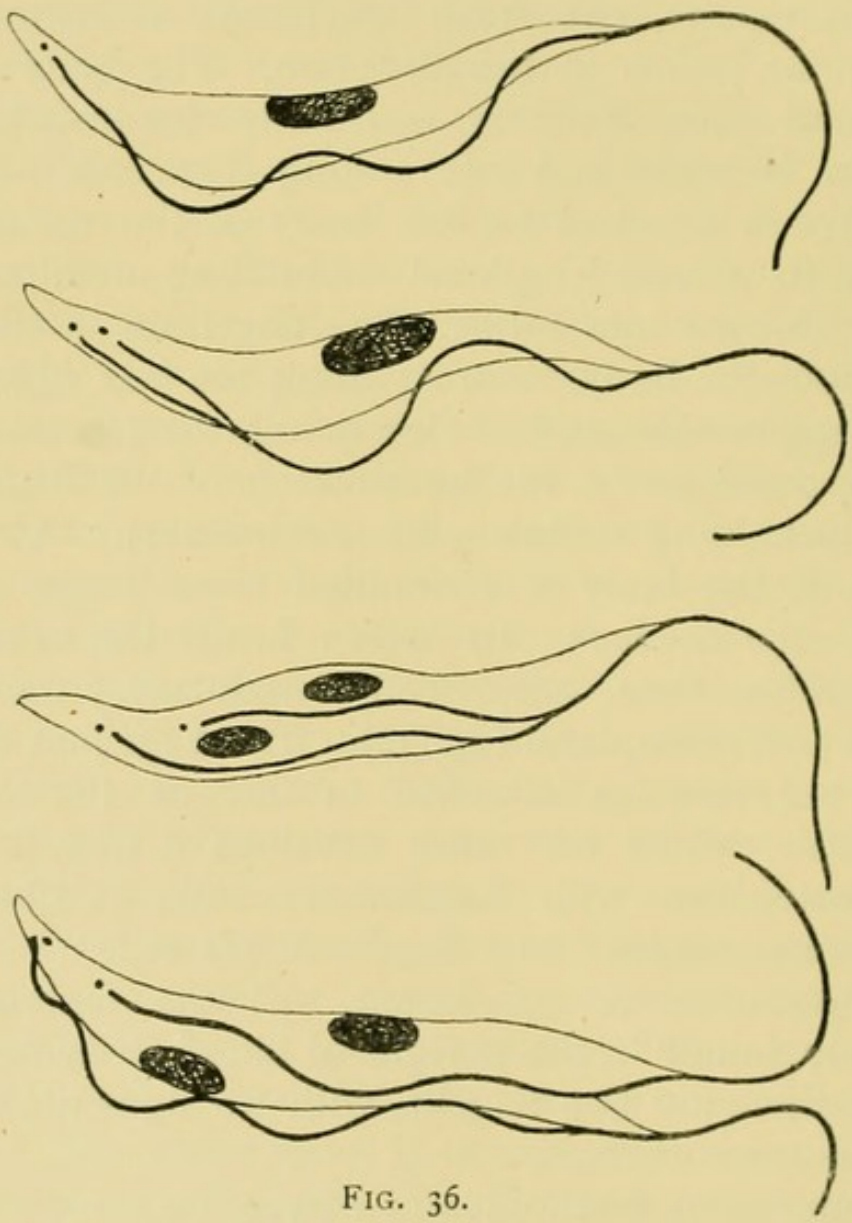


FIG. 36.

It has now been shown that a glossina very shortly after feeding on an infected animal becomes incapable of transmitting the infection. If, however, the fly be

kept alive, after a period of eighteen days or so it again becomes infective, thus pointing to some cycle of development taking place in the fly. Nothing is known definitely of the phases of this supposed developmental cycle. No sexual phase has been observed in the trypanosomes. Further work is much required on this subject.

In three cases of trypanosomiasis in man, in which the infection seemed to have been contracted in Rhodesia, it was noted that not only were the parasites much more virulent than *T. gambiense* to man and to lower animals but also that in one case the form of some of the parasites was different, the nucleus being situated close to the micronucleus, and in some instances even between the micronucleus and the antiflagellar end. The parasite in man in the Rhodesian cases seems to be very resistant to atoxyl, though this peculiarity is not retained when the parasites are inoculated into lower animals. Stephens suggests that in his case the morphological differences are sufficient to be considered specific, and describes the parasite as *T. rhodesiense*.

SPIROCHÆTA.

The spirochæta of relapsing fever—*Spirochæta recurrentis* (fig. 37)—is now generally believed to belong to the *Mastigophora*. They can be seen in fluid blood films made as for malarial blood. The organisms are very transparent and can only be seen in fresh fluid preparations with the diaphragm nearly closed. They are then seen as fine, transparent, thread-like bodies, which are in active movement and coil and uncoil themselves. They are also seen in the corkscrew-like forms which are commonly drawn as representing them.

Dried films are best thin. In such films the spirochætæ are seen in the undulating form (fig. 37*b*). In thicker films they appear more frequently coiled up (fig. 37*a*). The spirochætæ stain with all basic stains, but not intensely, and are best demonstrated by the use of the

stronger basic stains, such as carbol fuchsin diluted 1 to 3 of water (Plate VI., 23).

They stain well by Leishman's method or with Giemsa's. Ordinarily no definite structure can be made out.

The disease can be reproduced in monkeys, and less readily in rats. African tick fever has been shown by Ross and Milne to be caused by a similar spirochæta—

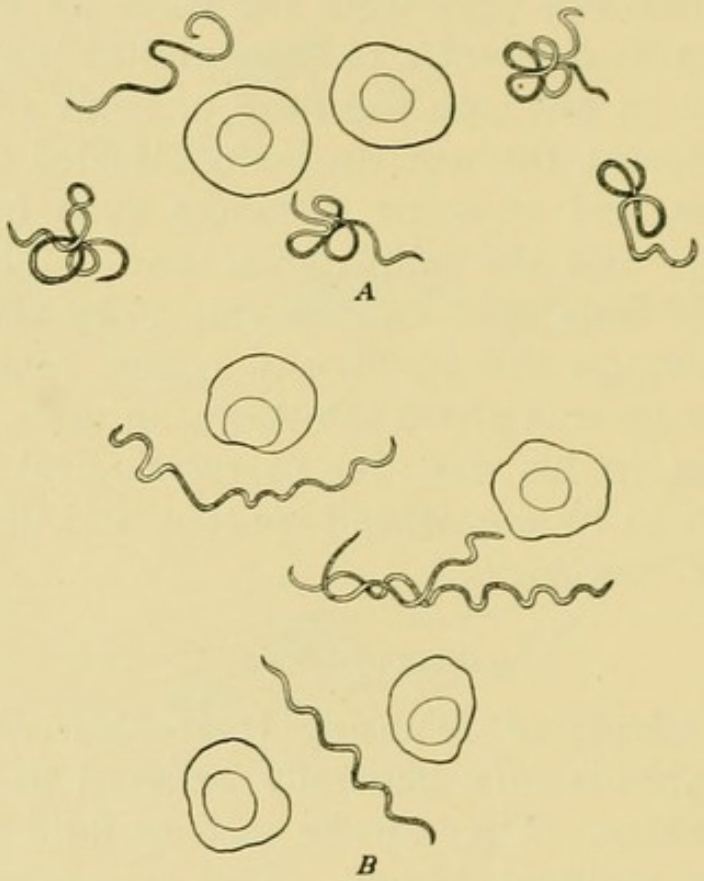


FIG. 37.

S. duttoni. In appearance it closely resembles *S. recurrentis*, but is more readily inoculated into lower animals, rats, guinea-pigs, &c. The pathogenicity of the two parasites differs, and infection with one does not affect the susceptibility to infection by the other spirochætæ.

In man there is leucocytosis and marked relative increase of the polymorphonuclear leucocytes. This increase persists to some extent in the periods of apyrexia,

so that a differential count of the leucocytes may exclude malaria.

The spirochæta shows no signs of longitudinal division in the blood, and in human blood has no tendency to great variation in length. It is found in the plasma, never in the red blood corpuscles. The spleen enlarges, and in fatal cases spirochætæ are found in large numbers in that organ. The organisms are found in greatest number during the first pyrexial period. In the apyrexial period they are not to be found, and in the subsequent pyrexial

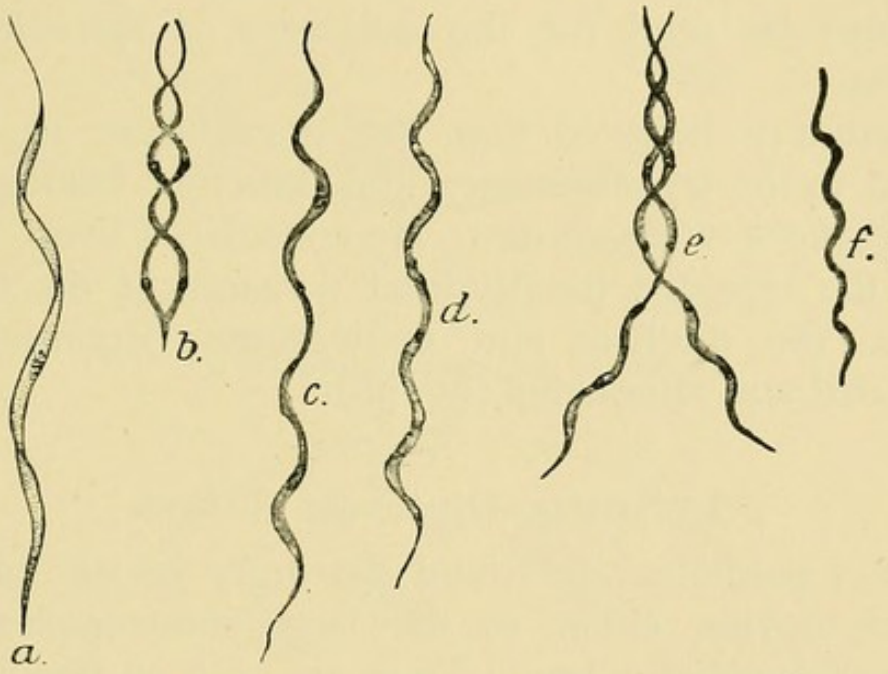


FIG. 38.—*a*, Single trypanosome much enlarged; *b*, stage of fission; *c*, parasites still attached by posterior ends; *d*, same, both parasites commencing to divide, nucleus and blepharoplast divided; *e*, resultant stage of division; *f*, one of the four spirilla into which *e* has divided.

attack they are found in smaller numbers than in the primary attack. In some cases the disease passes on into a chronic condition of irregular pyrexia—secondary fever; it is exceptional to find the parasites during that period.

Spirochætæ are found in the mouth and sometimes in expectoration and fæces. In various syphilitic lesions, *S. pallida*, in yaws, *S. pertenius*, in sclerosing granuloma and in many ulcers spirochætæ are found. The best known of these is *S. pallida*, found in syphilis. Spiro-

chætæ are found in the blood of many of the lower animals.

A convenient way of demonstrating *S. pallida* is by the Indian ink method. The surface of the lesion is scraped until a drop of serum is obtained. This is transferred to a slide and there mixed with two drops of Indian ink (Gunther's). The mixture is then spread with another slide as in making a blood film. The film is allowed to dry and can be examined with the oil immersion lens without applying a cover slip. The spirochætæ are seen as bright spirals on a dark brown field. This method can also be used for the detection of spirochætæ in sputum.

Schaudinn believed that the spirochætæ are closely related to the trypanosomes, and therefore belong to the *mastigophora* or *flagellata*. He considered them to result from the repeated longitudinal division of the trypanosomes, the nucleus and centrosome becoming both elongated and attenuated (fig. 38).

Leishman-Donovan Bodies.

These parasites are found sparingly in the blood, in the leucocytes, either in the large mononuclear or in the polymorphonuclear. They are said to become very numerous just before death.

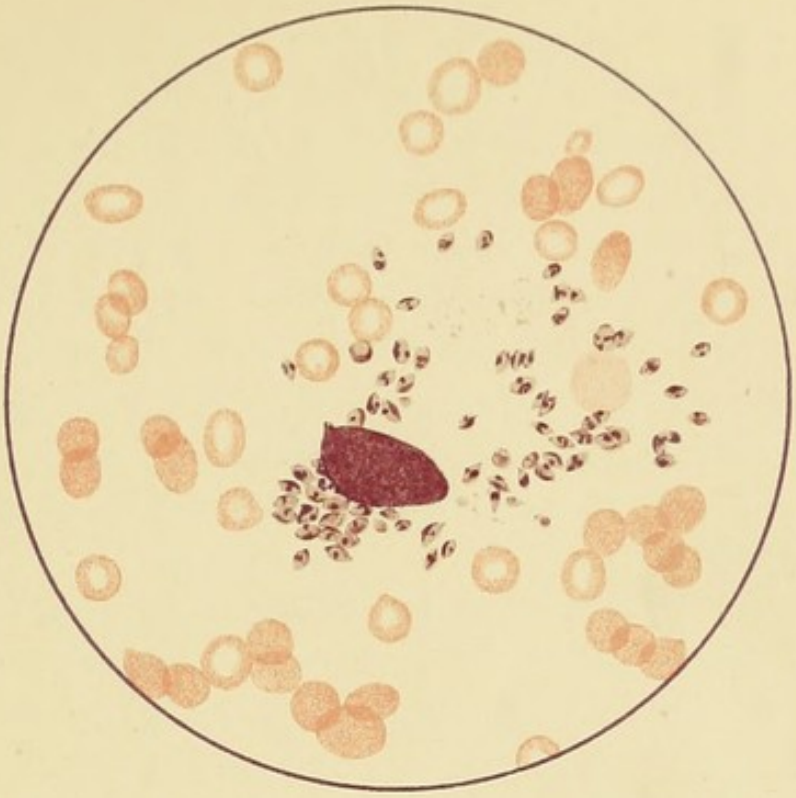
Possibly a free flagellate form may, in time, be found in the plasma, as in cultures the bodies develop flagella. These flagellate forms have not been observed in man.

The bodies are found in the spleen, liver, lymphatic glands, lungs and submucosa, and occur in these situations, particularly in the spleen and liver, in enormous numbers. They are contained in the endothelial cells, and in masses imbedded in a hyaline matrix between the cells.

They can be observed in smears from the organs taken after death, or by puncture and aspiration, with a hypodermic syringe, of the spleen or liver. Fatal accidents have resulted from puncture of the spleen, and punctures

Plate II.

b.—Cryptococcus.



a —Leishman-Donovan bodies.

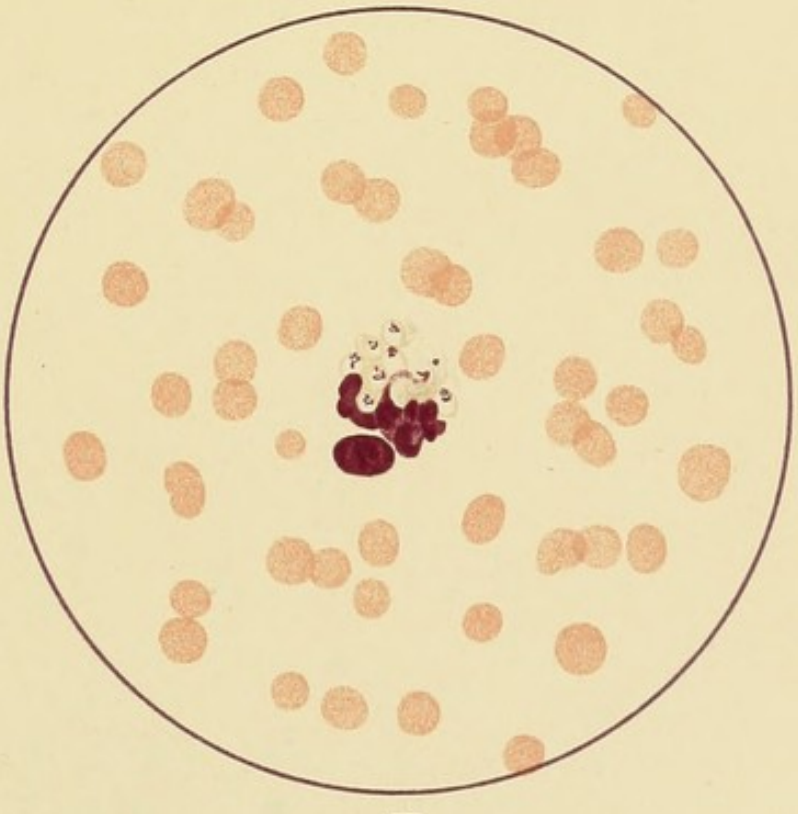


Fig. 39.

Bale & Danielsson, L.td del. et lith.

of the liver should therefore be made, though the parasites are not found so readily in the fluid drawn from the liver. In some cases they may be obtained by puncture of the superficial lymphatic glands. (Cochran).

A large all-glass syringe is convenient for the purpose. The needle should not be too fine. The skin must be carefully sterilized over the selected place and the syringe and needle sterilized dry. The needle should be plunged right into the liver, so that it moves with the movements of the liver. It should be rotated and withdrawn a little, but still kept in the liver before aspiration. The aspiration should not be too forcible.

If much blood comes this will so dilute the fluid containing the Leishman-Donovan bodies that a prolonged search may be necessary to find them.

They swell up and break down on the addition of water, so that thick decolorized blood films cannot be used. It is for this same reason that the syringe used for aspirating must be dry.

The bodies are small, round, or oval masses of protoplasm, which stain faintly with basic stains, and contain two chromatin masses, which stain deeply with ordinary basic stains, and with the polychrome methyl blue a deep red. These two chromatin masses are unequal in size. The larger is oval, is situated to one side of the parasite, and stains with Leishman a decided red, but not very deeply. The smaller is rod-shaped, and stains intensely red with Leishman. It usually is directed pointing obliquely towards the nucleus. (Plate II., *b*).

These bodies are much the same size as blood platelets, but the peculiar chromatin masses render them easy to recognize. They are frequently found in clumps.

In the peripheral blood they may be found in thin films within leucocytes, but as many leucocytes as possible should be present, as only 1 in 100 to 500 will contain the bodies. The edges and ends of the films contain most leucocytes, and the number can be increased by suddenly lifting the upper slide off the lower one, in making a film by the ordinary method. There

is always some anæmia, and degenerate red corpuscles are common. The leucocytes are scanty, only 2,000 to 4,000 per cubic millimetre. The mononuclear elements are relatively increased.

Leishman-Donovan bodies were considered by Leishman to resemble, in the arrangement of the chromatin masses, degenerate forms of trypanosomes. Laveran suggested a resemblance to piroplasmata.

Rogers and others have shown that in cultures in sterile citric acid solution, ½ per cent., to which sodium citrate 2·5 per cent. has been added, the bodies become much elongated and form a flagellum, showing that they are the resting stage of a flagellate (fig. 40*b*, 1, 2, 3).

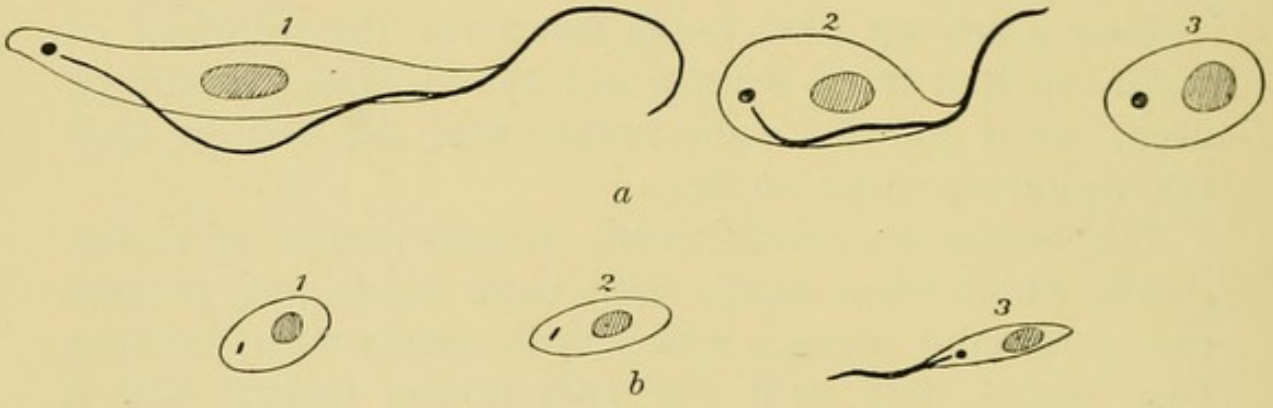


FIG. 40.—*a*, Trypanosomes and the altered forms found in culture; *b*, Leishman-Donovan bodies and the altered forms found in culture.

It had previously been shown that trypanosomes could be kept alive for some time in a medium rich in hæmoglobin, and that forms (? degeneration forms) were produced which had no flagellum, whilst the form of the organism became round and the centrosome was brought nearer to the nucleus (fig. 40*a*, 1, 2, 3).

It is now considered to be established that the bodies belong to the *Mastigophora* or *Flagellata*, but not to the genus *Trypanosoma*, as there is no undulating membrane and the flagellum emerges from the end at which the centrosome is placed. It is probably a *Herpetomonas*.

Flagellate forms may be discharged from the ulcers in

the intestines in kala-azar, the disease due to the Leishman-Donovan bodies.

Delhi Boil.—Scrapings from the raw surface in these ulcers show a considerable number of bodies closely resembling Leishman-Donovan bodies. The two diseases are probably distinct, and therefore the parasites in Delhi boil are probably of a different species from those found in kala-azar.

The parasites of Delhi boil have been cultivated in a medium similar to that used for the cultivation of the Leishman-Donovan bodies. Exactly the same phases of development were observed as in the parasites of kala-azar.

Cryptococcus.—A parasite, somewhat resembling the Leishman-Donovan body, has been described under the above name in horses suffering from epizootic lymphangitis. The parasites are small ovoid or spherical bodies 3 to 5 μ in diameter, found either free in the pus of abscesses or contained in the large mononuclear or polymorphonuclear leucocytes. They resemble the parasites of kala-azar in man, but differ from them in that no micronucleus can be made out, and the single macronucleus seems to be less compact and the individual granules of the same to be more loosely arranged, giving somewhat of a rosette appearance. Some observers assert that these bodies are not protozoa but a variety of yeast.

These parasites stain well with Leishman or Giemsa's stain and will be found frequently in large numbers in the body of leucocytes and even invading the nucleus. (Plate II., *a*).

CHAPTER VII.

Parasites other than Protozoal found in Human Blood.

Animal parasites belonging to higher orders of animal life are found in human blood.

Schistosomum hæmatobium (Bilharzia) and *S. japonicum* frequent the veins of the portal system and pelvis (vide *Trematoda*).

Nematodes.—One species of filaria in the adult form has been once found in the circulatory system of man by Megalhães in Brazil, but no further observations have been made. The worms were found in a blood-clot on the left side of the heart.

In the lower animals nematode worms are not uncommon in the blood. *Filaria immitis* is found in the right side of the heart and pulmonary vessels of the dog, and in the East and in some of the Pacific Islands it is exceptional to find a dog free from these parasites. When the worms are in large numbers cardiac dilatation and death result. Various nematode worms in horses and other animals cause "verminous aneurisms."

Of the human filaria the adults are found in various parts of the body, whilst the embryos may be discharged through an aperture in the skin, as in *F. medinensis* (guinea-worm), and probably also in *F. volvulus* which is found in subcutaneous cysts in patients in West Afrca. In those filaria in which we are at present more specially interested the embryos find their way into the blood and circulate with that fluid.

The filarial embryos, or *microfilaria*, as seen in fresh blood, are clear, transparent, worm-like bodies, which are

in active movement. They are most readily found in a fresh fluid blood film, as the active movements and the disturbance in the red corpuscles set up by their movement catch the eye. An inch or two-thirds inch objective is quite sufficient magnification for the detection of the commonest micro-filariæ, but it is better to use a half-inch, as the smaller species may be overlooked with the two-thirds objective. The film must not be so thin as that required for examination for malaria parasites. No special precautions are required, and sufficient blood should be taken to completely fill the space between the slide and cover-glass. As the slide must be kept for a sufficient period to enable the movements of the worm to cease, the cover-glass should be ringed with vaseline to prevent evaporation of the blood.

To examine the embryos in detail, higher powers, including a one-twelfth oil immersion, are required. At first the movements of the microfilaria are so active that it is impossible to examine it with these objectives, but after some hours the movements become much more sluggish, and finally cease. The best time for examination is just before the cessation of movement and the death of the embryo. The points to observe in the examination of the fresh embryos are :—

(1) The character of the movement and whether active locomotion takes place or whether the movement, however active, leads to no progression.

(2) The size of the embryo. This is of the greatest importance, as measurements of dried specimens vary greatly with the rapidity with which the film has dried.

(3) The shape of the embryo and that of the two ends.

(4) The presence or absence of a loose sheath.

(5) Any details of structure, and particularly the presence, position and character of any contractile vesicles, the so-called V spots, and the cephalic movements and any appearance of armature.

Embryos can also be readily observed in dried films.

The blood films for diagnostic purposes should be as thick as posible. A convenient way of making them is to allow three or four large drops of blood to fall on a slide close together and smear them together into a space about two-thirds of an inch in diameter (fig. 41). Allow to dry face upwards, protecting the films from insects during the process. Such a film will be so thick

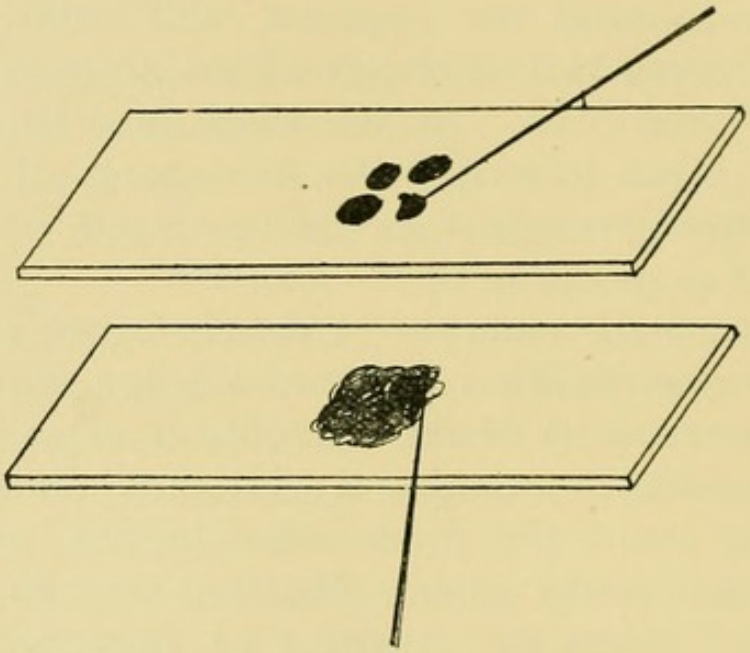


FIG. 41.

as to be almost opaque. It must not be fixed. When quite dry place in water and leave there till the hæmoglobin is all dissolved out. It is best to have the film side downwards in the water, but not resting on the bottom of the vessel. As the hæmoglobin dissolves out it will fall to the bottom of the vessel. It will be found better after a few minutes to transfer the slide to clean water, so that it is easy to observe when the hæmoglobin is all removed.

Remove the slide from the water and examine at once whilst still wet. The white corpuscles will stand out from the film as refractile spots and the white colourless worms will also stand out brilliantly.

If it is preferred to stain the specimen it should be

allowed to dry and fixed in alcohol and ether. Any basic stain gives good results, and weak carbol fuchsin is perhaps the best of the aniline stains. Hæmatoxylin gives good and permanent results, but the sheathed microfilariæ do not stain rapidly. If the hæmalum mixture is used it should be warmed, and five or ten minutes will be required for satisfactory staining purposes; the slide should then be flushed and left in water for ten minutes. A good many slides can be stained at the same time. For this the staining vessel (fig. 42) is convenient.

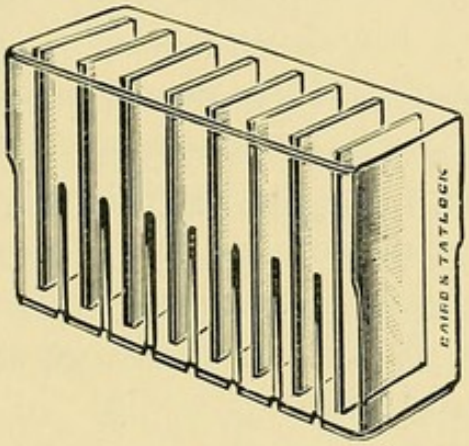


FIG. 42.

Counter-staining brings out nothing more, but eosin may be used for this purpose.

The shape of the worm, and also the sheath if present, are well shown in a specimen stained with hæmatoxylin. The body of the worm is found to contain a core of deeply-staining points or nuclei. These do not extend to either extremity, nor do they completely fill the worm, as a clear, unstained portion is left on each side. This unstained portion must not be mistaken for the sheath. The sheath will be faintly stained and only clearly seen at the two ends, where it will be found flattened on itself and often folded sharply like a piece of ribbon.

In the nuclear core complete or incomplete gaps in the mass of nuclei will be seen in most micro-filariæ. For each species the position of these gaps is constant, or

nearly so, and consequently the exact position of these gaps is important for the differentiation and identification of species from the examination of these embryos.

The arrangement of the nuclear core at the blunt cephalic end is of particular importance, as it is an additional point used in the diagnosis of species. In the *Microfilaria bancrofti* the nuclei are loosely arranged at this end, whilst in *Microfilaria loa* they form a compact mass terminating almost as a straight line.

Embryos of some species of filaria are not found in the same number all through the twenty-four hours. During a part of this period they may be found in numbers, whilst a few hours later they are found with difficulty or not at all. Thus one species has a periodicity which is called *nocturnal*, because the embryos are found in largest numbers in the peripheral blood at night; in other species embryos are only found in the daytime and are said to have a *diurnal* periodicity. Embryos of other species are found in fairly equal numbers at all times of the day and night.

In any investigation of the periodicity of filarial embryos it is essential that measured quantities of blood should be examined.

The periodicity can be altered in the case of *Microfilaria bancrofti* by changing the habits of the host, and cases are fairly common in which the periodicity is reversed without known cause. It is still more common to find small numbers of *Microfilaria bancrofti* during the day and larger numbers at night.

The chief points of difference in the various embryo filariæ are indicated in the subjoined table.

These points require no detailed explanation.

It is well to draw the embryos accurately with a drawing camera or camera lucida. By substituting a scale for the object, a scale can also be drawn on the same paper and measurements made from this, which is easier and usually more accurate than measurements made with a micrometer eyepiece.

Name	Length	Greatest thickness	Sheath	Shape of tail	Periodicity	Distance of head gap from head	Adult (known or suspected)
	mm.	mm.				mm.	
Microfilaria nocturna or Microfilaria bancrofti	·317	·0075	Present	Sharply pointed	Nocturnal in peripheral blood	·052	*F. bancrofti.*
Microfilaria diurna or Microfilaria loa	·317	·007	Present	Sharply pointed	Diurnal in peripheral blood	·052	*F. loa.*
Microfilaria perstans	·195	·0045	Absent	Blunt, truncated	None	·03	*F. perstans.*
Microfilaria demarquayi	.21	·005	Absent	Sharply pointed	None	·03	*F. demarquayi.*
Microfilaria ozzardi	·21	·005	Absent	Sharply pointed	None	·03	*F. ozzardi.*

Periodicity refers to the time of appearance of embryos in the peripheral blood. With regard to this periodicity, it was for a long time not definitely known what became of the embryos during the time they were absent from the peripheral blood. *Post-mortem* examinations, however, have shown that in the case of persons harbouring *F. bancrofti* where death has occurred during the day, the embryos are found in greatest numbers in the lungs and large vessels, though some may be found in the vesssels of other viscera.

In sections of the organs of such a person the microfilaria are found in numbers. The material may be imbedded in either celloidin or paraffin, and should not be too thin, or such short lengths of the microfilariæ will be cut that they cannot be easily identified.

Hæmatoxylin solution, two minutes, is quite sufficient to stain the embryos in section, and there is no need to counter-stain. Transverse and oblique sections of numerous embryos will be found. In places longer lengths, or even complete embryos, which were lying in the plane of the section, may be seen.

As far as is known, no developmental changes take place in the human filarial embryos in the blood or human tissues, but there is evidence that some degree of growth

does take place in some of the avian microfilaria whilst they are circulating in the blood.

In the case of the human *Microfilaria nocturna*, the next stage of growth occurs in several species of mosquitoes of different genera—*Culex*, *Anopheles*, *Mansonia*, &c.—and when a certain stage of maturity is reached the embryos escape from the proboscis of the mosquito and pass through the skin into man. At this stage the embryos in the case of *F. bancrofti* are 1·5 mm. in length, the alimentary canal is complete, but the sexual organs are not developed.

The further development in man has not been traced, but the adult forms of the species *F. bancrofti* have been found by many observers always in, or in connection with, the lymphatic system. The other human adult filariæ, *F. perstans*, *F. demarquayi*, *F. ozzardi*, and *F. loa* (the adult form of *Microfilaria diurna*), are found in connective tissue, either subcutaneous or in the subperitoneal tissues.

The adult human filariæ are not very readily found. *F. bancrofti* are found in lymphatics in almost any part of the body, but as a rule, in the cases of elephantiasis, the adults are long dead and the positions they once occupied only indicated by lymphatic obstruction. *F. perstans*, though smaller, are more readily found, as they occur, at any rate in greatest numbers, in subperitoneal connective tissue, particularly at the base of the mesentery. *F. demarquayi* has been found by Dr. Galgey in the same position, and *F. ozzardi* has been once found in the subserous connective tissue of the anterior abdominal wall.

Adult filaria, when found, are occasionally in a condition of partial or complete calcification. These calcified filaria occur fairly commonly in the pelvis of the kidney, in lymphatic glands, and occasionally in lymphatics elsewhere in the body. This should be borne in mind when a search is being made for the adults.

F. loa can be seen when it passes under the skin or

conjunctiva. It is difficult to extract, for as soon as an incision is made in the skin it rapidly moves away.

F. immitis, the "worm in the heart" of dogs, is found in the cavity of the right side of the heart and the pulmonary vessels. When only one or two worms are present they are usually in the smaller pulmonary arteries. Avian filariæ occur in many positions. Some species are found in loose connective tissues, as in the neck, others in the limbs, and particularly in thickenings about the claws; others in the submucous tissues, as in the crop; and others in the blood-vessels, and even in the pouches formed by the semi-lunar valves. Adult filariæ are easily mistaken for empty blood-vessels, small nerves and shreds of fibrous tissue. They are more readily recognized with slight magnification, and for this purpose a watchmaker's glass of about five-inch focal length is very useful. Those mounted in horn are best, and should be perforated at the sides, otherwise moisture condenses on the lens. The advantage of these glasses is that both hands are free, and it is easy to learn the use of this simple lens.

In searching tissues for filaria a dark surface, such as a slab of slate, makes a good background, and the rough surface of the slate prevents the specimen slipping about. The tissue should be kept wet with normal saline solution, as this keeps it transparent. The dissection should not be made with the tissues *floating* in water or salt solution, as strands of tissue are much more readily twisted or ravelled out if floating, and would be mistaken for filaria.

Description of Adult Filaria.—Some authors construct a formula for the description of filariæ based on the relative positions of various structures and the measurement of the worm at these places. The unit of measurement is the one-hundredth part of the length of the worm, so that the measurements are percentages of the length. Five measurements are taken by the author of the method —Cobb—commencing from the head: the base of the œsophagus, the nerve ring, the cardiac constriction, the

fourth at the vulva in the female and the middle of the male, and the fifth at the anus (fig. 43).

Many of these points are very difficult to make out in the human filariæ. In living filariæ the first of them is very variable in the same individual. As the head and neck are capable of considerable contraction, the head cannot be taken as a fixed point to serve as the basis of a series of measurements. Also the whole formula is based on the assumption that the proportions of various parts of the body are constant in different individuals, which, according to Shipley, is not certain.

Though we do not consider that this graphic method is applicable in many cases, still, where possible, it may be given.

The human filariæ resemble each other rather closely in their adult forms, and some of them require very careful examination for differentiation. Measurements

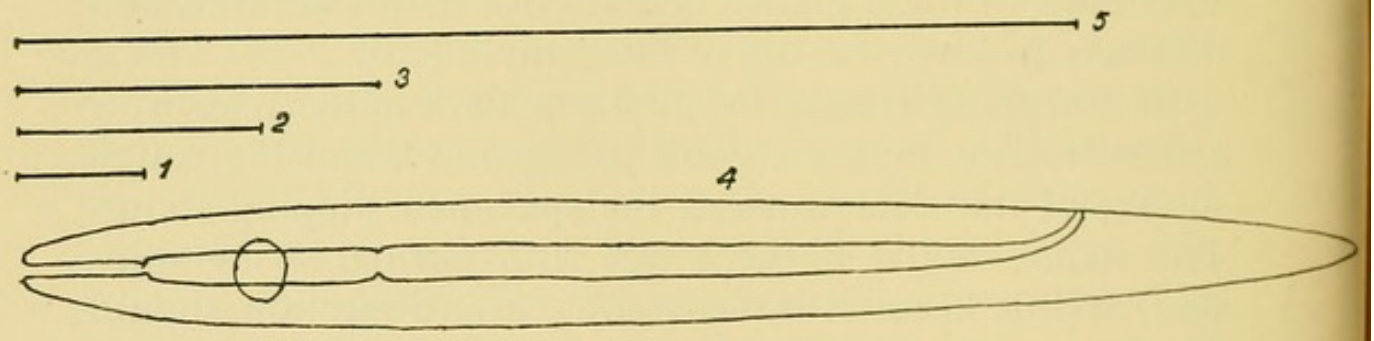


FIG. 43.

of the head and tail, making due allowance for the contractility of the worms, are of great importance. Particular attention must be paid to the transparent cuticle, as there are important differences in its arrangement in different species, and these differences are constant for the individuals of each species.

The measurements should be made, where possible, in the fresh worms, as serious shrinking and distortion occur with most reagents. Alcohol and spirit cause great distortion. This can be diminished by placing the

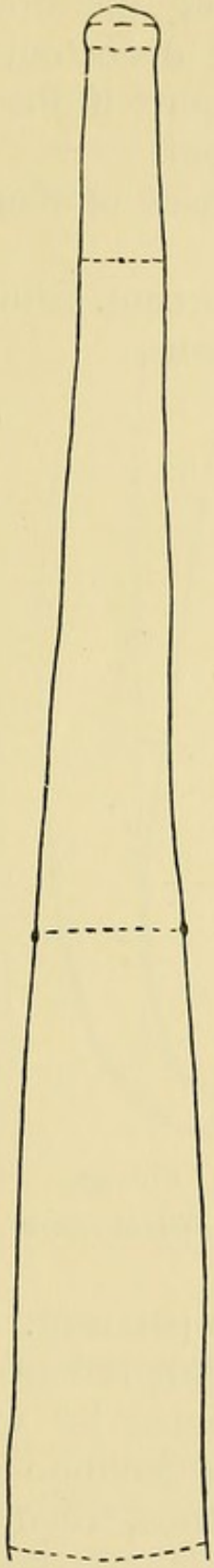


FIG. 44.
Head of *Filaria bancrofti*, ♀.

FIG. 45.
Head of *Filaria ozzardi*, ♀.

specimen first in dilute spirit, 1 to 3 of water, for a few hours, and then gradually increasing the strength, but however carefully this is done the distortion is great. Much less distortion is caused by spirit if the specimen is first hardened in formalin 2 per cent.

A general method for the treatment of nematodes is as follows :—

(1) Place the worms alive in a 1 per cent. saline solution and shake up. This removes all mucus.

FIG. 46.
Tail of *Filaria bancrofti*, ♀.

FIG. 47
Tail of *Filaria ozzardi*, ♀.

(2) Have ready alcohol (60 to 70 per cent.) which has been heated to the boiling point. This is best done in a porcelain dish over a flame protected by wire gauze. Transfer the worms from the saline solution to the hot alcohol, dropping them in one at a time, when the worms will die in an extended position.

They may be preserved in 70 per cent. alcohol till required for examination.

(3) For examination they are placed in a vessel containing a mixture of 95 parts alcohol and 5 parts pure

glycerine and placed on a water bath. The alcohol is thus evaporated. In pure glycerine the worms become transparent. They may be studied in glycerine or mounted in glycerine jelly.

If placed at once in glycerine without any preliminary treatment, there is, at first, some swelling, though when

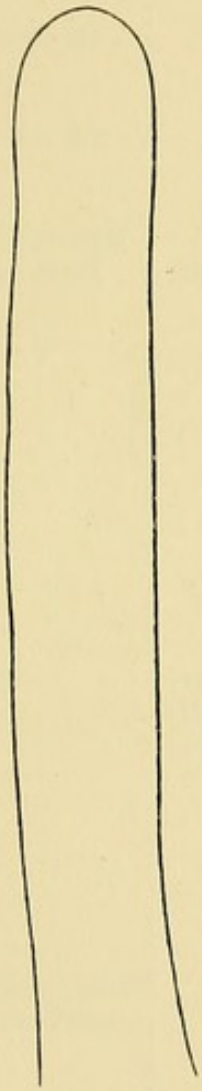


FIG. 48.
Head of *Filaria demarquayi*, ♀.

FIG. 49.
Head of *Filaria perstans*, ♀.

left long in the glycerine there is a return to a more natural condition. The specimens so prepared are much softened and can very readily be flattened out, and whilst thus gently compressed between two slides be hardened in methylated spirit and finally in alcohol, and mounted after clearing in oil of cloves. Such specimens are very transparent and do not show much detail; if, however,

	F. bancrofti		*F. perstans*		*F. ozzardi*	
	Female	Male	Female	Male	Female	Male
Length	80—90 mm.	44 mm.	70—80 mm.	45 mm.	81 mm.	38 mm.
Greatest breadth ...	·23 mm.	·1 mm.	·12 mm.	·06 mm.	·21 mm.	·19 mm.
Diameter of head ...	·055 mm.	·05 mm.	·07 mm.	·04 mm.	·05 mm.	?
Character of cephalic end	Unarmed. Rounded	Unarmed. Rounded	Unarmed. Rounded	Unarmed. Rounded	Unarmed Rounded	?
Distance of genital pore from head (female)	·66—·75 mm.	—	·6 mm.	—	·71 mm.	—
Diameter at point of genital pore	·14 mm.	—	·07 mm.	—	·12 mm.	—
Distance from tail of anus	·225 mm.	—	·145 mm.	—	·23 mm.	—
Cuticular thickening on tip of tail	None ...	None ...	Double terminal cuticular thickening	—	None...	None ...
Spicules (male) ...	—	Two unequal, anterior and posterior, both retractile	—	Two unequal spicules	—	?
Papillæ (caudal) ...	None ...	Minute flat papillæ have been described by Leiper	None ...	Four preanal and one postanal. Very close to opening of cloaca	None...	None ...
Habitat	Lymphatic system... ...		Connective tissue, usually subperitoneal ..			
Geographical distribution	In most tropical regions ...		Africa (West Coast and Central), British Guiana		British Guiana ...	

F. demarquayi		*F. loa*		*F. magalhãesi*	
Female	Male	Female	Male	Female	Male
65–80 mm.	Not known ...	50—55 mm.	30—35 mm.	155 mm.	83 mm.
·21—·25 mm.	—	·55 mm.	—	·6—·7 mm.	·3—·4 mm.
·1—·09 mm.	—	—	—	·06 mm.	·04 mm.
—	—	Rounded with papillæ	—	—	—
·76 mm.	—	2·35 mm.	—	2·56 mm.	—
·1 mm.	—	—	—	·58 mm.	—
·25 mm.	—	·3 mm.	1·75 mm.	·13 mm.	—
Cuticular thickening over tip. Knobby and irregular in outline	—	No thickening over tip. Two lateral alæ. Cuticular bosses not found at tip, but over the greater part of the body	Thickening over tip. The "bosses" so abundant over the cuticle in the body of the worm are not found at the tip	None ...	None.
—	—	—	Two unequal, anterior and posterior	—	Two spicules.
—	—	—	Three preanal pairs and two postanal. The last are very small	—	Four preanal and four postanal.
Subperitoneal connective tissue		Connective tissues, subcutaneous, subconjunctival, or in the deeper parts of the limbs.		Left side of heart.	
West Indies		West Africa		Brazil.	

they are slowly stained with very dilute solutions of stains, such as dilute borax carmine, before placing in glycerine, many details of structure are brought out well. They can also be stained with well-diluted hæmatoxylin, and subsequently slightly decolorized with dilute acid spirit ½ per cent. to show eggs and embryos *in situ*.

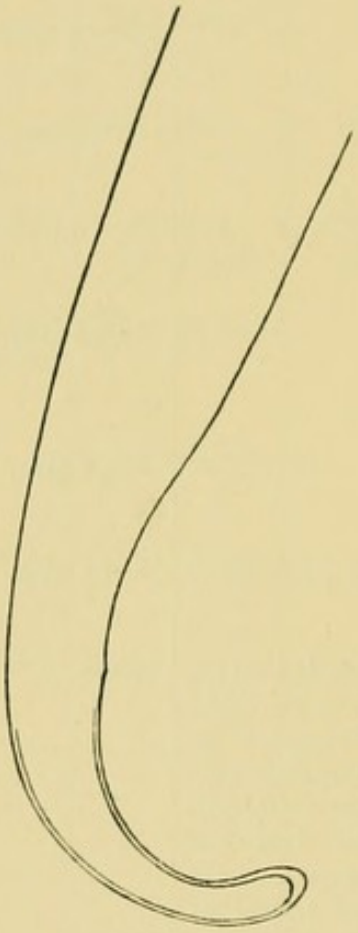


FIG. 50.
Tail of *Filaria demarquayi*, ♀.

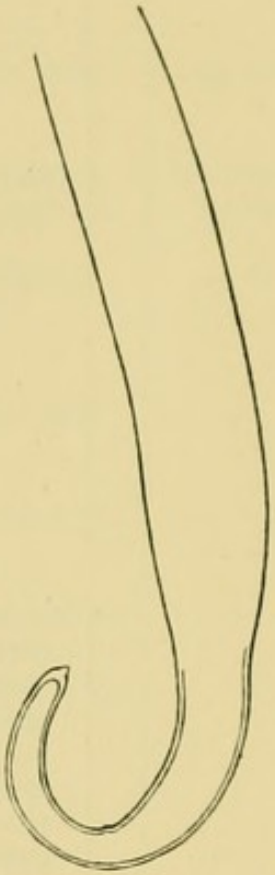


FIG. 51.
Tail of *Filaria perstans*, ♀.

Many filariæ show fairly well when mounted direct in glycerine jelly, but these, after a time, become distorted. If previously hardened by placing first in 1 per cent. formalin for two days and then in 2 per cent. formalin for two days, and then kept in 5 per cent. formalin for some days, they can be mounted in glycerine jelly, or even in Farrant's solution, and retain their natural size and appearance.

The different points enumerated in the tabular form can usually be made out, but to see either the genital pore

or the anal opening that portion of the worm must be viewed in profile. It is therefore necessary to turn the worm gently before mounting so that they can be seen. This can generally be effected by slightly moving the cover-glass by pressure on its edge with a needle so as to roll the worm over slightly.

The points of difference and resemblance are shown in the table (pp. 136 and 137 and figs. 44-51) for the known adult human filariæ. The description of *F. ozzardi* is from a single specimen. The embryos or microfilariæ of *F. ozzardi* and *F. demarquayi* correspond in every respect. As the single adult found of *F. ozzardi* differs from the specimens of *F. demarquayi* in some respects, the worms cannot be considered as the same, but further specimens are required before the question of the identity of the species can be considered to be settled.

EXAMINATION OF THE BLOOD FOR PATHOGENIC BACTERIA.—Most of the organisms found in blood films are due to contamination with skin organisms during the preparation of the film. To avoid this, the finger, which for this purpose is the most convenient part to examine, should be well washed with 2 per cent. lysol, and then wrapped in a 1 in 500 sublimate compress covered with gutta-percha tissue for twelve hours. The first drop of blood should be rejected as the most likely to be contaminated. Thick and thin films should be taken and rapidly dried. The thin films can be stained by Louis Jenner's or Leishman's stains, or the film can be fixed and stained by any of the methods used for bacteria. In thick films, after drying, the hæmoglobin can be removed by placing in sterilized water, and the film afterwards fixed and stained.

In most cases organisms are present in such minute numbers that they will not be found by this method, and the drops of blood obtained should be used for making cultures. The organisms of plague, septicæmia, tubercle, &c., may be found in the blood, but great caution must be exercised as mistakes are frequent. Cultivation of the

organisms from blood drawn from a vein by a hypodermic syringe is more satisfactory. For this purpose a few drops of blood should be placed in each of a series of flasks containing some 20 cc. of broth, so that great and rapid dilution of the blood takes place. The leucocytes will fall to the bottom of such flasks, and will not destroy so many of the organisms which may be present in the blood.

CHAPTER VIII.

Certain Properties of Blood Plasma and Blood Serum.

The living blood in the body is composed of a fluid element—the plasma—in which are suspended the solid elements, red and white corpuscles, blood platelets, and at times parasites. After death, and when the blood is shed, coagulation occurs, the blood plasma being converted into a jelly-like mass which soon contracts, and a fluid—the blood serum—separates. Parasites are usually included in the coagulum as well as the more solid elements of the blood, such as the corpuscles.

Blood Plasma.—For certain purposes it is desirable that the blood should be kept fluid and coagulation prevented. This is requisite when we wish to inject blood containing living parasites, such as filariæ, or trypanosomes, &c., or to obtain certain constituents of the blood, such as the white blood corpuscles. If the blood is allowed to coagulate, parasites contained in it are usually entangled in the blood-clot. To prevent this, the blood should be quickly mixed with a 10 per cent. solution of citrate of sodium. One part of this solution, if rapidly and thoroughly mixed, will prevent the coagulation of twenty-five parts of blood. Twice the quantity of a 5 per cent. solution is somewhat easier to work with and equally effective—others use a normal citrate of soda solution, a much larger quantity being required. In these mixtures the blood corpuscles are not destroyed.

To obtain blood in quantity such as is required for injecting into animals, it is best to plunge a hypodermic

needle into a distended vein. The median basilic or median cephalic will be found most convenient. An all-glass syringe should be used. No powerful suction is required; if the point of the needle is in the vein, by holding the syringe horizontally very little suction is necessary. When possible injections should be made at once with unmixed blood; if this is not possible the blood should be citrated.

To study the characters and special properties of either the red or white corpuscles, coagulation must be prevented in the same way and the corpuscles rapidly separated by centrifugalizing the blood. Such corpuscles may then be transferred by means of a pipette to normal saline solution, and will retain their properties for a considerable time.

Coagulation Time.—Blood varies greatly in the rapidity and firmness with which it coagulates, and the time required is influenced by various diseases. Methods of estimating the coagulation time for clinical purposes are not very satisfactory, and all determinations must be made at a constant temperature.

Wright's method is to draw up blood into a series of capillary tubes of uniform calibre, and attempt, by blowing at intervals of half a minute, to dislodge the blood. When it cannot be dislodged it has coagulated, and the time it has taken is the coagulation time. This simple method appears to give as good results as any.

Specific Gravity.—The specific gravity of the blood is another valuable element, and is not easily determined accurately with the small amounts of blood that can be used for clinical purposes.

Blood is dropped into a series of fluids of known specific gravity varying from 1035 to 1068, and the specific gravity of the fluid in which the blood neither sinks nor rises is of the same specific gravity as the blood. The fluids chiefly used are glycerine and water or chloroform and benzol in varying proportions.

Chemical Reaction.—The reaction of the blood can be

determined either by using glazed litmus paper previously soaked in chloride of sodium solution, or a plaster of Paris disc soaked in neutral litmus solution.

Spectroscopic Examination.—In addition to hæmoglobin we may have in the blood in some cases derivatives or modifications of hæmoglobin. A small direct vision spectroscope is the most satisfactory method of determining the presence of these substances. The blood should be laked by the addition of distilled water to render it sufficiently translucent. If it be desired to determine the presence or absence of hæmoglobin from the serum another specimen of the blood should be allowed to coagulate, and when the serum has separated that should be examined separately. The diluted blood should be placed in a small vessel with two plane sides inclined towards each other at an acute angle, so that varying thicknesses of the fluid can be examined.

Either ordinary daylight or a lamp can be used, and the spectrum should first be focussed as sharply as possible and the slit closed as much as is convenient to bring out Fraunhöfer's lines distinctly.

The spectra of oxyhæmoglobin and reduced hæmoglobin can be readily obtained from the same specimen, either by shaking up with air to oxidize or reducing by the addition of ammonium sulphide (Plate III.).

Methæmoglobin gives two additional lines, as seen in the diagram, and the two lines between the D and E are further apart and faint; on the addition of alkali the spectrum changes and becomes more like that of oxyhæmoglobin (Plate III.). Methæmoglobin is of considerable importance, as it colours the urine brown and not red. If no spectroscopic examination is made it will usually be overlooked. In some of the mildest cases of "Blackwater Fever" methæmoglobin and not hæmoglobin may be found. Urobilin is shown by the single broad band between E and F (Plate III.). Bile in human urine causes no definite banding.

The coloured plate gives the spectra of hæmoglobin

and its derivatives ; some of these are only formed under artificial circumstances and consequently are of little practical clinical value.

The colouring matter of blood is hæmoglobin, it forms some 90 per cent. of the red corpuscles, and is not found in blood plasma, nor, when the blood coagulates, in the serum. It can, however, be readily removed from the red corpuscles by the addition of water either to the fluid blood or to the freshly dried blood.

Advantage is taken of this property when thick films are made, as in examining for filaria, in order to render a thick flim transparent. To prevent the occurrence of this solution in making preparations for the examination of thin films, "fixing agents," such as alcohol, perchloride of mercury, formalin solution or vapour, or heat, are employed.

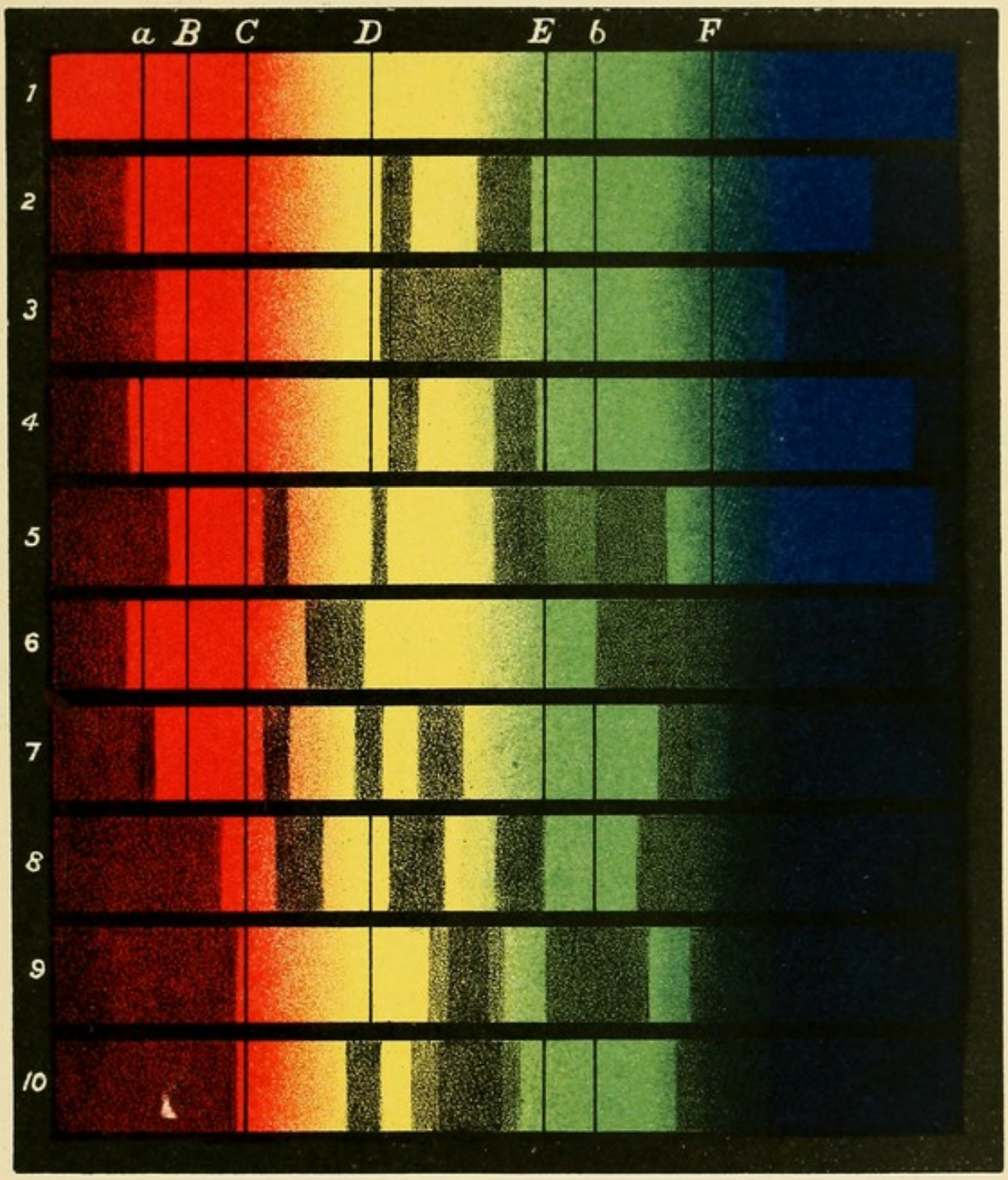
Estimation of Tonicity.—Different specimens of blood vary in the power of the corpuscles to retain their hæmoglobin. Though distilled water will remove the hæmoglobin completely from the corpuscles in fluid blood, saline solutions over a certain strength will not remove it. This resistance or "tonicity" of the blood corpuscles is measured by the strength of saline solution, which is just sufficient to prevent the solution of the hæmoglobin. Such a solution is said to be "isotonic." Normal saline solution ·75 per cent. prevents the solution of hæmoglobin in blood. A series of weaker solutions differing in the amount by ·02 per cent. is made, and a drop of blood is dropped into each, and after shaking allowed to stand.

The weakest salt solution that does not cause solution of the hæmoglobin is the index of the "tonicity" of the blood used.

The strength of that solution gives the isotonic strength, which is the measure of the resistance of the blood, normally 0·46 to 0·48 per cent. A less accurate but more convenient method is to mix a measured amount of the blood with a measured amount of a solution, such as a

Plate III.

BLOOD-SPECTRA COMPARED WITH SPECTRUM OF ARGAND-LAMP.



1 Spectrum of Argand-lamp with Fraunhofer lines in position.
2 Spectrum of Oxyhæmoglobin in diluted blood.
3 Spectrum of reduced Hæmoglobin.
4 Spectrum of carbonic oxide Hæmoglobin.
5 Spectrum of acid Hæmatin in etherial solution.
6 Spectrum of alkaline Hæmatin.
7 Spectrum of Chloroform extract of acidulated Ox-bile.
8 Spectrum of Methæmoglobin (alkaline.)
9 Spectrum of Hæmochromogen.
10 Spectrum of Hæmatoporphyrin.

Most of the above Spectra have been drawn from observations by Mr. W. LEPRAIK, F.C.S.

Fig. 52.

3 per cent. solution, which is well above the isotonic strength, and add gradually measured amounts of water till the solution of the hæmoglobin takes place ; from this the strength of the fluid which just causes solution can be calculated.

Wright's tubes with the air and mixing chambers are convenient for the purpose. The tonicity of the blood is of considerable importance, as a decrease in the tonicity often precedes a hæmolytic attack. This occurs in black-water fever, and persons whose blood is of a low degree of tonicity should not be allowed to live in countries where this disease is endemic.

These methods assume that the red corpuscles in the blood are all equal as regards their tonicity, or nearly so. This is not the case, as even in healthy blood an occasional corpuscle will be found, that will be decolorized in a stronger solution of salt than the others, and also a few will retain their hæmoglobin when all the others are decolorized. With healthy blood the great majority of the corpuscles may be equal, but with blood in some diseases, a much larger number of red corpuscles are of a lower degree of tonicity than the average.

To determine the range of variation of tonicity in the corpuscles the blood should be first well diluted with a strong salt solution, 4 per cent., which is hypertonic to all corpuscles. The mixing can be done in one of Wright's tubes. The tube is allowed to stand and the corpuscles will fall to the bottom, and by heating the air chamber can then be expelled into a clean watch glass.

Hanging drop preparations of this blood, diluted with one, three, or seven parts of distilled water, will be equivalent to 2 per cent., 1 per cent. and ·5 per cent. of salt solution. An examination of these hanging drops will show if any considerable proportion of the corpuscles have lost their hæmoglobin. If none or very few are decolorized with the 1 per cent. salt solution the remainder of the red corpuscles in the watch glass in 4 per cent. salt solution

should be diluted with three parts of distilled water in one of Wright's tubes and well mixed in the mixing chamber. The fluid can be expelled into a clean watch glass and a series of dilutions, as hanging drops, made. One part diluted with one of water will give ·5 per cent., with two of water ·33 per cent. Two parts of the diluted blood with one of water will give ·66 per cent., and so on. In this manner, by examining a series of these hanging drops, and determining the proportion of the "shadow" corpuscles which can be easily seen with an oil immersion lens if the light is cut off, the proportion of corpuscles of lower index of tonicity than represented by these solutions can be determined.

When the hæmoglobin is dissolved in the serum the blood is said to be "laked." Dissolved hæmoglobin is found in the serum in acute hæmolytic processes, but appears to be rapidly removed either by the hepatic or renal cells, converted by the liver into bilirubin, or deposited in the subcutaneous tissues. The yellow tint of skin and conjunctiva in some diseases which simulate jaundice is of this nature, and is called hæmatogenous jaundice. The yellow tinge round old bruises is due to the solution of the hæmoglobin of the extravasated blood.

Normal blood serum is "hypertonic," that is, not only is it sufficient to prevent the solution of hæmoglobin from the red corpuscle, or is isotonic, but is considerably above that strength. This excess of tonic value is not simply due to the amount of salts; it varies considerably and is estimated by determining the dilution with water required to render it isotonic as regards normal red corpuscles. This can be determined either by using a series of dilutions and dropping (with distilled water, or as a series of hanging drops in Wright's tubes) blood into each, or by diluting to a known extent a mixture of blood and serum.

Blood Serum.—Blood serum is required for several purposes, notably for the demonstration of the presence or absence of specific agglutinins.

The glass tubes devised by Wright afford a ready method of obtaining and diluting serum. The simplest form is to draw out a piece of glass tubing of about one-quarter inch diameter. In drawing out the tube it is well to rotate it in the flame until it is quite soft at the required place, then removing it from the flame, pull steadily. In this way tubes of more uniform size are obtained than if the traction be exercised while the tube is still in the flame. The thin tube thus formed should be broken, and at a convenient distance another portion of the tube should be heated and pulled out in the same manner, or bent as shown in fig. 53.* One of the capillary extremities should

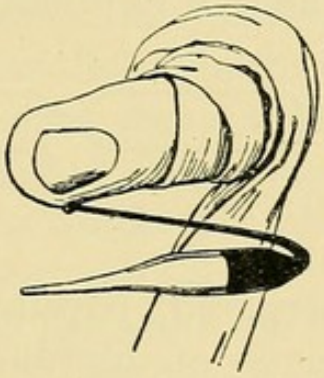


FIG. 53.

be sealed. A puncture with a broad needle or small knife should be made in the skin and the *upper* half of the unexpanded tube, that towards the sealed end should be heated in the flame of a spirit lamp, which is lighted and placed close at hand before the skin is punctured. Holding the lower part of the tube which has not been drawn between the fingers to make sure that it is not too hot, the open drawn-out end is placed in the exuding blood. As the air in the tube cools and contracts the blood will be drawn up into the tube. If there is not enough or the blood is not drawn entirely up into the thick part of the tube by the time it is cool, the sealed

* We are indebted to the kindness of the proprietors of the *Lancet* for the use of fig. 53.

end can be broken off and the upper end of the thick tubing again heated and the same end again sealed. The contraction of the air will be sufficient to draw more blood up, and the blood already in the tube higher up. When sufficient blood is in the tube and the tube is cool, the lower end through which the blood entered can be sealed. The tube is now placed on its side horizontally till the blood coagulates, and is then placed vertically, so that as the serum is expressed by the contraction of the clot it will run down into the narrow part of the tube. In this way clear serum, free from blood corpuscles, can be obtained without using a centrifuge. Capillary vaccination tubes can be used to collect the blood, but will require to be centrifugalized to obtain clear serum.

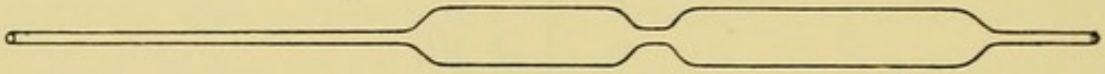


FIG. 54.

Serum, however prepared, requires dilution for most purposes, and the degree of dilution is important. The graduated pipette of a hæmocytometer may be used for this purpose. A very convenient method of obtaining any degree of dilution is by Wright's tubes. A piece of glass tubing is drawn out sharply in the middle so as to make a short, sharp constriction. About an inch and a half from this constriction on each side the tubing is drawn out into a long, thin capillary tube. One of these is broken off and sealed, and the other, preferably the more uniform and thicker, is touched with a file, broken off square and left open. The part above the middle constriction with the sealed capillary tube attached is called the air chamber, and that below in connection with the open capillary tube is called the mixing chamber. A narrow mark is made on the open capillary tube with a grease pencil about an inch or less from the open end. This distance depends on the calibre of the tube, the greater this is the shorter the distance from the open end

to the mark, as the volume of the column of fluid between the open end and the mark is the unit of measurement. The finger or ear is pricked, and from the blood obtained the serum is allowed to separate as above and blown out into a sterile watch glass. The air chamber is then well heated, and the open end of the tube is placed in the blood serum till the serum runs up to the mark. The tube is then removed from the serum, and a little air enters the tube as the air in the hot air chamber contracts, the open end is then placed in the diluting fluid and withdrawn as soon as the fluid reaches the grease pencil mark; it is then withdrawn, but as soon as air has entered the tube, it can be replaced in the fluid and again withdrawn when the mark is reached. This can be repeated as long as the air in the air chamber contracts. If repeated nine times, there will be nine parts of the diluent to one of the blood serum. As the fluid by contraction of the air in the air chamber is all drawn up into the mixing chamber, it can there be well mixed by rapidly rotating between the palms of the hands.

This procedure would give a dilution of 1 in 10, and by continuing the process, greater dilutions would be obtained; but it is better, if high dilutions are required, such as 1 in 100 or 1 in 1,000, after well mixing the serum and diluent to expel a part of it into a sterilized watch glass by heating the air chamber.

The expelled diluted serum is further diluted in a second tube in exactly the same manner as the first dilution, and from this a third, and in turn a fourth, dilution can be made. The tubes with the diluted serum may be sealed up and kept for some time if necessary.

If it be desired, a known amount of a broth culture of an organism can be in the same manner drawn up into the mixing chamber and there mixed with diluted or undiluted serum.

Wright uses india-rubber teats to draw up the fluid (*vide* fig. 55), but as in the Tropics india-rubber does not keep well, the air chamber drawn out into a long capillary

tube is more satisfactory. Wright, when using an air chamber, blows it out into a bulb so as to have a larger volume of air, but a smaller air chamber is sufficient in most cases, and if it cools too rapidly, so that the air ceases to contract, the sealed end may be broken off, and whilst the tube is still open, the air chamber can be heated and the tube again quickly sealed. This can be repeated as often as one wishes if a large volume of serum or blood is required in the mixing chamber.

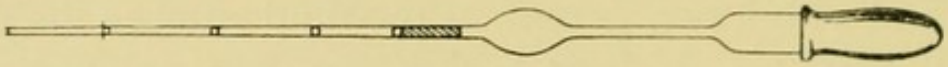


FIG. 55.

Instead of serum, the blood itself can be mixed with a diluent in a similar manner, and the diluted blood used for counting leucocytes or red corpuscles. It is necessary that the diluent should be one that will prevent coagulation and will not cause destruction of the red corpuscles. Gower's solution is fairly satisfactory, or if it be desired to stain the leucocytes, Toisson's fluid may be used. The mixing for uniform and successful results must be done quickly, otherwise part of the blood may coagulate or the corpuscles adhere together in masses.

In addition to agglutinins other substances may be formed in serum as a result of infection by micro-organisms. These include the toxins and antitoxins, *i.e.*, the poisonous products of the growth of organisms and substances that neutralize these poisonous products; also hæmolysins or substances that have a destructive action on blood corpuscles.

Precipitins.—A class of substances which promise to be of much practical importance are the precipitins. It is found that if blood of one animal, as, for instance, man, be repeatedly injected into a rabbit, the constitutional disturbance set up by the injections becomes less and less, and after a few injections they cease to cause any

disturbance. It is further found that the blood serum of this rabbit, immunized as to human blood, will give a precipitate when added to a solution of human serum or of closely related animals, such as the ape, when much diluted, but not with solutions of serum of other animals, such as the Rodentia.

Similarly, if a rabbit be immunized by repeated injections of the blood of any animal of a different genus, horse, rat, pig, &c., the serum of the rabbit will give a precipitate with solutions of the serum of the horse, rat, and pig respectively, or animals closely related to them.

This gives a new means of grouping animals and promises to be of practical and medico-legal value. The immunized or test sera can only be made where proper appliances are available, but appear to keep well.

The application of the test is easy. Clean white filter paper is soaked in the fresh uncoagulated blood of the animal and allowed to dry in the air. A portion of this paper is treated with normal saline solution. The clear solution thus obtained is placed in a small test tube (Durham's tubes are suitable) and a few drops of the test serum are added. A precipitate indicates that the blood was either that of the animal against whose blood the rabbit was immunized or a closely related one.

Nuttall has made observations on a large series of animals, and the results obtained have been consistent, and in many ways have thrown light on the relationship of different animals. The filter papers, soaked in blood and well dried in the air, keep well and give the reaction after many months. It is important that blood should be obtained from any rare animal and examined in this way to aid in its classification. The filter paper or white blotting paper should be soaked in the blood and clots removed. The blood must be fresh. The paper should then be allowed to thoroughly dry, and, if possible, wrapped in wax paper and sent to England to be tested with the prepared sera.

Culture Medium.—Blood serum makes an excellent cul-

ture medium for many organisms, and some will not grow, or only grow with difficulty, in more artificial media.

For the cultivation of trypanosomes the blood serum obtained after the blood has been "laked" is the most satisfactory medium. Such a fluid is a solution of hæmoglobin in diluted blood serum.

Opsonins.—Another property of blood serum is the power it possesses of acting on pathogenic micro-organisms, and so altering them that they can be taken up and destroyed by the leucocytes. The substances to which the blood serum owes this property have been named by their discoverer, Wright, *opsonins* (Latin *opsono*, I prepare food for). Wright believes that the leucocyte is a constant factor in the phenomenon of phagocytosis, and that the variable and most important factor is the amount of opsonin in the blood serum. It is further considered probable that these opsonins have a high degree of specificity, that is to say, that there is a corresponding opsonin for each organism ; that for the tubercle bacillus, for example, has no action on staphylococci, and *vice versâ*.

In order to estimate the amount of opsonin present in a blood serum for a given organism there is required :—

(1) The serum to be tested.

(2) The serum of a healthy person to be used as a control.

(3) An emulsion of the given organism in normal saline solution. This emulsion may contain either living or dead organisms, and they should be present in such numbers as to admit of their being readily counted in the leucocytes.

(4) Leucocytes washed free from plasma.

To obtain these allow about 1 c.c. of blood from a healthy person to drop into 10 c.c. of normal saline solution containing 1½ per cent. of sodium citrate. This mixture is now centrifugalized until the corpuscles have fallen to the bottom. The clear supernatant fluid is

then pipetted off and replaced by normal saline solution and the whole shaken up. After further centrifugalization the supernatant fluid is again removed, and the leucocytes, which form a creamy layer on top of the red blood cells, are ready for use.

(5) Two pipettes prepared as described on p. 150 (fig. 55).

As described under serum dilutions, draw up equal parts of (1) emulsion of organisms ; (2) emulsion of leucocytes ; (3) serum to be tested in the order named. Expel these on to a glass slide and mix thoroughly. Afterwards draw the mixture into the pipette and seal off the tip. Remove the rubber teat and place the pipette in an incubator, or in a vessel of water at 37° C., carefully noting the exact time. Now prepare a second pipette in the same way, using this time the normal serum instead of the serum to be tested. Place this under precisely the same temperature conditions.

At the end of fifteen minutes in the case of each pipette expel the contents on to a slide, mix well, and prepare smears. The smears are then fixed and stained by a method suitable for the demonstration of the organisms.

The stained smear should then be examined with a lens of low power, and a part selected where leucocytes are present in greatest numbers.

On examination with the oil immersion lens, it will be found that organisms have been taken up by the polymorphonuclear leucocytes. The number of organisms in eighty such leucocytes should be counted, in the one case where the serum to be tested was used, and also in the other case where the control serum was used.

The ratio between these two figures gives the *opsonic index*. For example, if in the smear prepared from the mixture in which the serum to be tested was used, it was found that 80 polymorphonuclear leucocytes contained 95 organisms, and in the other smear the same number of polymorphonuclear leucocytes contained 190 organisms, then the opsonic index of the serum under examination for that organism is $\frac{95}{190} = \cdot 5$.

CHAPTER IX.

ARTHROPODA—INSECTA.

WITH the progress of the enquiry into the causation of disease it has come to be of importance that the investigator of tropical diseases should possess some knowledge of the blood-sucking flies, mosquitoes, ticks, fleas, &c., which are known or suspected to be concerned in the transmission of various infections. A brief account is here given of the more important members of the zoological division *Arthropoda*, to which these carriers of disease belong.

The *Arthropoda* are bilaterally symmetrical segmented animals with a thick chitinous cuticle. To some of the segments are attached articulated ambulatory limbs—it is from this feature that the name of the phylum or sub-kingdom was derived.

The segments vary in number, and in some arthropods the segmentation is not obvious externally, but is inferred from the arrangement of internal structures, such as nerve ganglia. The cephalic segments, three in number, are always fused into one mass, the head—the cephalic appendages are modified for purposes of mastication or suction and sensation. The three or four segments behind the head are often fused more or less completely to form the thorax, which may be united to the head to form the cephalo-thorax. To the thorax are attached the legs. The abdominal segments are usually distinct, but may be fused and united to the cephalo-thorax, as in some of the *Arachnida*. The abdominal appendages are essentially those connected with excretion and repro-

duction, but may be so modified as to form "stings," or weapons of offence and defence.

The sub-kingdom or phylum *Arthropoda* is divided into four groups :—

(1) *Myriapoda* (centipedes, millipedes, &c.).
(2) *Insecta* (horse-flies, butterflies, mosquitoes, &c.).
(3) *Arachnida* (spiders, ticks, &c.).
(4) *Crustacea* (cyclops, &c.).

The Insecta and Arachnida include the most important carriers of disease, and will be considered first.

Class INSECTA *or* HEXAPODA.—The term *Insecta* was originally employed to embrace all those animals whose body is externally divided into segments, including butterflies, beetles, centipedes, scorpions, &c. It is now used in a much more restricted sense to apply to such arthropods as have six walking-legs. The Insecta have the body distinctly divided into three regions—head, thorax and abdomen. They take in air by means of tracheæ, a system of tubes ramifying throughout the body and opening externally by means of orifices placed in pairs at the sides of the body. The appendages forming the mouth-parts are paired, and consist of mandibles, maxillæ and labium, the pair in this latter part being combined to form a single body. To the head are also attached a pair of antennæ.

There are two pairs of wings, but one or both of these may be rudimentary or modified for purposes other than flight : sometimes even rudimentary wings appear to be absent. The wings are always placed on the thorax, and to this region also are attached the three pairs of legs.

Insects in many of the orders undergo a variety of changes of form in the course of their development.

In the system here adopted the insects are divided into eleven great groups or Orders, the characteristics of which we give in brief. By "mandibulate mouth" is meant one in which the mandibles or maxillæ, or both, are fitted for biting, crushing or grasping food. The term "suctorial" implies that some of the mouth-parts

are modified to form a tubular suctorial apparatus; this is frequently protected by a modification of other parts, which act as a sheath.

(1) *Aptera.*—Wingless insects. All wingless insects do not, however, belong to this order. Mouth mandibulate, or very imperfectly suctorial.

Two sub-orders.—(i.) *Thysanura;* (ii.) *Collembola.*

(2) *Orthoptera.*—Four wings, the front pair being leather-like, and usually smaller than the hind pair, which are membranous and contract after the manner of a fan. Mouth mandibulate.

This order includes earwigs, cockroaches, grasshoppers, crickets, &c.

(3) *Neuroptera.*—Two pairs of membranous wings, frequently with much network; the front pair similar to the hind; the latter with little or no fanlike action in closing. Mouth mandibulate.

This order includes dragon-flies, may-flies, termites, &c. There are some parasitic wingless forms, such as "bird-lice." Adult dragon-flies are destructive to other insects, and their larvæ are very destructive to aquatic larvæ, such as those of mosquitoes. Attention to the breeding of some species of dragon-flies, particularly of the *Agrionidæ*, is important, as these breed in places similar to those in which larvæ of Culicidæ are usually found.

The larvæ of *Agrionidæ* have short rounded bodies. Their mouth-parts are modified to form a protrusible mask with which they seize their prey. These larvæ lurk at the bottoms of pools, puddles and streams, and are the most important of the natural enemies of mosquito larvæ. The adult *Agrionidæ* can be distinguished by the peculiar position of the wings, which are always inclined backwards, never at right angles to the body as in other dragon-flies.

(4) *Hymenoptera.*—Two pairs of membranous wings, linked together with little hooks, the front pair being larger than the hind, which are always small, and do not fold up in repose. Mouth mandibulate, sometimes also provided with a tubular proboscis.

This order includes bees, wasps, ants, &c.

(5) *Coleoptera.*—Two pairs of wings, the front pair shell-like and forming cases which meet together over the back, so as to lose entirely the appearance of wings and to conceal the delicate membranous hind pair. Mouth mandibulate.

This order includes the beetles.

(6) *Lepidoptera.*—Two pairs of large wings covered with scales. Mouth suctorial.

This order includes butterflies and moths.

(7) *Diptera.*—One pair of membranous wings. Mouth suctorial, but varying greatly, sometimes penetrating as well as suctorial.

(8) *Thysanoptera.*—Two pairs of very narrow fringed wings. Mouth imperfectly suctorial.

This order includes thrips.

(9) *Hemiptera* or *Rhyncota.*—Two pairs of wings, the front pair either leather-like, with membranous apex, or entirely parchment-like or membranous. Mouth perfectly suctorial, and modified into a definite beak, which is bent so as to be flattened out under the head and thorax.

This order includes bed-bugs, scale-insects, cicada, &c.

(10) *Siphonaptera.*—Wings absent. Mouth suctorial and piercing. Thorax composed of three separate parts not fused together. Legs powerful and adapted for jumping. This order comprises fleas.

(11) *Anopleura.*—Wings absent. Mouth suctorial and of peculiar construction, of a totally different nature to the mouth parts of other orders. This order includes pediculi.

Metamorphosis.—Conspicuous changes after birth, or *metamorphosis*, is one of the most striking phenomena of insect life.

The more highly specialized insects, such as Lepidoptera, Diptera, Hymenoptera, Coleoptera, and most Neuroptera, undergo profound changes of form known as *indirect* or *complete* metamorphosis. For example, the egg of a mosquito or house-fly produces a *larva*. The

larva feeds and grows, casting its skin from time to time, but not changing markedly in form till it becomes a *pupa*. From this pupa, in which internal development only takes place and no food is taken, the *imago* or adult sexual form emerges. Thus these insects, at different stages in their life-history, assume very dissimilar forms.

In other less highly specialized insects, such as Orthoptera and Hemiptera, the imago or adult resembles in external form the young at birth, except that the latter is devoid of wings and mature sexual organs. This is known as *direct or incomplete* metamorphosis. As there is no distinction between larva and pupa, it is usual to employ the term "nymph" to describe the stage between egg and imago in such insects.

In cases of complete metamorphosis, it is usually possible to determine the order to which a larva belongs, though the actual form of the larvæ and adults is so dissimilar. Most dipterous larvæ have no legs; they may be aquatic, as in the case of mosquitoes, or headless maggots, as in those of the Muscidæ. Coleopterous larvæ have three pairs of legs, whether they are like maggots, worms, or wingless insects. Lepidopterous larvæ have not only three pairs of legs, but also supplementary legs or pseudopodia on the abdominal segments; these differ in appearance from the true legs and are not jointed.

CHAPTER X.

DIPTERA.

MANY members of the order *Diptera* are known to be concerned in the causation of diseases of man and of animals.

Diptera may be harmful to man and animals in a variety of ways, by biting, by living as parasites, especially in the larval stage, either internally or externally, and by carrying disease germs either as direct agents or as intermediate hosts for parasites.

(1) Certain species are noted for their virulent bite; such insects apparently secrete a poisonous or irritating saliva. Whether the virulence of flies' saliva varies at different times is not known; the dissimilar effects produced by particular species upon man at different periods and upon different individuals may be due to varying susceptibility. There is no doubt, however, that at certain times biting insects are more venomous than at others.

Diptera feed both by night and by day; as a rule each species, often each family, has its particular feeding time. The gadflies (*Tabanidæ*), for instance, only feed during the day; *Culicina* usually feed at night, but some species are day-feeders, and some may feed either by day or by night; *Anophelina* chiefly but not exclusively by night. Fleas, or *Pulicidæ*, are almost exclusively nocturnal.

(2) *Internal Parasitism* is fairly common in this order of insects, and man may be the host. Human dipterous parasites are nearly always found as such in their larval state. There are some notable exceptions in which the

adult is the parasite, as the Jigger Flea (*Sarcopsylla penetrans*). The larvæ of diptera parasitic in man and in animals produce what is technically called *Myiasis*. These parasitic larvæ may be situated internally (internal myiasis) or externally, or under the skin (cutaneous myiasis). Grubs such as the horse-bots, or larvæ of *Gastrophilus equi*, may live and develop in the stomach and intestines of the horse, the horse forming a definite host. Grubs may exist in the intestines of man, as the *Anthomyia* larvæ, by chance occurrence and not normally. There are no known dipterous larvæ which live and develop only in man's intestines. Cases of internal myiasis in animals are common, in man rare.

Cutaneous myiasis is much more common in man. The eggs of various diptera are deposited on sores and wounds, and the grubs feed in such places (*Lucilia* and *Calliphora*), or the larvæ may live under the skin (*Dermatobia*), or even penetrate the organs of sight. External myiasis refers not only to the skin but includes cases of insect invasion of the external openings of the body, such as the nose, orbits, ears, vagina, rectum, &c. (Screw-worm, *Compsomyia macellaria*).

Cases of internal myiasis require the most careful investigation, as diptera may deposit not only eggs but living young on fæces directly they are voided, and these maggots may be thought to have been passed *per anum*. There are, however, well-authenticated cases of internal myiasis, even in England.

(3) Diptera often feed indiscriminately upon man and animals. In this way a biting fly may carry germs of some disease from animal to man, such, for instance, as anthrax, or from man himself to a fellow-creature. Another source of infection of disease in man in which diptera play a prominent part is not due to biting diptera alone, but to germs being carried from fæcal matter in latrines, &c., by all kinds of carrion and foul-feeding flies, to man's food and drink (typhoid fever, &c.).

An important *rôle* is played by diptera as definitive

hosts of human parasites, such as the malaria parasites, and as intermediate hosts for the *Filariæ*.

Order DIPTERA.—Flies with the anterior pair of wings membranous, except in the case of certain parasitic forms, as the sheep "tick," which are wingless. The posterior pair of wings is transformed into a pair of club-shaped processes, the halteres or balancers. The head, thorax and abdomen are distinct. The head is very variable in shape. There are usually two large compound eyes, and ocelli may be present. The antennæ are very variable and present important characters which are of importance in classification. Certain families have antennæ which consist of a number of segments approximately similar to one another and arranged in a linear manner (fig. 56, 1 and 2). The number of the segments in this division varies in the different families, from eight to sixteen. Diptera having this form of antennæ are called *Nemocera* or *Nematocera*. The majority of insects popularly known as "flies" have antennæ of another form, namely, three segments, the third of which is of different form according to genus or species, and bears on its front a fine projecting bristle frequently feathered, the *arista* (fig. 56, 6). Between the two forms of antennæ described there exists a variety of intermediate forms. In these latter there are one to three segments, and a terminal appendage, which is frequently annulated (fig. 56, 5, *Tabanidæ*), or maybe hairlike (fig. 56, 3, *Asilidæ*). Flies with these forms of antennæ are called *Brachycerous*. Exceptional forms of antennæ are found in the parasitic flies of the series *Pupipara*.

The mouth is suctorial, and in some the parts are adapted for piercing. The normal mouth-parts are :—

(1) The labrum or upper lip.
(2) The mandibles.
(3) The maxillæ.
(4) The labium or lower lip.
(5) The hypopharynx.

Jointed appendages, the maxillary palpi, are also present; the labial palps are represented by the labellæ, which are

jointed on to the distal end of the labium. The form and sometimes the function of each part varies in each group. The labium in many species is more or less fleshy and acts as a sheathing organ; the labrum and

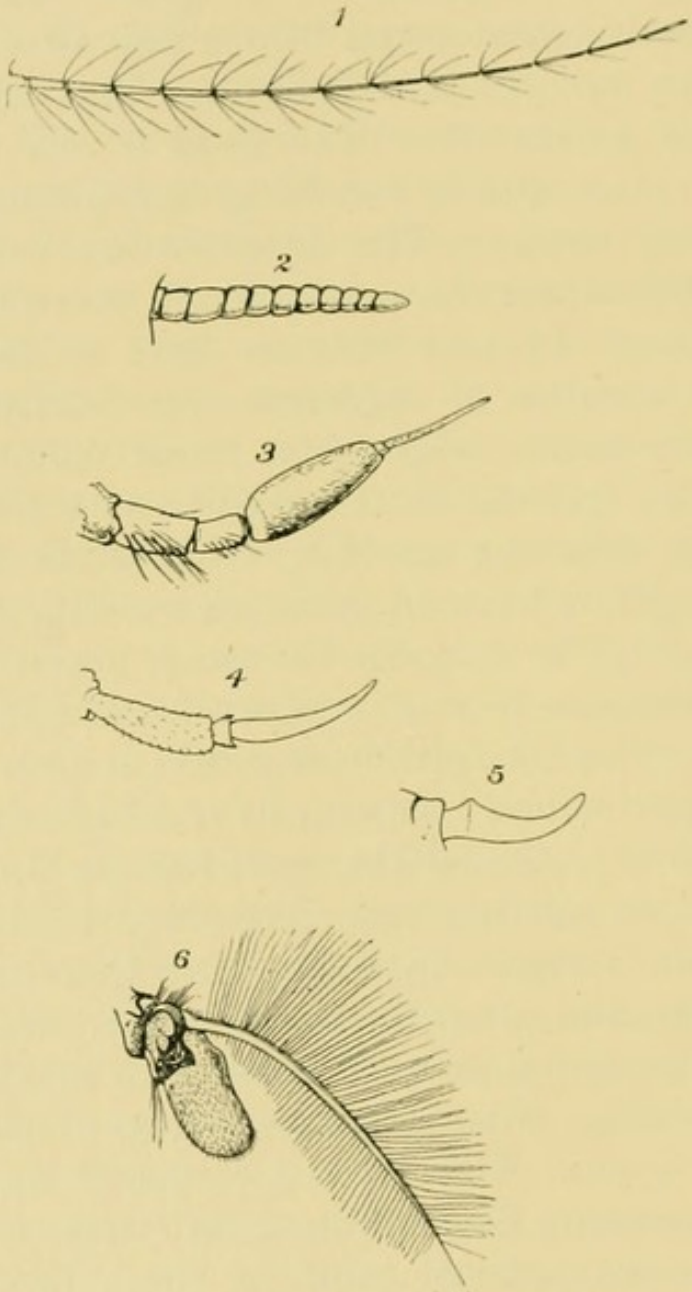


FIG. 56.—Antennæ. 1, Culicid; 2, Simulid; 3, Asilidæ; 4, Hæmatopota; 5, Tabanid; 6, Muscid.

hypopharynx are often much elongated, and together may form a more or less perfect tube.

The space between the eyes is called the *vertex*, that part in front of the eyes the *frons*, the part behind the *occiput*; the sides the *genæ*, or cheeks; and the mouth parts arise from a projection in front, the *clypeus*.

Many diptera have a peculiar structure in the form of a vesicle on the head called a "*ptilinum*." This is a bladder-like expansion in front of the head, which appears as the fly emerges from the pupa. It serves to rupture the hard shell in which the fly is enclosed. This ptilinum becomes completely inverted in the mature fly, being represented externally by a space, the "*lunula*," under an arched suture, extended over the point of insertion of the antennæ.

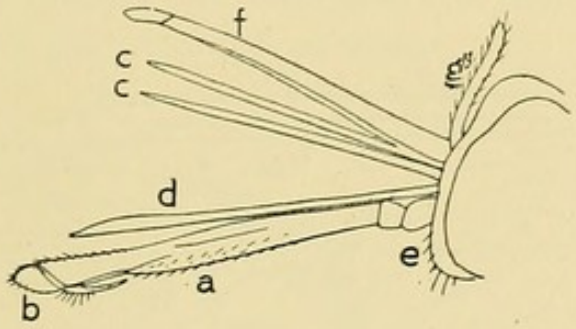


FIG. 57.—Mouth of an *Empis*. *a*, Lower lip or labium; *b*, labella; *c*, stylets or maxillæ; *d*, hypopharynx; *f*, upper lip or labrum; *g*, maxillary palp. (After Meinert.)

The head is joined to the thorax by a narrow neck at the back of the head called the *nape*. The thorax may have all three segments distinct, or the pro- and meso-thorax may fuse; the former is usually small, the latter large; the metathorax, more commonly known as the metanotum, is small. The prothorax is most pronounced in the *Nematocera* and forms either two prothoracic lobes or a narrow collar; a portion of the mesothorax is cut off behind by a depressed line, forming the *scutellum*; a transverse suture may sometimes be seen on the meso-thorax running across from the base of the wings, and there is also a prominent groove above the root of the wings, along which there are often characteristic bristles.

The *pleura* or sides are built up of several pieces, and lie below the meso- and metanotum.

The *wings* have a variable number of veins, which are

both longitudinal and transverse. The figure given here is of a Daddy-long-legs (*Tipula*).

In the centre will be seen a space surrounded by veins —the discal cell (fig. 58, 9). On the fourth longitudinal vein that bounds this cell in front will be seen a short connecting vein—the anterior cross-vein ; this always connects the fourth longitudinal vein behind with the third in front, and the cell behind is always the discal cell (9) ; between the second and third longitudinal veins are the marginal cells. The other cells are shown in the figure.

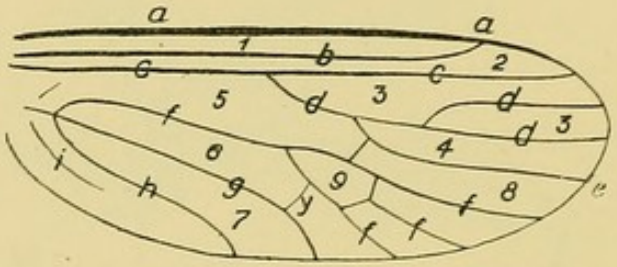


FIG. 58.—Wing of *Tipula*. *a*, Costal vein ; *b*, mediastinal vein ; *c*, first longitudinal vein ; *d*, second longitudinal vein ; *e*, third longitudinal vein ; *f*, fourth longitudinal vein ; *g*, fifth longitudinal vein ; *h*, sixth longitudinal vein ; *i*, seventh longitudinal vein. 1 and 2, Mediastinal cells ; 3 and 4, sub-marginal cells ; 5, anterior basal ; 6, posterior basal ; 7, anal ; 8, posterior marginal ; 9, discal cell. (After Loew.)

The longitudinal veins are known as follows :—

The costal (*a*) ;
Auxiliary, mediastinal, or subcostal (*b*) ;
First longitudinal vein (*c*) ;
Second longitudinal vein or radial (*d*) ;
Third longitudinal vein or cubital (*e*) ;
Fourth longitudinal vein or discoidal (*f*) ;
Fifth longitudinal vein or postical (*g*) ;
Sixth longitudinal vein or anal (*h*) ;
Seventh longitudinal vein or axillar rib (*i*).

On the hind margin of the wing near the base there is often a more or less free lobe, the "alula," and still nearer the base or placed on the sides of the body two

other lobes, the one nearer the alula, called the "anti-squama," the other the "squama," which covers the haltere.

The halteres or balancers may be hidden by the squamæ, as in certain *Muscidæ*. In such cases the fly is said to be *calyptrate*.

The *legs* are attached to pro-, meso- and meta-thorax; they usually terminate in *ungues* or hooks, and *pulvilli* at the base of the ungues, in the form of two pad-like fleshy cushions, but the latter may be absent. In certain flies we find between them the *empodium*, a median appendage in the form of a pad, bristle, or spine.

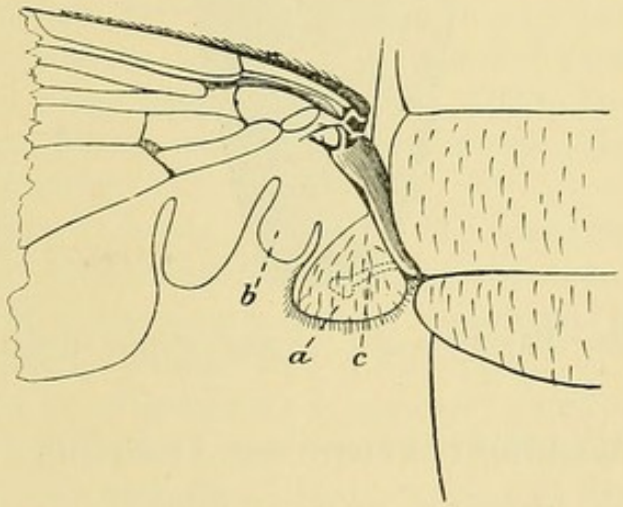


FIG. 59.—Base of wing, calyptrate diptera. *c*, Haltere hidden by (*a*) squama.

The abdomen is composed of nine segments, but they are not as a rule all shown. The male genitalia or hypopygium is of importance for differentiation of genera and species.

All parts of the body may bear bristles (*chætæ*) which are important in classification (*chætotaxy*).

The larvæ of all diptera have neither true nor false legs, but may or may not have a distinct head.

The pupæ may be either naked or enclosed in the hardened larval skin or puparium (fig. 60).

The production of living young occurs in some groups. In the forest-flies and sheep-ticks, or "keds," the young

may be born as fully matured larvæ in a puparium case, which is at first white, but soon darkens. In the *Glossina* the larva is passed fully mature, and travels into suitable ground, and there becomes a pupa.

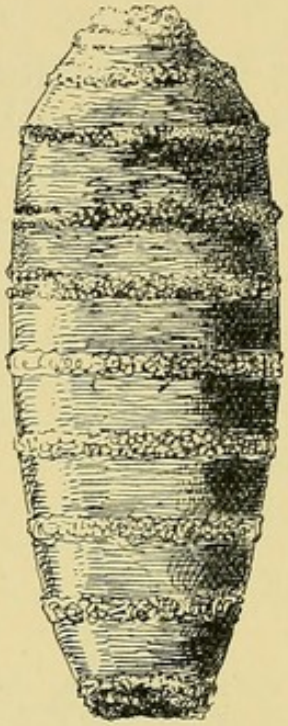


FIG. 60.—Puparium of a "Screw-worm" (enlarged).

CLASSIFICATION OF DIPTERA.

A satisfactory classification of Diptera is not yet agreed upon by entomologists. At one time it was suggested that the order should be divided into two great groups, *Nemocera* and *Brachycera*, according to the structure of the antennæ, as described above. Later Brauer proposed a classification based mainly on development as shown by larval and pupal characters, into two sub-orders, *Orthorrhapha* (straight-seamed) and *Cyclorrhapha* (circular-seamed). The characters of these groups were defined as follows :—

Sub-order 1. ORTHORRHAPHA.—Larva with a distinct head; pupa either free or encased in the larval skin (puparium); the pupal skin always bursts, for the exit of the imago, in a T-shaped opening on the back of the anterior end, rarely in a transverse slit between the eighth

and ninth segments. Imago without the frontal lunula and ptilinum.

Sub-order 2. CYCLORRHAPHA.—Larva without any distinct head; pupa always in a puparium (fig. 60 and fig. 79, A); imago always escapes *via* a more or less circular opening at the anterior end (fig. 79, C). Frontal lunula always present in the mature fly, as there is a ptilinum when it first emerges.

Sub-order I. ORTHORRHAPHA.—The pupa escapes from the larval skin either through an anterior T-shaped opening, or (rarely) through a posterior transverse slit: adults without a frontal lunule:—

1. NEMATOCERA. Flies with four- or five-jointed palpi and many-jointed antennæ, the segments of which, except the basal two, are similar and are often fringed with long hairs:—
   i. *Nematocera vera.* Antennæ long and frequently with whorls of hairs: legs long and slender: abdomen usually long and slender. Examples: Craneflies, Midges, Gnats, Mosquitoes, Chironomidæ.
   ii. *Nematocera anomala.* Antennæ of many small segments, but short and without whorls of hairs: abdomen usually stoutish: legs shorter and stouter than in *Nematocera vera.* Examples: March-flies, Buffalo-gnats. Simulidæ.
2. BRACHYCERA. Flies with one- or two-jointed palpi, and usually short three-jointed (sometimes four- or five-jointed) antennæ:—
   iii. *Brachycera anomala.* Third segment of antennæ ringed as if composed of several small segments fused together: body without strong bristles. Examples: Horse-flies, Soldier-flies. Tabanidæ.
   iv. *Brachycera vera.* Third segment of antennæ not ringed, but usually bearing a bristle or style (antennæ sometimes four- or five-jointed): usually with strong bristles. Examples: Snipe-flies, Robber-flies, Dance-flies.

Sub-order II. CYCLORRHAPHA.—The pupa escapes from the larval skin through an anterior circular opening: adults with a frontal lunule: antennæ short, usually three-jointed, the third segment with a bristle or style:—

1. ASCHIZA. Flies without a frontal suture. Examples: Syrphus flies, Big-eyed flies.
2. SCHIZOPHORA. Flies with a frontal suture. Examples: Bot-flies, Muscids, Glossina, Stomoxys.

Sub-order III. PUPIPARA.—Larva nourished within the parent and not born till it is ready to change into a pupa. Examples: Tick-flies, Hippoboscidæ, Bat-ticks, Bee-louse.

ORTHORRHAPHA NEMOCERA.

NEMOCERA VERA.

Family CECIDOMYIDÆ (Gall Midges).—Small, slender flies with long antennæ, with bead-like segments; proboscis short, elongated in one genus only. Abdomen composed of eight segments. Wings usually hairy; no alula; never more than five longitudinal veins, usually only three, the first, third and fifth; fourth and sixth may be present. Costal vein encloses entire wing;

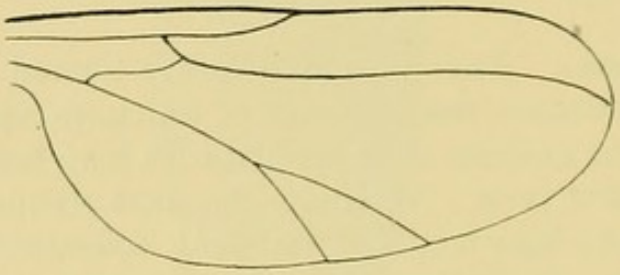


FIG. 61.—Wing of a *Cecidomyia*.

fifth vein forked; only one basal cell. Larvæ vegetable feeders; most produce galls. A few live as parasites in society of plant lice. Larvæ with fourteen segments and possess an "anchor process" under the head end of body. The proboscis is elongated in the genus *Clinorrhyncha* (Loew), and directed downwards. They are often injurious to crops, but only exceptionally cause annoyance to man by biting.

Family CULICIDÆ (Mosquitoes).—Proboscis elongated for piercing. Eyes reniform; ocelli wanting. Antennæ usually plumose in the male (except *Sabethes*, *Wyeomyia*, &c.). Thorax with large mesothorax, narrow scutellum, rounded metanotum. Abdomen composed of eight segments. Wings (figs. 62 and 63) with six longitudinal veins, exclusive of the sub-costal, and two fork-cells; veins clothed with scales; costal vein continued round the border of the wing, fringed with scales. Head, thorax and abdomen usually but not always scaly. Palpi short or long in the female and male. The females are bloodsuckers in many species. The larvæ and pupæ

are aquatic. This family is dealt with in more detail in a subsequent chapter.

Family BLEPHAROCERIDÆ. — These little flies have broad wings and long legs. The proboscis is elongated, and the females in some species (*Curupira*) are blood-suckers. The thorax has a distinct transverse suture. The hind legs are longer than the front ones and there

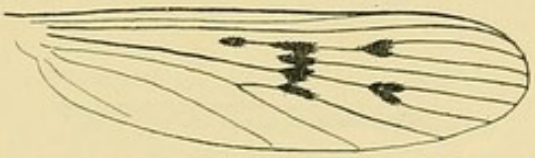


FIG. 62.—Wing of *Anopheles maculipennis*.

FIG. 63.—Wing of a *Culex*.

are no pulvilli. The broad wings are quite bare, there is no discal cell, they are iridescent, and have a secondary set of fine network of veins. They perform aerial dances like midges, especially near the spray of waterfalls. The larvæ live in rapidly running water fixed to stones by suckers. Some forms of larvæ (*Curupira*) are composed of only six or seven segments, with widely projecting side lobes and small tracheal gills near the suckers. The pupæ are flattened, inactive, and enclosed in a semi-oval shell, the anterior end having horny erect breathing tubes and suckers on the ventral surface.

Family CHIRONOMIDÆ (Midges).—This family includes the majority of midges which are frequently taken for *Culicidæ* or mosquitoes. They are all small, delicate, gnat-like flies, with small head, partly concealed by the cowl-like thorax. The antennæ in the female are thread-like and composed of from six to fifteen segments; in the male they are densely plumose. Ocelli wanting or rudimentary. Proboscis short. The oval thorax has no transverse suture, is bare, and projects more or less over the head. The long, narrow abdomen is com-

posed of segments and is often semi-transparent and pilose. The legs are slender and rather long and not spinose, but very hairy in some tropical species. The wings (fig. 64) are narrow, long, and bare or hairy, never scaly; the anterior veins darker than the rest; the subcostal vein complete but small; second longitudinal vein small or wanting; third longitudinal vein sometimes forked close to its origin, the upper branch often rectan-

FIG. 64.—Wing of *Chironomus*

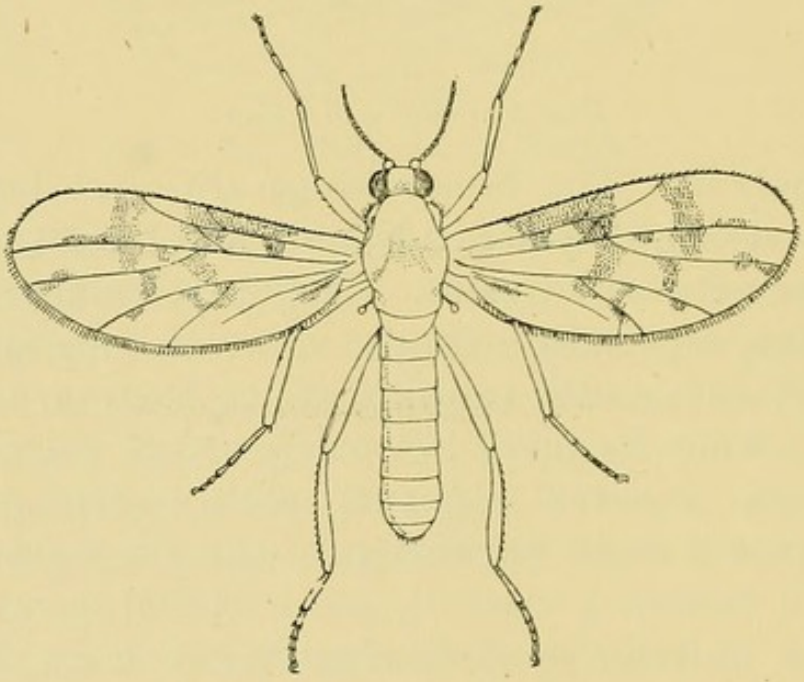


FIG. 65.—A *Ceratopogon*.

gular; fifth long vein forked, sometimes the fourth; the costal vein always ends near the tip of the wing.

Great numbers of this family occur in all parts of the world. The members of one European genus (*Ceratopogon*) (fig. 65) bite severely. They often occur in swarms, dancing in the air. When at rest they wave their forelegs in the air. The dorsum of the thorax is not produced over the head; the palpi are four-jointed; the wings are usually spotted (figs. 65 and 66). *Ceratopogon* occur in

most countries. They are known as "punkies" or "no-see-um," and cause great annoyance by their bites. Many tropical genera bite severely, and on account of this and their small size are frequently misnamed "sand-flies."

FIG. 66.—Wing of *Ceratopogon*. (After Leonardi.)

The larvæ of *Chironomidæ* are mainly aquatic and worm-like, often red in colour, and the pupæ are active, and the respiratory tubes frequently plumose; they also live in damp earth and in decaying vegetation, and in the Tropics in stumps of bamboos, pitcher plants and other open accumulations of water in plants. Those of *Ceratopogon* and allied genera live in the sap of trees, under fallen leaves, and in decaying vegetation, or are aquatic, and are long, slender, delicate, whitish creatures.

Family PSYCHODIDÆ (Owl-midges). — Small, densely hairy, thick-set insects. Proboscis usually short, but in one European genus (*Phlebotomus*) it is long and horny; palpi hairy and composed of four segments. The short abdomen is composed of six to eight segments, hairy. The legs are often short and densely hairy and the claws small. The wings are broad and usually pointed at the tip, and when at rest lie roof-shaped over the body; they are densely covered with long hairs and are fringed with hairs; neuration mostly composed of longitudinal veins; the costal vein completely encloses the wing; the first longitudinal vein near the costa, the second arises near the origin of the first and is usually twice forked, third vein simple, fourth forked, fifth, sixth and seventh usually distinct, the latter sometimes wanting. These small flies can at once be told by their moth-like appearance. They run well, but their flight is weak. Owl-midges are found frequently on windows and in out-buildings, especially in

privies. The genus *Phlebotomus* and some tropical genera bite severely. The larvæ live in stagnant water and decaying vegetation. They are cylindrical and have a short terminal breathing tube. The inactive pupæ have two long tubular stigmata.

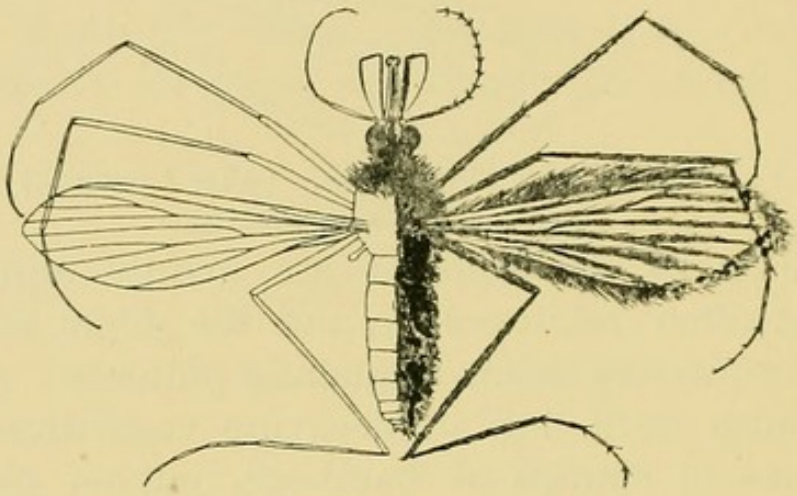


FIG. 67.—*Phlebotomus*, sp. (From Giles's "Gnats or Mosquitoes."

NEMOCERA ANOMALA.

Family SIMULIDÆ (Sand-flies). Usually called sand-flies, black flies, brulots, buffalo and turkey gnats, and sometimes mosquitoes. All small, with oval thorax devoid of any suture. Cylindrical abdomen composed of seven or eight segments. The eyes are holoptic, *i.e.*, the two eyes meet in the middle line in the male, and there are no ocelli. The male is darker and more velvety than the female. The short antennæ are composed of ten or eleven bead-like segments, the two basal ones distinct, the rest closely united and having no whorls of hairs at the joints of the segments. Palpi composed of four segments, the basal joint short, the next two equal, the last longer and narrowed. The legs short, thick; femora broad and flat. Wings (fig. 68) large and broad, the anterior veins thickened, remainder delicate, costal vein terminates near tip of the wing; the sub-costal terminates in the costa about half the length of the wing; first and third longitudinal veins lie close together; fourth vein forked nearly opposite the anterior cross-vein; forks

terminate near the tip of the wing. Proboscis short with strong horny lamellæ, consists of two resisting bristles for puncturing, and on its sides two four-jointed maxillary palps. These small flies bite very severely and cause much annoyance. They especially attack the eyes, nostrils and ears of both animals and man. Sand-flies occur in all climates. The larvæ are all aquatic and some live in rapidly flowing water; they attach themselves to stones, plants, &c., and form elongated cocoons,

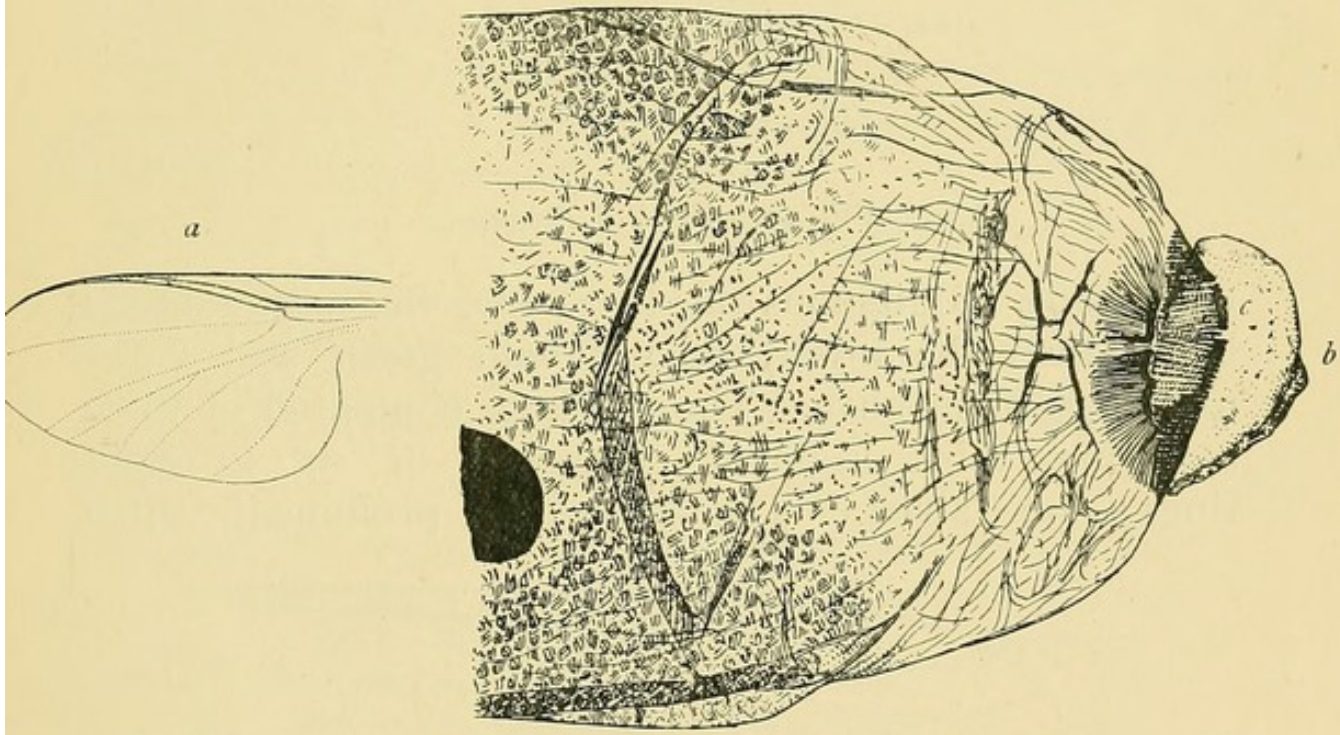


FIG. 68.—*a*, Wing of *Simulium*; *b*, Hinder end of *Simulium* larva; *c*, fixative sucker.

open above. They are soft skinned, with thickened ends, a cylindrical head, and on the first segment a prominence with bristly hooks. The end of the abdomen is provided with appendages, by which the larvæ attach themselves (fig. 68, *b*). The pupæ have the anterior end of the body free, and from it pass out a number of thread-like breathing tubes. The flies are accused of propagating anthrax and septic diseases. Their punctures give rise to severe inflammation, which sometimes results in depilation in animals.

ORTHORRHAPHA BRACHYCERA (Antennæ short).
BRACHYCERA ANOMALA.

Family TABANIDÆ (Gad-flies).—This family includes a number of genera, the popular names being gad-, breeze- or horse-flies, brimps and sneggs. They are mostly large and stout; the head (fig. 69) large; eyes

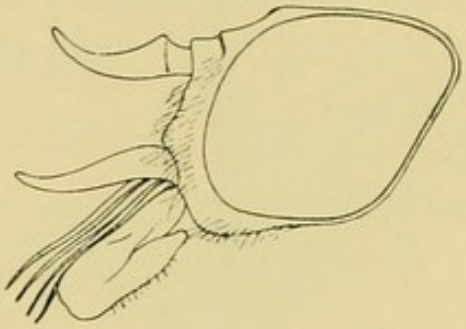


FIG. 69.—Head of *Tabanus*.

very large, contiguous in the male, the upper facets larger than the lower, usually with green and violet markings when alive. The antennæ composed of three segments, third segment composed of six to eight rings; no stylet. The proboscis prominent, often

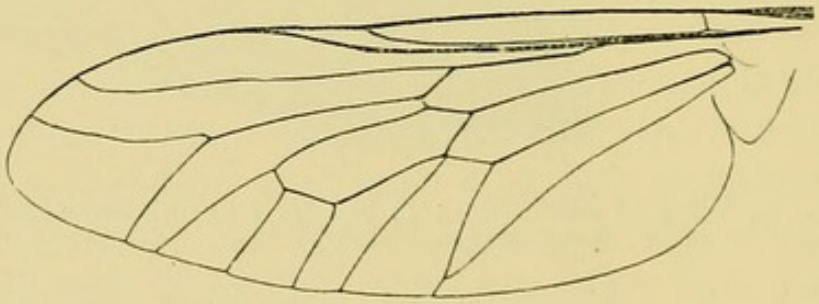


FIG. 70.—Wing of a *Tabanus*.

greatly elongated, fleshy, with pointed horny processes; the female with six, the male with four stylets; the former only suck blood. Palpi two-jointed, the second joint large. The abdomen is broad, often flattened, never slender, composed of seven segments (*vide* fig. 71). The legs are rather thick, mid-tibiæ always with spurs; tarsi with three membranous pads at the tip. There are never any bristles. The third longitudinal vein forked.

Two submarginal and five posterior cells present; anal cell closed at or near margin of wing. Tegulæ large.

Mostly large flies which occur during hot weather and have remarkable powers of flight. The bite of the female is often severe. The eggs are spindle-shaped and dark, and are laid on leaves and stems of plants, and on water plants. The larvæ are carnivorous and feed upon snails, insect larvæ and also roots; elongated, composed of eleven segments, jointed, often with retractile fleshy protuberances; the last segment has a breathing pore, or the last two segments may form a breathing tube. The pupæ are free, and live in earth and water.

The worst biting species are found in the following genera: *Pangonia*, *Chrysops*, *Lepidoselaga*, *Hæmatopota*, *Therioplectes*, *Atylotus* and *Tabanus*.

There are two sections, distinguished as follows :—

Hind tibiæ with spurs at the tip ..... *Pangoninæ*.
Hind tibiæ without spurs ..... *Tabaninæ*.

The following characters separate the above-mentioned genera :—

Pangoninæ.

Third joint of antennæ eight ringed, the first ring slightly the longer; the fourth posterior cell open; proboscis often very long ..... *Pangonia*.

Third joint composed of five rings, the first of which is much longer than the following; the second joint of antennæ as long as the first; wings with dark areas; three ocelli; brilliant eyes with purple lines and spots ..... *Chrysops*.

Tabaninæ.

Third joint of antennæ without or with only a rudimentary basal process; thorax and abdomen with iridescent tomentum; tibiæ dilated..... *Lepidoselaga*.

Thorax and abdomen without iridescent tomentum; front of ♀ as broad as long..... *Hæmatopota*.

Third joint of the antennæ with well-developed basal process.

First antennal joint short; body broad.

Eyes pubescent, small ocelligerous tubercle present *Therioplectes*.
Eyes pubescent, but no ocelligerous tubercle..... *Atylotus*.
Eyes bare ..... *Tabanus*.

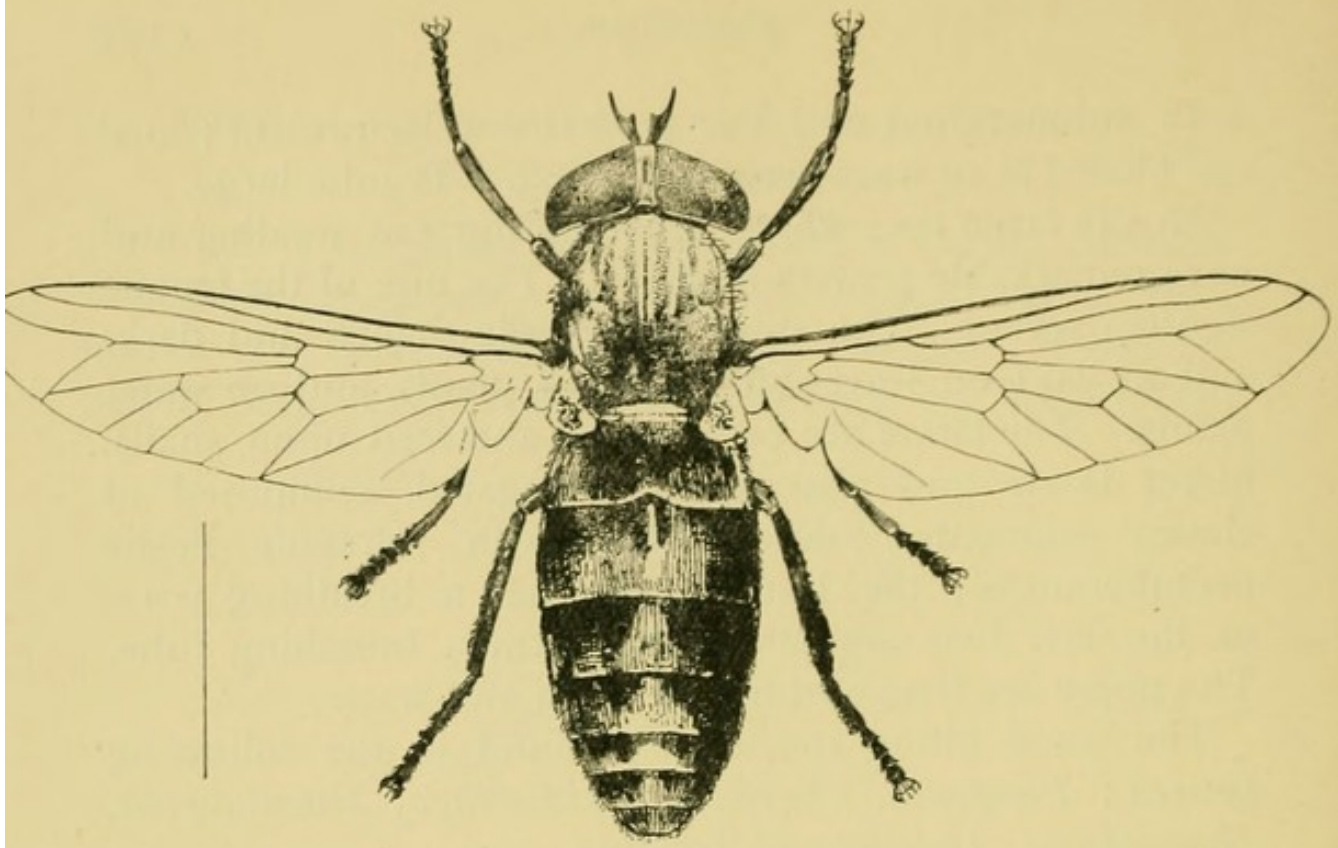


FIG. 71.—*Tabanus bovinus.*

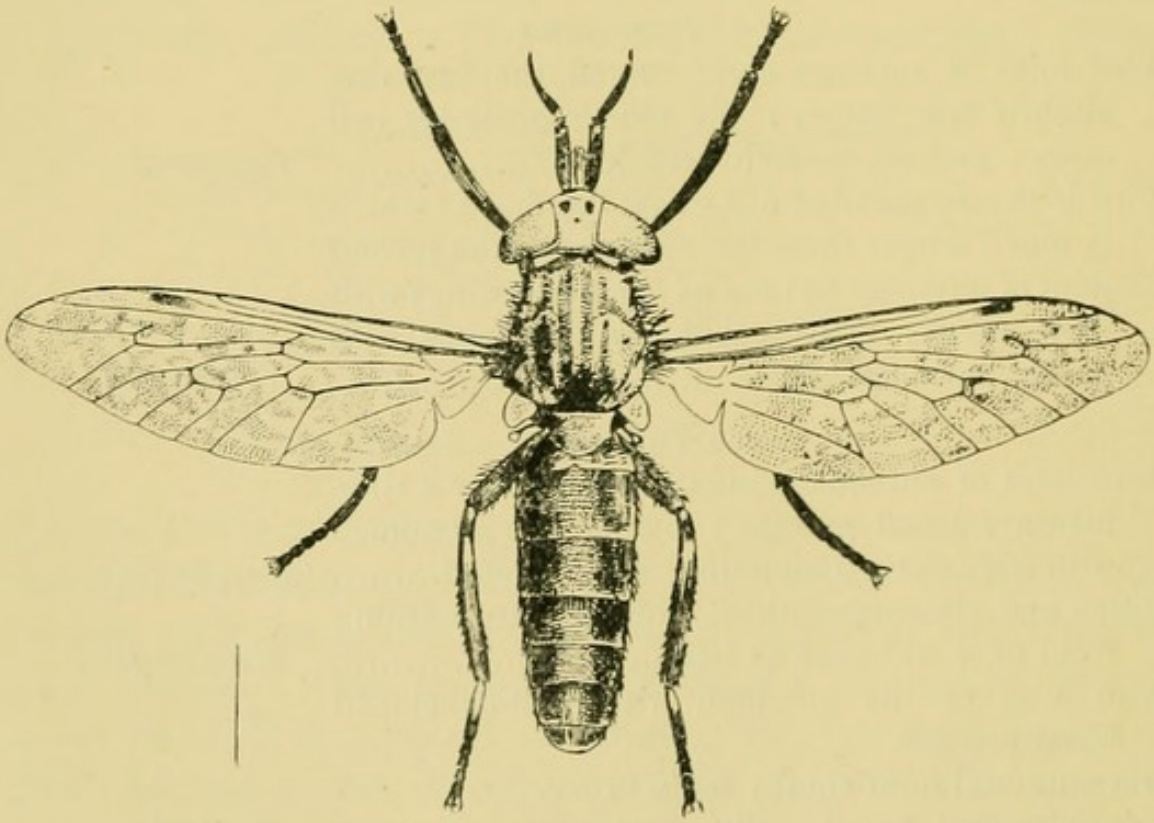


FIG. 72.—*Hæmatopota pluvialis.*

The *Pangonia* are found in woods, forests and pastures; their flight is rapid. The proboscis may be greatly elongated, even to three times as long as the body, so that they can pierce through even thick clothes. An epizootic of anthrax in Pine Islands, New Caledonia, was traced to this genus. The genus *Tabanus* (fig. 49) is world-wide; the short, thick, salient proboscis and the last joint of the antennæ being annulated and notched in crescentic form, and their large size render them easily

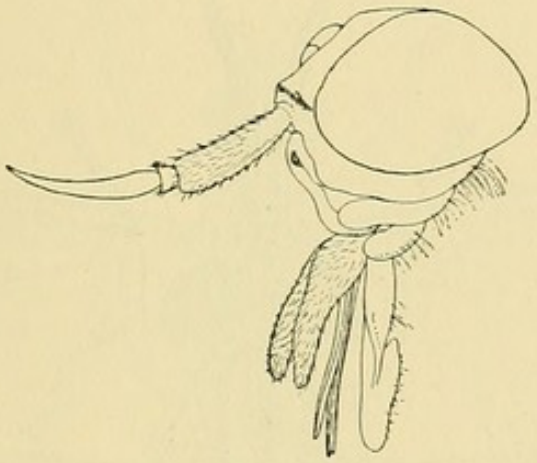


FIG. 73.—Head of *Hæmatopota*.

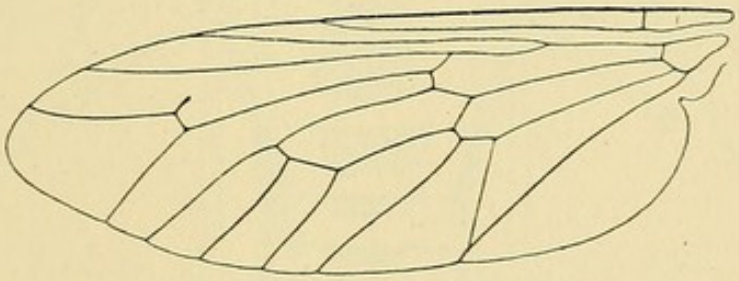


FIG. 74.—Wing of *Hæmatopota pluvialis*.

identifiable. The genus *Hæmatopota* (fig. 72) has no crescentic antennal notch (fig. 73), and the wings (fig. 74) overlap; the abdomen is also narrower than in *Tabanus*, and the wings have hyaline spots. The second joint of the antennæ is very short.

The genus *Chrysops* can usually be told by their wings (fig. 75) being marked with dark areas and their eyes with purple lines and spots. They bite severely and

usually attack round the eyes. An example of *Lepidoselaga* is the Motuca fly of Brazil, which causes deep wounds.

BRACHYCERA VERA.

Family ASILIDÆ (Robber-flies).—Mostly large flies, usually more or less elongated in form, and often thickly hairy and with strong bristles. Head broad and short with a freely movable neck; eyes separate in both sexes, with a deep notch between. Antennæ

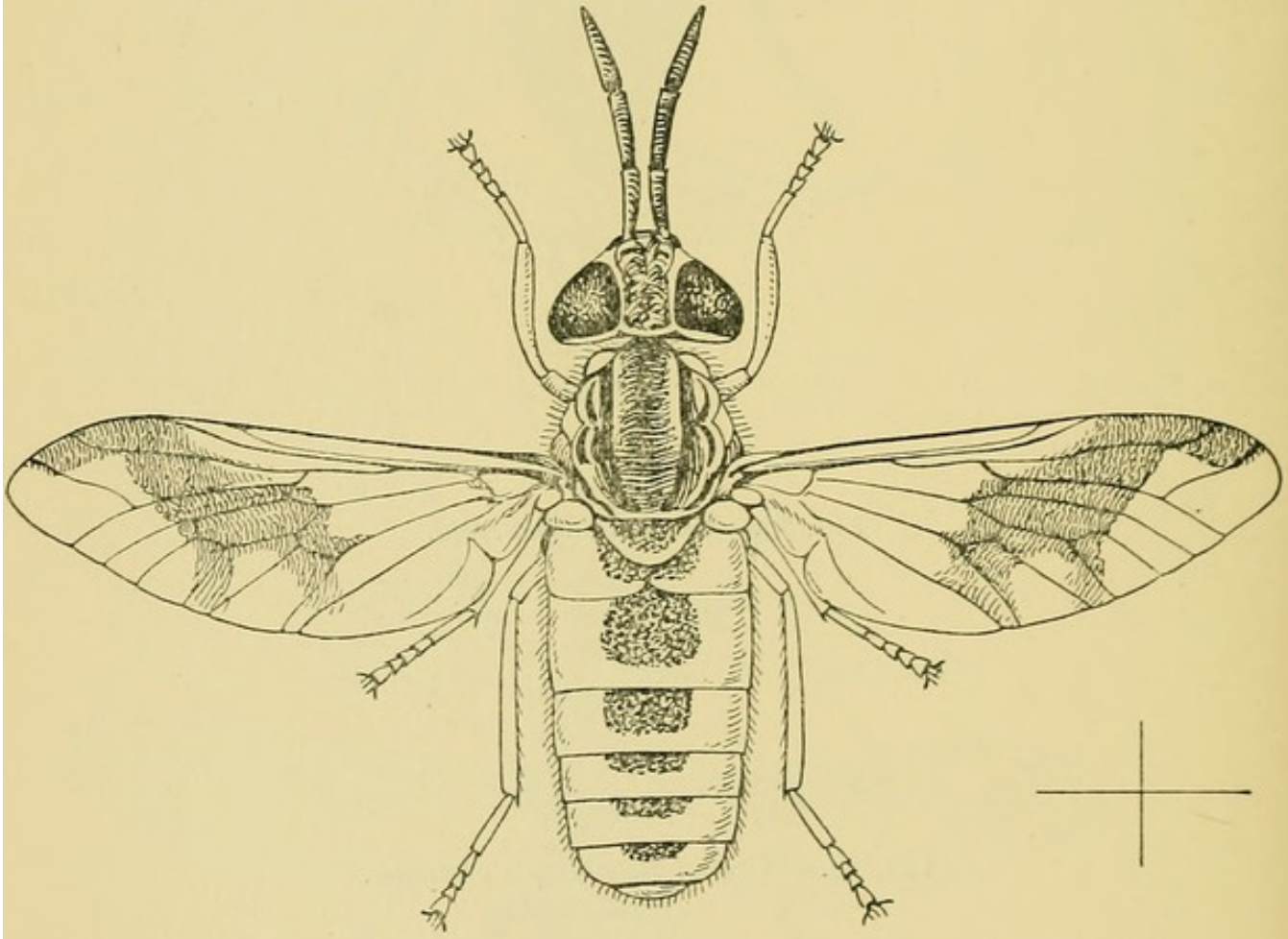


FIG. 75.—*Chrysops distinctipennis.*

composed of three segments, the third elongated, generally simple, with or without a terminal style or bristle (fig. 56-3); style sometimes thickened and forming one or two apparent antennal joints. Proboscis firm; upper lip horny, used for piercing; labella not fleshy. Legs strong and bristly. Wings when closed lying parallel over the

abdomen; three long basal cells, two or three submarginal cells and five posterior cells; third longitudinal vein forked. These flies usually feed upon insects. Some attain as much as two inches in length. The larvæ live in rotten wood and in the soil, and feed upon other

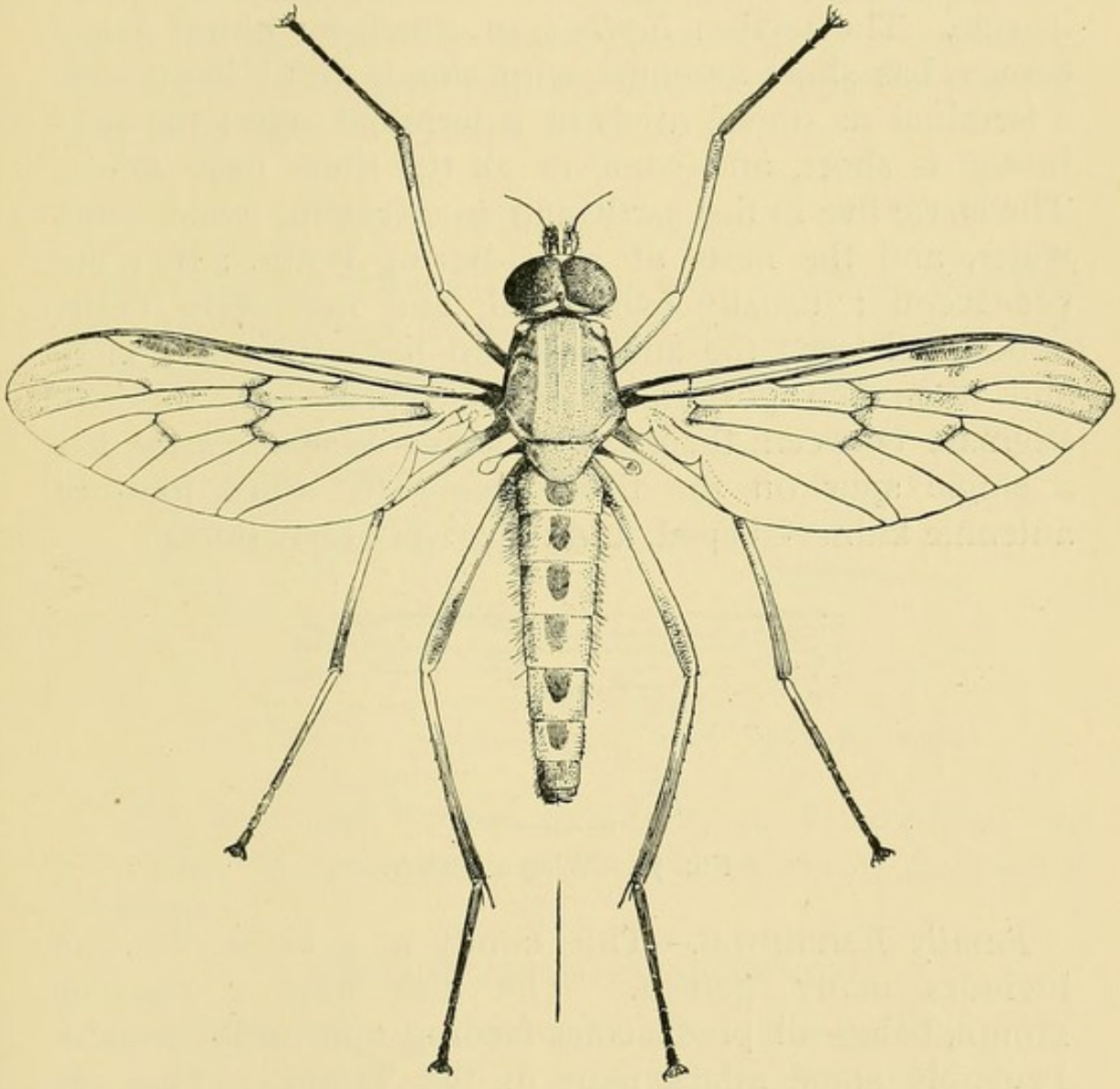


FIG. 76.—*Leptis scolopacea.*

larvæ. There do not seem to be any authentic records of these Robber-flies biting man, but some of the larger tropical species are said to do so; animals are also attacked by them. There are over 150 genera in this family.

Family LEPTIDÆ.—This family includes a number of

elongated flies of moderate or large size (fig. 76). The veins of the wings distinct, not crowded anteriorly; third longitudinal vein forked, basal cells large; five posterior cells usually present. Third joint of antennæ complex or simple, with or without a terminal or dorsal arista or a terminal style. One genus only (*Symphoromyia*) bites, the rest being predaceous upon insects. The section *Leptina*, in which the biting genus occurs, has short antennæ with simple third joint, with a terminal or dorsal arista or a terminal style; the proboscis is short, and some or all the tibiæ have spines. The larvæ live in the earth and in decaying wood, sand, water, and the nests of wood-boring beetles; they are predaceous; usually cylindrical, and may have fleshy abdominal legs; the anal segment has a transverse cleft, and often two posteriorly directed processes, and two stigmata between them. The genus *Symphoromyia* has a single spur on the third tibiæ; the third joint of antennæ kidney-shaped, and the arista nearly dorsal.

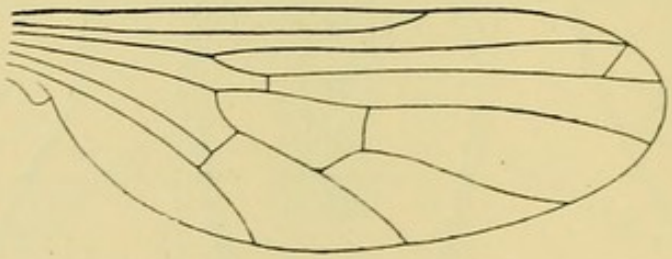


FIG. 77.—Wing of *Empis*.

Family EMPIDIDÆ.—This family is a large one and includes many genera. The flies have a piercing mouth, being all predaceous, feeding upon other insects. Probably some attack man in the Tropics. They are mostly small to moderate-sized species, with small head, provided with either a short or long proboscis. The proboscis (fig. 57) consists of two stylets (*c*), a hypopharynx (*d*), and an upper (*f*) and lower lip (*a*). The antennæ three jointed, the first two joints often small, third joint very variable, with or without a terminal arista or style. Abdomen of from five to seven segments; male

genitalia very prominent. The legs are of peculiar structure, the femora thickened and spiny; metatarsi flattened. Neuration (fig. 77) of wing variable; there are three or four posterior cells; the anal cell is closed remote from the border, sometimes wanting, at other times it is closed near the border (*Hilarimorpha*); then the discal cell is wanting. Tegulæ small. The larvæ are cylindrical, with small ventral swellings on the mesothoracic segments; they live in earth and amongst decaying vegetable matter. The pupæ have two points at the anterior end.

CYCLORRHAPHA—ASCHIZA.

This section does not contain any members that bite man, but some are the agents of intestinal myiasis, *e.g.*, rat-tailed larvæ of syrphidæ, and phoridæ (fig. 77A).

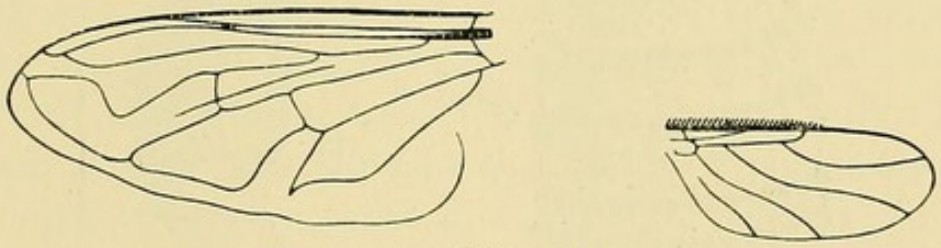


FIG. 77A.

Wing of *Syrphid.* Wing of *Phoridæ.*

The chief family, the *Syrphidæ*, or Hover-flies, are noted for the good some of their larvæ do in destroying Aphides.

CYCLORRHAPHA—SCHIZOPHORA.

I. MUSCIDÆ ACALYPTRATÆ.—Mostly small flies with the antennæ composed of three segments bearing a non-terminal bristle; halteres never covered by a squama or basal scale; nervuration of wings simple, few cells.

This group contains a large number of sub-families. None annoy man to any noticeable extent. The following families are of economic importance, agriculturally and otherwise: *Chloropidæ*, *Trypetidæ*, *Psilidæ* (as vegetable feeders), *Scatophagidæ* (dung-flies).

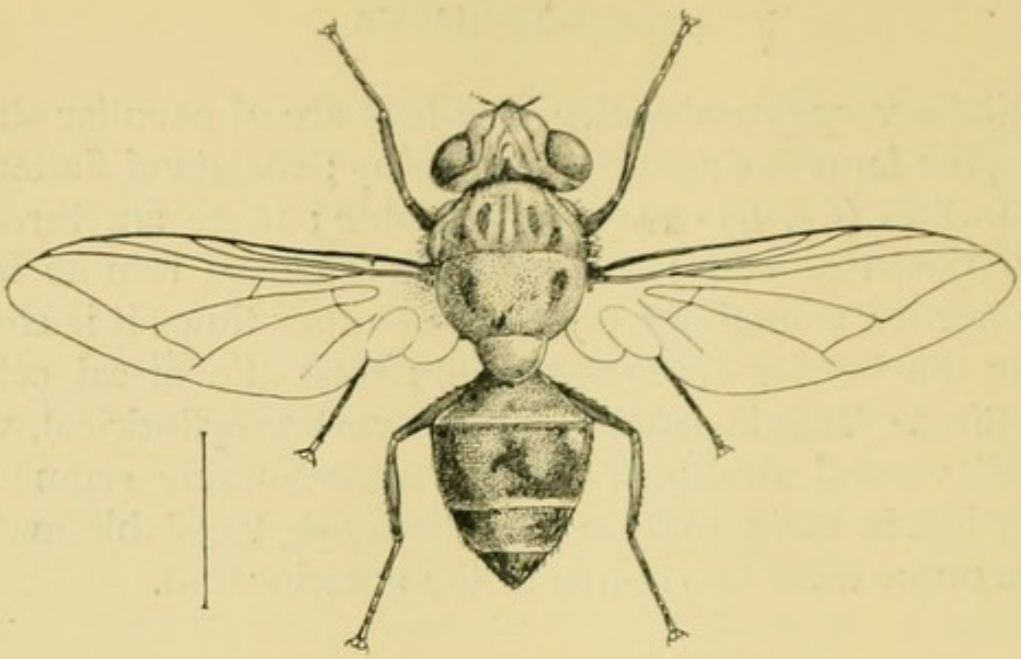


FIG. 78.—*Dermatobia noxialis.*

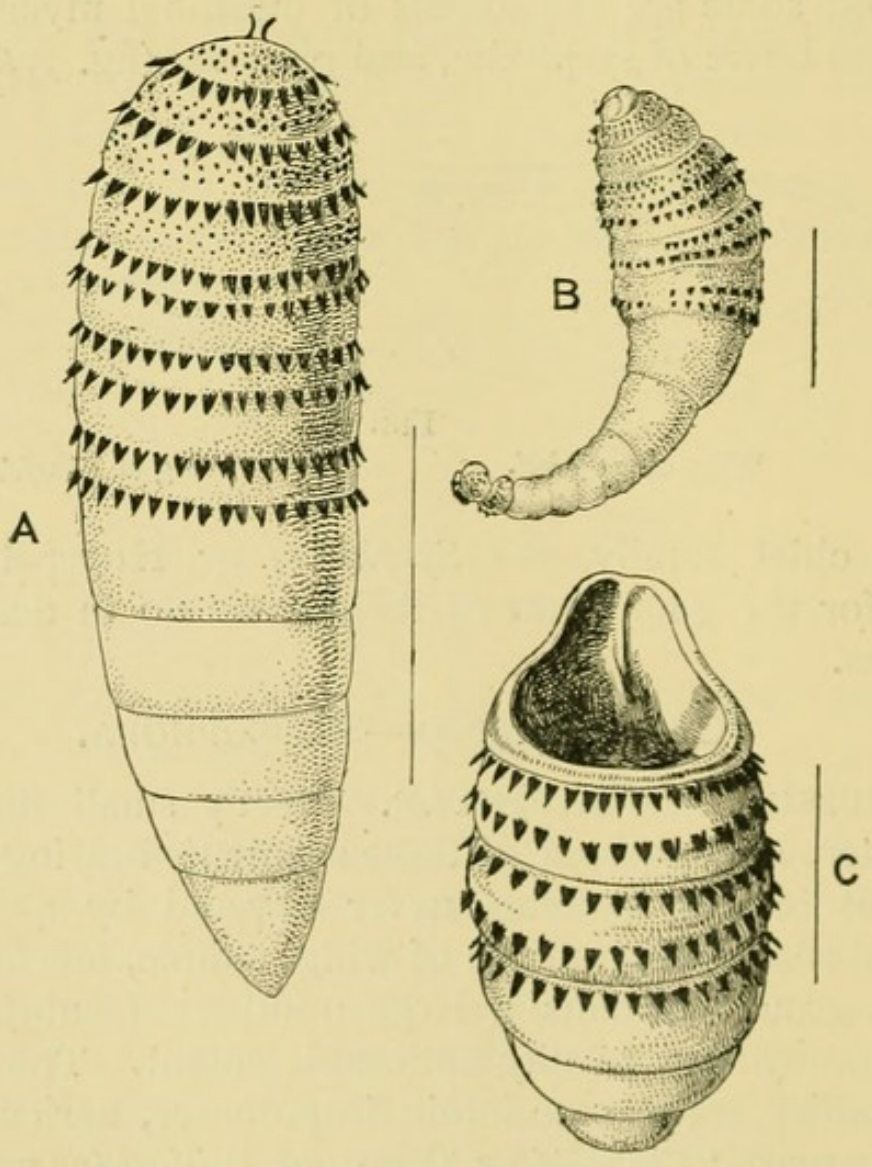


FIG. 79 —*Dermatobia noxialis.* A and B, Larvæ ("bots"); C, puparium. (After Brauer.)

II. MUSCIDÆ CALYPTRÆ.—Halteres concealed by a squama, or large transparent scale (fig. 59).

Family OESTRIDÆ.—(Warble-flies) (fig. 78).—Flies of large size, thick-set, and often very hairy. Mouth small parts rudimentary, palpi usually wanting; eyes rather small, bare. Head large; the antennæ small, composed of three segments, more or less hidden; arista simple or plumose. Thorax broad, with distinct transverse suture. Abdomen short and thick. Legs of moderate length, the hind pair often longer than the rest. Wings with or without markings; anal cell, small discal cell, may be absent. The larvæ or bots (fig. 79, A and B) are provided with circles of spines, two hooked mandibles and anal breathing pores; parasitic. They live in three ways—(1) under the skin, (2) in nasal and pharyngeal cavities, and (3) in the alimentary canal. Man as well as animals may be attacked (*Dermatobia*). The larvæ of *Dermatobia* (fig. 79) live under the skin of man, apes, cattle, dogs, &c. In the adult *Dermatobia* the arista is plumose on the upper side and the tarsi slender; the proboscis is bent at the base and is concealed in the buccal cavity; squamæ large; first posterior cell closed; body hairy. Larvæ club-shaped, slender posteriorly, and surrounded with rows of prickles on the borders of the segments of the apical half. The chrysalis stage is formed in a hard puparium case (C). The common species, *D. noxialis* (Goudot), occurs from Mexico to Brazil, and is known as the "macaw worm," "ura," "torcel," and "moyoquil worm."

Family SARCOPHAGIDÆ (Flesh-flies). Usually thick set and of variable size. Abdomen composed of four visible segments, with bristles which are confined to the anal end, but sometimes elsewhere. Arista plumose to the middle, *apex always bare.* Some are metallic (*Cynomyia*). Larvæ feed on decaying animal and vegetable matter, and may live as parasites in the flesh of animals and in the orifices of man, also in wounds and ulcers. Those of *Sarcophaga* often occur in wounds in man, and

are sometimes produced alive. The larvæ are rounded, and thin anteriorly; abdominal segments distinct, each with a circle of spines; mouth with two-curved mandibles; posterior stigmata placed in a deep cavity, and there are two pointed anal swellings. The pupa lies in a brown oval puparium.

The genus *Sarcophaga* (Meigen) has the first posterior cell open; the tibiæ with a few bristles; the mid and posterior cross-veins nearly in the same line.

Sarcophaga carnaria, the common British flesh-fly, may be taken as an example.

Cynomyia (Desvoidy) has a metallic coloured abdomen and the tibiæ with short hairs.

Cynomyia mortuorum is a bright blue fly about the size of a blow-fly, and, like it, lays its eggs in decaying animal matter, and may possibly do so on wounds.

Sarcophila (Rondani), like others in the *Sarcophagidæ*, are viviparous. The females deposit their larvæ in wounds in animals and man.

The larvæ of the genus *Ochromyia* are also parasitic under the skin of animals and man—Cayer or Senegal fly (*O. anthropophaga*).

Family MUSCIDÆ (House-flies, Tsetse-flies, &c.).—A large family, easily told from the former by the arista being plumose at the tip (now and then it is bare); there are no bristles on the abdomen except at the tip, and the first posterior cell is very narrow. The eyes of the ♂ contiguous, bare or hairy in both sexes. Abdomen composed of four visible segments. This family contains the house-fly (*Musca*), blue- and green-bottle flies (*Lucilia* and *Calliphora*), stable or "stinging flies" (*Stomoxys*), horse-flies (*Hæmatobia*), and tsetse-flies (*Glossina*. The larvæ are variable; some live in decaying vegetation, in decaying animal matter and fæces; others, as the screw-worm (*Chrysomyia*), as parasites in animals and man: so also may *Calliphora* and *Lucilia*. The *Stomoxyinæ*, which include the stable-fly, tsetse-fly and the horn-fly, have elongated, piercing probosces, and are blood-suckers.

The following characters will separate the more important genera :—

Proboscis long, used for piercing ; palpi shorter than proboscis	*Stomoxys.*
Palpi nearly as long as proboscis	*Hæmatobia.*
Proboscis very long, straight	*Glossina.*
Proboscis short, not adapted for piercing ; arista plumose on both sides ; curvature of fourth vein angular ; mid tibiæ without bristles on inner side ; abdomen non-metallic ; blackish species with more or less yellowish markings	*Musca.*
Mid tibiæ with bristles on the inner side ; abdomen &c., with metallic colours.	
Thorax blackish	*Calliphora.*
Thorax black with whitish longitudinal stripes, more or less metallic.....	*Chrysomyia.*
Thorax unicolorous, metallic	*Lucilia.*

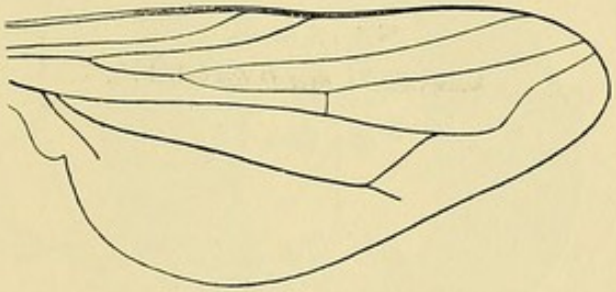


FIG. 81.—Wing of *Stomoxys calcitrans.*

GENUS STOMOXYS.

In the genus *Stomoxys* the solid, elongate proboscis, jointed at an angle near its base, is the obvious characteristic. The type species of the genus is *Stomoxys calcitrans*, which is very like the common house-fly in general appearance.

Mouth-parts in Stomoxys.—The proboscis beyond the angle projects horizontally. The palpi are very slender and scarcely one-third the length of the proboscis ; they spring from above the angle and are not in contact with the proboscis.

The proboscis consists of three parts—labium, labrum and hypopharynx.

The *labium* is the only part seen in the ordinary resting position. It is a solid-looking chitinous structure, ovoid in cross-section, with a narrow, shallow groove on its anterior or upper surface, within which lie the *labrum* and *hypopharynx*. The lateral walls of the labrum are incurved below to form a tube, with a slit along the lower side. This slit is closed by the apposition of the *hypopharynx*, a delicate rod within which lies the excretory tube of the salivary ducts.

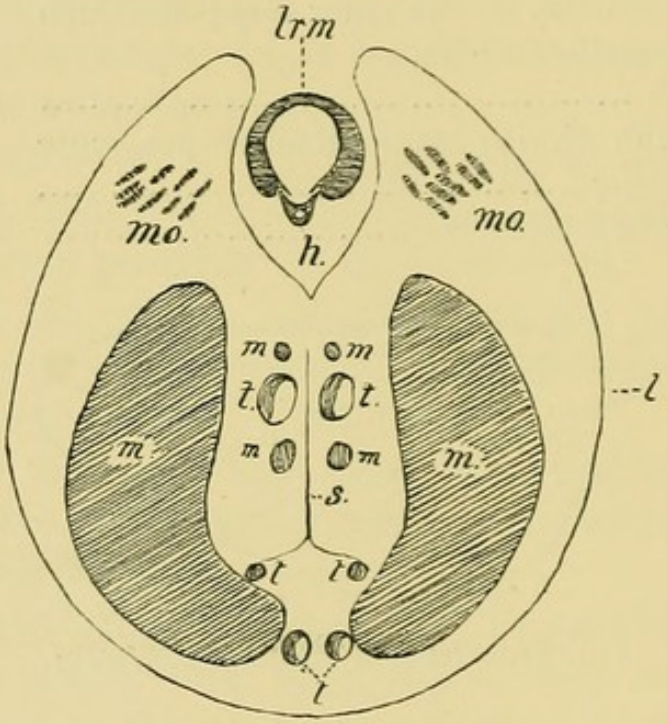


FIG. 82.—Cross-section of proboscis of *Stomoxys* (after Giles). *h*, Hypopharynx ; *l*, labium ; *lrm*, labrum ; *m*, muscle ; *t*, tracheæ.

Dissection.—To expose the structures in the thorax in such flies, snip off with sharp scissors the legs with a little of the ventral wall of the thorax. There will then be seen a number of vertical bundles of muscle fibre (the coxal muscles). Separate these muscle bundles in the middle line and expose the great thoracic ganglion, a sausage-shaped mass of considerable size situated opposite the first pair of legs. Clean this away, and just under its anterior end will be seen a nodule about the size of a small pin's head ; this nodule is the proventriculus. Extending backwards from it is a glistening tube, the mid-

gut, and lying upon it three delicate tubes, the crop duct centrally placed, and at the sides the commencement of the salivary glands.

The abdominal viscera can be exposed by snipping through the chitinous covering of the dorsum in a longitudinal direction and floating the contained structures in normal saline solution.

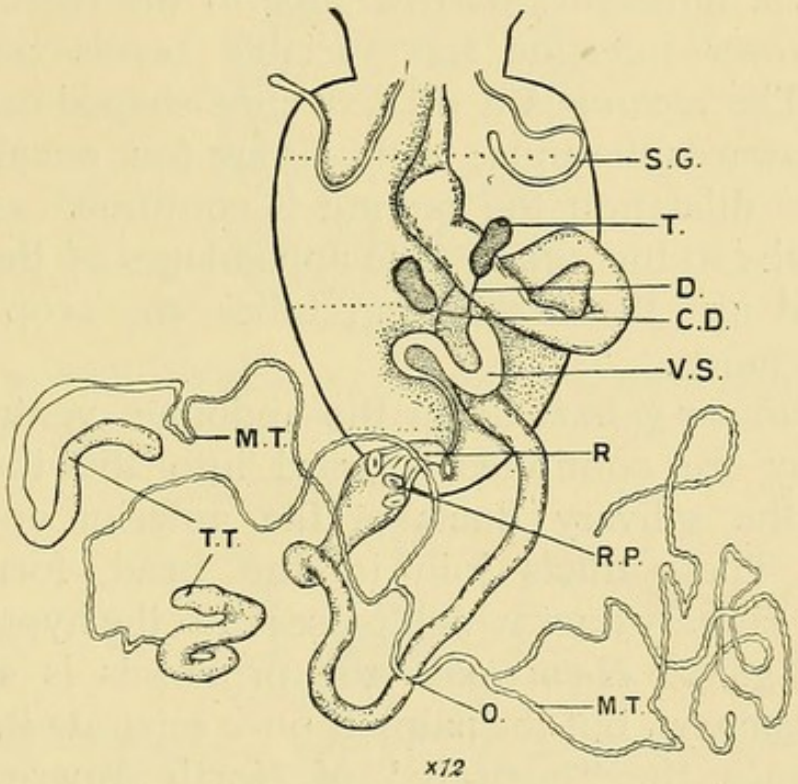


FIG. 83.—Dissections of the abdomen of *Stomoxys*, after Lieut. Tulloch, seen from above, with the alimentary canal unravelled. CD, Common seminal duct; D, seminal duct; MT, Malpighian tubes; O, junction of distal intestine and mesenteron; R, rectum; RP, rectal papillæ; SG, salivary glands; T, testis; TT, dilated ends of left Malpighian tubes; VS, vesiculad seminalis.

Internal Anatomy.—The buccal cavity, which is a narrow tube, is contained in the base of the proboscis. From it the *pharynx* runs almost vertically upwards in the head, then bends sharply backwards to become the œsophagus, the latter being continued backwards as the mid-gut. At the junction of the œsophagus and mid-gut is situated the *proventriculus*, into which opens also the duct of the crop, a large hollow sac situated in the

anterior part of the abdomen between the mid-gut and the salivary glands. The mid-gut runs backwards into the abdomen as a narrow tube until it reaches nearly to the posterior border of the crop, where it becomes dilated. This dilated portion has three simple coils, which lie superposed in the middle of the abdomen. The tube then gradually narrows and into this region the four Malpighian tubes open. The alimentary canal is continued as a uniformly narrow tube to the rectum. The narrow lower intestine has variable bends but is not coiled. The *rectum* is a dilated cone-shaped cavity with its apex towards the anus; within it are four rectal papillæ. Below the dilatation the rectum is continued as a short narrow tube to the *anus*. The appendages of the alimentary canal are the Malpighian tubes, the crop and the salivary glands.

The *salivary glands* lie in the abdomen ventral to the crop, they are continued forward into the thorax and become the salivary ducts at the anterior end of the thorax. These ducts join in the head, forming the common salivary duct which passes into the hypopharynx.

In the genus *Hæmatobia* the proboscis is similar to that in *Stomoxys*, but the palpi at once separate it. *Hæmatobia serrata*, the "horn-fly" of North America, causes much annoyance to cattle and bites man, but in Great Britain it seems harmless.

Genus Glossina.

In the genus *Glossina* the proboscis is long and straight. Palpi are the same length as the proboscis and form a sheath for it. Arista plumose to the tip, the hairs being on upper surface only and compound. The wings when at rest are crossed over one another "scissors like," and project well beyond the abdomen. The wing venation is characteristic, especially in the course of the fourth longitudinal vein, which makes two bends, one before it meets the anterior transverse vein and another before it reaches the costal margin.

The females of this genus produce their larvæ full grown, the larvæ changing to pupæ without feeding. The larvæ are deposited in the neighbourhood of decaying vegetation, particularly about the roots of certain trees, such as the banana tree and others of its class, and burrow into this before pupating.

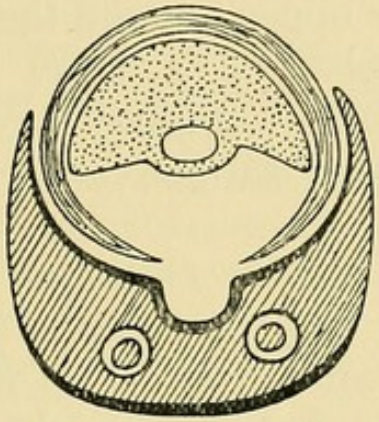


FIG. 84.—Transverse section of the proboscis of *Glossina palpalis* at almost mid-length.

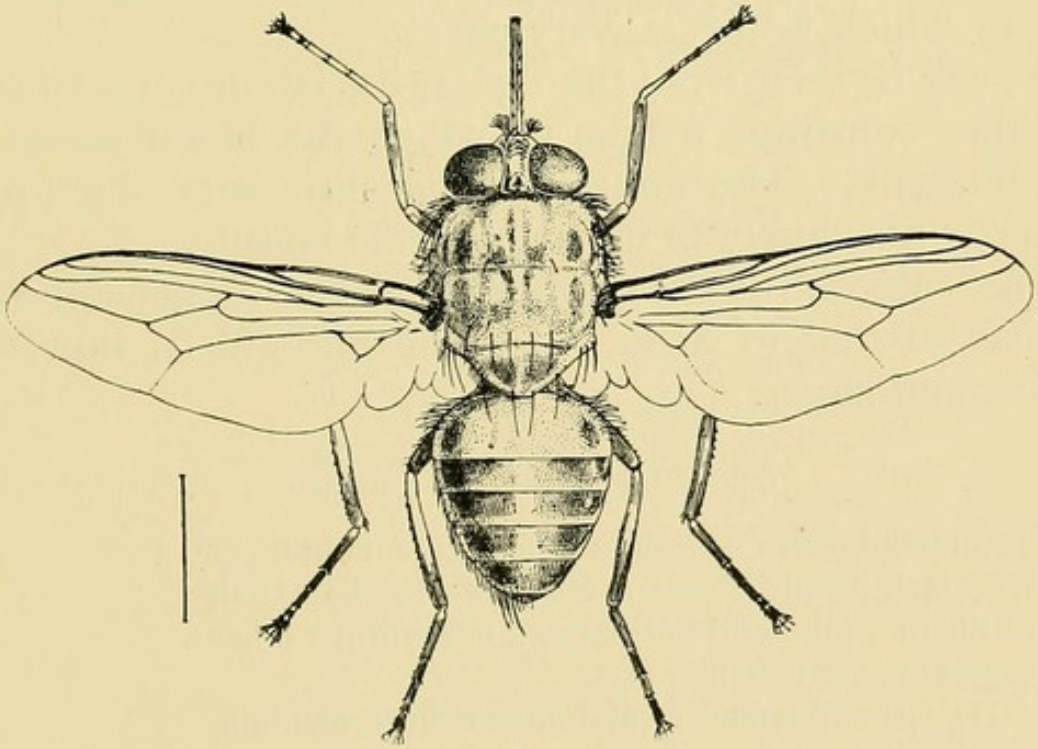


FIG. 85.—*Glossina morsitans*.

Glossina are found in belts usually in the vicinity of streams or rivers on the edge of forest land. In such situations they may be present in large numbers for a few hours, at other times none or very few can be found.

Mouth-parts in Glossina.—The maxillary palpi project horizontally and being grooved on the inner aspects act as ensheathing organs for the proboscis.

The proboscis is expanded at the base, the remainder being proportionately very slender; it reaches almost to the end of the palpi and is slightly curved, with the convexity beneath. It consists of three parts—*labium* or lower lip, *labrum* or upper lip, and the *hypopharynx*.

The *labium* consists of a large basal bulb and a terminal long, slender, chitinous rod. It is deeply grooved on its upper surface and bent upwards and inwards.

The *labrum* is a narrow chitinous rod, the prolongation of the exterior wall of the pharynx. Its edges are turned downwards and inwards, so that it forms about two-thirds of a tube. It terminates in a sharp point.

The *hypopharynx* is a solid rod, semilunar in cross-section, with a rib running down its ventral surface, within which is the salivary canal.

As will be seen from the figure, the labium and labrum together constitute a tube up which the blood passes to the pharynx. The convex dorsal surface of the hypopharynx fits closely to the sides of the labrum.

The internal anatomy in *Glossina* is in general very similar to that in *Stomoxys*. The mid-gut is, however, longer and larger.

Synoptic Table of Species.

1. Hind tarsi dark, or at least all the segments more or less dark (in the ♀ of *G. tachinoides* the basal half of the first joint and the extreme bases of the other segments are usually pale).
   (1) Ground colour of abdomen ochraceous buff, with interrupted dark brown deep transverse bands and sharply-defined pale hind borders to the segments, a very conspicuous square or oblong pale area in the centre of the second segment; small species, not exceeding 8 mm. in length, exclusive of proboscis, the males much smaller ..... *tachinoides.*

(2) Abdomen very dark, or for the most part uniformly brown, hind borders of segments if lighter extremely narrow and cinereous; pale area in centre of second segment usually triangular, with the apex directed backwards and continued into a cinereous median stripe; larger species.

(*a*) Third joint of antennæ dusky brown to cinereous black.

(α) Thorax, pleuræ and coxæ more or less uniform in colour or with stripes only ..... *palpalis.*

(β) Thorax with elongated transverse black spots; pleuræ, coxæ and femora with conspicuous black spots ..... *maculata.*

(*b*) Third joint of antennæ pale (orange-buff) ..... *pallicera.*

II. Hind tarsi not entirely dark; last two joints alone dark, remainder pale.

A. Smaller species; length rarely reaching 11 mm., often considerably less; wing expanse not exceeding 25 mm.

(*a*) Last two joints of front and middle tarsi with sharply defined dark brown or black tips.

Generally distinctly larger; head wider; front darker and narrower in both sexes, sides parallel in ♂; abdominal bands deeper, leaving hind margins only narrowly pale; hypopygium in ♂ smaller, darker and more hairy; tip of abdomen more thickly clothed laterally with short black hair, bristles on sixth segment finer and less prominent ..... *longipalpis.*

Usually smaller; head narrower; front paler and wider; eyes in ♂ as well as in ♀ distinctly converging towards vertex; abdominal bands less deep; pale hind margins of segments

therefore deeper; hypopygium in the ♂ larger, paler, somewhat more oval in outline, and clothed with fewer fine hairs; tip of ♂ abdomen less hairy laterally; bristles on the sixth segments in ♂ stouter and more conspicuous ..... *morsitans.*

(*b*) Last two joints of front and middle tarsi without sharply-defined black or brown tips; front and middle tarsi entirely yellow, or last two joints of former faintly tipped with pale brown *pallidipes.*

B. Large species; length at least 11 mm., wing expanse at least 25 mm.

Dorsum of thorax with four sharply-defined small dark brown or oval spots arranged in a parallelogram, two in front of and two behind transverse suture; bulb at base of proboscis brown at the tip..... *longipennis.*

Dorsum of thorax without such spots, though with more or less distinct longitudinal stripes; bulb at base of proboscis not brown at the tip..... *fusca.*

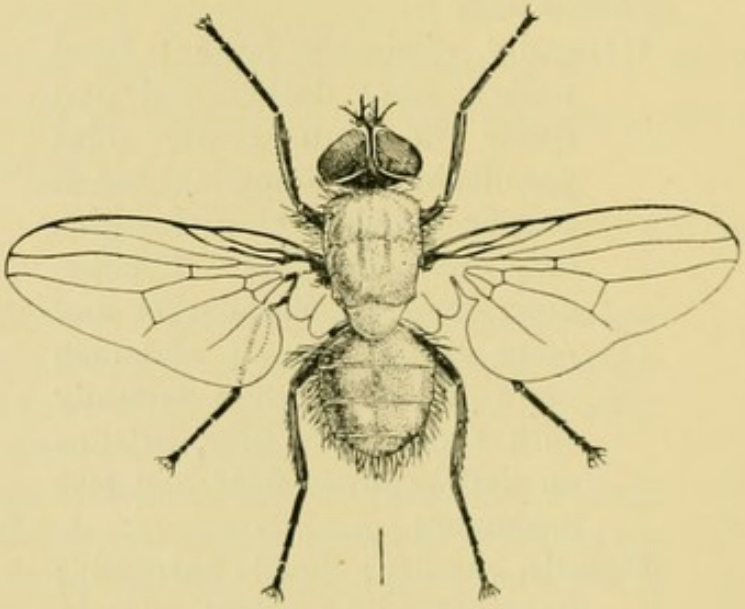


FIG. 86.—*Lucilia cæsar.*

Genus *Lucilia* (fig. 86), the so-called green-bottle flies, have a soft proboscis (fig. 87). They are all of metallic

colour and the abdomen is short and round; the third segments of the antennæ are quadruple the size of the second. Basal half of third longitudinal vein carries

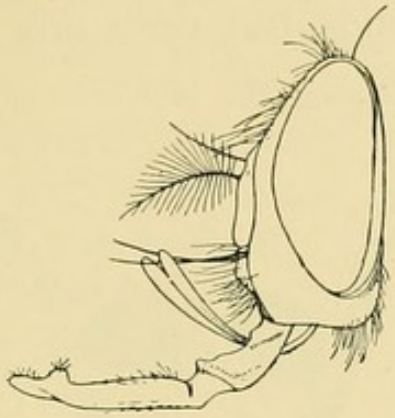


FIG. 87.—Head of *Lucilia cæsar*.

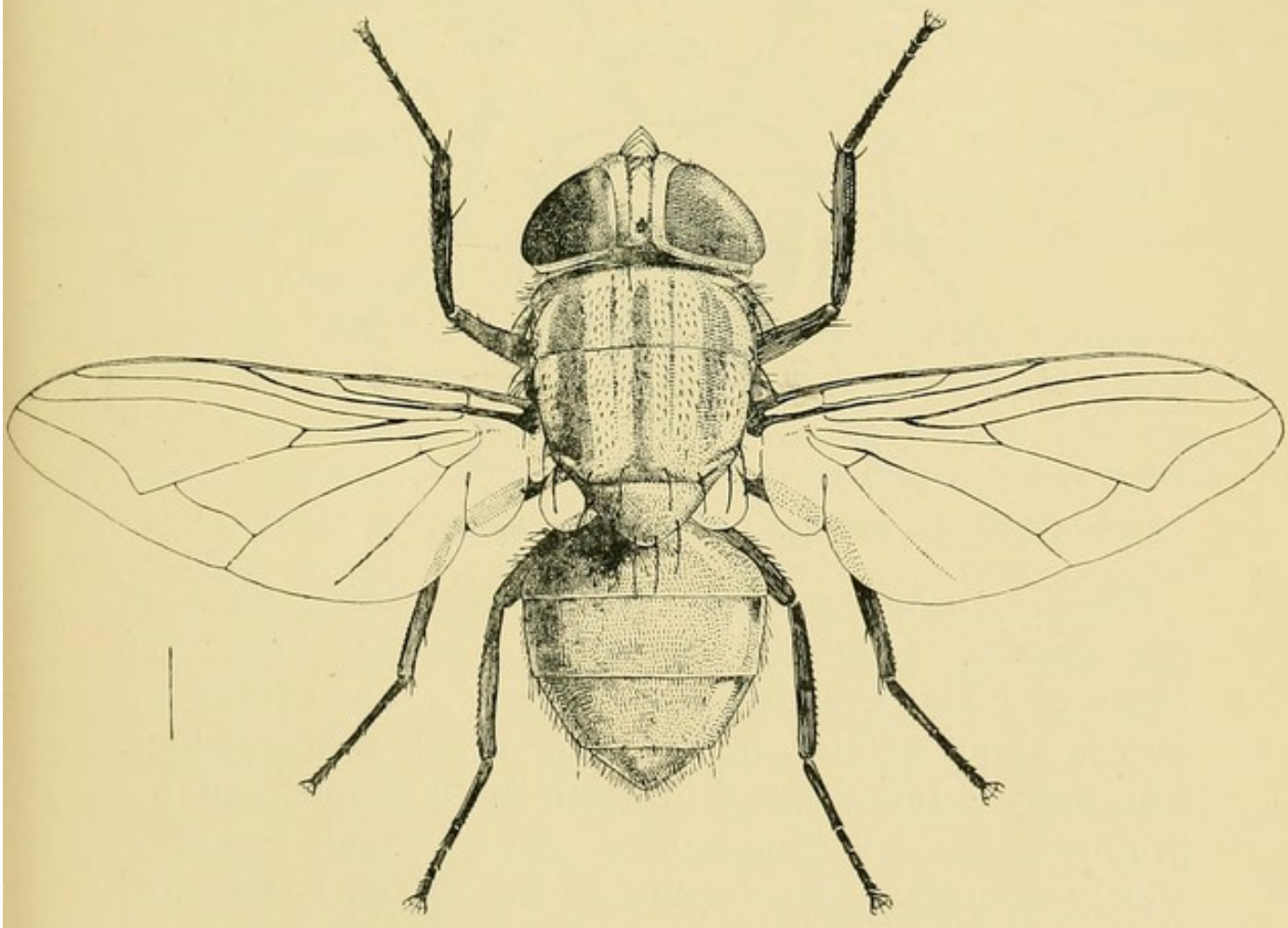


FIG. 88.—*Chrysomyia macellaria*.

spines. The ova and larvæ are often deposited on wounds and ulcers in animals and man (*L. sericata*). This species causes the well-known "maggot" in sheep.

Genus *Chrysomyia* (Compsomyia).—This genus also contains metallic-coloured flies which differ from *Lucilia* in that the thorax is striped. The screw-worm fly (*C. macellaria*, fig. 88) is found in North and South America and the West Indies, but does not attack man farther north than Kansas.

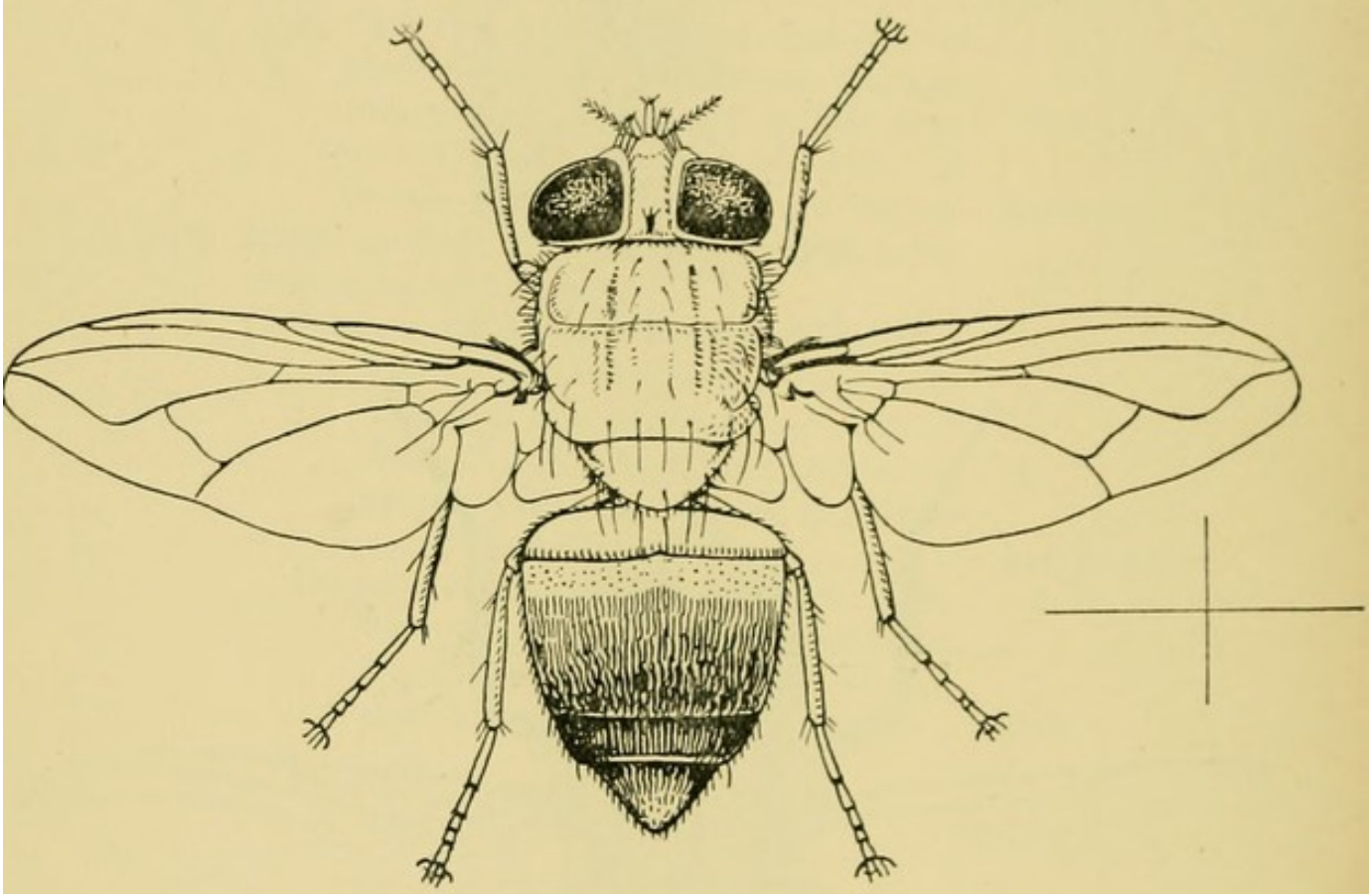


FIG. 89.—*Auchmeromyia luteola.*

Genus *Auchmeromyia*.— The blood-sucking larva of one species, *A. luteola*, has been described under the name of "The Congo Floor Maggot." The perfect insect is about 11 mm. in length, of a pale yellow colour. The head is broad, equal in width to the thorax, and the proboscis lies in a deep groove. Eyes in both ♂ and ♀ widely separate. The dorsum of the thorax is flattened and marked by longitudinal dark stripes. The abdomen consists of five segments, the second being much the longest and broadest. This segment is dark brown or black in its posterior half, the anterior half being pale and bounded in front by a dark narrow line on the

posterior edge of the first segment. The third and fourth segments are dark brown to black in colour. The fifth segment is small and contains the genitalia.

The larva is dirty white in colour, about 15 mm. in length, the body comprising eleven segments. The anterior segment is conical in shape and carries the mouth, which is armed with two black hooklets and paired teeth. They live under mats or in the cracks of the earthern floors of native huts. They feed at night.

This fly has a wide distribution from Northern Algeria to Natal, and is especially common in the Congo and about Lake Chad.

Genus *Cordylobia*.—The larva of one species (*C. anthropophaga*) lives beneath the skin of man and animals, and feeds on the tissues. The fly itself closely resembles *Auchmeromyia luteola*, but differs from it in that the second segment of the abdomen is not so large or clearly defined, and the eyes are much closer together, those in the male nearly meeting in the middle line. Widely distributed in Africa.

Family ANTHOMYIDÆ.—These are mostly moderate-sized, dull-coloured flies resembling the common house fly. The arista is plumose, pubescent, or bare. Abdomen composed of four or five segments; sometimes there are no bristles on the body, but they are usually present. The first posterior cell of the wings broadly open; tegulæ of considerable size. Male eyes usually contiguous. It is closely connected on one hand with the *Muscidæ*, and on the other with the *Sarcophagidæ*. None are metallic. The open first posterior cell is the chief character. The following genera have been connected with man either as parasites or by causing other annoyance—viz., *Hydrotæa* (Desvoidy), *Homalomyia* (Bouche), and *Hylemyia* (Desvoidy).

They may be told as follows :—

Eyes of ♂ close together; tegula large, larger than ante-tegula; fore femora of ♂ with processes, tubercles, &c., below; arista always somewhat pubescent; eyes bare; black or blue-black, and pilose ... ... *Hydrotæa* (Desvoidy).

Eyes of ♂ close together, bare ; tegula large ; abdomen nearly bare, unspotted : head almost composed of eyes ; antennæ short third joint elongated ; arista bare; mid-legs of ♂ often with peculiar structures ; black and grey ..... *Homalomyia* (Bouche).

Arista plumose ; eyes bare ; elongated species, grey or black ..... *Hylemyia* (Desvoidy).

The genus *Homalomyia* (fig. 90) has often occurred in human beings in the larval state in the intestines, being passed alive in the fæces. Most larvæ in this family are vegetable feeders. They are normally slender

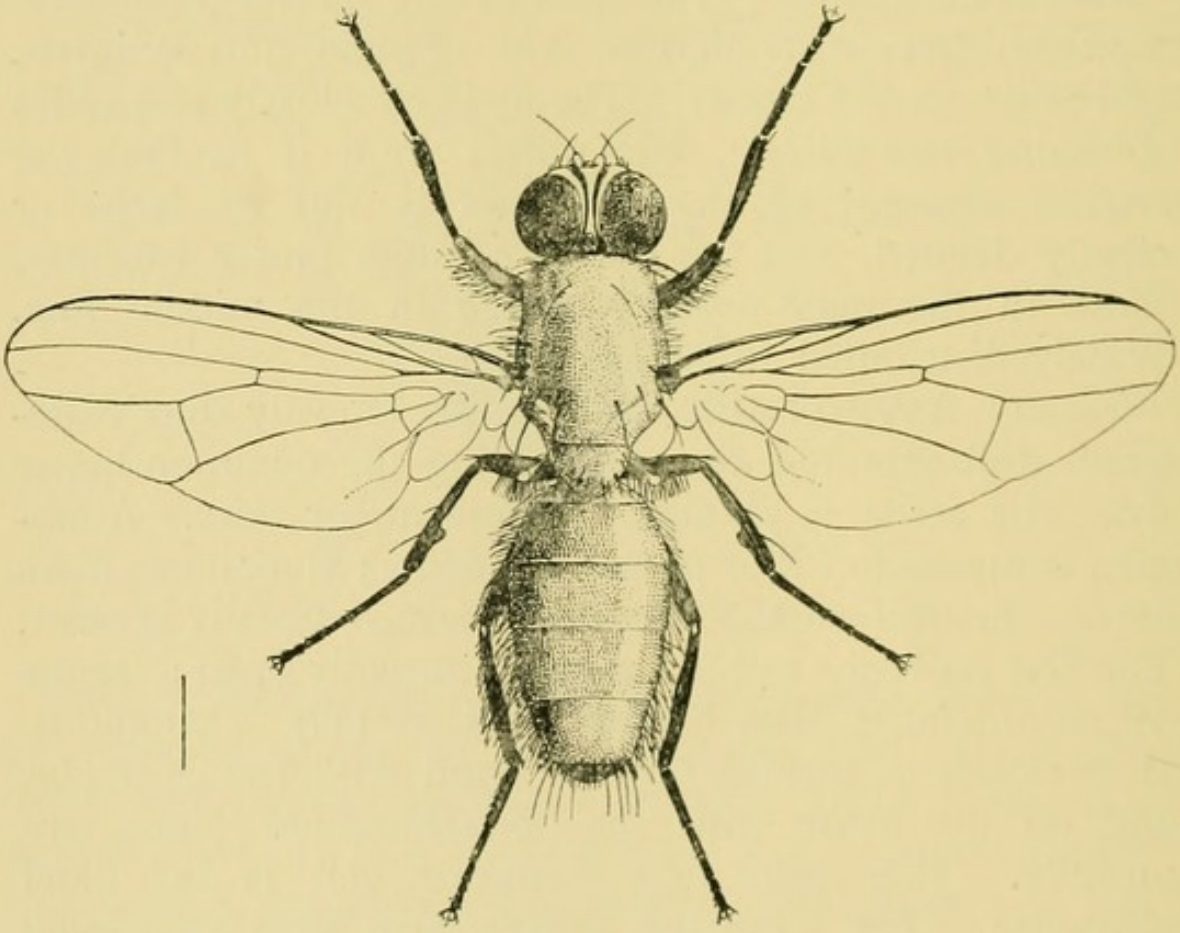


FIG. 90.—*Homalomyia canicularis.*

and cylindrical, or flat and oval, with four rows of thread-like processes on the segments, and have two mouth hooks. The puparium may be oval or flattened. In *Homalomyia* they have curious branched processes (fig. 91). The genus *Hydrotæa* also occurs in the larval

form in human beings. The characteristic neuration is shown in fig. 92. *Hylemyia* larvæ have also occurred in human excreta, having been passed *per anum*. Some are dung frequenters and produce living young.

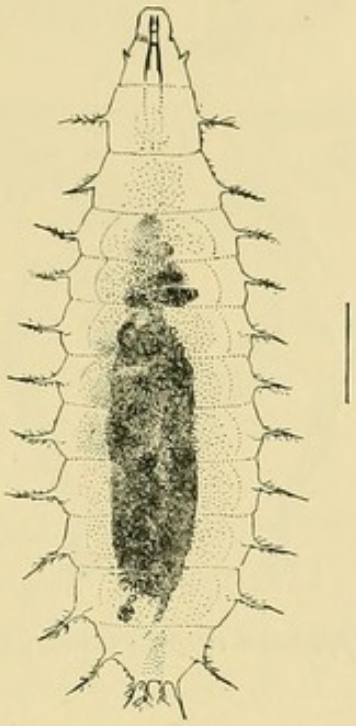


FIG. 91.—Larva of *Homalomyia*.

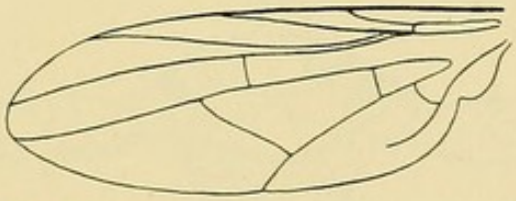


FIG. 92.—Wing of *Hydrotæa ciliata*.

Family TACHINIDÆ.—Like *Anthomyidæ*, but always bristly. Arista bare. Palpi formed of one segment. All veins of the wings simple; basal cells large; three posterior cells; first posterior cell closed or only just opened. Squamæ large. Larvæ parasitic in insects, especially in the larvæ of certain *Lepidoptera*.

PUPIPARA.

Blood-sucking, parasitic on vertebrates (except *Braula*).

Family HIPPOBOSCIDÆ.—Parasites upon birds and mammals when mature. Proboscis may be long and

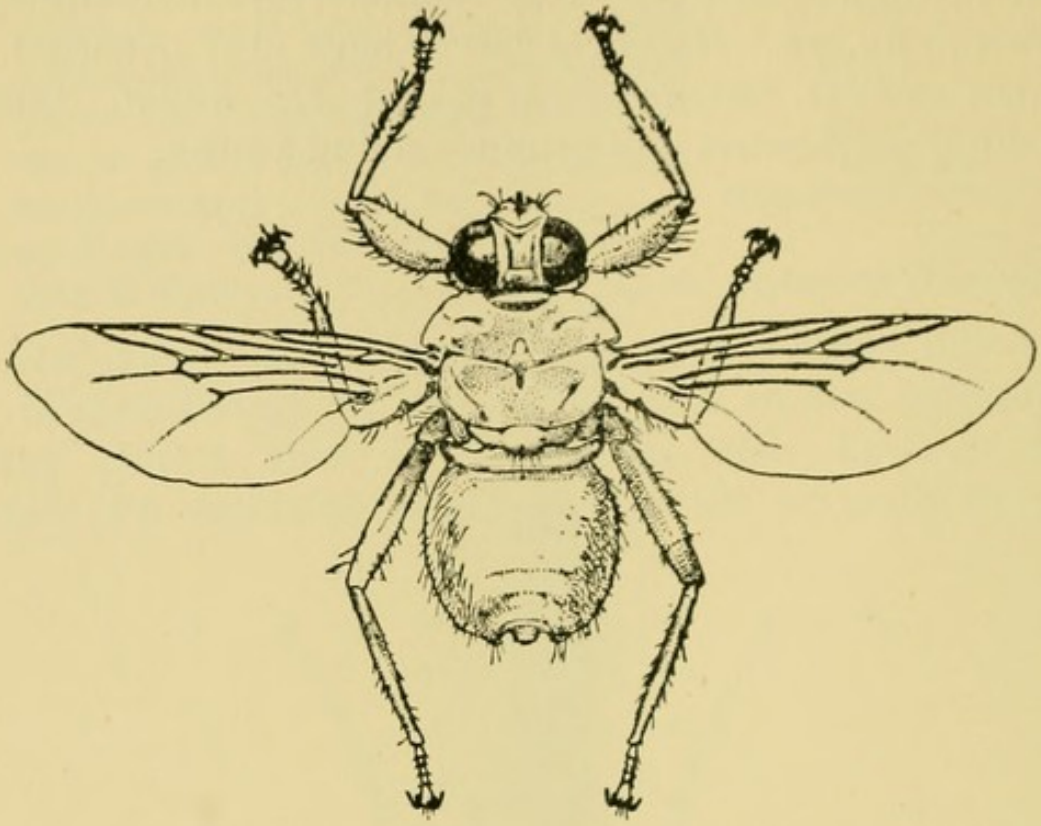


FIG. 93.—*Hippobosca equina* (enlarged four times).

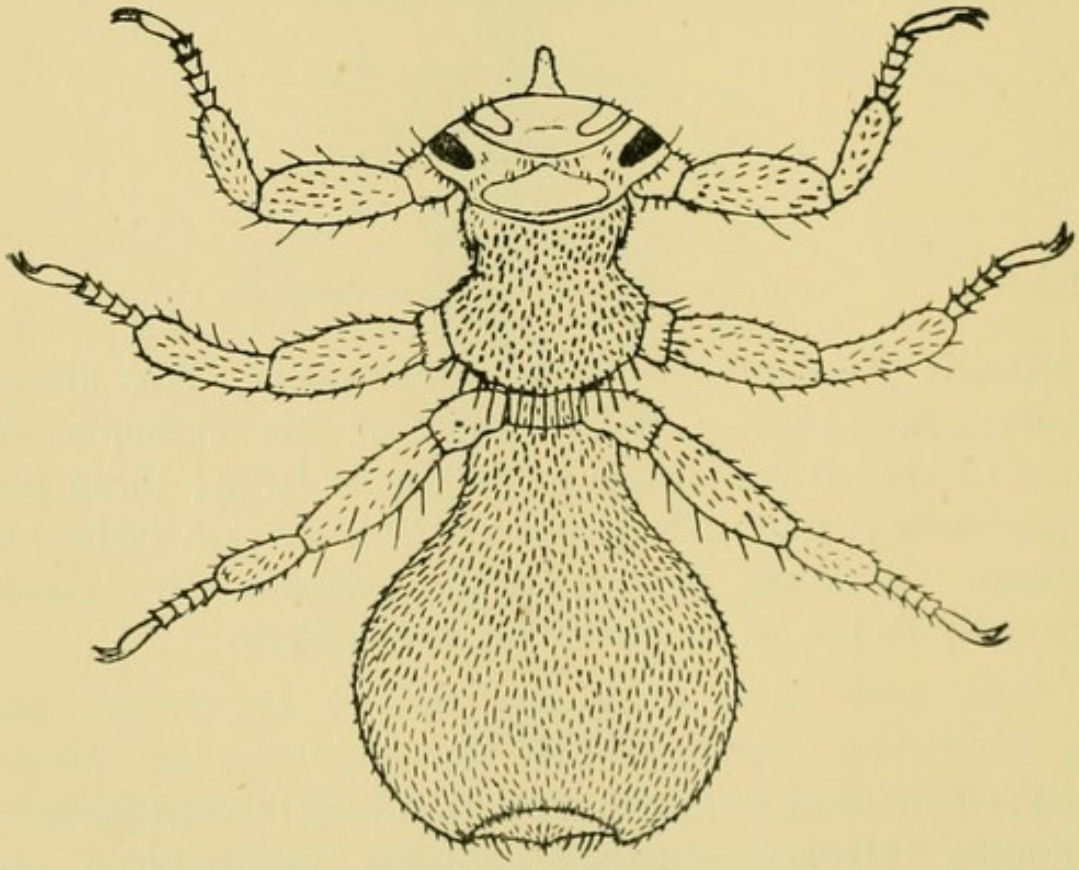


FIG. 94.—*Melophagus ovinus* (enlarged twelve times).

sharp. Palpi absent; antennæ placed in pits, composed of one segment, with or without terminal bristle or hairs. Eyes round or oval, often very small. Thorax flat, leathery; scutellum broad and short. Abdomen leathery, inflated, no sutures visible. Legs short, strong; claws large and dentate; empodia distinct. Wings present or absent (*vide* figs. 93, 94). The larvæ are born nearly matured in the puparium case, passing most of their development in the body of the parent. Of general louse-like form. This family contains the forest-fly (*Hippobosca equina*) (fig. 93), and the sheep ked (*Melophagus ovinus*) (fig. 94). The proboscis is composed of elongated, hard, closely applied flaps and an inner tube between. They live on horses, cattle, and birds, and now and then attack men.

Family NYCTERIBIDÆ.—Found exclusively on bats. Spider-like; no wings. Eyes and ocelli indistinct or wanting. Legs long, femora and tibiæ flattened.

Two other families, *Braulidæ* and *Streblidæ*, occur; the former live on bees, the latter on bats.

CHAPTER XI.

MOSQUITOES.

MOSQUITOES are the hosts of many parasites, and some of these are injurious to man or the lower animals. As these diseases include malaria and yellow fever, at least one of the human filariæ, as well as the *Proteosoma* of birds and the *Filaria immitis* so fatal to dogs, a good working knowledge of the structure, life-history, and modes of classification of these insects is required for tropical work.

Mosquitoes, or *Culicidæ*, belong to the order of dipterous insects, as they have the anterior pair of wings membranous, whilst the posterior pair are represented by a pair of club-shaped processes, the halteres or balancers.

The insect is divided naturally, into three regions : (1) head, (2) thorax, (3) abdomen. To the head are attached the sensory and biting organs, consisting of two compound eyes, two antennæ, two palpi, and a complex suctorial and piercing organ, the proboscis.

To the thorax are articulated a pair of wings, a pair of balancers, and three pairs of legs ; whilst the abdomen is segmented and terminates in the anus and external organs of generation.

In the *Culicidæ* the head, thorax, abdomen and legs are thickly covered with scales in most of the genera, whilst on the wings scales are found only at the edge and on the veins. The character and arrangement of the scales are important points in the differentiation of genera. The absence of scales on the wings or the presence of scales on other parts of the wings than those mentioned, or the substitution of hairs for scales, are valuable aids

in the differentiation of other insects from the *Culicidæ*. The type of venation of the wings and the characters of the cephalic appendages are of value both for identification of the family *Culicidæ*, and for differentiation of genera, species, and sexes.

Scales lose their colour and become too transparent for proper examination unless the specimens are mounted dry, so that for identification of species it is advisable that the mosquitoes should be mounted dry and so arranged that all the surfaces can be examined. It is best to examine or send for examination young mosquitoes, as in older specimens many scales are rubbed off and the insects otherwise injured.

Mosquitoes hatched out from pupæ should be kept alive for at least twenty-four hours, as if killed when too young they become much distorted when they dry. They should be kept in a dark place, as they then are less liable to have the scales rubbed off.

The mosquitoes must be killed rapidly, and a cyanide pot is invaluable in this connection, though chloroform vapour, formalin, or even tobacco smoke, may be used. The dead mosquito must be mounted without delay, as the limbs soon lose their pliability. To mount they should be placed on their backs on a piece of cork felt. A small square or a circle of thin card should be taken, and on one side of it the date and place of capture of the mosquito should be written, with a distinguishing number if the name is not known.

A fine entomological pin (No. 20) should be taken up with forceps *near the point*, and the piece of card with the blank side downwards should be placed on a piece of cork felt. The pin, still held in the forceps, should be pushed through the card. The hold on the pin should then be shifted higher, and the pin pushed still further through the card till about half of it is through. The pin, still held in the forceps, with the card transfixed on it, should then be pushed through the thorax of the mosquito. On lifting up the pin the mosquito, which

has been transfixed, will remain on the pin, and on turning the card upside down the legs and wings can, with a few touches of a clean needle, be arranged so as to be readily visible, and will not hide any part of the back of the insect. A stout pin should then be run through

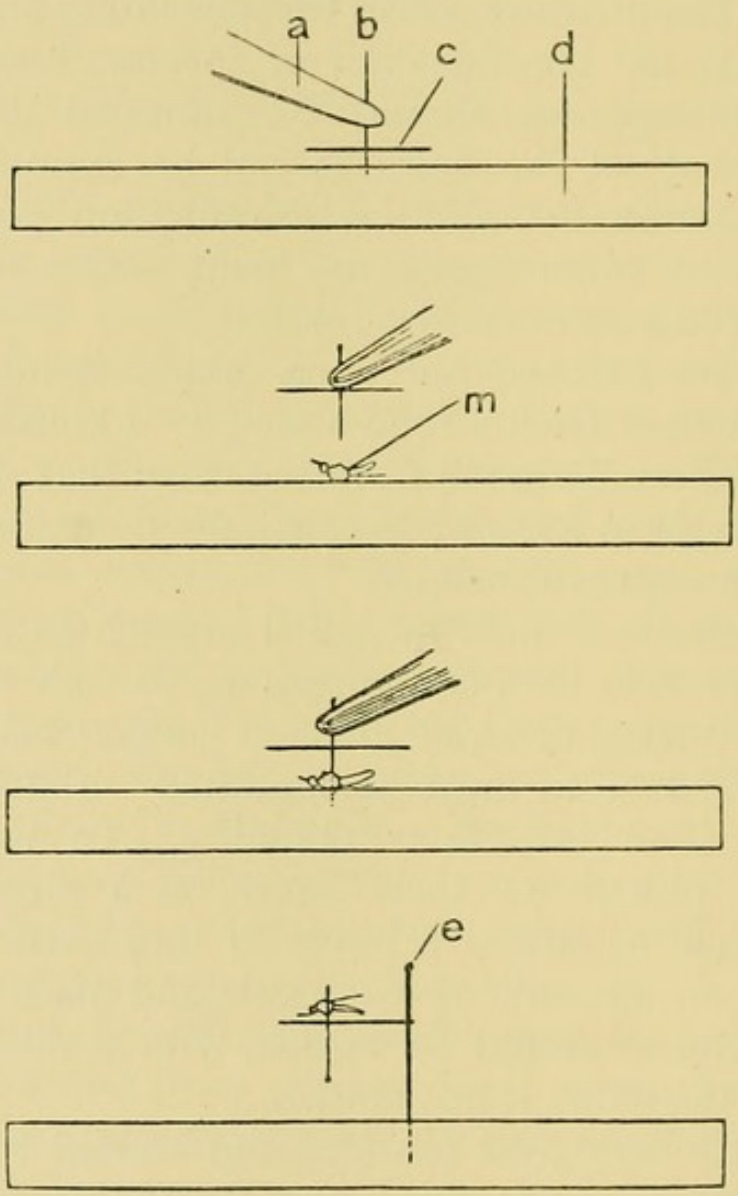


FIG. 95.—*a*, Forceps; *b*, pin; *c*, disc; *d*, cork; *m*, mosquito; *e*, large pin to carry disc.

the corner of the piece of card into the cork felt floor of the collecting box. To prevent insects attacking the specimens some powdered naphthalin enclosed in a cloth bag should be placed in the box, and securely fixed to the side of the box with strong drawing-pins, or melted naphthalin may be poured in the corners of the box. An

alternative plan is to pin securely into the corners of the box some small pieces of sponge and on these to place a drop or two of creosote.

To examine such a specimen a low power, one inch or two-thirds of an inch, is required. With such a power the character of the scales on each part of the insect can be examined. The examination should be made by reflected light and the insect so rotated that the part to be examined is horizontal. This can be done best by altering the inclination of the large pin and using a strip of cork felt as a slide (fig. 96). In this way each part of the upper surface can be examined in succession.

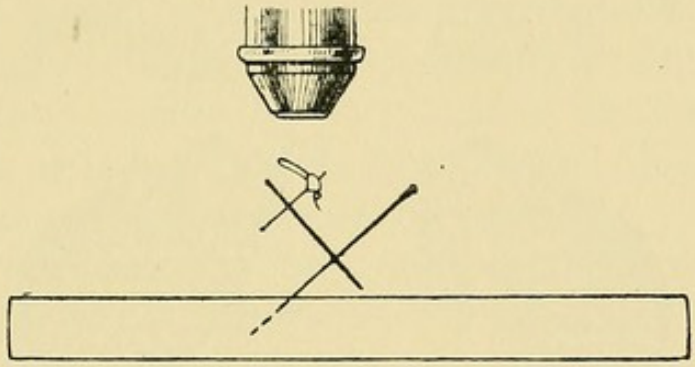


FIG. 96.

To examine the under surface, a second mosquito mounted with its back towards the card is required.

The main types of scales found in the *Culicidæ* are represented in the drawing (fig. 97).*

These scales can for descriptive purpose be reduced to the small number of types represented :—

(*a*) Broad, flat, spade-shaped or tile-shaped scales.
(*b*) Broad, expanded, asymmetrical scales.
(*c*) Narrow, asymmetrical scales.
(*d*) Narrow, hair-like scales.
(*e*) Narrow curved scales or crescents.
(*f* & *g*) Spindle-shaped scales.
(*h* & *i*) Upright fork scales or darts.
(*j*) Long twisted scales.
(*k*) Pyriform scales.

* Figs. 97 and 99—101 are reproduced by kind permission of the Editor of the *Journal of Tropical Medicine*.

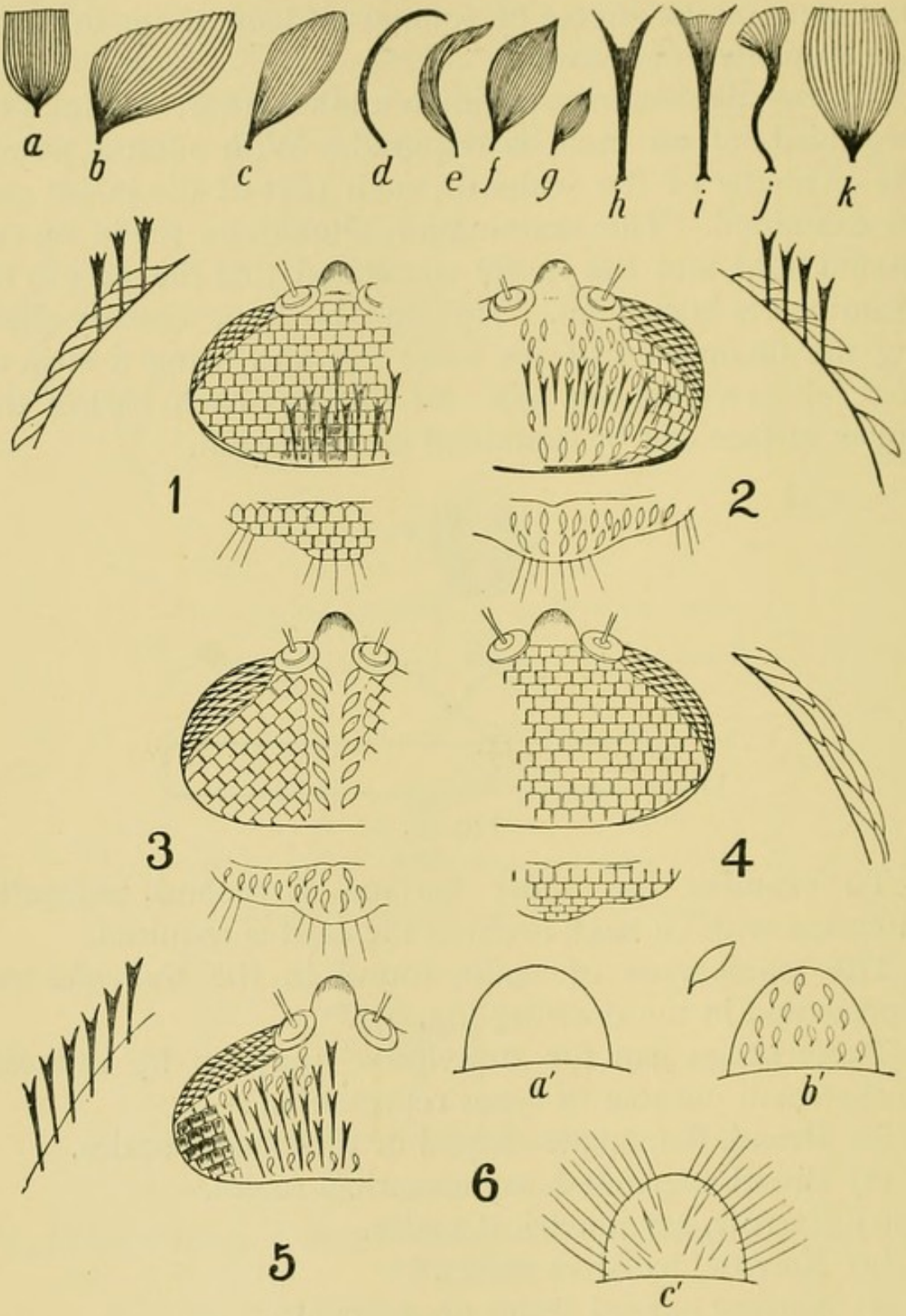


FIG. 97.—Types of scales, *a* to *k*. Head and scutellar ornamentation, 1 to 4; forms of clypeus, 6. 1, Head and scutellum of *Stegomyia*, &c.; 2, of *Culex*; 3, of *Ædes*, &c.; 4, of *Megarhinus*, &c.; 5, Head ornamentation of *Cellia* and some other *Anophelina*; 6, clypeus, *a′*, of *Culex*; *b′*, of *Stegomyia*; *c′*, of *Joblotia*. (Theobald.)

On the wings other types of scales, either lanceolate, long, narrow scales pointed at the free end, or long and narrow and with square free ends, are met with (fig. 101).

Head Appendages.—The head appendages can be easily seen in most specimens mounted as described, but for more minute examination it is better to cut off the head and mount it in a shallow cell either as a dry specimen or in glycerine jelly. In this way the parts are not much distorted, and if a thin slide be used both sides of the specimen can be examined. Canada balsam can be used, for the examination of the scales, hairs, &c., but not satisfactorily, as they become too transparent.

Proboscis.—To examine the component parts of the proboscis it is better not to use the shallow cell, but to forcibly compress the head after soaking in liquor potassæ for twenty-four hours, so as to cause the various component parts of the proboscis to separate ; in one or more specimens all the elements can be seen.

Palpi.—The points to be noted in the palpi are their length relative to the proboscis, the number of joints, and the colour, shape and arrangement of scales and hairs. To determine the number of joints it is necessary to remove the scales from the palpi.

The Antennæ.—Their length, and the relative lengths of the different joints. The number, length and arrangement of hairs, and the presence or absence of scales.

The different regions of the mosquito are shown in the diagram (fig. 98). To the head are attached the appendages already mentioned, and, in addition to these, the back part of the head, or occiput, requires close examination.

The thorax is composed of three segments fused together. The greater part is formed by the second segment, or *mesothorax*. Anteriorly on each side are two rounded projections, the *prothoracic lobes*, the remnants of the anterior segment. The posterior edge of the mesothorax is a narrow, overhanging, often trilobed plate—the *scutellum*. The scales on this part of the thorax are of generic value.

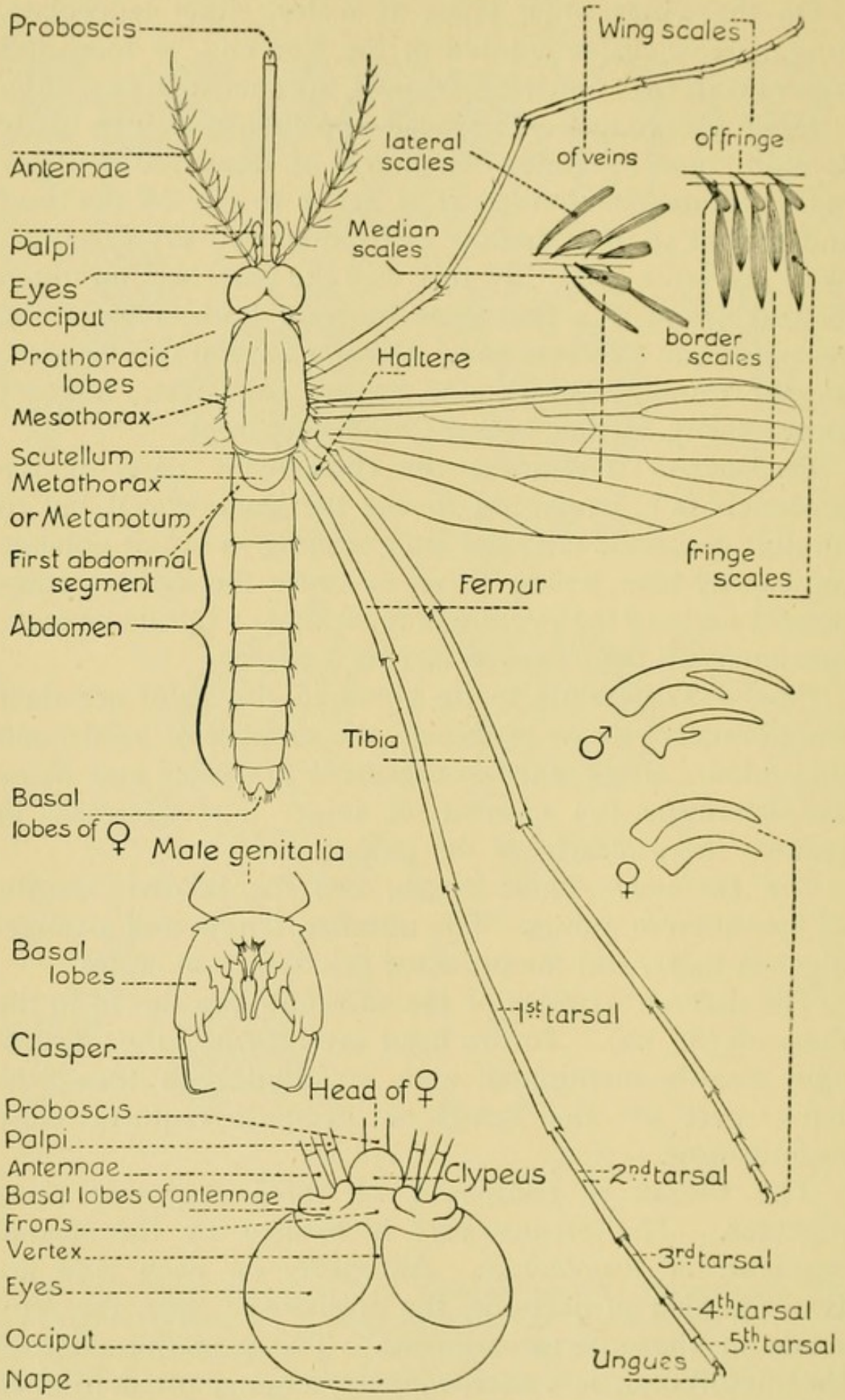


FIG. 98. (After Theobald.)

Partly overlapped by the scutellum is a rounded mass connecting the thorax and abdomen, known as the *meta-thorax* or *metanotum*. This is the third segment of the

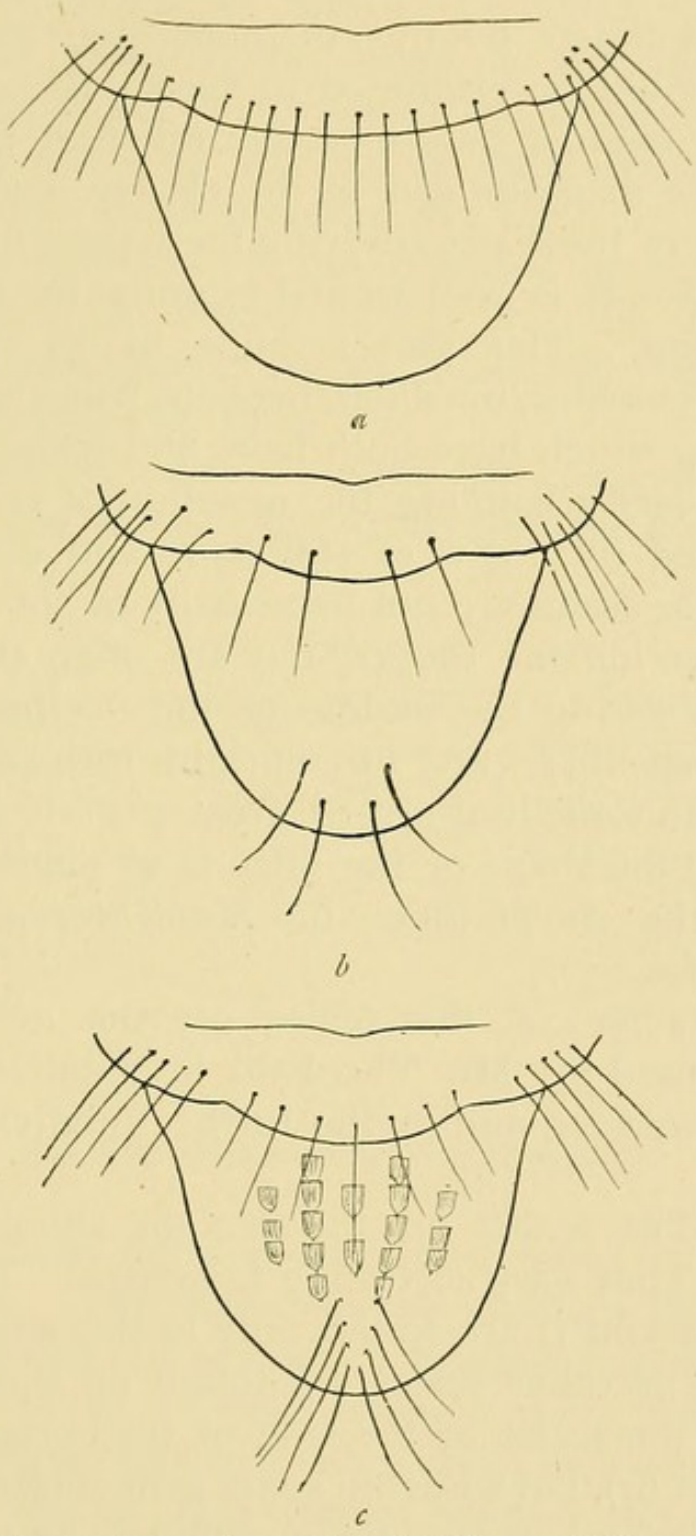


FIG. 99.—Types of metathorax (Theobald). *a*, *Culicina*; *b*, *Dendromyina*; *c*, *Joblotina*.

thorax. On each side of the metathorax are the halteres, which arise from the sides of the mesothorax.

The abdomen is segmented and has no lateral append-

ages. The last segment terminates in the external genitalia. These are of specific but rarely of generic value.

Thorax and Abdomen.—In the examination of the dry mounted specimen by this method, each part of the mosquito should be examined in turn. By altering the angle in the manner described, the different parts represented in the diagram can all be clearly made out and the character of the scales covering these parts investigated.

Metathorax.—It is well to first examine the metathorax or metanotum. This part is nude in all sub-families except *Dendromyinæ*, in which there are hairs, in *Toxorhynchitinæ* and *Limatus*, which have both hairs and scales (fig. 99).

Scutellum.—Overhanging the metathorax is the scutellum, bordered by a row of stiff hairs and covered with scales. These scales are not necessarily of the same type as those covering the thorax, but are often the same as the scales covering the middle of the occiput, with the exception that there are no upright fork-scales. The scales on the scutellum are of great generic importance (fig. 97), and the shape of the edge is of similar value in separating the *Anophelinæ* and *Megarhininæ* from the other *Culicidæ*.

The character of the scales on the *abdomen* and *thorax* are used by Mr. Theobald to subdivide the old genus of *Anopheles*, and in the further subdivision of the *Culicinæ*.

Occiput.—The scales on the occiput should next be examined. They vary according to genera. The upright fork scales (*h* and *i*) are found only in this situation. In some genera no other scales are found on the head. In most genera the scales at the side of the occiput are tile-shaped scales (*a*), but whilst in some genera (*Megarhininæ*) these scales extend all over the middle line of the occiput, and are the only scales found, in others, as *Stegomyia*, they are found also with fork-scales; in others, again, they are not found in the middle of the occiput, but are replaced by spindle scales (*f*), either alone as in *Ædes*, or

with narrow-curved and upright fork-scales as in *Culex*, *Mansonia*, &c. (fig. 97).

Wings.—The type of wing venation can be seen in a specimen mounted as described above, but is better seen in the wings when detached, flattened out and examined dry. The character of the scales covering the longitudinal veins on the wings must be observed, as these are of generic importance (fig. 101).

The wing venation of the *Culicidæ* is comparatively simple. Here we follow closely in this, as in other respects, the description by Mr. Theobald. It is an easy one to work with (fig. 100).

The thickened edge is called the costa, it forms the free edge of the wing. The scales on it are of no generic

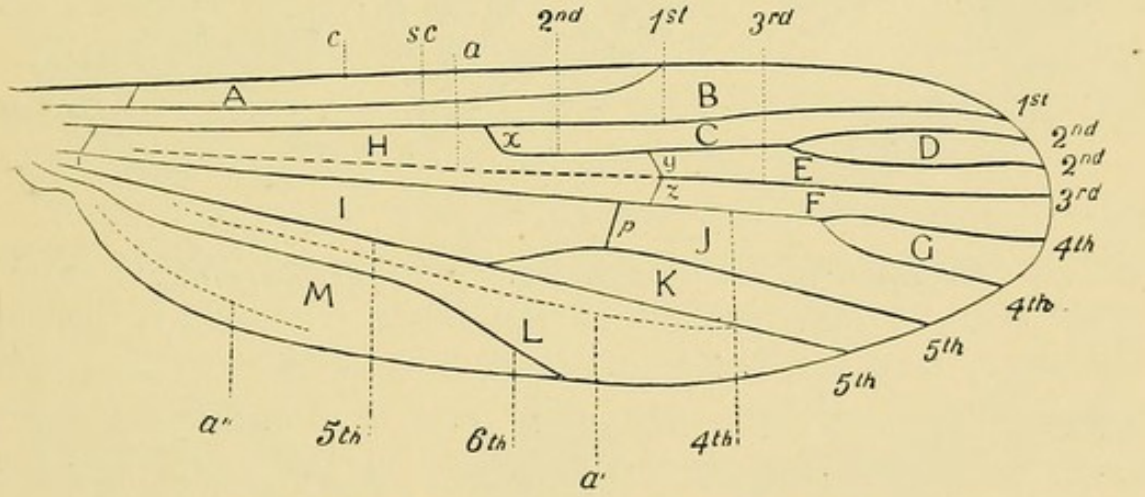
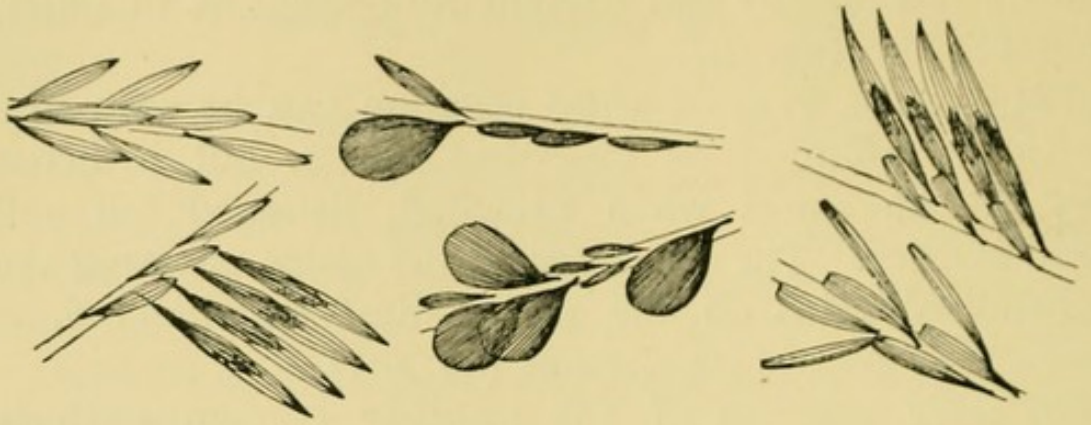
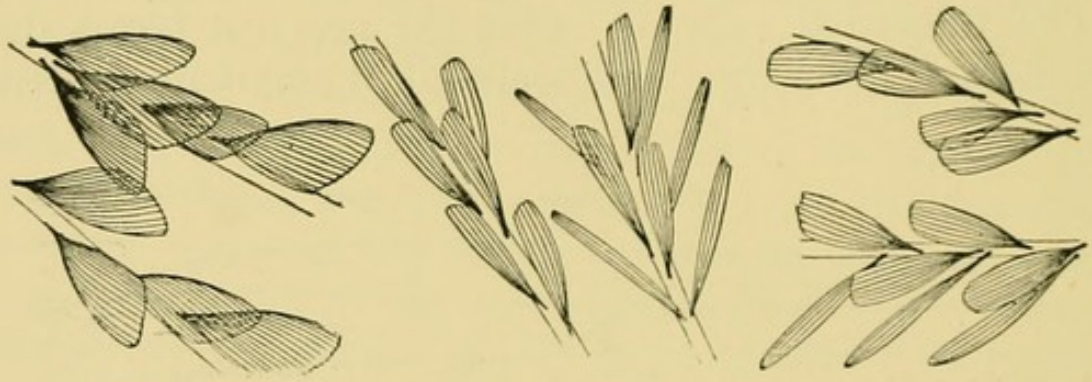


FIG. 100.—Neuration of Wing (Theobald).

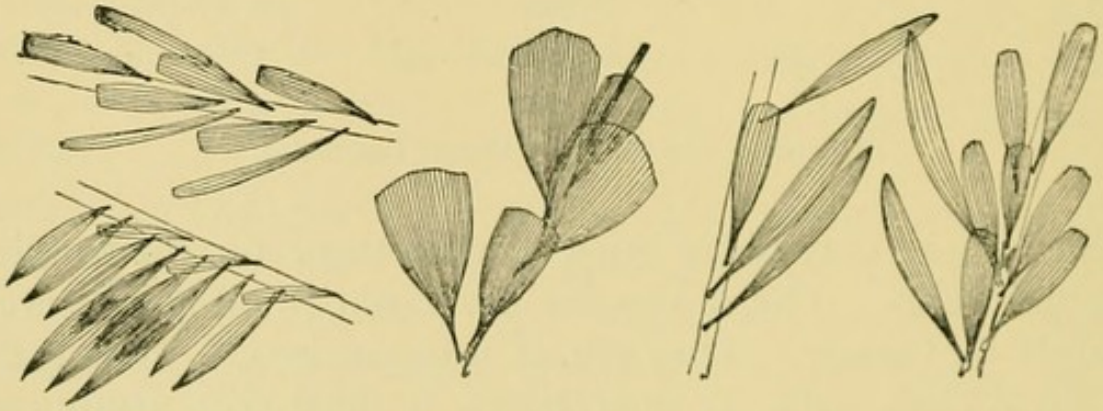
value; these may differ greatly from those on the longitudinal veins. The scales on the costa in all genera are mainly lanceolate. They are of unequal length, arranged in two tiers with, at their bases, a third row arranged obliquely; these last are more like the scales on the longitudinal veins. The straight edge of the wing, with the wing expanded, is the anterior edge, and is therefore so described. Next to the costa is a vein running from the base or attachment of the wing to rather more than half-way to the tip, terminating in the costa; this is called the sub-costal vein (*sc*).



1 *Anopheles.* 2 *Cyclolepteron.* 3 *Janthinosoma.*



4 *Mansonia.* 5 *Stegomyia.* 6 *Eretmapodites.*



7 *Culex.* 8 *Mucidus.* 9 *Psorophora.*

FIG. 101.—Various Forms of Wing Scales (Theobald). 1, Scales on veins and on costa in *Anopheles;* 2, scales on veins in *Cyclolepteron;* 3, scales on veins and on costa in *Janthinosoma;* 4, scales on veins in *Mansonia;* 5, scales on veins in *Stegomyia;* 6, scales on veins in *Eretmapodites;* 7, scales on veins and on costa in *Culex;* 8, scales on veins in *Mucidus;* 9, scales on veins and on costa of *Psorophora.*

The other veins running from the base towards the tip are known by numbers, the most anterior being the *first longitudinal*. This is a single vein running the whole length of the wing and terminating at the tip. It is covered with scales in its whole extent.

The *second longitudinal* arises from the first nearly half-way from the base, and bifurcates before reaching the tip. The space enclosed in the bifurcation (D) is known as the first fork-cell.

The *third longitudinal* arises in the base of the wing, but is not covered with scales for nearly the first two-thirds, and therefore appears merely as a yellowish line. It does not bifurcate.

The *fourth longitudinal* arises from the base, is covered with scales in its whole extent, and bifurcates near the tip, forming the second fork-cell (G).

The *fifth longitudinal* arises at the base, is covered with scales in its whole extent, and bifurcates half-way up the wing, enclosing the third fork-cell (K).

The *sixth* does not bifurcate, and terminates in the costa about the middle of the posterior border of the wing. There are markings or thickenings on the wing between the fifth and sixth longitudinal and posterior to the sixth, which have no scales and are not regarded by Mr. Theobald as veins. The most constant of these is scaled in some species of mosquito, which are placed by Theobald in a separate sub-family *Heptaphlebomyina*. Connecting the second and third longitudinal veins is the transverse vein. The third and fourth are connected by the middle transverse vein, and from the fourth longitudinal to the anterior division of the fifth is the posterior transverse vein. They are definite bands of considerable thickness and often contain air. They are not scaled. The relative positions of these three transverse veins is of some importance in the separation of species. Variations cannot be relied on implicitly for this purpose, as in some species the arrangement of the transverse veins varies considerably in different individuals.

Classification of Mosquitoes.

In a work of this kind it is not necessary to consider more than the identification of the commoner and more important genera into which the *Culicidæ* are divided.

The *Culicidæ*, as has been indicated in the previous chapter, constitute a family in the sub-order *Orthorrhapha nemocera* of the order *Diptera*. Their systematic classification has been attempted by several writers, but that of Mr. Theobald, which is based largely on the scale characters of the mature insect, has been here, in the main, adopted.

The main characteristics of the family have been already given. In brief, it may be stated that the *Culicidæ* may be told by the venation of the wing and by the arrangement of scales on the head, body and veins of the wing. Other insects which are often mistaken for these have a different form of wing venation, and the wings are either bare or ornamented with hairs.

The male external genitalia are by some considered to be of great importance in differentiation of genera. The external genitalia vary greatly in different species. The general type (see fig. 98) may be described as consisting of two large fleshy basal lobes, each with a terminal chitinous clasp segment, always curved and often ornamented with spines. Between the claspers, arising internally and ventrally to the claspers, are other chitinous processes, the *harpes*, which may be well developed, formed of two segments, or rudimentary.

Between the harpes and the claspers are a pair of clasping organs, the *harpagones*.

The chitinous lobes above the cloaca, the setaceous lobes, are part of the rudimentary eighth segment.

The variations of each part are great. They should be examined both in the dried specimen, in order to see the relative positions of the various parts, and after treatment with liquor potassæ, or in flattened specimens, rendered transparent in order to make out the details.

The family *Culicidæ* is divided into various subfamilies.

(1) Sub-family *Corethrinæ*.*—Proboscis short and not adapted for piercing, palps dependent. These insects are incapable of biting man and animals, and play no part in the transmission of disease (fig. 102, E).

(2) Sub-family *Megarhininæ*.—Large, brilliantly coloured insects. Proboscis long and bent downwards. Palps thin towards the extremity and bent upwards, those of the male being composed of five segments, those of the female varying according to genera. Head clothed with flat scales only (fig. 102, F, and fig. 97, 4).

These mosquitoes are found as a rule only in the vicinity of the jungle. They rarely attack man.

Larvæ are larviverous. They have a very short respiratory siphon.

Head, thorax and abdomen thickly covered with flat, square-ended scales. Scales of wings small and square-ended. First sub-marginal cell much shorter than second posterior. Caudal tuft of hairs on each side of abdomen in some species.

(3) Sub-family *Anophelinæ*.—Proboscis straight and adapted for piercing. Palps very nearly as long as the proboscis in both sexes, composed of five segments in the male and four in the female; the last two joints in the male are expanded. Antennæ plumose in the male and pilose in the female.

Head has numerous upright forked scales, and a few narrow curved scales and flat, square-ended scales at the sides in some of the genera. *Thorax* and abdomen ordinarily have few scales as compared with other *Culicidæ*. Scutellum simple, never trilobed. The varied covering of thorax and abdomen is the basis of the sub-division into genera. Wings in most genera marked with black or brown patches; wing scales long and lanceolate or fusiform.

The female has only one spermatheca. The eggs are

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* In Theobald's latest classification the *Corethrinæ* are not considered as belonging to the *Culicidæ*.

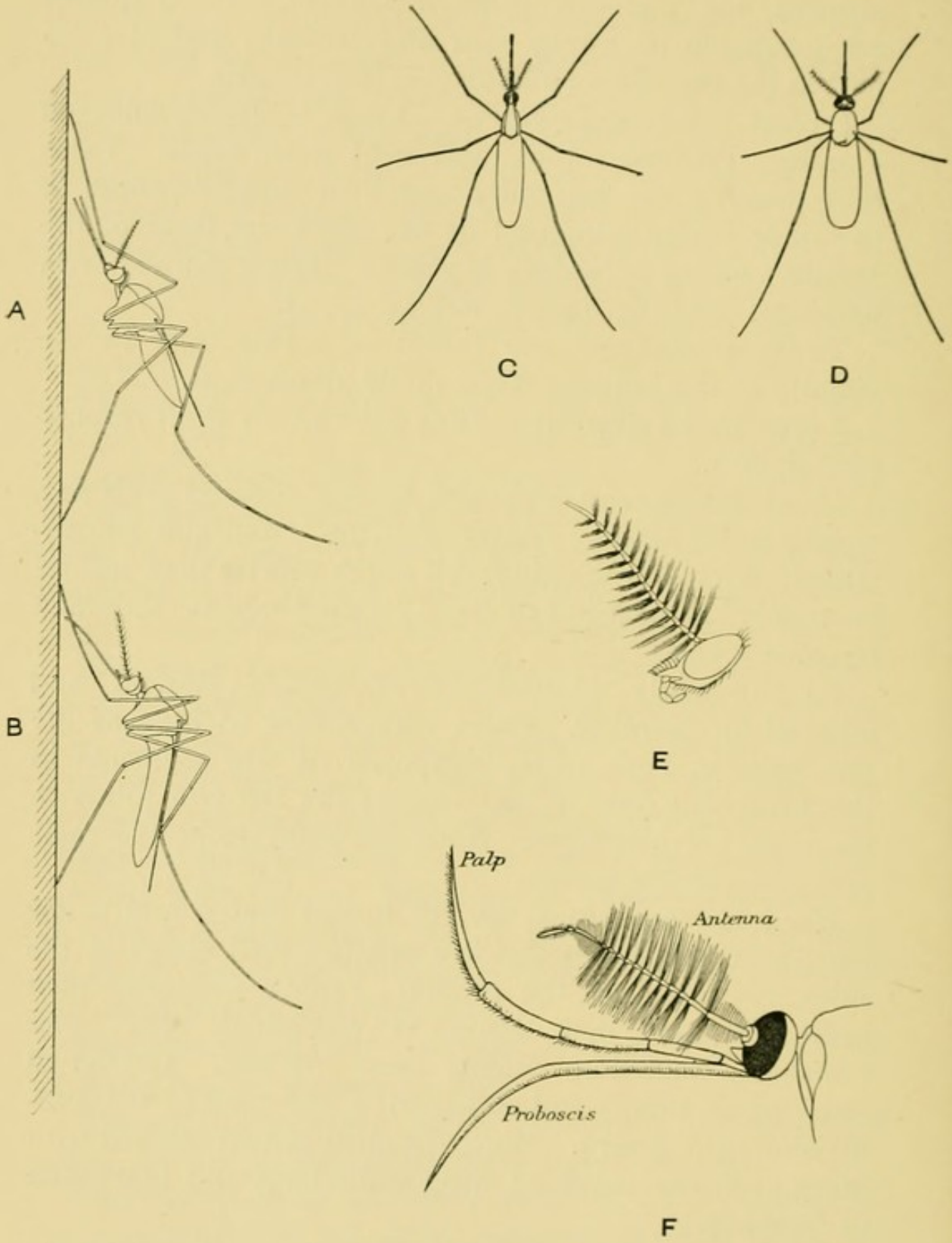


FIG. 102.

A—Lateral view of Anopheline.
B—Lateral view of Culicine.
C—Anopheline viewed from above.
D—Culicine viewed from above.
E—Head of Corethra.
F—Head of Megharinina.

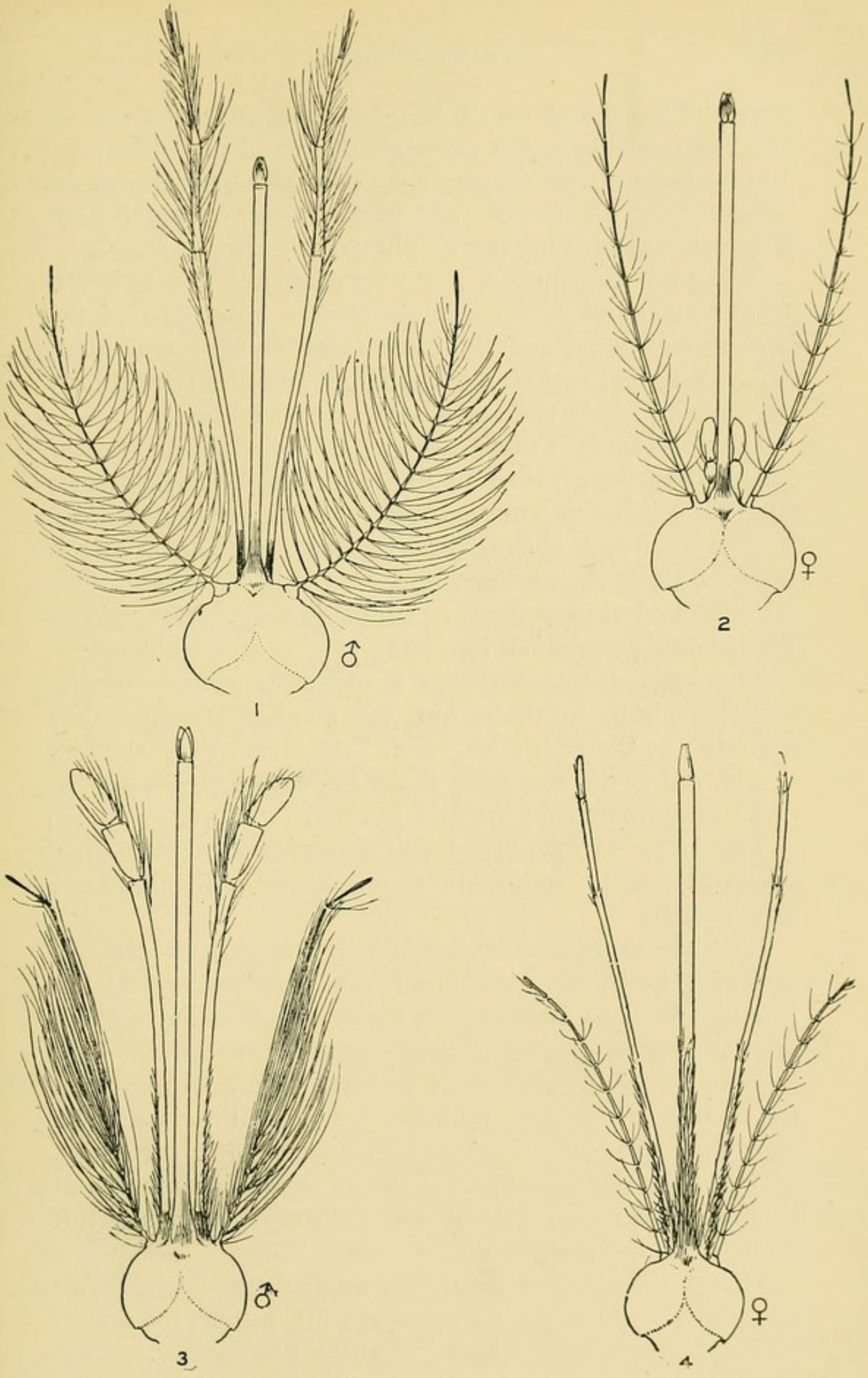


FIG. 103.—1, Culicine male; 2, Culicine female; 3, Anophelina male; 4, Anophelina female.

laid singly, are more or less boat-shaped and float on the water, owing to the presence of lateral air chambers. Larvæ have no respiratory siphon, and when at rest lie horizontally on the surface of the water. Pupæ have trumpet-shaped respiratory siphons.

The adult mosquito appears very narrow when viewed from above, and as seen in profile the long axes of the proboscis, thorax, and abdomen form an almost straight line. When at rest on a flat surface, the insect commonly presents an appearance as if standing on its head.

(4) Sub-family *Culicinæ*.—Palps in the ♂ as long or longer than the proboscis ; in the ♀ always much shorter than the proboscis ; metanotum nude, scutellum trilobed.

Viewed from above, these mosquitoes appear much broader than *Anophelinæ*, and from a lateral aspect have a hunchbacked appearance, which is very different from that of the *Anophelinæ* (fig. 102).

The eggs differ markedly in different genera of the Culicines, both in shape and the manner in which they are laid ; in the genus *Stegomyia* and others they are laid singly, but never resemble those of *Anophelinæ*, while in *Culex* and others they are laid in rafts.

The larvæ are provided with a respiratory siphon, and when at rest lie obliquely in the water with the head downwards.

(5) Sub-family *Ædinæ*.—Many of the genera formerly included under this sub-family are now separated from it, and Theobald makes several new sub-families. These are : *Ædinæ, Limatinæ, Deinoceratinæ, Uranotæninæ, Dendromyinæ*. Palps very short in both sexes. Antennæ in the males not always plumose. The proboscis may be very long, and is sometimes clubbed at the extremity. Metanotum nude, without hairs or scales in most, but in the sub-division *Dendromyinæ* there is always a tuft of hairs on the metanotum.

This sub-family has been less studied than *Anophelina* or *Culicina*. Most of its members are jungle, mangrove or forest mosquitoes. Many of them bite by day. They

breed in natural collections of water, often in the hollow axils of leaves, in pitcher plants or in perforated bamboos. They may also breed in swamps, slowly moving water, roadside trenches, or in streams.

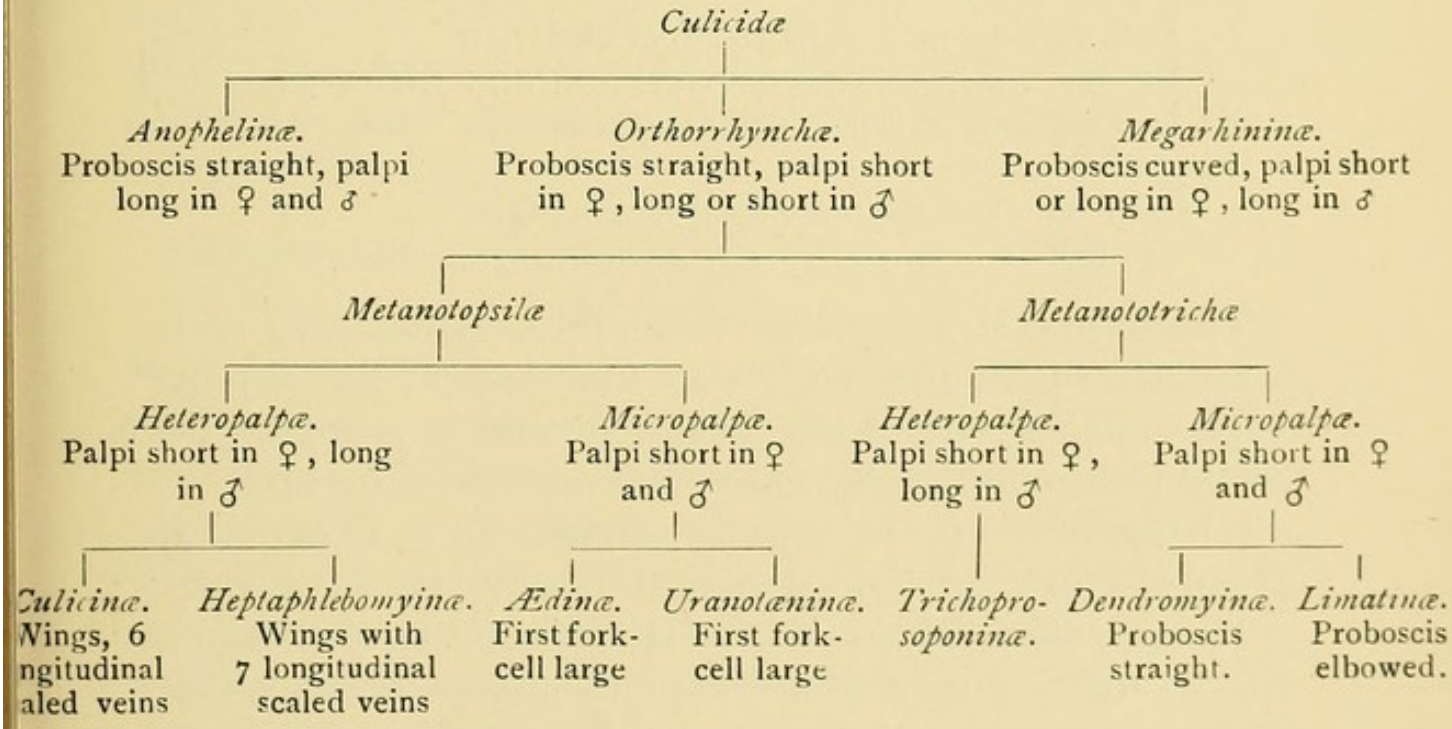
The remaining sub-families are represented by a few rare species and are of little importance to medical workers.

(6) Sub-family *Toblotinæ* (*Trichoprosoponinæ*, Theobald). Head, thorax and abdomen covered with square-ended scales. Palps short in both sexes. Metanotum adorned with hairs and square-ended scales.

(7) Sub-family *Heptaphlebomyinæ*. Head, thorax, scutellum, metanotum and abdomen as in *Culex*. Wings have a seventh scaled longitudinal vein.

Four species known. *H. simplex* (Theobald, 1903) from Central Africa (Mashonaland) was the first described.

Theobald's latest grouping of the *Culicidæ* is modified from that of Lutz, and differs in some respects. He divides the *Culicidæ* as follows :—



There are many points in favour of this classification.

(1) *Corethrinæ* are separated off from the *Culicidæ* and considered as a distinct family, the *Corethridæ*.

(2) *Anophelinæ* and *Megarhininæ*, two groups which differ at all stages of their development from the rest of the *Culicidæ*, are separated off from the other groups.

(3) The old group *Ædeomyinæ*, containing several markedly dissimilar sub-groups, is broken up so that the *Ædinæ*, *Uranotæninæ*, *Dendromyinæ*, *Limatinæ* and *Deinoceratininæ*, which are naturally distinct groups, are now widely separated.

Though we consider that the scheme is one which will be of practical value and a useful aid in the subdivision of the *Culicidæ*, we have not thought it advisable to adopt it in the present unsettled condition of the classification of these insects, as it has not received the general approval of those working with mosquitoes.

(1) Genera of sub-family *Corethrinæ*.

Metatarsus longer than first tarsal joint ..... *Corethra.*
Metatarsus shorter than first tarsal joint ..... *Mochlonyx.*

(2) Genera of sub-family *Megarhininæ*. This sub-family is divided by Theobald into three genera.

A. Palpi long in both sexes.
  (*a*) Last segment of ♀ palpi round or blunt as if broken ..... *Megarhinus.*
  (*b*) Last segment of ♀ palpi long and pointed... *Ankylorhynchus.*
B. Palpi of ♀ short.
  Palpi not more than one-third length of the proboscis ..... *Toxorhynchites.*

The differences between these three genera are comparatively slight, and a division founded on the absence or presence of lateral caudal tufts would be simpler, but would not quite correspond with Theobald's division.

(3) Genera of sub-family *Anophelinæ* (Theobald). Theobald now subdivides this sub-family into 21 genera.

The practical value of the subdivision of the *Anophelinæ* into so many genera is disputed by many medical men. There are few branches of natural history in which such enormous advances have been made as in our knowledge of the *Culicidæ*, and each year there are additions to this knowledge.

Of the *Anophelinæ* about 130 species are now known. This is too large a number to be conveniently grouped into one genus. A subdivision is necessary, and some grouping will be made automatically by any one working with these insects. It is better that a uniform system should be adopted than that each worker should make his own groups.

The subject is so large that it is now a special branch, and we propose merely to deal with selected parts of it and select such groups as are represented by common species, and by species known to carry malaria.

It is unfortunate that the grouping into genera based on the external characters of the adults does not correspond with grouping according to the power of carrying malaria, or with the class of breeding place or the habits of the mosquitoes. In one genus, *Myzomyia*, are included the harmless *M. rossi* and the harmful *M. funesta*, the first essentially a foul or stagnant water breeder, the second breeding in fresh water, preferably in streams.

In another genus, *Anopheles*, are included *A. maculipennis*, which hibernates through an English winter, whilst the larvæ are killed by cold, and *A. bifurcatus*, which is killed by cold, though the larvæ can survive an English winter.

ANOPHELINÆ.

Of the 21 genera, ten are represented by one species only; one, *Stethomyia*, is represented by four species, including *Stethomyia fragilis* (*Anopheles treacherii*). This genus is very similar to *Anopheles*, in that the scales on the wings are all of the same colour and that the mosquitoes have a peculiarly "bald" appearance. The flat scales on the head on which the separation is now based are very scanty. The mammilation of the prothoracic lobes, which was formerly described as the characteristic, is not present in all species. There seems little good reason for separating this group from *Anopheles*.

The ten genera, represented each by a single species,

and in some cases by a single specimen, are separated off on various grounds. They are :—

Feltinella, in which the basal lobe of the male genitalia is divided into two segments. One species, *F. pallidapalpi*.

Chrystya, similar to *Myzorhynchus*, but separated by the possession of long lateral tufts of hairlike scales on the abdomen.

Lophoscelomyia, resembles *Nyssorhynchus*, but differs in that there are long tufts of scales on the femora of the hind legs. Bamboo breeder.

Kerteszia. — Intermediate between *Myzorhynchus* and *Cellia*.

Bironella.—Male only known.

Chagasia.—Antennæ of female have whorls of scales as well as hairs, and dense outstanding scales at the side of the thorax.

Aldrichia.—Thorax and abdomen scaled as in *Culex*. One specimen.

Neomyzomyia.—Is allied to *Myzomyia* and *Pyretophorus*, but is at once told by the dense tufts of scales at the posterior angles of the head and on the prothoracic lobes. One species known, *N. elegans*.

Calvertina.—Closely allied to *Chagasia*. *Antennæ* with outstanding scales on the second segment, more appressed ones on the first. At least one segment of the abdomen with long, flat, more or less spatulate scales. One specimen known.

Manguinhosia.—Thorax with narrow hair-like curved scales, and some of them broad straight scales, others spatulate on the sides. Abdomen covered with fine hairs, except the three last segments, which are covered with scales. Tufts of scales on hind femora. Wing scales lanceolate.

The more important of the groups or genera of *Anophelinæ* are *Anopheles*, *Myzomyia*, *Cycloleppteron*, *Stethomyia*, *Pyretophorus*, *Myzorhynchus*, *Nyssorhynchus*, *Cellia*.

1. Thorax and abdomen with hair-like curved scales.
   (*a*) Only upright fork scales on head, wing scales lanceolate and uniform in colour ..... *Anopheles*.

(*b*) Some flat scales as well as upright fork on head. Otherwise like *Anopheles* ..... *Stethomyia.*

(*c*) Only upright fork scales on head. Scales on wings mostly long and narrow and of two colours ..... *Myzomyia.*

(*d*) Only upright fork scales on head. Scales on wings partly large and inflated. Wing scales of two colours ..... *Cycloleppteron.*

2. Thorax with distinct narrow curved scales : abdomen hairy. Wing scales of two colours.

(*a*) Wing scales small and lanceolate : head with ordinary upright fork scales ..... *Pyretophorus.*

(*b*) Wing scales broad, lanceolate : head with broad scales, not closely appressed, but not forked or fimbriated ..... *Myzorhynchella.*

3. Thorax with hair like curved scales. Wing scales of two colours. Scales on head upright fork.

(*a*) Some narrow curved scales in front of thorax : abdomen with apical lateral scale tufts and scaly venter, no ventral tuft ..... *Arribalzagia.*

(*b*) Abdominal scales on venter only, no lateral abdominal tufts, but a distinct ventral apical tuft. Palps in ♀ densely scaled ..... *Myzorhynchus*

(4) Thorax and abdomen with scales. Wing scales of two colours. Upright fork scales on head.

(*a*) Thoracic scales narrow curved to spindle-shaped : abdominal scales as lateral tufts and small dorsal patches of flat scales ..... *Nyssorhynchus.*

(*b*) Abdomen nearly completely covered with irregular scales and lateral tufts..... *Cellia.*

(*c*) Similar to *Cellia* but no lateral scale tufts ... *Neocellia.*

Some authorities object to such subdivision and it has been urged that as in any one country the number is comparatively small a complete description of the species found in each country would suffice for practical purposes, but with this argument we do not agree.

On the other hand, some of Theobald's points are very difficult to make out, and this is particularly so as regards the wing scales. Some groups are easily separated, and between *Anopheles* on the one hand and *Cellia* on the other the differences are very marked. A division into four to six genera would probably suffice.

Genus *Anopheles*.—Thorax and abdomen clothed with hairs only; the palpi in the female are thin, not densely scaled, and generally unbanded. Wing veins covered with long lanceolate scales, which may or may not form spots. These spots, if present, are never so numerous as in other genera, and are not formed by different colouring of the scales. In the female a single spermatheca only is present.

These mosquitoes may be said to be characterized by their extreme baldness. They are of comparatively large size.

The genus includes two well-known carriers of the parasites of malaria in sub-tropical and temperate climates, *A. maculipennis* and *A. bifurcatus*.

Anopheles maculipennis, the type of the genus, is easily recognized. It is a yellowish-brown mosquito; neither legs, proboscis, nor palps are banded. Four black spots on the wing formed by accumulation of scales. It does not assume the Anopheline position as markedly as most Anophelines.

This mosquito is widely distributed in Europe, from the borders of the Mediterranean to Scandinavia. It is found also in Algeria, Palestine, the United States and Canada. In England it is common; according to Nuttall its distribution in England agrees to some extent with the old malarious districts.

A. maculipennis is the most active propagator of malaria in Europe, Algeria, Tunis, and the United States. It has been shown to be capable of serving as a definitive host for all forms of the malaria parasite. It is also an intermediate host of *Filaria immitis*.

In the winter in temperate climates the larvæ die, but the imago can hibernate all through an English winter.

Anopheles bifurcatus is easily distinguished from the preceding species by having no spots on the wings. Its distribution corresponds in a general way with that of *A. maculipennis*, but is a less common species and does not frequent houses.

This mosquito is also an active carrier of malaria, and

has been shown to be more easily infected experimentally than even *A. maculipennis*. In an English winter the adult forms are killed, but the larvæ remain alive even if the water be frozen throughout the winter. *Stethomyia*, with a limited number of genera, would be included in Anopheles.

Genus *Myzomyia*.—Thorax and abdomen with hair-like curved scales. The wings are spotted, and have mostly long, thin, or narrow lanceolate lateral vein scales. They are usually small or moderate-sized mosquitoes. Head has only a few upright forked scales.

Myzomyia rossii is distinguished by the ornamentation of the wings. Along the costa are four large patches of dark brown or black scales. The large middle spot has a small dark spot below it in the centre, giving it a T-shaped appearance.

This mosquito is the "large dappled-winged mosquito" which Major Ross, in his work in 1899, in Calcutta, failed to infect with malaria. It is a common mosquito in various parts of India and the East, chiefly in the neighbourhood of towns. It breeds in muddy pools or shallow tanks, even in cesspools.

This mosquito has never been found infected with the parasites of human malaria in nature. It may serve as an intermediate host for *Filaria bancrofti*.

Myzomyia funesta.—A small, rather dark mosquito. The black costa is marked by six pale spots; there is always a pale costal spot near the base. The black scales upon the wing veins are also interrupted by white spots. There are pale spots on the fringe of the wings at the points of insertion of the longitudinal veins.

This mosquito is widely distributed in Central and West Africa, and is an important carrier of the parasites of human malaria in those regions. It frequents houses, but does not leave them in the daytime, hiding in dark corners high up out of the reach of the breeze from doors and windows. It feeds in the early hours of the evening by preference, but at other times as well.

Myzomyia culicificacies.—Has unbanded legs, and the

largest light costal area near the base of the wing. There are only three light areas on the fringe of the wings. It appears to be a carrier of malaria in India. It assumes the position of a culex when at rest.

Pyretophorus.—Thorax with narrow curved scales, not hairlike as in *Myzomyia.* Abdomen with hairs, and a few scales on the genital lobes. Wings with small, short, lanceolate or narrowish scales, much spotted as a rule. Legs banded, sometimes spotted. The differences from *Myzomyia* are not obvious.

Pyretophorus costalis.—Told by the curious mottled character of the femora and tibiæ. The wings may be said to be white spotted with black, in contrast to *M. funesta,* which are black spotted with white.

This species is also widely distributed over the whole of Central Africa and the West Coast. The larvæ are found in abundance during the rainy season in puddles in West Coast towns. It is the most common Anopheline on the sea-coast.

Like *A. maculipennis* it serves as a definitive host for the parasites of all forms of human malaria. It also is an occasional intermediate host of *F. bancrofti.*

Genus *Myzorhynchus.*—Thorax with hair-like scales. Abdomen with ventral scales and a ventral apical tuft of black scales. Wing scales moderately broad and lanceolate. Palpi and proboscis densely scaled. In some species this scaling is so thick that the proboscis and palps appear to be very thick as seen with the naked eye.

Myzorhynchus sinensis.—A brownish mosquito. Thorax, slaty-grey background with purplish-brown longitudinal stripes, adorned more or less with pale golden scales. Found in China, Formosa, Malay Archipelago, &c.

Myzorhynchus barbirostris.—In this species the palpi are densely covered with deep black scales. The prothoracic lobes have dense tufts of large black scales projecting forwards. Found in Malaya and Old Calabar.

Both these species can carry filaria. They are difficult to infect with parasites of malaria, and are probably not

important carriers, as they may be numerous in towns where malaria is not prevalent.

Genus *Nyssorhynchus*.—Thorax with narrow curved and spindle-shaped scales. Abdomen with flat ventral scales, and sometimes latero-dorsal patches. Wing scales bluntly lanceolate. (The legs are always banded or spotted with white).

N. fuliginosus.—Probably the "small dapple-winged mosquito" of Ross.

Genus *Cellia*.—Thorax with flat spindle-shaped scales. Abdomen scaled, the scales irregularly disposed on the dorsum and on the venter. Two dense bifid tufts on the ventral aspect of each segment. Palpi densely scaled. Wings covered with large bluntly lanceolate scales.

Cellia pharœnsis is found in Africa, *C. kochii* in Asia, *C. argyrotarsis* and *C. albimana* in the West Indies and South America.

These mosquitoes may breed in fairly dirty water. *C. kochii* is commonly found in outlying villages or suburbs of towns. They are night biters as a rule.

C. argyrotarsis is one of the carriers of malaria in Tropical America, but according to Darling, *C. albimana* is of more importance. It differs from *C. argyrotarsis* in that the last tarsal joint of the hind legs is not completely white. *C. kochii* probably acts as a carrier in the Malay Peninsula and Archipelago. These mosquitoes are common in the most malarial settlements in these countries.

CULICINA.

Genera of the sub-family *Culicina*.—This sub-family includes a large number of mosquitoes. Numerous genera* have been created by Mr. Theobald with a view to simplifying the identification. It is not proposed to deal here with all of these genera, but only such as are known to be of importance from the medical point of view. A table is appended, but those who wish to go into

* Theobald now makes eighty-three genera of Culicina.

the matter more fully should consult Theobald's monograph.

The type, *Culex pipiens*, has narrow curved scales on the head and scutellum. On the head there are also upright fork-scales at the back, and flat tile-shaped scales at the sides (see diagram, p. 210). The lateral scales on the veins of the wings are long and narrow, those running along the vein are shorter and broader.

1. Certain genera can be told at once by the characters of the scales on the wing, though in other respects they may resemble the genus *Culex*.
   (*a*) Wing scales broad and asymmetrical (see fig. 101, p. 210) ..... *Mansonia.*
   (*b*) Wing scales broad-ended or pyriform, symmetrical, and often parti-coloured (see fig. 101, p. 210) ..... *Mucidus.*
   (*c*) Wing scales thick and elongated, ending either diagonally or convexly, more or less bluntly pointed..... *Tæniorhynchus.*
2. Other important genera are characterised by having the head and scutellum entirely covered with flat, square-ended scales arranged like tiles on a roof (see fig. 97, 1).
   (*a*) Palpi of ♀ short, of ♂ thickened apically and tufted..... *Stegomyia.*
   (*b*) Palpi of ♀ longer than in *Stegomyia*, of ♂ long, thin, acuminate and without tufts ..... *Desvoidea.*
3. Other genera have head and scutellar scales of the *Culex* type, but are differentiated on various grounds.
   (*a*) Wing scales long, narrowly lanceolate, and collected in spots; palpi clubbed in the ♂ *Theobaldia.*
   (*b*) Wing scales at apex of veins dense and rather broad; femora swollen. Small dark mosquitoes ..... *Melanconion.*
   (*c*) Wings with short, thick, median scales, and short, broad lateral ones on some of the veins; scales mottled ..... *Grabhamia.*
4. Peculiarly twisted scales arranged in whorls on the sixth, seventh, eighth, and sometimes ninth joints of the antennæ in the males. The females show no similar scaling. Fairly common mosquitoes throughout Malaya and in Brazil ..... *Lophoceratomyia.*

For purely diagnostic purposes Theobald arranges the genera somewhat differently. The table subjoined is based on this arrangement.

A. Legs more or less densely scaled.
- (*a*) Head not entirely clothed with flat scales.
  - 1. All the legs densely scaled.
    - Wings with large pyriform scales ... *Mucidus.*
    - Wings with narrow scales ..... *Psorophora.*
  - 2. Hind legs only densely scaled ..... *Janthinosoma.*

B. Legs uniformly scaled with flat scales.
- (*a*) Head and scutellar scales all flat and broad.
  - 1. Palpi of ♀ short, of ♂ thickened apically and tufted ..... *Stegomyia.*
  - 2. Palpi of ♀ longer than in *Stegomyia*, and in ♂ thin, acuminate, simple... *Desvoidea.*
- (*b*) Head scales mostly flat, but a median line of narrow curved ones; scutellar scales flat on mid-lobe, narrow curved on lateral lobes; ♂ palpi longer than proboscis ... *Macleaya.*
- (*c*) Head scales mostly flat, irregular, narrow curved ones behind; mid-lobe scutellum with flat scales, lateral lobes with narrow curved; ♂ palpi shorter than proboscis *Catageiomyia.*
- (*d* Head scales mostly flat, but a few narrow curved ones in middle in front; scutellar scales all flat ..... *Scutomyia.*
- (*e*) Head with flat scales, except a small median area of narrow curved ones; scutellar scales all narrow curved ..... *Howardina.*
- (*f* Head with all flat scales, except a thin line of narrow curved ones behind; scutellar scales all narrow curved ..... *Danielsia.*
- (*g*) Head with small flat scales, over most of surface, with median line and line around eyes of narrow curved ones; scutellar scales bluntly spindle or club-shaped..... *Hulecœteomyia.*
- (*h*) Head and scutellar scales narrow curved.
  - 1. Wing scales long, narrowly lanceolate, collected in spots; palpi clubbed in ♂, five-jointed and rather long in ♀ *Theobaldia.*
  - 2. Wing scales (lateral) long and narrow; palpi in ♂ not clubbed, or hairy, in ♀ three-jointed ..... *Culex.*

3. Wing scales at apex of veins dense and rather broad; femora swollen. Small dark species ..... *Melanoconion.*
4. Wings with short, thick median scales and short, broadish lateral ones on some of the veins; scales mottled; fork-cells rather short ..... *Grabhamia.*
5. Wings with dense, broadish, elongated, truncated scales ..... *Tæniorhynchus.*
6. Wings with broad, short, asymmetrical scales ..... *Mansonia.*

(*i*) Head covered with rather broad, flat, spindle-shaped scales; scutellum with small flat-scales to mid-lobe..... *Gilesia.*

(*j*) Head clothed with flat, irregularly disposed scales all over, with patches of narrow curved ones; ♂ palpi clubbed ... *Acartomyia.*

(*k*) Abdomen with projecting, flat lateral scales with deeply dentate apices; wings not ornamented ..... *Lasioconops.*

(*l*) Wings ornamented; scutellum with flat and narrow curved scales ..... *Finlaya.*

(*m*) Head flattened laterally. Palps in female nearly half the length of the proboscis. Spindle-shaped scales round the eyes, otherwise scaled as in *Stegomyia.* Common jungle mosquitoes in Malaya ... *Leicesteria.*

Genus *Culex.*—Head with narrow curved and upright fork-scales only on the occiput and flat tile-like scales at the sides. Narrow curved or spindle-shaped scales on the scutellum. On the wing veins short truncated median scales and long, thin lateral ones.

The genus *Culex* has been of late much subdivided, but is still a very difficult one and includes a large number of species that rather closely resemble each other.

The type mosquito of the genus *Culex pipiens* is a common English variety and is widely distributed in the temperate regions, and may be met with in houses at any time of the year. The females hibernate in cellars and outhouses.

Culex fatigans.—Abdomen dusky black with basal pure white bands and basal white lateral spots; pleuræ and metanotum chestnut-brown, thorax with two dark parallel lines.

This mosquito is found everywhere in tropical and sub-tropical countries. It is a domestic species and passes its life in the vicinity of houses.

It is the chief carrier of *Filaria nocturna*, and also serves as the definitive host of *Proteosoma* of birds.

Genus *Stegomyia.*—For the most part black and white mosquitoes. Head completely covered with broad, flat scales, some upright forked scales (fig. 97). Mesothorax with narrow curved or spindle-shaped scales. Scutellum always with broad flat scales on the middle lobe, and usually with similar scales on the lateral lobes. Abdomen completely covered with flat scales, banded or unbanded, with white spots on the lateral aspect. Wings similar to *Culex*; fork-cells shorter. Eggs laid singly. Larvæ with short, broad respiratory siphon.

Mosquitoes of this genus have a wide distribution in the tropical zone. They are all hardy mosquitoes, *Stegomyia calopus* (*S. fasciata*) being especially so.

Stegomyia calopus (*S. fasciata*) is distinguished by the marking on the thorax, which has a curved silvery line on each side, and two dull yellow narrow parallel ones in the middle. This marking is, however, subject to some variation.

This species is widely distributed in the Tropics, and is also found in temperate climates, owing probably to the ease with which it may be carried in ships as ova, larvæ, or adults. It is the carrier of Yellow Fever and therefore most important.

Stegomyia scutellaris is another member of this genus which is very common in some districts: it is important to distinguish it from *S. calopus*. It is easily recognized by the presence of a single broad white band down the centre of the thorax. Abdomen and legs banded black and white in both species.

This species is widely distributed in Asia and is a severe biter. It is common in the jungle and may be found where there are no human habitations, but is also very common in small and large settlements, and may infest houses and breed in similar places to *S. fasciata*.

Genus *Mansonia*.—Head clothed with narrow curved scales and numerous long upright forked scales. Thorax and scutellum with narrow curved scales and many hairs. Wings densely scaled with characteristic broad, asymmetrical scales (fig. 101, 4). Two spermathecæ in the female. Eggs are in the form of a bottle with an elongated neck.

This genus includes several species commonly found in the Tropics. No representative of the genus has yet been found in Europe.

The wing scales are characteristic, but in the genus *Ædeomyia* scales of very similar appearance are found. It is of importance, therefore, that the sub-family should be accurately determined, and in case of doubt both male and female should be examined.

Members of this genus are found in the neighbourhood of marshes and along the course of rivers and streams with sedge-grown banks and edges.

One species, *M. uniformis*, a brownish variety, is the common carrier of filariasis on the Zambesi and in parts of Central Africa. *M. annulipes* also can carry filaria.

M. annulipes is a banded black and white mosquito having a superficial resemblance to a Stegomyia. Examination with a hand lens will show the characteristic marking of the thorax, three whitish spots on the front margin of the thorax and three others about the middle.

The colours are not those of a Stegomyia, as the dark is a dark brown, and the white is not the silvery white of *Stegomyia*. All the *Mansonia* are specially liable to be infected with larval ticks.

Genera of Sub-family *Ædinæ* :—

Theobald now makes nine genera in this sub-family, of which only the more important are here given.

A. Antennæ plumose in male. Head clothed with narrow curved and flat scales. Middle lobe of scutellum with six border bristles. Scutellum with narrow curved scales, palpi in ♀ four-jointed, in ♂ two-jointed ..... *Ædes.*

B. Head clothed with flat scales only.

(*a*) Fork cells normal length.

(1) Mid-lobe of scutellum with four border bristles: palpi of ♀ two-jointed. Small dark species ..... *Verallina.*

Palpi of ♀ five-jointed, metallic ..... *Hæmogogus.*

(2) Mid-lobe of scutellum with six border bristles: palpi of ♀ three-jointed ..... *Skusea.*

(*b*) Fork cells small.

Scutellar scales narrow curved, wings with Mansonia-like scales ..... *Ædeomyia.*

Genera of Sub-family *Limatinæ*:—

Only one genus, viz., *Limatus*, occurs in this sub-family.

Head, thorax, and scutellum with flat scales. Metanotum with both hairs and scales. Wings with broad, elongated conical scales. Proboscis in ♂ elbowed with two scaly tufts..... *Limatus.*

Genera of Sub-family *Deinoceratinæ*:—

Second segment of antennæ very long. Palpi short in both sexes. Both ♂ and ♀ antennæ pilose.

(1) Eyes well separated: clypeus with bristles ..... *Deinocerites.*

(2) Eyes contiguous, clypeus without bristles, second joint of antennæ very long indeed. ..... *Dinomimetes.*

Genera of Sub-family *Uranotæninæ*:—

A. First fork cell very small.

(1) Clypeus nude.

(*a*) Male ungues normal. Wings with broad lanceolate lateral scales: no inflated ones ..... *Uranotænia.*

Wings with some inflated vein scales ..... *Pseudouranotænia.*

(*b*) Male ungues broad and plate-like *Anisocheleomyia.*

(2) Clypeus with long, dense scales ..... *Squamomyia.*

B. Fork cells moderate size.

(1) Proboscis normal.

(*a*) First fork cell normal : nearly as large as the second fork cell ..... *Mimomyia.*

(*b*) First fork cell expanded basally ... *Pseudograbhamia.*

(2) Proboscis swollen apically, elbowed with complex arrangement of hairs, clypeus elongate, almost covering palpi ..... *Harpagomyia.*

(3) Proboscis and clypeus normal : lateral vein scales forked apically..... *Hodgesia.*

C. Fork cells short but first longer than second. Proboscis normal, like *Uranotænia :* no flat thoracic scales ..... *Ficalbia.*

Genera of Sub-family *Dendromyinæ :—*

A. Legs with paddle-like structures ..... *Sabethes.*

B. Legs without paddle-like structures.

(1) Lateral vein scales linear. Proboscis longer than body ..... *Phoniomyia.*

Proboscis shorter than body, swollen at apex ..... *Wyeomyia.*

Metanotum with white scales ..... *Menolepis.*

(2) Long, dense lateral vein scales on fifth vein *Bolbodeomyia.*

(3) Lateral vein scales obovate or spatulate.

Proboscis fine at apex, same length as abdomen : posterior and mid-cross veins in one line ..... *Sabethoides.*

Proboscis short, apex swollen : posterior cross vein slightly nearer base than mid. Scales of mesonotum very brilliant. Metathorax with scales : proboscis same in ♂ and ♀ *Sabethinus.*

Scales of metanotum dusky metallic, clypeus without scales ..... *Dendromyia.*

Clypeus with scales ..... *Prosopolepis.*

Metanotum nude. Resembling *Dendromyia*, but head with narrow curved scales in middle and scutellum with narrow curved scales..... *Philodendromyia.*

Head scales flat except a row of narrow curved ones behind : scutellum with flat scales. Culex venation and scales : apex of abdomen bristly ... *Polylepidomyia.*

These sub-families include a large number of genera and species which differ very markedly from each other as well as from the *Culicinæ*. Few of them frequent towns or houses, and many of them are bush, jungle, or swamp mosquitoes. Some of them attack man in their free state, and many bite only or mainly in the daytime. They have been less studied than *Culicinæ* and *Anophelinæ*, and are not known to carry disease. The frequency with which malaria is acquired in some jungle districts where Anophelines are not abundant suggests the possibility that some of these mosquitoes may act as carriers of malaria. Experimental evidence is wanting and is difficult to obtain, as most of these mosquitoes will not feed in captivity or when bred from larvæ, and many of them die rapidly in captivity. Some species are so susceptible to a dry atmosphere that they will die in a few hours after their removal from the jungle.

In nature they rarely leave the jungle in which they live. This is very marked with some species, so much so that standing at the edge of a clearing, one hand thrust into the jungle will be covered with mosquitoes, whilst the other hand in the open will not be attacked at all.

The eggs of only a few species of these mosquitoes are known. Frequently they form raft-like masses, differing from those of *Culex fatigans* in that they are less compact.

The known larvæ all have a respiratory siphon, which may be very short and broad, and resemble *Stegomyia* larvæ, or may be very long and thin. In some instances the respiratory siphons are profusely ornamented with simple or compound hairs. The abdominal and thoracic segments may be similarly adorned.

The larvæ of some genera lie nearly horizontally at the surface of the water when at rest, and are frequently mistaken for Anopheline larvæ if the respiratory siphon is not observed.

Many of these mosquitoes have very special breeding places, such as natural collections of water in various plants, pitcher plants, &c., the water collected in the

joints of growing bamboos which have been perforated by coleopterous and other larvæ, or in the axils of leaves. Others will breed freely in swamps, puddles, streams, or at the edges of rivers.

The synopsis should enable the reader to distinguish the chief genera common in any part of the world, but is far from being complete. For full information the reader is advised to consult the "Monograph of the *Culicidæ*," Theobald, vols. iii., iv. and v. For distinction of species, the size, colouring, and particularly the markings on the legs, thorax and wings, and slight modifications in the arrangement of the cross-veins of the wings, become important. With the *Anophelinæ* the markings on the posterior pair of legs are in some instances sufficient for the identification of species.

The reader is warned not to be alarmed at the apparent magnitude of the subject. It is true that this table does not give all the genera, but, on the other hand, many of the genera are of limited distribution, and in few places will there be more than some thirty common species of mosquitoes, which can readily be subdivided into their respective genera; and often the species can be easily identified by reference to the standard books—if not they should be forwarded to England to one of the schools of Tropical Medicine for identification. For the distinction of species the amateur will often be much helped by examination of the eggs or larvæ, as these sometimes show more obvious differences than the adults.

For examination of the external characters of mosquitoes the method of mounting on entomological pins is in every way preferable. They may be mounted in Canada balsam, but this method has some disadvantages, as the scales are rendered too transparent, and the non-scaled veins, particularly the cross-veins, are difficult to make out. Theobald mounts wings and other parts *separately* in Canada balsam.

To mount in Canada balsam the insect should be placed on its back with the legs separated and the wings

spread out. A small drop of thick Canada balsam is placed on a slide. The slide is then held in the hand so that the drop of Canada balsam is on the under surface, and this is gently pressed against the thorax of the mosquito, and the mosquito adheres to it on lifting it. The slide should then be turned over so that the mosquito rests on it. The wings and legs should be arranged to taste, and a drop more of fluid Canada balsam placed on the mosquito; as this flows over the mosquito it will cause the head appendages to spread out. A mosquito so arranged will keep indefinitely. To complete the process a glass ring should be cemented round the mosquito, and when firmly set the cell so formed should be filled with balsam and a cover-glass placed over it.

Mounted in glycerine jelly the mosquito retains its natural colouring. The best method of mounting in glycerine jelly is to make a deep cell with a glass ring and slide. Lay the mosquito at the bottom and fill the cell with the jelly previously melted by placing in hot water. Place a cover-glass over the jelly, taking care to avoid air bubbles. It is very difficult to arrange a mosquito in the jelly. Specimens mounted in this manner often have their legs interlaced. It is a useful method for mosquitoes which have been kept in spirit, as such mosquitoes are too brittle to stand any handling and cannot be pinned out.

Specimens so mounted should be ringed with some varnish as soon as the jelly is set, otherwise evaporation will take place and the jelly shrink, resulting in the formation of air bubbles. If this accident should happen the cover-glass must be removed from the jelly and fresh glycerine jelly added. This is easily done by dissolving off the cement and gently warming to melt the jelly. The cover-glass can then be removed and fresh jelly melted and added. The cover-glass is to be replaced, and when the jelly has set cemented on.

Another method of mounting mosquitoes, known as the Bentley-Taylor method, is as follows:—

Prepare (1) a solution of 1 per cent. celloidin in absolute alcohol; and (2) a solution of celloidin in absolute alcohol of the consistence known as "thick" in ordinary histological work.

Catch the mosquito, chloroform it, and when dead or narcotised, place a drop of solution 1—thin celloidin—on a cover-glass. Place the insect back downwards on the cover-glass. In the majority of cases wings and legs spread themselves out in the orthodox exhibition position, but if not they may be adjusted with a needle.

When the thin solution has become "tacky," *i.e.*, in about eight or ten minutes, place a drop of the thick solution, No. 2, over the insect. Invert the cover-glass over a hollow slide to which it may be fixed by a ring of balsam. The specimen is then complete and in that condition both ventral and dorsal surfaces can be examined.

CHAPTER XII.

Dissection of Mosquitoes.

The internal anatomy of the mosquito is not very complicated, and the more important parts are easily dissected out.

The alimentary canal is a tube with dilatations running from the proboscis to the anus, which is terminal. The proboscis is suctorial and piercing and composed of the

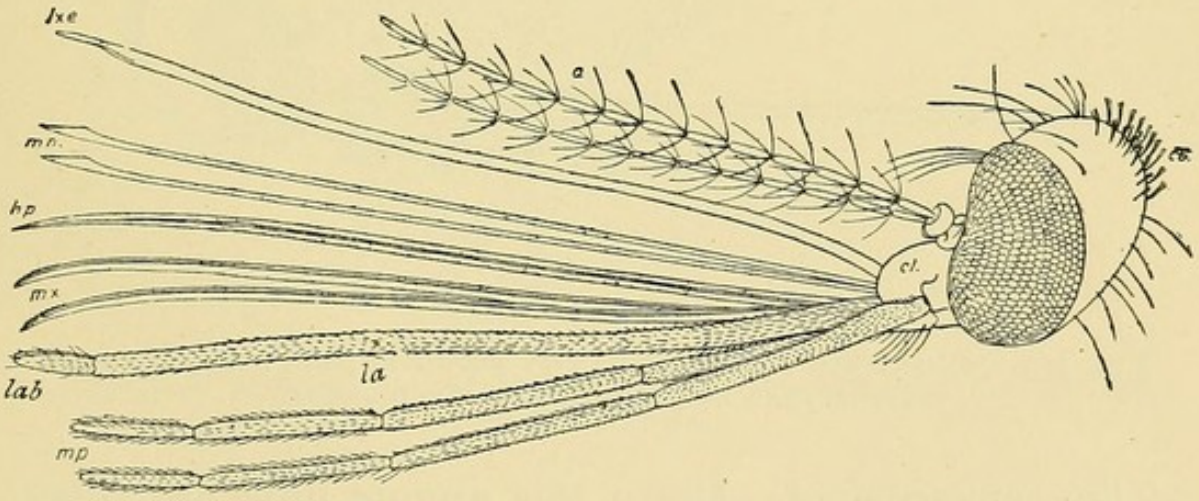


Fig. 104.—*a*, Antennæ; *cl*, clypeus; *lxe*, labrum-epipharynx; *mn*, mandibles; *hp*, hypopharynx; *mx*, maxillæ; *la*, labium; *mp*, maxillary palps; *lab*, labella. (After Nuttall.)

following parts (fig. 104): (1) Below is a deeply-grooved, fleshy *labium*; this contains air tubes, is covered with scales, and forms what appears to be the proboscis, as when the mosquito is at rest the other elements are contained in the groove. It terminates in two small jointed lobes—the *labellæ*. (2) Above is the *labrum*, or more correctly, the *labrum-epipharynx*, as this part results from the fusion of the labrum and epipharynx. The labrum

is deeply grooved on the under surface and terminates in a sharp point. (3) This groove is, by the apposition of the *hypopharynx*, converted into a tube, up which the food is sucked; this tube is continuous with the cavity of the pumping organ. The *hypopharynx* is a flattened chitinous rod terminating in a sharp point; it is strengthened in the middle by a ridge, and in this thickening is contained a minute tube, the termination

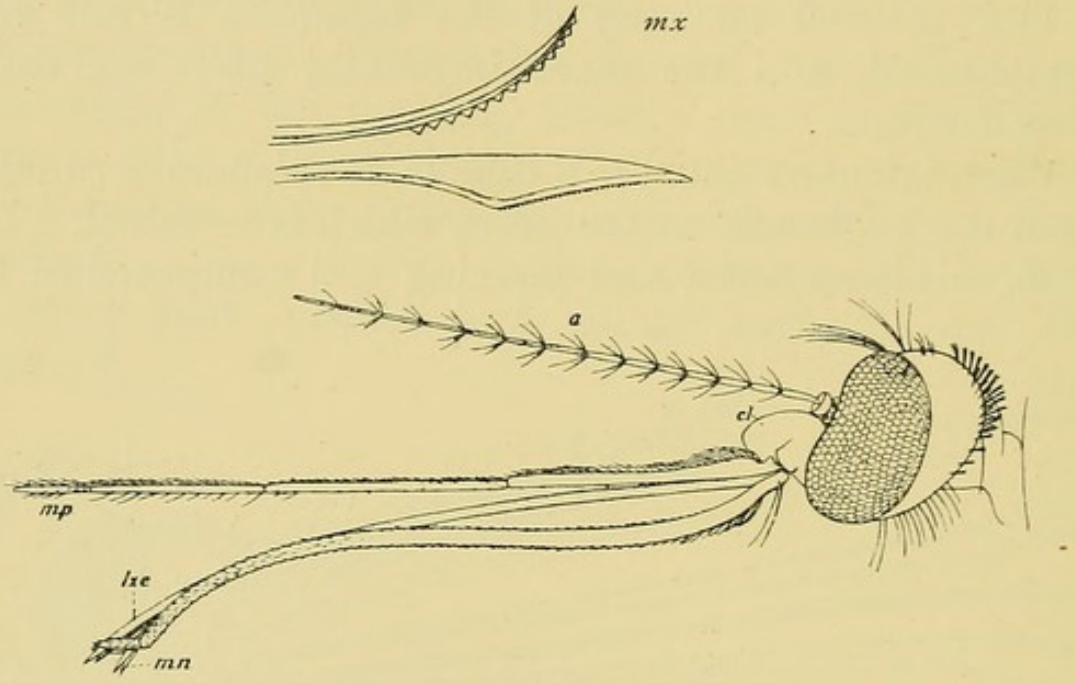


FIG. 105.—*a*, Antennæ; *cl*, clypeus; *mp*, maxillary palps; *lxe*, labrum-epipharynx; *mn*, mandibles; *mx*, maxillæ.

of the salivary duct. Down this minute tube the saliva is ejected into the depths of the wound made by the penetrating parts of the proboscis. (4) There are two pairs of piercing organs, the *mandibles* and *maxillæ*; both of these are thin strips of chitin with sharp cutting edges terminating in a lancet-like point. The cutting edge may or may not be serrated. In most species the maxillæ only have a serrated edge (fig. 105).

In the male the mandibles are well developed, but less so than in the female; the maxillæ are absent in some species; the hypopharynx is closely adherent to the labrum in its whole length.

Of these elements the labium mainly acts as a sheath and protects the more delicate parts of the proboscis from injury. It does not penetrate the skin. The tip is applied firmly to the skin, and in the angle between the two labellæ all the other elements of the proboscis are thrust into the skin (fig. 106). No doubt it aids in penetration by keeping together and rendering more rigid the other elements, and as it is supplied by nerves aids in the selection of a suitable place for puncture.

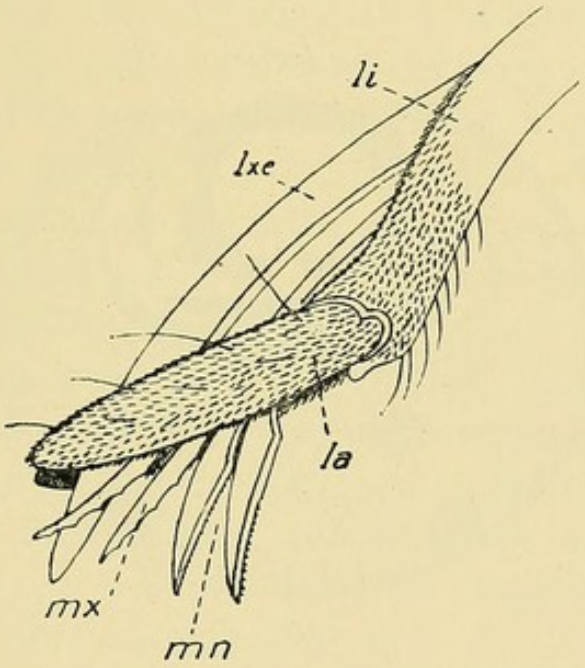


FIG. 106.—*li*, Labium ; *la*, labella ; *lxe*, labrum-epipharynx ; *mx*, maxillæ ; *mn*, mandibles.

As the other elements penetrate the skin the labium becomes bent on itself, as depicted in the diagram.

The penetrating elements form two tubes, with the mandibles and maxillæ at the sides. Up the superior tube formed by the groove of the labrum epipharynx and the flat hypopharynx the blood is sucked, whilst the saliva is ejected through the small tube in the hypopharynx (fig. 107).

The main points in the anatomy of the proboscis can be readily demonstrated. The hypopharynx often closely adheres to the labrum-epipharynx, so that it is the most difficult component to separate and identify.

To demonstrate the two tubes formed by the apposition of these elements, transverse sections of the proboscis are requisite. From the tube thus formed by the labrum-epipharynx and hypopharynx the blood is conveyed into the pumping organ, which is composed of three chitinous plates, to which muscles are attached. This in turn forces the blood into a membranous tube which is continuous with the commencement of the œsophagus. These parts also can only be satisfactorily demonstrated in sections.

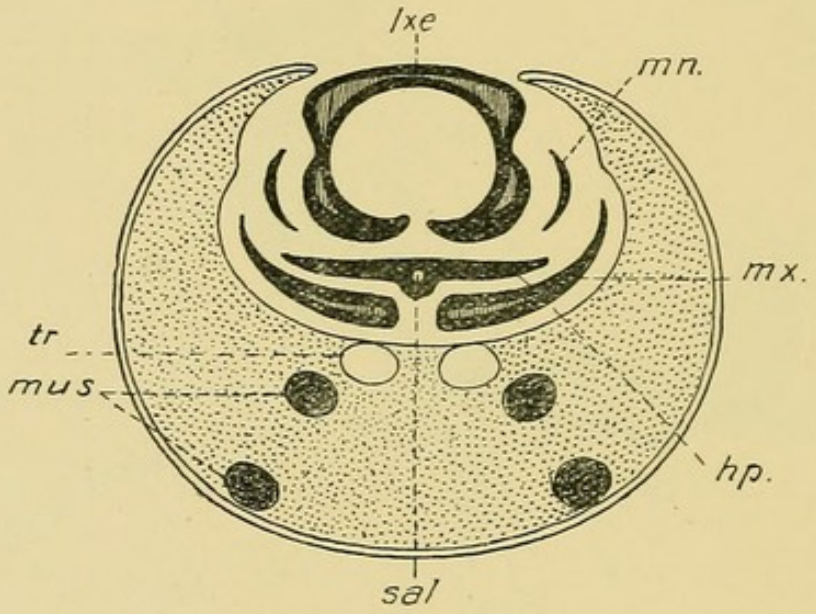


FIG. 107.—*lxe*, Labrum-epipharynx ; *mn*, mandibles ; *hp*, hypopharynx ; *sal*, salivary duct ; *tr*, trachea ; *mus*, muscle ; *mx*, maxillæ.

The rest of the alimentary canal is best shown by dissections.

Dissection.—Only freshly killed mosquitoes are suitable for dissection. They can be killed in many ways. With those required for dissection no great precaution need be taken, as it is immaterial if the scales are knocked off. They can be killed with tobacco (cigarette) smoke, chloroform vapour, or stunned by concussion.

Dissection of Alimentary Canal.—The mosquito should be caught in a test tube. This is done by placing the test tube slowly over a resting mosquito. If it is done rapidly the mosquito will take alarm and usually escape.

It is important to so approach the mosquito that no shadow falls on it. By proceeding cautiously mosquitoes are readily caught in this way, and they then fly to the closed end of the tube. With practice nine or ten mosquitoes can be caught in this manner in one tube.

Six clean slides and cover-glasses should be prepared, and on three of them a drop of normal saline solution should be placed; the other three should be left dry. Two sharp needles are also required.

After killing or stunning the mosquito it should be transfixed through the thorax with a mounted needle and the legs and wings pulled off and dropped on a clean dry slide. This can be easily done with the fingers, but there is no objection to the use of forceps. These can be examined dry by covering with a cover-glass and fixing this with gummed paper, or they can be mounted in glycerine jelly or Canada balsam.

The mosquito, denuded of its limbs, is placed in the saline solution on one of the slides. The posterior part of the abdomen is gently flattened with the shaft of a needle and two nicks made, one on each side, about the junction of the second and third last segments (fig. 108). This weakens the exoskeleton at that point so much that when traction is made on the last segment the exoskeleton breaks.

Traction is best exercised by fixing with the point of one needle the thorax and laying the other flat on the last segment and steadily and slowly dragging away from the head.

In the space between the broken ends of the exoskeleton a series of white strands will be seen—the intestine and Malpighian tubes. On further traction the stomach and part of the œsophagus will appear (fig. 109).

If the traction be continued from the end there is a risk that the stomach may break off. It is better to shift the needle from the posterior segments to the œsophagus at the point of emergence from the broken end of the abdomen, and pull slightly obliquely on this so as to drag

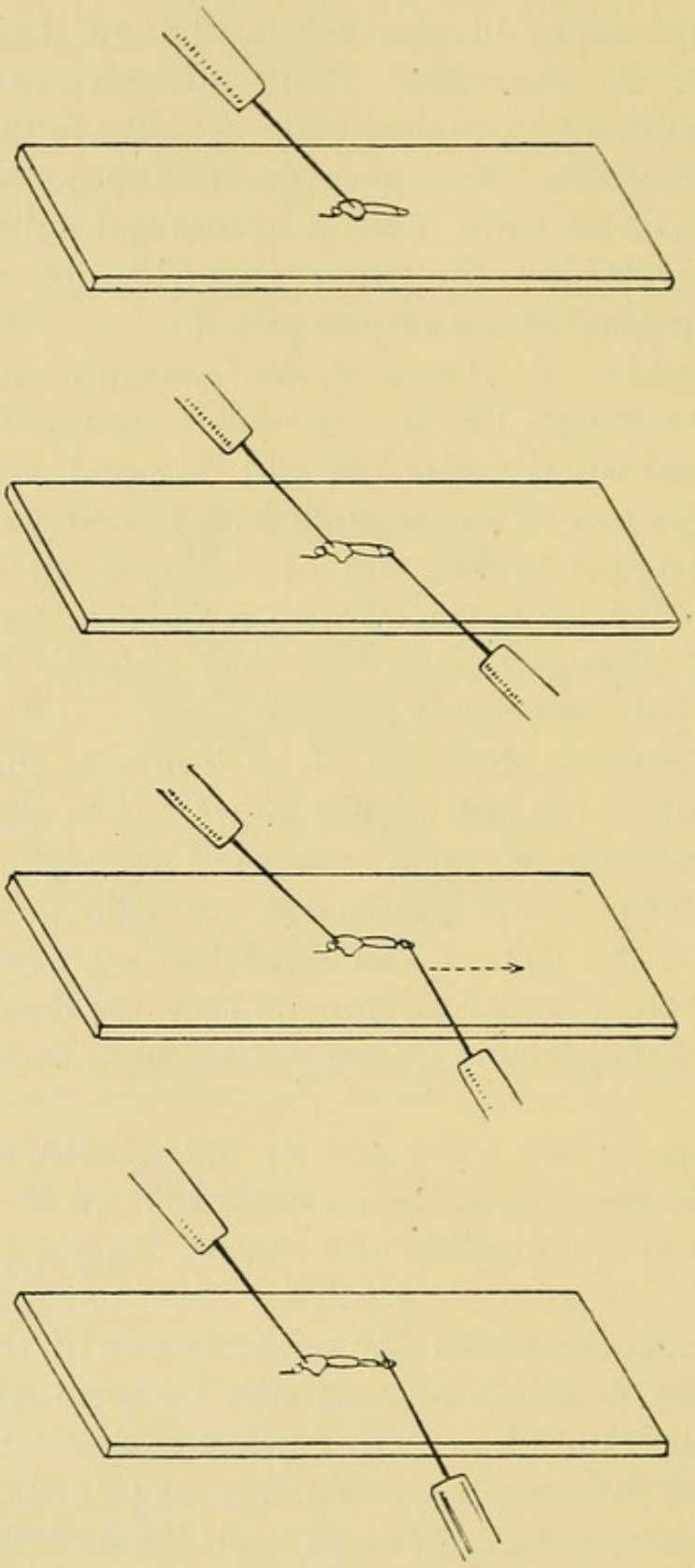


FIG. 108.

the rest of the œsophagus out of the abdomen and thorax. It should be covered with a cover-glass.

The stomach with its appendages can now be examined directly. The genital organs will be still attached to the terminal segments of the mosquito and can be examined at the same time. To show them completely it is better under the microscope to tease off the remainder of the exoskeleton of the last two segments.

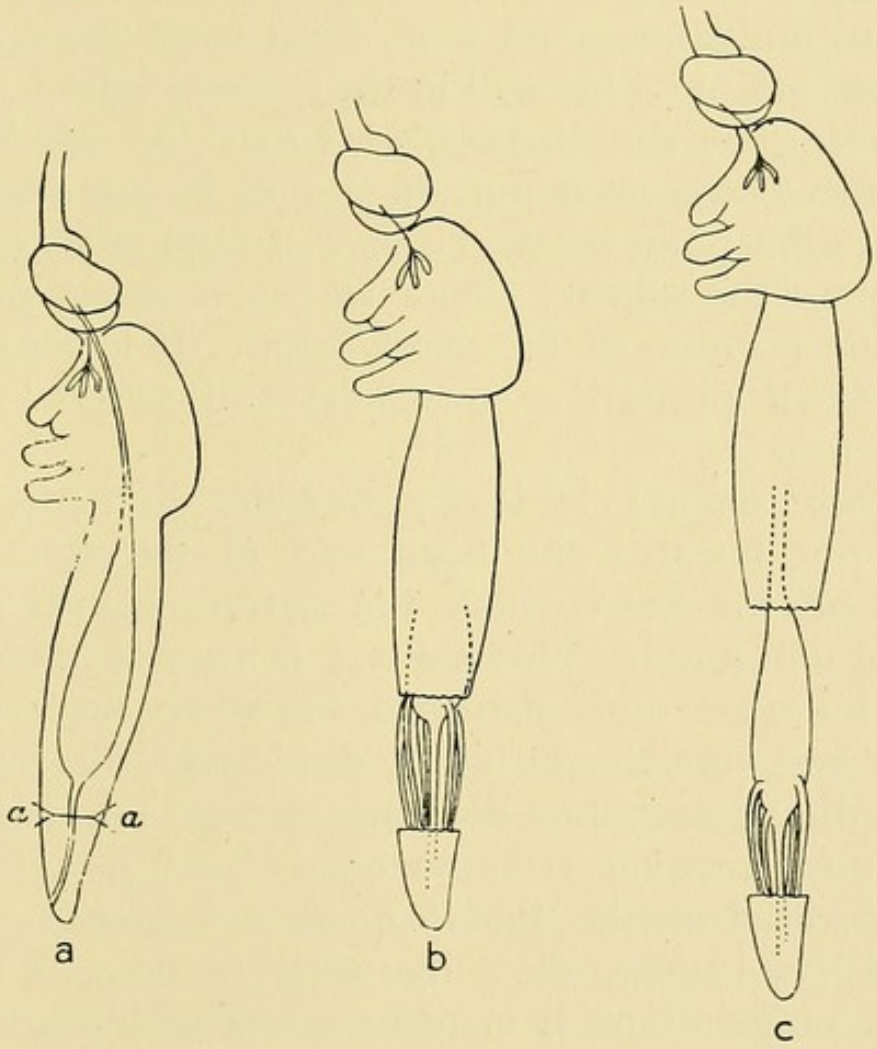


FIG. 109.

The remainder of the mosquito should be placed in the drop of saline solution on one of the other slides for the dissection of the salivary glands.

To dissect the salivary glands there are several methods. The one described here is that which is most readily learnt and by which uniform results can be obtained

fairly readily. It has the disadvantage that other tissues are present in the dissection and may conceal more or less of the lobes.

The principle is to take as small a portion of the mosquito as is possible, with the certainty that the portion contains both salivary glands.

The chitinous portion of this remnant, which includes the bases of two pairs of legs, is sufficient to conceal the glands ; it must therefore be broken up with the points of the needles into four or five fragments, which should be about a quarter of an inch from each other. These fragments are of course still in the saline solution.

A cover-glass should be placed over the whole series of fragments and each portion should be compressed in turn with the point of the needle. In the great majority of instances the salivary glands will be squeezed out from under the portions of the exoskeleton. It is common to find a small portion of one lobe still covered by the exoskeleton.

Another method is to squeeze the contents of the thorax out towards the head end of the thorax, after cutting off the head, when the salivary glands may be shot out uninjured. This method is uncertain, and some of the lobes are often damaged, but when successful the glands are sometimes better displayed and have less surrounding tissues than the other method (fig. 110).

The first method recommended here has the very decided disadvantage that the salivary glands are not isolated but surrounded by other tissues. For permanent preparations it is not a good method, as other tissues are present. The glands do not dry quickly and become fixed to the slide as isolated glands do.

For mere examination it is satisfactory, but when confidence has been acquired by this method, if permanent preparations are desired the salivary glands must be isolated.

In the best method for isolation of the glands the head is not cut off, but the back of the thorax is separated

by a longitudinal incision. A sharp edge, such as is provided by a surgical needle or cataract knife, is better than an ordinary needle. A second incision at right angles to the first is made at the level of the second pair of legs. The head is now transfixed as near the neck as possible with one needle and the remnant of the thorax fixed with another. On pulling on the head the salivary glands will be pulled out of their bed in the thorax and can be seen attached to the head. Microscopic examination under a low power objective is necessary at this stage. A final cut will separate the head, and the salivary glands are left isolated (fig. 111). It is not uncommon to find that the ends of some of the lobes have been left behind in the thorax or the glands otherwise damaged, but perfect specimens can be obtained in this way.

FIG. 110.

The excess of salt solution should be removed with blotting-paper, the specimen air-dried, fixed in alcohol, and stained on the slide.

To show the relations of these parts and other structures in the mosquito serial sections are requisite. The mosquito can be cut, embedded either in celloidin or in paraffin wax.

To show structure, young mosquitoes which have only been hatched for a few hours are best, and they should be placed alive in spirit and hardened in absolute alcohol.

With older mosquitoes it is better to puncture the thorax and abdomen with the point of a fine sharp knife or needle, so as to facilitate the entrance of the paraffin or celloidin.

To Cut and Stain Sections of Mosquitoes.—According to Dr. Low the best method is to kill the mosquitoes by dropping them into 60 per cent. alcohol alive, so that some spirit may be drawn into the interior. Keep them five days in this spirit. Remove the wings. and legs from the mosquito and place the trunk in 95 per cent. alcohol for twenty-four hours, then in absolute alcohol for twenty-four hours, then in alcohol and ether equal parts twenty-four hours. After this thin celloidin one day, thick celloidin one day. Mount on blocks,

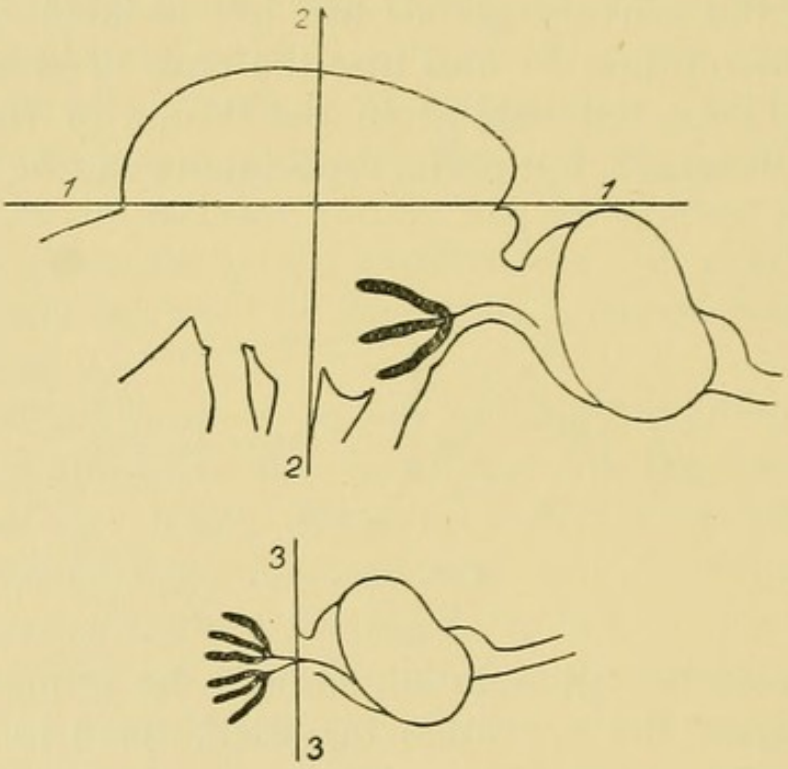


Fig. 111.

hardening the celloidin on them in 60 per cent. spirit; then cut serial sections, keeping the sections in 60 per cent. spirit. For staining float out in water. Stain in watch glasses with hæmalum or hæmatoxylin for five to ten minutes so as so overstain decidedly. Decolorise with 1 per cent. hydrochloric acid in 70 per cent. alcohol till, when replaced in water, only a faint violet colour is retained by the mosquito. Replace in 60 per cent. spirit, then in 95 per cent., and from that to carbol-xylol—25 per

cent., till the section appears perfectly clear and transparent. Transfer to slide, press firmly with clean filter paper, and mount in xylol balsam.

For mosquitoes celloidin is to be preferred, particularly for the demonstration of filaria *in situ*. For structure, good results can also be obtained with paraffin sections. For this purpose recently hatched mosquitoes are the best, and they should be placed alive in the spirit, passed through the usual processes, embedded in paraffin and serial sections cut. These small sections are easily detached from the slide. The method recommended by Annett and Dutton to prevent this is to lay the paraffin section on a thin layer of two parts of liquid glucose and one part of a thick syrup of pure dextrin, spread on a slide and kept in the hot incubator till the glucose mixture has dried hard. The paraffin is then removed by xylol and alcohol, and a solution of photoxylin is poured over the slide so as to form a film over the sections. This is allowed to set till the edges of the photoxylin film crinkle. On placing the slide in water the film comes away with the sections, which can then be stained in the usual way as recommended for celloidin sections. Carbol-xylol must be used for clearing.

In dissections the points to be observed are as follows :—

At the commencement of the œsophagus are three diverticula, of which one, the ventral, is much larger than the others. These diverticula usually contain air and sometimes food. They vary greatly in size. They are often pulled out of the thorax with the œsophagus, but to show them satisfactorily it is necessary to tear off the back of the thorax and break through the upper segments of the abdomen before exercising traction on the œsophagus. Bacteria in large numbers are found in these diverticula.

The stomach is seen as a clear translucent expansion of the œsophagus. The cells lining the intestine appear to be polygonal, and the outlines can be clearly made out by using central light only.

At the hinder end of the stomach just before the junction

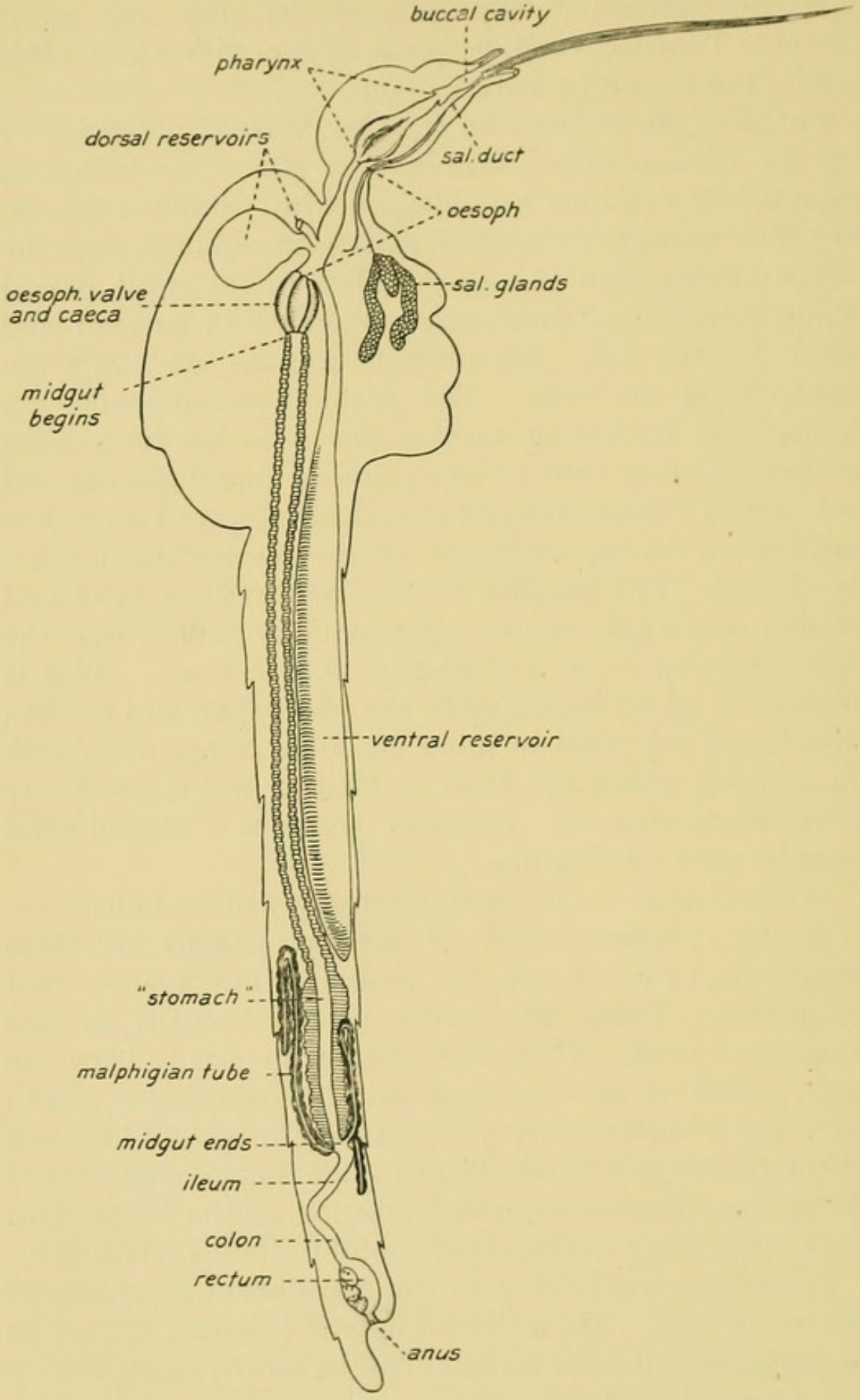


FIG. 112.—Internal Anatomy of the Mosquito.

with the hind gut, are seen the five Malpighian tubes, which are much more opaque and are lined by large nucleated cells, and these cells contain granules or droplets of a refractile oily nature. The lumen of these diverticula is difficult to make out (fig. 112).

The continuation of the intestine is a tube which is not straight—the hind gut or rectum. The cells vary in different parts of the tube and the variation differs in different species. Parasites of various kinds may be found. Examination with a high power, one-twelfth inch, is necessary, as the youngest forms of the malarial parasite cannot be readily seen with lower powers, and therefore familiarity with the normal appearance of the cells of the mosquito's stomach with this power is essential.

Some confusion may be caused by the air tubes which ramify over the surface of the stomach. These appear to be black when seen by transmitted light, on account of the air they contain, but silvery white when seen by reflected light for the same reason. They can be recognized by the spiral thickening and their repeated branching.

The cells seen in the stomach form the epithelial lining of that organ. They are detached by pressure on the stomach. By making a nick in the side of the stomach and alternately floating up the cover-glass with water and abstracting it on the other side of the cover-glass with blotting paper, the detached epithelium can be removed. By repeating this process several times the epithelial lining can not only be detached but in great part washed away. This measure may be required to wash out the contents of the stomach, particularly when they are dark and opaque with altered blood. It is also necessary for satisfactory staining of malaria parasites in the wall of the stomach.

When the epithelium is washed out the stomach is reduced to a clear transparent bag. Longitudinal and transverse markings are often seen in this, and are indications of the muscular bands.

In a stomach with the epithelium thus removed the

developed malaria parasites can be stained by running the stains under the cover-glass. Picrocarmine gives fair results. When sufficiently stained the excess of stain can be washed out in the same manner, and finally Farrant's solution run in to displace the water.

The stomach with the epithelium intact can also be stained in this manner, but more uniform staining is obtained by removing the cover-glass and allowing the stomach to dry on the slide. It can then be fixed in alcohol and stained with any basic stain, and after washing, dehydrated in alcohol, cleared with xylol, and mounted in Canada balsam. By this method the developed malarial parasites are not well shown, as they will not stand drying or dehydration without great distortion.

The salivary glands can be mounted in the same way, but in the Farrant's solution the cells wrinkle and poor results are obtained. Somewhat better results are obtained by removing extraneous tissues under the microscope and drying the slide in the air. The salivary glands can then be fixed, stained and mounted. In the fresh preparation the cells will be found to vary greatly, and they are often distended with refractile droplets. These may be so numerous as to fill many or all of the cells. The cells in the middle lobe are smaller and often differ in appearance from those in the lateral lobes. The main duct has cubical epithelium, which is continued for some distance down the lobules. In *Anopheles* the ends of the ducts in the lobules are dilated, whilst in most of the genera the ducts maintain the same calibre in their entire length. Occasionally a diverticulum is met with. This may be terminal, so that the lobule bifurcates at the end, or it may be found in any other part. In *Psorophora* each gland has five lobes.

At first there may be difficulty in finding these glands with a low power. The point to search for is the main duct and its trifurcation, as this is most readily seen even if the gland is embedded in muscular or other tissues. To see the character of the glandular cells in detail a $\frac{1}{12}$

oil immersion must be used, and the diaphragm nearly closed, as the cells are very transparent.

Sporozoa have been described in the ovaries of the mosquito, and we know that the piroplasma of cattle and dogs are transmitted by an infected tick to its offspring.

In the present state of our knowledge it is therefore advisable to study the internal genital organs of the mosquito to some extent. These are usually removed with the stomach, but in part are hidden by the exo-skeleton of the last two or three segments of the mosquito which remain still attached to the stomach. This exo-skeleton can be teased off with a pair of needles ; this can be done under a dissecting or other microscope with greater certainty of not at the same time injuring the genital organs.

The female genital organs consist of a pair of ovaries opening into a common tube by the ovarian tubes. Into this common tube opens a mucous gland and also the *spermathecæ* by a long narrow duct. The spermathecæ are chitinous sacs and store up the spermatozoa received from the male. In this way by a single act of coitus by the male sufficient spermatozoa are stored up to enable many series of eggs to be fertilized. The number of spermathecæ varies. In most genera there are three, but in the *Anophelinæ* there is only one and in *Mansonia* two.

The male genital organs consist of two testicles joined by vasa deferentia to the ejaculatory duct formed by their union. Just before this junction each vas deferens is connected by a short tube with a sac-like receptacle—the vesicula seminalis.

The ejaculatory duct leads to a short fleshy penis situated between two internal claspers, internal gonapophyses, and on each side of these are the large conspicuous external claspers.

The spermatozoa are rounded bodies with a flagellum. According to Giles they do not reach their full development in the male, but in the spermathecæ of the female.

CHAPTER XIII.

Demonstration of Development of Parasites in Mosquitoes.

In the freshly shed blood we saw that so-called sexual forms of the parasites of malaria—*gametocytes*—occurred which flagellated, and that forms differing little from these did not. However much the blood was altered by exposure to air, water, or in the mosquito's stomach, a proportion of non-flagellating bodies was always present. Both those that flagellate and those that do not are the gamete or sexual forms of the parasite. They are only easily recognized in autumno-æstival fever (sub-tertian), where they appear as the crescent bodies. It is simpler to follow the development in that species of parasite on this account, though the same changes occur in the other species of malaria.

In the shed blood the crescents rapidly undergo changes if the blood be exposed to the air or moisture be added to it. If, on the other hand, air and moisture be excluded no change occurs in the crescents till they die and break up. To exclude the air a drop of vaseline is placed on the finger-tip, and the finger is pricked through it so that a drop of blood exudes into the centre of the oil. The oil and the contained drop of blood are transferred to a slide and the whole compressed under a cover-glass. The blood can be watched indefinitely and no change will be found to occur in the crescents till they disintegrate.

If, however, a drop of blood is taken up on a cover-glass and exposed freely to air for two minutes and then

placed on a slide, flagellating forms will rapidly appear and the crescents which do not flagellate—the females—will become round.

Instead of freely exposing to air, admixture with water leads to the same result. This can be conveniently done by breathing on the slide before placing the cover and drop of blood on it.

In short, a change in the environment of the sexual forms of the parasites which does not kill them leads to transformations due to their becoming sexually active. The same changes take place in the stomach of the mosquito with greater certainty and rapidity.

To demonstrate satisfactorily these changes it is necessary to have a fairly good crescent infection. As has been already seen, the flagella from the males are actively motile, and these are the sexually active agents which enter the female and fertilize it. The process is one of conjugation, and the product is a *zygote*. At first it is an actively motile body, termed the travelling vermicule or *oökinet*. This travelling vermicule contains the pigment of the female crescent and is pointed at one end.

In the stomach of a suitable mosquito—several species of *Anophelina* for human parasites and *Culex fatigans* for proteosoma—the vermicule passes out of the stomach cavity and the zygote becomes encysted in the stomach wall. About thirty-six hours after feeding on an infected person these encysted zygotes will be found, and can be readily recognized by their pigment, which at this stage can be seen to be little changed from the pigment of the parasites from which they were derived. They are best seen in fresh specimens, but can be stained with any basic stain and seen after the epithelium has been removed from the stomach. The youngest forms are a little larger than a red blood corpuscle, but they rapidly increase in size, though at a rate varying with the temperature of the air. At about 80° F. they attain their full development in *Myzomyia funesta* in twelve days. In some species of *Anophelina*, under the most

favourable conditions, the full development may take place in eight days.

With this increase in size there is, of course, no increase in the pigment, as the zygote does not derive its nutriment from the blood. The pigment therefore is relatively scanty and absorption or solution of it must take place, as it frequently disappears completely.

When fully grown the zygotes attain the size of 50 or 60 μ. The growth of the parasites is entirely outwards into the body cavity of the mosquito and away from the *lumen* of the intestinal tube, so that when mature they appear to be globular excrescences stuck on to the stomach. The proportion of gametocytes that form zygotes varies a great deal. When several mosquitoes have fed at the same time and all apparently fed well, in some there will be no zygotes, in others two or three, whilst some may have fifty or more. Darling used blood in a case in which twenty-two crescents were present to 100 leucocytes. He found that the average increase of the mosquitoes after feeding was about ·001 grm. and as the leucocytes were 6,500 per c.mm., which gives some 1,088 gametes ingested, or as a result of three feedings if half were males and half females there should have been 1,632 zygotes. There were only 50, indicating a loss of 97 per cent. The loss appears to be mainly due to phagocytosis by polymorphonuclear leucocytes.

The contents of the zygote first divide into a series of segments called *sporoblasts* or *blastophores*. These blastophores soon lose their smooth outline and have an irregular shaggy appearance, which as they become more mature is seen to be due to the conversion of the outer part of the blastophore into a mass of filaments attached by one end to a small central residual mass. When quite mature these filaments break off and the cyst is then filled with these filaments, which are narrow bodies pointed at both ends and about 14 μ in length. These bodies are known as *sporozoites*. Zygotoblasts, blasts, exotospores, are names that have also been employed.

In the fresh state they can be seen only in specimens immersed in saline solution or in weak 1 per cent. formalin solution.

To observe them the freshly dissected stomach in one of these solutions is covered with a cover-glass, and by gently moving this cover-glass with a needle the stomach can usually be rolled over a little so that one of the mature zygotes is seen in profile projecting from the edge of the stomach (fig. 113). Pressure with a needle on the cover-glass will now cause the rupture of the capsule of the zygotes and the contents, the blasts or sporozoites

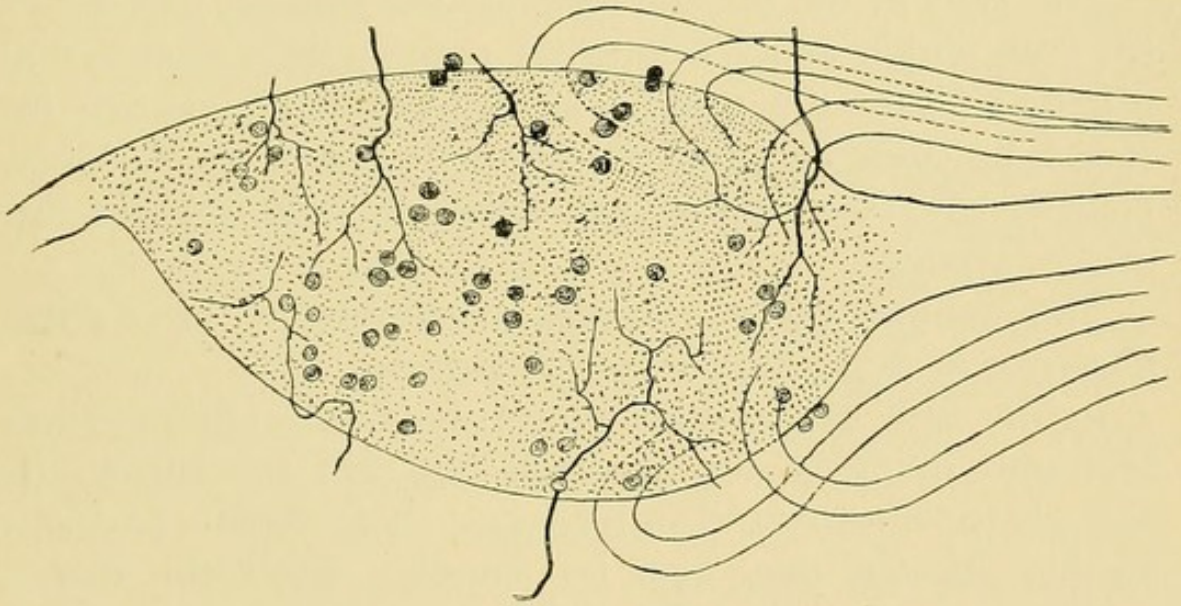


FIG. 113.

will be poured into the surrounding saline solution and can then be examined. If quite mature the contents will be entirely composed of sporozoites with a few small round masses of residual protoplasm, and in some cases a few small grains of pigment that have escaped absorption.

If not quite mature some of the sporozoites will remain attached to the protoplasmic residue which formed the centre of the blastophore, forming a tangled mass round this centre.

If empty cysts are found attached to the stomach

detached sporozoites will be found in the fluids from any part of the body of the mosquito, and in some of the cells in the salivary glands they will be found in large numbers.

Even with a low power the invaded cells in the salivary gland can usually be detected, as they present a granular appearance, and with a high power, oil immersion, the individual sporozoites can be made out unless they are too numerous. In such a case by pressure on the cover-glass the cells may be ruptured and sporozoites will be poured out in a manner similar to that in which they were poured out on rupture of a mature zygote.

The cells in the middle lobes of the salivary glands are the ones which most frequently contain sporozoites, and usually cells in the middle lobes of both glands are invaded, but they may be found in cells in any of the lobes. Usually when scanty they are found in cells near the tips of the lobules.

The demonstration of the development of *Filaria nocturna* in mosquitoes is even simpler.

In the first twenty-four hours the filaria embryos will be found living in the stomach and will be seen to be actively locomotive and to have cast their sheaths (*ecdysis*). Empty sheaths may also be found. Later the filariæ will be found by teasing out the muscular masses, after the removal of the stomach, especially those of the thorax. Normal saline solution should be used, as pure water is apt to destroy the worms.

Every stage in the development can be traced by dissecting daily one or two of a number of mosquitoes found to carry this filaria and fed at night on a person harbouring *F. nocturna*.

F. nocturna has been shown to be carried by several species of mosquitoes belonging to several genera ; *Culex fatigans*, *Mansonia uniformis* and *albipes*, *Cellia argyrotarsis*, *Pyretophorus costalis*, *Myzorhynchus sinensis* and *barbirostris* are amongst these.

So far experiments with *Stegomyia fasciata* have always

failed. Occasionally the filariæ make their way into the muscles and become encysted, but development is slow and incomplete and the larvæ die and become absorbed without reaching the full larval development. *Myzomyia funesta* does not carry *F. nocturna*.

Temperature has an important influence, and at low temperature, even with a suitable species of mosquito, no development takes place, and at intermediate temperatures development is much retarded.

The points to observe in the larvæ are the alterations in size and shape, the variations in motility, and the formation of intestinal and other structures.

The larva as found at first in the muscles is exactly like the embryo freshly escaped from the sheath. It soon becomes less actively motile and thicker. The extreme tail of the worm does not become thicker, so that we soon have a body like an elongated sausage with a small thin tail. This tail retains its mobility longer than any other part of the worm. The embryo increases in length, an alimentary canal with a terminal mouth and subterminal anus is formed, and the mobile tail disappears. At this stage only very sluggish occasional movements can be observed. The larva continues to elongate and again becomes actively motile. At this stage the alimentary canal is complete, and there are three small projections developed at the tip of the tail.

The actively motile young filariæ now escape from the muscles and pass towards the head of the mosquito and from there into the labium, where they can be found in pairs, or in larger numbers, stretched out with their heads towards the tip of that organ.

It will be remembered that the labium is the only part of the proboscis that does not penetrate the skin.

In order that the young filariæ may obtain access to man it is therefore necessary that they must escape from the labium and find their own way down the puncture made by the other elements of the proboscis.

The most probable supposition as to the course taken

is that the worms make their escape through the thin membrane stretched between the bases of the labella, known as Dutton's membrane. This membrane is the weakest part of the labium and is put on the stretch when the two labella are pushed against the skin and separated as the piercing elements of the proboscis are plunged into the skin. In the angle between the two diverging labella the young worms would readily burst through this membrane and enter the skin, passing through the glandular ducts as has been shown by Fulleborn and others. That the filariæ escape from the labium in this way was surmised by Drs. Annett and Dutton, and Bancroft has shown that by pressure on the proboscis of a mosquito in which these filaria larvæ are present the larvæ are extruded between the labella.

The further development of the young filariæ in man is not known. At the last stage of development in the mosquito the worms are not only small, 1·6 mm., but sexually immature.

Further growth and impregnation of the female must take place in man before embryos are again formed, appear in the blood, and are, in turn, taken up by mosquitoes.

It is obvious, from the above, that a patient harbouring adult filaria and with embryos in his blood can not only cause infection of others, but continued and repeated reinfection of himself.

Another filaria known to be carried by mosquitoes is *F. immitis* of dogs. This is a sheathless filarial embryo, but instead of passing through the walls of the stomach of the mosquito it passes up the lumen of the Malpighian tubes and there it further develops and passes through its non-motile stage. When the larvæ again became motile they burst through the Malpighian tubes and work their way through the tissues of the mosquito to the head and enter the proboscis just as the young *F. bancrofti* does.

The demonstration of the development of the filaria is

best done with fresh specimens of infected mosquitoes by teasing them out on a slide in normal saline solution. The young worm in the proboscis can be demonstrated by breaking across the proboscis. The various changes in the motility of the embryo and young worm can only be demonstrated in the living state.

Sections (*vide* sections of mosquitoes) are the best for permanent specimens, as the worms can then be seen in their proper position.

Nothing is known of the mode of development of the other human filaria. Experiments with many species of mosquitoes have failed. Other blood-sucking arthropods may be the carriers or intermediate hosts, as is known to be the case with filaria of some of the lower animals.

A large number of species of filaria have been described in birds. The intermediate hosts are unknown, and much information as to the possible methods of the propagation of filaria might be obtained by systematic experiments on some of these birds.

Various protozoa, such as gregarines and sporozoa, have been found in mosquitoes or in their larvæ.

Bacilli swarm in the intestinal tubes of mosquitoes; they are particularly abundant in the air sacs or diverticula from the upper end of the œsophagus. Yellow fever has been shown to be carried by mosquitoes (*Stegomyia fasciata*). The organism of the disease is not known, and though there is reason to believe that some development of the unknown organism takes place in the mosquito, nothing is known of the changes that must take place.

Dengue Fever.—It is stated that this disease is carried by mosquitoes, but the evidence is unsatisfactory.

Mosquito larvæ cannot be bred in sterile water but special colour-producing organisms can be introduced into the water in which the larvæ breed and are swallowed by them. When pupation occurs the pupæ can be transferred to many changes of sterile water so that as few organisms as possible are on the surface of the pupa.

When the imago emerges in a sterilized vessel it will be found to contain some only of the organisms that were present in the water in which the larvæ lived, and others are absent.

From this it appears that bacteria imbibed by a larva can subsequently be distributed by the adult or imago.

In this way *Stegomyia fasciata* is shown to distribute *Bacillus pyocyaneus*, but not *B. prodigiosus* or *violaceus*.

An old observation of Ross on the development of certain gregarines in *Stegomyia fasciata* is an excellent instance of the manner in which protozoa may be acquired by the aquatic larvæ and distributed by the adult.

These gregarines are found in the intestines of the young larvæ and pass up the Malpighian tubes. By the time the larvæ are ready to pupate these gregarines have become encysted in the Malpighian tubes and the cysts are full of young gregarines.

During pupation the cysts rupture and the gregarines are set free to pass into the stomach.

When the imago emerges these gregarines are in the stomach of the mosquito and are passed with the first excrement deposited by the mosquito.

The dissemination of protozoa and bacteria by insects which in the larval stage have such abundant opportunities of acquiring them is worthy of very close investigation.

CHAPTER XIV.

Eggs, Larvæ and Pupæ of Mosquitoes.

The eggs of mosquitoes of different genera vary greatly. In most cases they are laid on the surface of water. A few species of mosquitoes will lay eggs in other situations. Some of these, whilst in captivity, can be induced to lay on many damp surfaces—wet blotting paper, the cut surfaces of apples, potatoes, and the like. *Grabhamia dorsalis*, and some, at least, of the *Stegomyia*, lay eggs in this manner.

Most, if not all, of the species belonging to the restricted genus *Culex* lay their eggs in masses or rafts. Other genera of the *Culicina*, and many of the *Ædinæ*,* form similar egg-rafts. Each individual egg has its long axis vertical to the surface of the water, or nearly so, and as the lower end is slightly the larger, the mass formed by the aggregation of these eggs rests with a convex surface downwards on the water and a concave surface upwards. The egg masses, when first laid, are white, but soon darken, usually to a black or dark brown colour, but in some species to a bright bronze. The individual eggs vary according to species. In all the upper end is plain, but the lower may be plain, spiked, or ornamented with a whorl. There is no definite operculum, that can be seen, in the unopened egg, but when the larva bursts through, the eggshell ruptures in a cir-

* In this chapter where the word *Ædinæ* is used it must be understood to include the various sub-families into which the old sub-family *Ædinæ* has now been divided.

cular manner round the broad end of the egg, and the lid thus formed is pushed aside by the larva. When the eggs hatch the raft breaks up.

In *Anophelinæ* the eggs are quite different. They are never laid in rafts, but deposited in little groups on the surface of the water. After a time, when disturbed by superficial currents in the water or in the air, they become scattered and arranged in patterns, which vary according to the nature and proximity of the sides of the vessel, or to floating bodies, such as blades of grass, pieces of stick, &c. The eggs lie horizontally on the surface of the water and are irregularly spindle-shaped, with the upper surface flattened. They are covered with a thin reticulated membrane, which is closely adherent to the upper and under surfaces and at the pointed ends, but is thrown into loose folds at the sides so as to form a projecting ridge running a distance, varying according to species, towards both the pointed ends. This fold is strengthened by tranverse thickenings, and air is contained between the folds. The *Anophelina* egg, therefore, has on each side an air chamber or float attached, which prevents the egg from sinking. If the egg does sink, or if, when it has become adherent to the sides of a vessel, it is submerged, it does not hatch, nor does it if once thoroughly dried. When the larva hatches the eggshell splits obliquely towards the thicker end, and pushing aside the cap thus formed the larva makes its escape.

The eggs of *Stegomyia*, of some other genera of *Culicinæ*, and of the *Megarhininæ*, are also laid separately. They are oval eggs and are covered completely with a reticulated membrane, or are bare. No large air cells are present, but at first there is air in some of the small reticular spaces. The eggs may remain floating and hatch, but more frequently sink and hatch after remaining some hours, or even days, submerged. Such eggs are highly resistant, and will withstand prolonged desiccation, or complete immersion in water.

This is most important from the point of view of

prophylaxis. The eggs of *Stegomyia fasciata* may be deposited in shallow puddles at the end of a wet season, and if the puddle dries the eggs lie in the dried mud at the bottom, and retain their vitality for months. With the onset of the next rains, when the puddle is re-formed, the larvæ rapidly hatch out. The eggs of some of the mosquitoes, *e.g.*, *Grabhamia dorsalis*, with similar thick shells, will retain their vitality all through the winter.

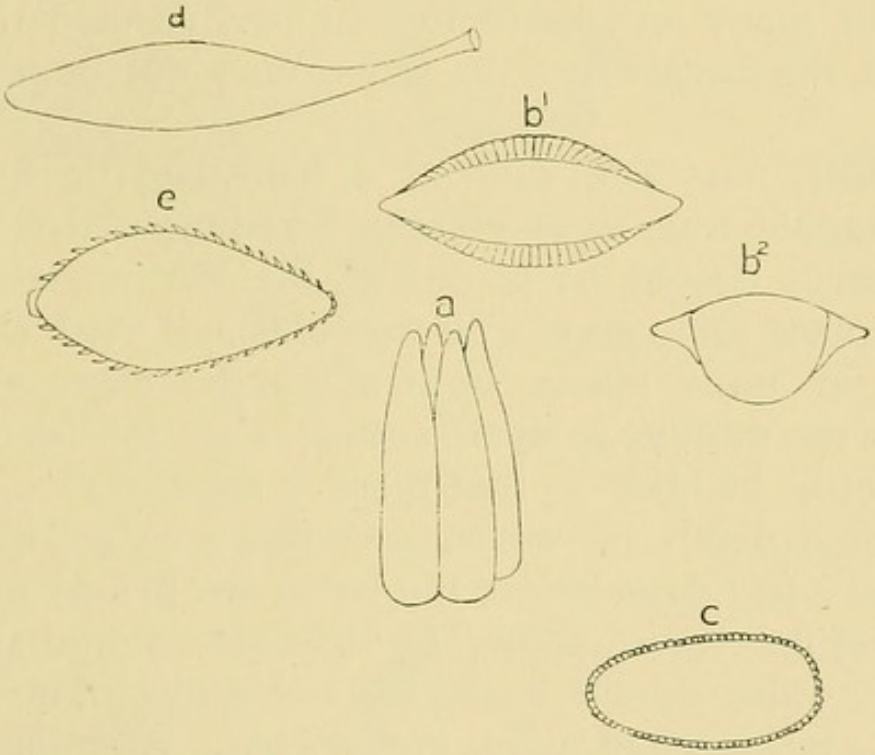


FIG. 114.—*a*, Egg of *Culex*; *b*¹, *b*², egg of *Anopheles*; *c*, egg of *Stegomyia*; *d*, egg of *Mansonia*; *e*, egg of *Psorophora*.

The species of *Mansonia* most frequently observed rarely lay eggs in captivity. The eggs are oval, and projecting from one end, have a long tube, terminating in a slightly expanded, trumpet-shaped opening.

The eggs of *Psorophora* are not unlike those of *Stegomyia* in shape, but are rather more pointed. According to Dr. W. N. Berkeley they are "prickly."

Eggs are best obtained by collecting adult female mosquitoes and keeping them in a small cylindrical vessel, a wide-necked 4-oz. bottle is suitable, containing water. It is well to have some twigs or fragments of

grass floating on the water on which the mosquitoes may rest. The top of the vessel should be covered with mosquito netting to allow air to have free access. They should be fed on blood as often as they will feed.

Larvæ can be obtained by keeping the eggs in water at suitable temperature. When first hatched they are small and quite white, but they soon increase in size, and either in part or as a whole change colour.

They are voracious and require abundance of food, but with many species of mosquitoes, particularly with some of the *Anophelines*, the water must not be putrid or peaty.

A white, flat dish, such as a half-plate or full-plate photographic tray, is as good a breeding place as any. Some earth should be placed at the bottom, and it is well to place some grass with the roots and earth attached in two or three places, both along the edge and also towards the middle, so as to form at least one islet. The dish should be filled so that there is about three-quarters of an inch depth of water, and these dishes are best prepared a few days before the larvæ are placed in them. A little of the "green slime" or other algæ found growing in fresh water should be added and a few grains of dry rice may be scattered about the bottom. Abundant food will thus be supplied, but the water must not be overstocked with vegetation, as if this decomposes the water will be unsuited to many larvæ. Great care must be taken that none of the natural enemies of mosquito larvæ are introduced into the water. Those most frequently introduced are the larvæ of *Agrionidæ*, one of the groups of the dragon-flies. These are short, squat, six-legged larvæ with the characteristic protrusible prehensile mask. They are often introduced with mud or in muddy water and are most destructive to other larvæ. Cannibal culicid larvæ should be looked for, as they also are very destructive. They can be recognized by the stiff row of curved bristles instead of fine hairs on each side of the mouth.

These dishes must not be kept in the dark, must be

well lighted, and are best exposed for short periods to direct sunlight if there is sufficient grass growing to provide shelter for the larvæ. They must not be left long enough in the sunlight to warm the water.

When pupæ have formed they must not be exposed at all to direct sunlight.

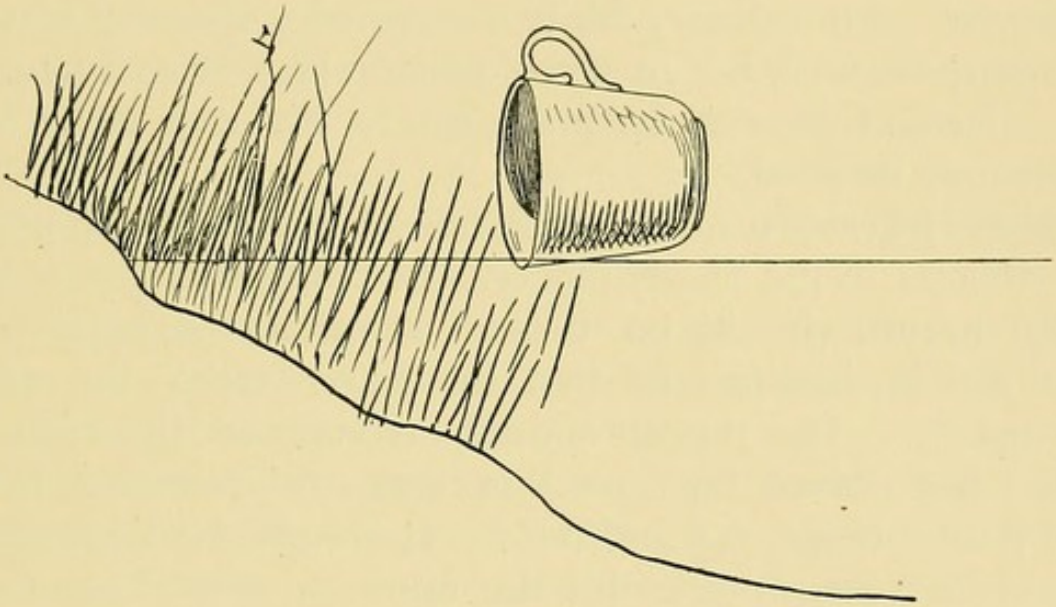


FIG. 115.

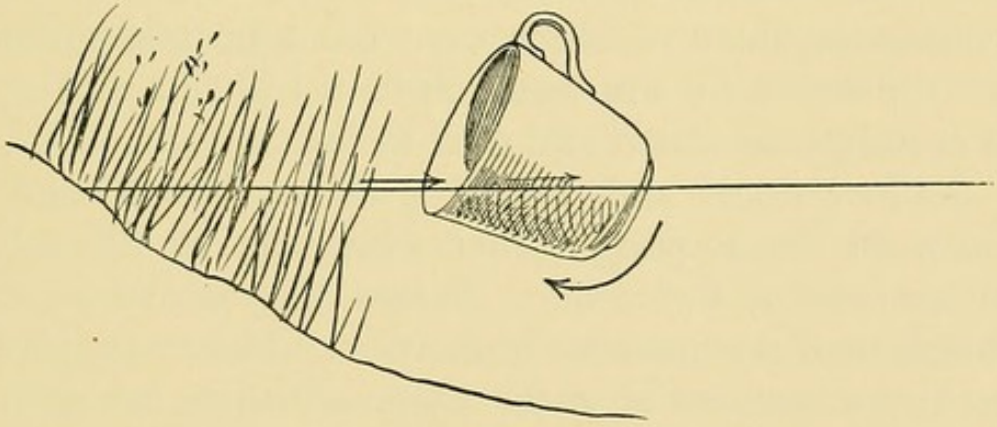


FIG. 116.

The water must not be overstocked with larvæ, as they are all, at times, carnivorous. The larvæ should all be about the same age, but may be of different species. Some large larvæ will destroy the young of both their own and other species.

The tops of the dishes should be covered to prevent the entrance of dust, a plate of glass, or better, a larger

glass dish inverted over the dish containing the larvæ, will suffice.

Larvæ can either be raised from the eggs or caught from natural waters by scooping up the water in any receptacle. When large numbers of larvæ are required any receptacle from a bucket downwards will do, but where larvæ are scanty they are best caught by using a dipper. An ordinary white enamelled coffee-cup serves the purpose well, but in some situations a longer handle is better and this can be fitted on to the cup, or a soup ladle may be used.

Some larvæ are most numerous at the edges of pools or streams in the shady places. In using the dipper the open mouth should be turned towards the bank and plunged in, inclined so that the water from the edge rushes in. The dipper should, as soon as the rush of water has ceased, be turned upright and removed from the pool. (Figs. 115 and 116.) It should be allowed to stand for a few minutes till the mud has settled and then examined. A hand lens is useful as the very young larvæ can easily be overlooked.

In obtaining the specimens care must be taken not to disturb the water in any way before using the dipper, as larvæ readily take alarm and dart to the bottom.

In shallow pools and small puddles, larvæ can be readily seen by looking rather obliquely at the undisturbed surface of the water. When they occur in such situations they are usually numerous. In running water and in larger masses of water they can rarely be seen in this manner, and unless a dipper is used will be overlooked. In such situations it is not common to find them in large numbers in any small surface of water, and consequently the dipper may have to be used frequently to demonstrate their presence. Though in any small area of water examined they may be scanty the total area of this class of breeding place is so great that these places are of the highest practical importance.

Some species of mosquitoes are more easily found as

larvæ because the adults do not frequent human habitations. During certain seasons, particularly cold and dry seasons, larvæ of all species will be found more readily than adults.

In making collections of mosquitoes it is well both to breed from adults collected in as many different classes of place, houses, cattle-sheds, grass and forest, as possible, and also to rear adults from larvæ or eggs found in still and running waters, natural pools, small and large, and also in artificial collections of water.

No water, even that in cesspits, is too foul for some species, whilst others will not breed in water that Europeans consider fit to drink.

The pupæ are found in the same situations as the larvæ; they appear as small black objects which are usually motionless unless disturbed.

The larvæ and pupæ can be transferred from the dipper to a wide-mouthed bottle for carriage. Both larvæ and pupæ are easily destroyed if the water is kept in motion, as they do not rest on the surface sufficiently long for proper respiration. If it is necessary to carry them for long distances it is well to make frequent halts every half hour to an hour and place the bottle containing the larvæ upright in a shady place for a quarter of an hour or so.

The character of the breeding place must be carefully noted. The special points are: (1) Whether it is fresh, or foul, or brackish. (2) If the water is still or in motion. (3) Vegetation in the water. (4) Other larvæ or animals present. Special attention should be paid to animals that may prey on the mosquito larvæ, especially fish, coleopterous and neuropterous larvæ, &c. (5) Any special features either in the natural or artificial receptacle for the water. (6) Exposure to light, wind, &c., of the surface of the water. Many of the *Stegomyia* larvæ will thrive in darkness. Ædine and Megarhinine larvæ often have peculiar breeding places, such as the cups of pitcher plants, the hollows in trees, or the interior of bamboo

joints or crab-holes. Some species will only be found in one kind of breeding place.

Duration of larval stage varies with the amount of food, temperature, and the species of the mosquito. Under the best conditions of food and temperature, with many mosquitoes the larval stage is seven or eight days, but may be indefinitely retarded by cold or insufficient food. Other mosquitoes, under the most favourable conditions, require several weeks for their development. Of these, Megarhinine larvæ are instances. The pupal stage is not affected by the food supply, as the pupa does not require food. It is prolonged by a low temperature. The pupal stage is about two days with most species, but with Megarhinine pupa and a few others is six days.

Anatomy of Larvæ and Pupæ.—The larvæ vary in colour in different species, but even in the same species variations occur according to the degree of exposure to light and the nature of the food. In the more transparent larvæ the colour of the intestinal contents, green or brown, is more obvious than that of the larva itself.

The larvæ of the *Culicidæ* conform to a general type. The head is joined to the thorax by a narrow neck. In the head are a pair of compound eyes and two simple eyes or ocelli. There are a pair of short antennæ and a mouth composed of an upper lip, a pair of mandibles, a pair of maxillæ, and a lower lip or labial plate.

The thorax is composed of three fused segments. There are no ambulatory legs, but sensitive and balancing hairs are abundantly supplied.

The abdomen is long, and composed of nine segments. The last is smaller, and inclined at an oblique angle downwards. At the termination of this segment is the opening of the anus, surrounded by four retractile papillæ, probably respiratory in function—anal gills or branchiæ.

On the upper surface of the eighth segment are the spiracles or openings of the two respiratory tubes, which run the whole length of the body, and supply the larvæ with air. In *Corethrinæ* these tubes are smaller, but have

dilatations on them in the thorax and abdomen—air-bladders.

The openings of these tubes are direct in the *Anophelinæ*, which can therefore be described as *asyphonate*. In the other sub-families the tubes are continued into a conical tube jointed on to the upper surface of the eighth segment, and at the apex of this tube are the external openings of the respiratory tubes. This projection is known as the respiratory syphon, and the larvæ of all the *Culicidæ*, except the *Anophelina*, are therefore said to be *syphonate*.

The head is composed of many chitinous plates, which are thicker and darker on the dorsal surface.

The opening of the mouth is directed slightly downwards in all, and almost directly downwards in many.

The upper lip consists of a middle portion or palate supported on either side by lateral plates covered with bristles.

The pair of mandibles are placed beneath the upper lip, and are usually toothed. The movements are lateral.

The pair of maxillæ are below and behind the mandibles; their movements are obliquely upwards and inwards.

The inferior lip, or labium, is a triangular plate, usually very dark in colour, and with a more or less serrated edge.

On each side of the mouth are chitinous plates attached to the mandibles and maxillæ, and from these arise the "brushes" or masses of long, stiff hairs, fine in most of the *Culicidæ*, but thick and curved in the larvivorous larvæ, which are so arranged that they can be moved laterally and folded completely over the mouth or thrown back so as to form a very oblique angle with each other (fig. 117). These brushes in life are in constant movement, and cause a sufficient current in the water to wash solid suspended particles to the open mouth of the larva.

There are great variations in the different parts which are of use in distinguishing larvæ of one mosquito from those of another. Much attention has been paid to the shape of the inferior lip plate, which is a conspicuous object, and varies in closely related species.

The antennæ are articulated to the head. They move slightly. They are not truly jointed, but in some there is an abrupt variation in thickness, probably indicating a joint. They vary in length and in the number and arrangement of the hairs and spines ornamenting them. They are of value in differentiating the larvæ of different species.

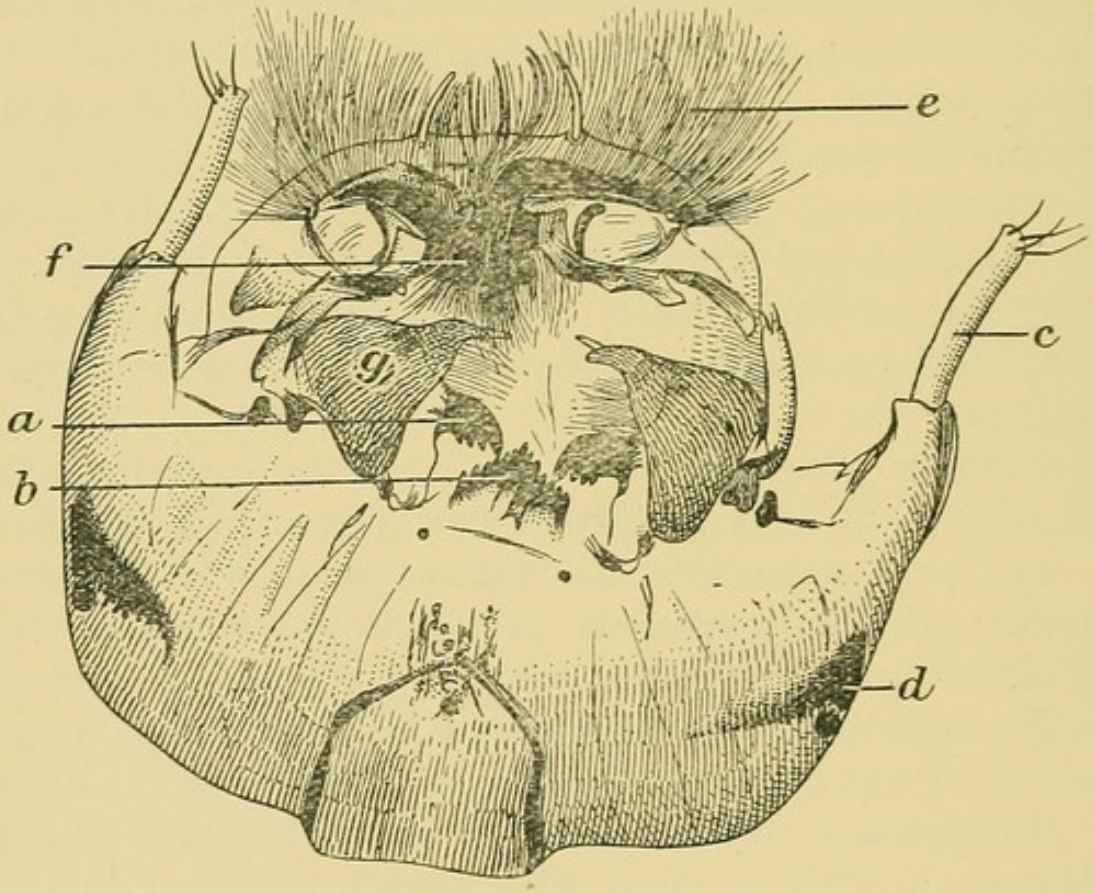


FIG. 117.—Head of mosquito larva. *a*, Mandible; *b*, lower lip; *c*, antenna; *d*, eye; *e*, brushes; *f*, upper lip; *g*, maxilla.

The head is very mobile in some species, and in the *Anophelinæ* so much so that they can turn the ventral surface upwards and feed in that position. As the ventral surface is light, and the dorsal black, a larva may at one moment appear to have a dark head, and at the next a light one.

The thorax is well supplied with simple or compound hairs. The longer and more conspicuous are arranged on each side. In a few species there are in addition

strong curved spines slightly below the lateral hairs. One or two pairs of these spines may be present.

On the abdomen are also hairs, varying greatly in genera and species. In the *Anophelines* are peculiar palmate hairs, the shape of which varies in different species.

In many of the *Ædinæ* compound hairs are very numerous. In the *Stegomyia* hairs are scanty and inconspicuous.

The shape of the respiratory syphon attached to the eighth segment in all but the *Anophelines* is of great importance. It varies in length, so much so that attempts have been made to classify the *Culicidæ* on the "syphonic index," or the relative length of the syphon. Generally speaking, all the *Megarhina*, all larvivorous larvæ, *Stegomyia* and *Desvoidea*, as well as some of the *Ædinæ*, such as *Uranotænia* and many other genera, have short respiratory syphons. Most species of *Culex*, in the restricted sense, and many others of the *Culicinæ* and *Ædinæ* have long respiratory syphons. A re-classification on the basis of the syphonic index would break up the present classifications founded on adult characters, whether those adult characters were on the character of the palps, proboscis, or scales. It would be less convenient than the classification founded on adult characteristics.

The openings of the respiratory tubes at the end of the syphon are often guarded by mobile flaps, and hairs and spines are present on the syphons in most species. In some of the *Ædinæ* these are very numerous.

The alimentary system of the larva consists of a tube, apparently structureless and of uniform calibre, running from the mouth to the anus. In the more fully grown specimens this tube is seen to be contained inside the true intestine, which is arranged as in the adult. In the space between is clear fluid not containing any food particles. The Malpighian tubes and other appendages of the alimentary canal of the adult are present at this stage.

The intestinal system, including the inner tube containing the food, and the outer tube and appendages, can

be pulled out of the larval case in a manner similar to that by which the intestine is removed from the adult, but it is more easily done by extraction through the anterior part of the larva than from the tail.

The space between the temporary and permanent intestine may contain gregarines and numerous micro-organisms, and it is to this space that attention should be paid in investigating the conveyance of parasites acquired by larvæ.

The respiratory system of the larva is comparatively simple. At the end of the respiratory syphon, if there be one, or from the dorsal surface of the eighth abdominal segment, are the openings leading into the two main tracheæ, which pass up the abdomen, giving off branches to each segment and inosculating freely in the thorax. They send off branches to the various parts here and to the head.

The Pupa.—When the larva has reached its full stage of development the thorax becomes swollen, the cuticle with all the appendages is detached and cast off and the larva becomes a pupa. The organs are already formed.

The pupa differs most materially from the larva in that there is no longer a mouth opening externally, and the respiration is conducted through two tubular openings arising on each side of the compound head and thorax. The change in appearance is great, the head and thorax are fused, and the only external appendages are the two respiratory tubes. The abdomen is still segmented and is usually curved, so that the termination is under the compound thorax. It terminates in two large fins.

The pupal stage is a comparatively short one. There is no possibility of feeding and the pupa remains quiet, breathing through the respiratory tubes unless disturbed, whilst the more complete development of the imago takes place within its sheath. The duration of the pupal stage is affected by the temperature, but is usually from two to five days. The pupæ of some species will not

remain alive longer than a few days if the conditions are not favourable for development.

In the examination of eggs, larvæ and pupæ, the points to be observed are as follows :—

Eggs.—(1) The size, shape, colour. (2) The manner in which the eggs are arranged and where deposited. (3) The character of any thickenings or other external markings. (4) The length of time required under stated conditions, temperature and so on, between the deposition of the eggs and the hatching of the larvæ, and any variations noted with variations of conditions. (5) The effect of desiccation, immersion and temperature on the vitality of the eggs.

Larva.—In the larva the relative sizes and shapes of the different divisions—head, thorax and abdomen. The character of the head appendages, the antennæ, mouth apparatus, &c. Any marked colouring. Much work has been done on the differences in the appendages of the head of *Anophelina* larvæ, and it has been shown that the differences are so marked in their arrangement that many of the species can be distinguished as larvæ.

In the thorax the character of the lateral hairs and any characteristic markings must be noted.

In the abdomen the points of greatest importance are the appendages on the eighth and ninth segments. The presence or absence of a respiratory syphon attached to the eighth segment is one of the most important generic differences. Where present it varies in length and shape in different genera. In different species it varies in colour and in the distribution of colour so markedly that it is often easier to distinguish between different species by the character of the syphon than it is to distinguish between the adults. Varying positions of larvæ are associated with the differences in length or the absence of the syphon.

The arrangement of bristles and hairs on the eighth and ninth segments presents marked differences in the different species. In *Anophelina* on the other segments,

in addition to the lateral hairs, there is on each side a row of stellate or palmate hairs. These are nearer the middle line than the simple bristles, and the stellate portion forms a kind of cup. This adheres to the surface film of the water and aids the larva in maintaining its horizontal position.

Colouring of larvæ is of less importance, as in some species the colour may vary from yellow to green, brown, or even black. In others variations are comparatively small, these are usually dark under all circumstances.

In noting the colour any conspicuous marking must be mentioned, the conditions under which the larvæ were grown, and whether or not change of conditions, such as greater light, different food, &c., results in a change of colour.

The nature of the food can be determined by the examination of the contents of the intestine, or by watching the larvæ feed in water containing a mixture of natural foods. It will be found to vary. The kind of food on which they thrive best should be noted.

The duration of the larval stage under as many diverse conditions as possible, including exposure to light, heat, and cold, and any observations as to the conditions predisposing to death or leading to an undue proportion of males in the imagines, should be noted.

In breeding from larvæ it is most important that the water should be properly oxygenated. Darling advises that a jet of air should be passed through by means of a Pacquelin cautery bulb, having a heavy glass perforated tip, once or twice a day.

The important natural enemies of the mosquito larvæ are fish, larvæ of other insects, particularly those of the dragon-fly, &c. Where possible the species of these enemies should be determined. If the larvæ are caught as larvæ and not reared from eggs particular care should be taken to observe the nature of the places in which they were found.

Breeding places of the known carriers of disease,

such as *Anophelinæ*, *Stegomyia* and *Culex*, require very detailed consideration. It is convenient to divide these into permanent waters such as will withstand a considerable period of rainless weather, and *temporary* waters, which require frequent renewal. They may be natural or artificial. Of permanent waters, rivers, large ponds and the edges of lakes under certain conditions are of the utmost importance. In such situations the larvæ are usually widely scattered, and without the repeated routine use of a dipper such places, often the most important, are usually overlooked.

The conditions favourable are the growth of grasses, reeds or sedges in the water. These growths check the stream, provide food, and protect to some extent the larvæ from their natural enemies.

There are two main classes of growths important :—

(1) Those growing from the bed of the river or lake, in the shallows and on shelving banks. The height of the water greatly affects the area suitable. The taller and thicker sedges are not so suitable as the lower and thinner ones, probably on account of the absence of light and too great stagnation of the water (fig. 115).

(2) Those growing from floating masses of roots and attached to the earth only near the edge of the river. The raft formed by the closely interlaced roots is submerged by the weight of the grass growing in the air, and in the shallow water lying above this raft of roots *Anopheline* larvæ breed freely. No alteration in the level of the water makes any material difference to this, a common class of breeding place (fig. 119). In flood times islets of this floating grass are torn off and carried down the stream, carrying with them larvæ, and in this manner they may be carried long distances down the river. It is not improbable that the cutting of the sudd in rivers may result in larvæ of mosquitoes being carried for long distances down the river and thus extending the area of distribution of these mosquitoes, but according to Balfour no such result seems to have taken place on the Nile.

Rivers are dangerous when variations in level are not too great or too rapid. Such streams as have a constant supply independent directly of the rainfall are particularly dangerous. Such sources are the melting of the snow from snow-covered mountains and the effluents of large lakes.

Springs which often arise on the slopes of hills are other important permanent breeding places. These usually commence as a small pool with a surrounding swampy

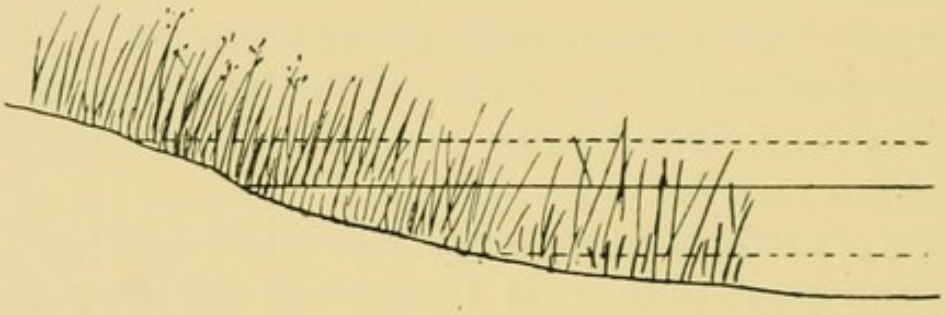


FIG. 118.

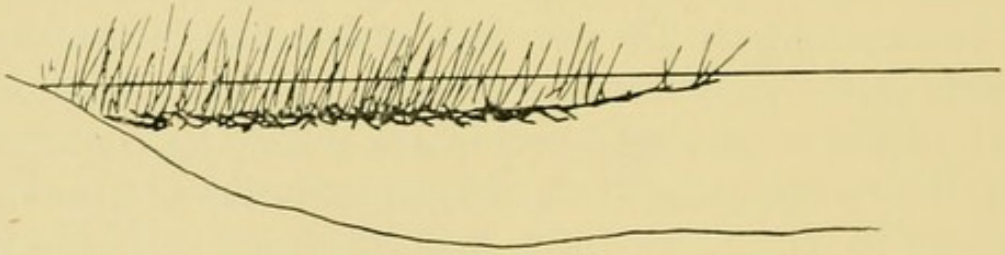


FIG. 119.

area. The grasses round are often of different species or grow more luxuriantly than elsewhere, and these places can therefore usually be identified with ease.

The streams arising from such springs are not of much importance during heavy rains, but when the water supply is diminished, wherever the streams spread into swampy areas, or form pools fringed with vegetation, or in backwaters, larvæ are usually to be found with the aid of the dipper. In some of these situations they are carried by the streams from the springs or other breeding places. In others the eggs may be deposited and hatch in the place in which the larvæ are found. Amongst the easiest

places to find larvæ are the pools left in the bed of such a stream when the spring commences to dry up, particularly if a small current connects the pools and keeps the water fresh. Some springs will dry up after a month's dry weather, others in three or four months, but some are usually permanent, though the water may be scanty, from one wet season to another.

Swamps, unless kept supplied by fresh water, are not suitable breeding places for many species. For other species they are suitable, provided the vegetation is not too rank.

A high-level subsoil water may lead to formation of natural permanent pools. On the sandy shores of great lakes the sand is usually thrown up into a ridge with a hollow behind it, and in this hollow, as long as the lake level is high, water will be present and forms a suitable breeding place. As the lake level reaches its greatest height at the end of the wet season and very slowly falls in the dry season, these pools may persist in the vicinity of such lakes for some months after the rains have ceased.

Temporary breeding places are of many different classes. Almost any hollow or hole that will contain water is a suitable breeding place during continuous rains. If the rain be intermittent, only such places as can retain water during the periods of intermission are suitable. Such places require a frequent and heavy rainfall and an impervious soil, and are not often found except under these conditions. The "*Anopheles*' pool" most often described belongs to this class, and is exceptional in many places where *Anophelinæ* are abundant.

A place that is frequently flushed is not a suitable breeding ground, but irrigation trenches or natural hollows are good breeding places for some species if the area of the trenches is such that the water supplying it is insufficient to flush it in its whole extent.

Artificial Breeding Places.—Borrow pits at the sides of railway embankments, the trenches so often made in

the course of road-making, and hollows or furrows made in native or other gardens, are common breeding places of *Anophelines* and some other mosquitoes. A high level of the subsoil water is necessary for these places to be of importance.

Irrigation systems where the water supply is continuous but insufficient to flush are important places. In any case, even with a well-designed system, if the source of the water be from a natural breeding place larvæ will be conveyed all over the irrigation system.

Instances occur in which larvæ are conveyed for over a mile by such a trench from a natural permanent breeding place to a European settlement.

Obstructions in the course of a stream, such as Irish crossings, dams, &c., may convert an inferior natural breeding ground into an excellent one.

Badly graded gutters, broken bottles, water-butts, empty tins and any artificial receptacle that will hold water are preferential breeding places for some species of mosquitoes, particularly those belonging to the genus *Stegomyia*.

Wells in many places do not seem to be breeding places, but in other places they certainly are.

On the whole, artificial breeding places are usually the work of Europeans, and the worker in the Tropics has rarely to go beyond his own grounds to find larvæ of several species of mosquitoes.

Too little attention has been paid to the breeding places of different species. We know that great differences occur in the preferential breeding places of different species as of different genera, but little exact work has been done on the subject.

For exact descriptions of the larvæ of one species which might serve as an example the reader is referred to the articles on *Anopheles maculipennis*, by Nuttall and others, in the first volume of the *Journal of Hygiene*.

The pupæ differ less from each other than the larvæ, and many insects form pupæ that are not unlike those of

the *Culicidæ*. The greatest differences are to be observed in the respiratory tubes. In all the *Culicidæ* they are simple tubes with one opening. In the *Anopheles* the opening of the tube is a wide, expanded, trumpet-shaped one; in the *Culex* the opening is more of a slit and the termination is little expanded. In *Mansonia*, according to Low, the tubes are very long and slightly bent forward. In the different species there are variations in the size of the pupa and in the colour. The majority are, after exposure to light, brown or black, though when first formed they are yellow. A few are green, though most of these become dark before maturity.

To hatch out the pupæ all that is required is that they should not be disturbed and that they should be kept in clean water. No food is needed. They should be kept in a half light.

Carriage of Mosquitoes.—Mosquitoes may be carried in any stage of their existence. As eggs they are not very easy to carry, as those that float are often washed on to the sides of the vessel and there are dried and killed. Eggs like those of *Stegomyia*, which sink and are not injured by immersion, are easily carried, and it is probably owing to this that these mosquitoes or their larvæ are so often found on board ship.

The carriage of larvæ we have already dealt with. For the development of many species light is a necessity, and consequently such species, including most of the *Anophelinæ*, are not carried far on board ship, as most of the fresh water is necessarily in closed casks or other dark receptacles.

The adult mosquitoes must be carefully carried as they are easily injured by rough handling or bruising. On the whole glass vessels should be avoided because of the hard surface of the glass. Mosquitoes cannot hold on to it. If fresh grass or other moist substances be placed in the glass vessel, water of condensation is often deposited on the glass, and the mosquitoes adhere by the wings to this wet surface and speedily die.

If glass vessels, test tubes, &c., are used, the mosquitoes must be carried very carefully, and no water be placed in the vessel. Jungle mosquitoes will be killed by exposure to dry air, and with them the plug of cotton-wool should be kept wet.

A light cage covered with mosquito netting is as good an arrangement as any, though at a pinch a small box

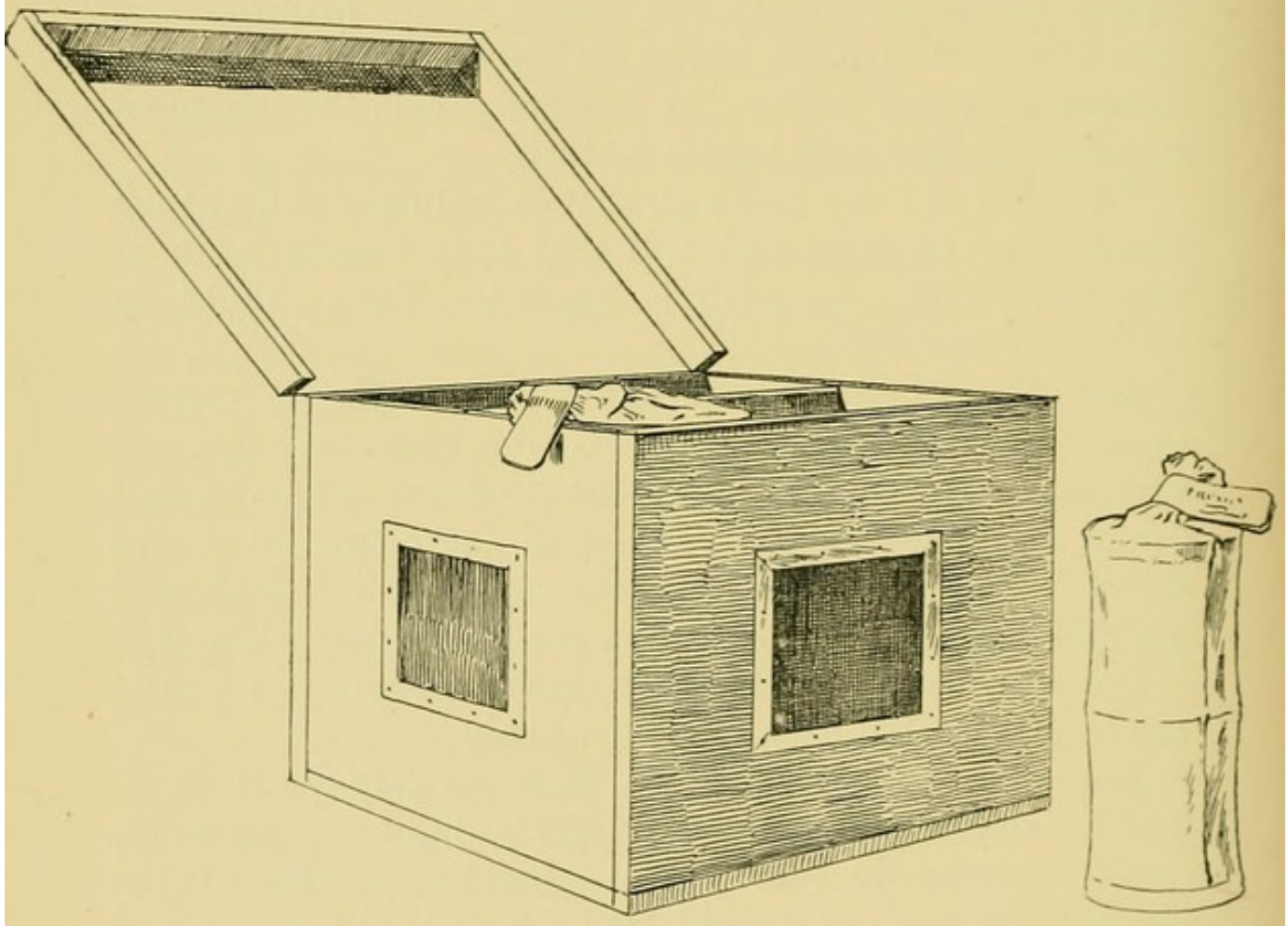


FIG. 120.

covered with netting on the open side will work satisfactorily.

The box designed by Dr. Sambon and containing four compartments, each containing a cylindrical wire cage covered with netting, is an excellent one (fig. 120). It was in such cages that infected mosquitoes were sent from Italy to the London School of Tropical Medicine for the well-known infection experiments, which resulted

in the practical demonstration that mosquitoes infected with the malaria parasite could infect men in a country where there was no other possibility of acquiring an infection.

A simple cage can be made by having a portable wire cage that can be folded flat, and bags of mosquito netting which are pulled over the framework. The open end is then tied in a knot (fig. 121).

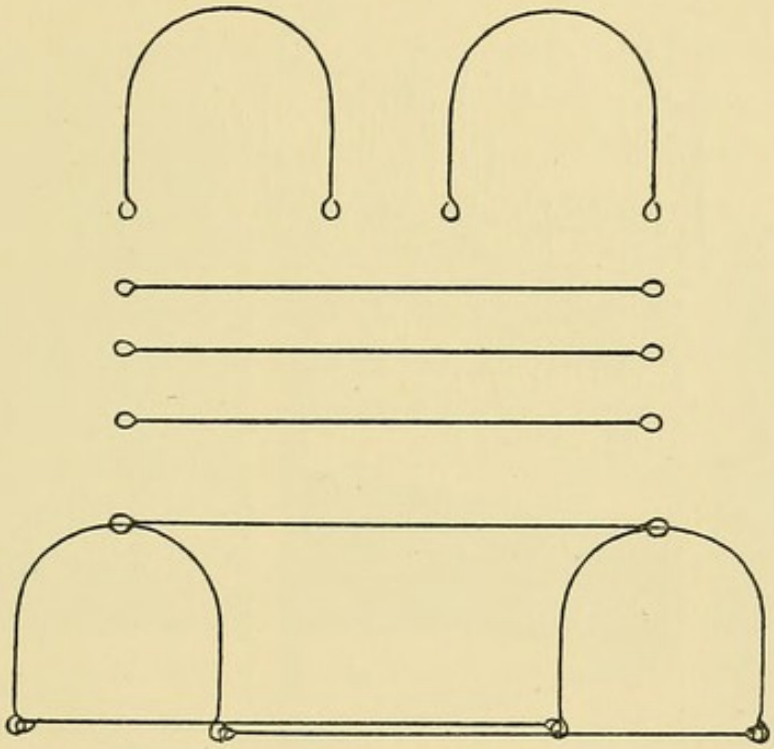


FIG. 121.

Adult mosquitoes can be kept in test tubes or wide-necked bottles covered with fine gauze or mosquito netting. A little water should be placed at the bottom and some resting place, such as a piece of stick, blade of grass, or folded card placed above the water.

Many mosquitoes will feed readily through the netting, others will not, though they feed readily in a larger space.

Mosquitoes thrive better if kept in a larger space. The box slightly modified from Dr. Sambon's cage is very convenient for this purpose. The front is composed of glass in two pieces for convenience in packing,

while the ends are of fine wire gauze to allow the entrance of air. The hole in the centre of this gauze is covered by cotton sleeves. These are convenient for the introduction of an arm for feeding experiments. Through these holes the hand and test tube can be introduced when we wish to catch a mosquito. The pieces of glass forming the face of the box slide in a groove, and can be removed when required (fig. 122).

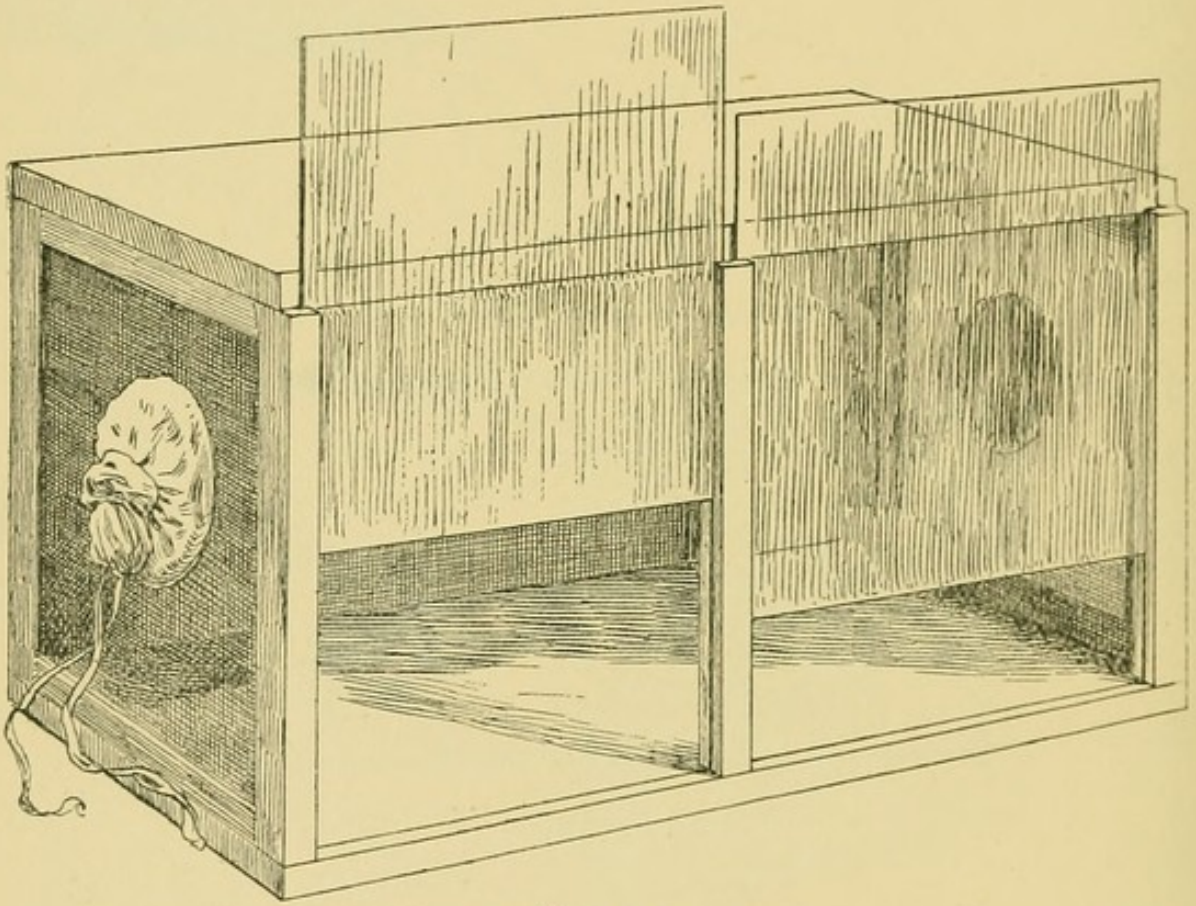


FIG. 122.

Ripe fruit, such as apples, dates, and bananas, serves as food for mosquitoes, but some will not lay eggs unless supplied with blood. As substitutes for fruit, sugar, syrups or jams will serve. Some species, *Stegomyia calopus* and *Culex fatigans*, for example, are easy to keep in captivity, and can be kept alive for months; others will die in a few days.

Ants of many kinds are very destructive to mosquitoes, particularly to those confined in small spaces. To avoid this the mosquito cage should be placed on legs, each of which rests in a small tin containing kerosene.

CHAPTER XV.

Fleas, Lice and Bed-bugs.

In view of the evidence that rats and their parasitic insects play an important part in the dissemination of plague, it is considered desirable to include here some notes on fleas that may aid the worker in recognizing the more common species.

It has long been known that fleas may serve as the intermediate hosts of certain parasites. The cysticercus stage of *Dipylidium caninum* is passed in the dog-flea, *Ctenocephalus serraticeps*, as well as in the dog-louse, and healthy rats have been infected with *Trypanosoma lewisi* by allowing fleas taken from infected rats to feed upon them. The work of the Indian Plague Commission, as well as observations elsewhere, show that fleas are the carriers of the *Bacillus pestis* from rats to rats and also from infected rats to man and other animals.

Fleas are usually included in the group *Insecta* as an order, the *Siphonaptera* (*vide* p. 157). They differ from most dipterous insects, not only in the absence of wings but also in that the three thoracic segments are distinct from one another, and that, beyond carrying legs, these segments differ but little from the abdominal segments. The antennæ also differ from those found in *Diptera*.

Capture and Examination of Fleas.—To collect fleas from rats, mice, &c., it is best to confine the animal in a vessel at the bottom of which is a piece of cotton-wool soaked in chloroform. The fleas are killed more quickly than the host, and may be picked from among the hairs or from the bottom of the vessel. If it is required to

examine the internal structure this method is not very satisfactory, as the fleas are frequently found filled with blood, and this obscures details. A preferable method is to capture them alive and keep them in a test-tube until they die of starvation.

They may be examined directly, or if it is desired to make permanent preparations they should be rendered

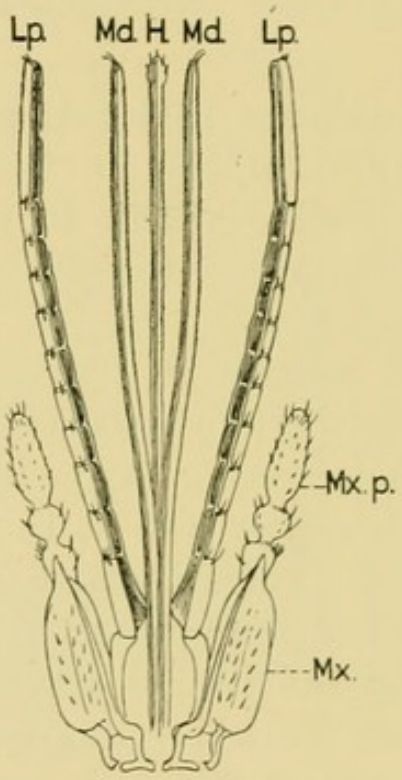


FIG. 123.—Mouth-parts of a Flea (*Vermipsylla alakurt*, ♂). (After Wagner.) *h.*, Median lancet; *lp.*, labial palpi; *md.*, mandibles; *mx.*, maxillæ; *mx.p.*, maxillary palpi.

transparent and mounted. There are many methods for clearing and mounting small insects. Glycerine is a good clarifying medium, but it takes a long time to penetrate. A method that gives good results is to fix in absolute alcohol, leaving them about one day to completely dehydrate, clear in xylol and mount in Canada balsam. Some authorities use turpentine or creasote for clearing. The preparations should be examined not only by transmitted light, but also by direct illumination to note the depressions, the direction of hairs, &c.

External Anatomy.—Mouth-parts.—These comprise a

perforating and suctorial tube and two free pieces, the maxillæ (fig. 123).

The *maxillæ* (mx.) have the form of a triangular pyramid, the apex of which projects downwards. Arising from the base of each maxilla is the *maxillary palp* (mx.p.).

The piercing and suctorial apparatus is formed by the mandibles (md.) and the epipharynx (h.). The labial palps (l.p.) act as sheaths.

The *mandibles* have the form of elongated styles with serrations along their distal two-thirds. Each contains on its mesial surface a salivary groove, which at the base widens out into a trough and towards the tip is nearly closed by the approximation of its edges forming a canal.

The *epipharynx* is a pointed, pricking organ, grooved ventrally. By the approximation of the epipharynx and the two mandibles a channel is formed along which blood is sucked.

The epipharynx makes a way through the skin, and the mandibles enlarge and lacerate the hole thus made, and convey into it the salivary secretion, and blood is aspirated from the wound along the channel formed by the epipharynx and mandibles.

The *antennæ* are contained in fossæ at the back part of the head, behind the eyes when these are present, and are directed obliquely downwards and backwards. It is important not to mistake the maxillary palps for them. The antennæ are three-jointed, the third joint being ringed.

The three thoracic segments known as pro-, meso-, and meta-thorax are freely movable on one another, and to each segment is attached a pair of legs. Each leg is described as composed of five pieces—coxa, trochanter, femur, tibia, and tarsus, the latter consisting of five segments, to the last of which are attached two claws. The hairs on the last joint of the hindmost pair of legs are of value in classification.

The abdomen is oval in shape and is composed of nine

segments. The ninth segment is the smallest and is known as the pygidium.

The males are recognized by their small size and by the presence of the coiled penis extending some distance inside the abdomen.

Metamorphosis of Fleas.—The metamorphosis in fleas is complete. The eggs are laid at all seasons of the year, but their development is more rapid in summer than in winter. The female does not attach the eggs to the skin or hairs of the host, but allows them to fall anywhere,

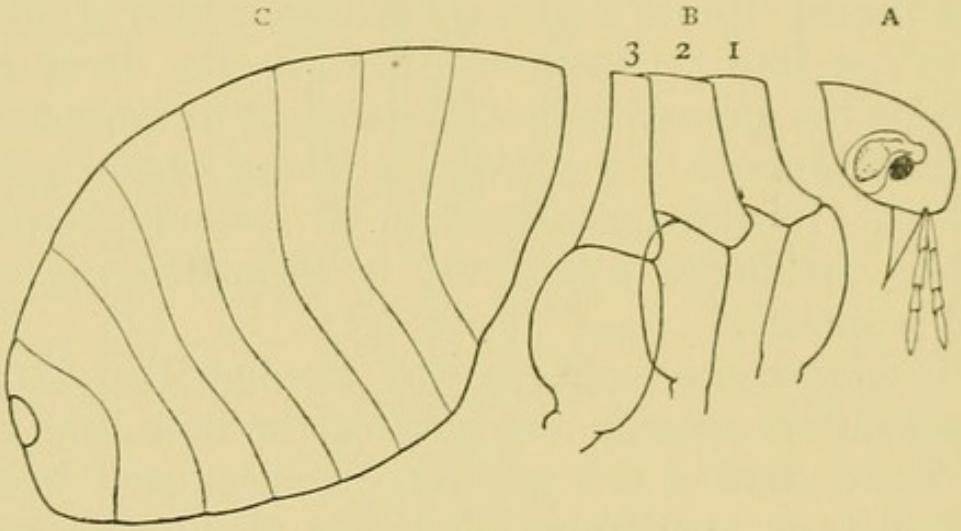


FIG. 124.—A, Head; B, thorax, (1) prothorax or pronotum, (2) mesothorax or mesonotum, (3) metathorax or metanotum; C, abdomen.

whether it be on the host, on the earth, or elsewhere. The ova are small, ovoid or rounded in shape, and usually eight to twelve are laid. The larva is hatched out in four to six days in summer and in nine to twelve days in winter.

The larvæ (fig. 125, *e*), are footless maggots, whitish in colour, and have thirteen segments, of which the first is provided with a buccal apparatus and antennæ. The buccal apparatus is formed for masticating. They are always found in dry places, dust, sand, or clothing. At the end of about ten days the larva ceases to feed and becomes immobile and forms about itself a small cocoon, to which dirt, sawdust, &c. adhere. In about eleven

days the larva is transformed into a nymph, which has three pairs of legs and resembles the perfect insect. These nymphs after a further period of about twelve days are transformed into perfect insects and come out of the cocoon.

The whole process is accomplished in about a month in summer and six weeks in winter.

Dissection.—This is best carried out in normal saline solution, using needles with very fine points. With a simple lens the dissection is very difficult—a stereoscopic microscope is almost an essential.

The flea is transfixed through the head and held by the left-hand needle. The point of the right-hand needle is then inserted under the edge of the third or fourth abdominal segment and the chitinous covering peeled off. The internal organs float out in the salt solution and may be further separated.

Internal Anatomy.—The *mouth* is situated at the base of the bulb of the ventral portion of the epipharynx and forms the commencement of the alimentary canal.

The *pharynx* extends from the mouth to the œsophageal commissure. It is aspiratory in function.

There are two *salivary glands*, each consisting of two lobes, and they lie on each side of the anterior end of the stomach embedded in the fat body.

At the junction of the pharynx and the stomach is an organ to which the name of "gizzard" has been applied. It is suggested that its function is to prevent regurgitation of fluids from the stomach.

The *stomach* consists of a basement membrane, two oblique layers of muscle fibre and a lining layer of cubical cells.

Classification.—Fleas are divided into three families, *Pulicidæ*, *Sarcopsyllidæ*, and *Vermipsyllidæ*, of which only the first two are of interest to us.

Family PULICIDÆ. — Generally speaking larger than unimpregnated *Sarcopsyllidæ*. Head relatively small, round above and frequently armed with combs upon

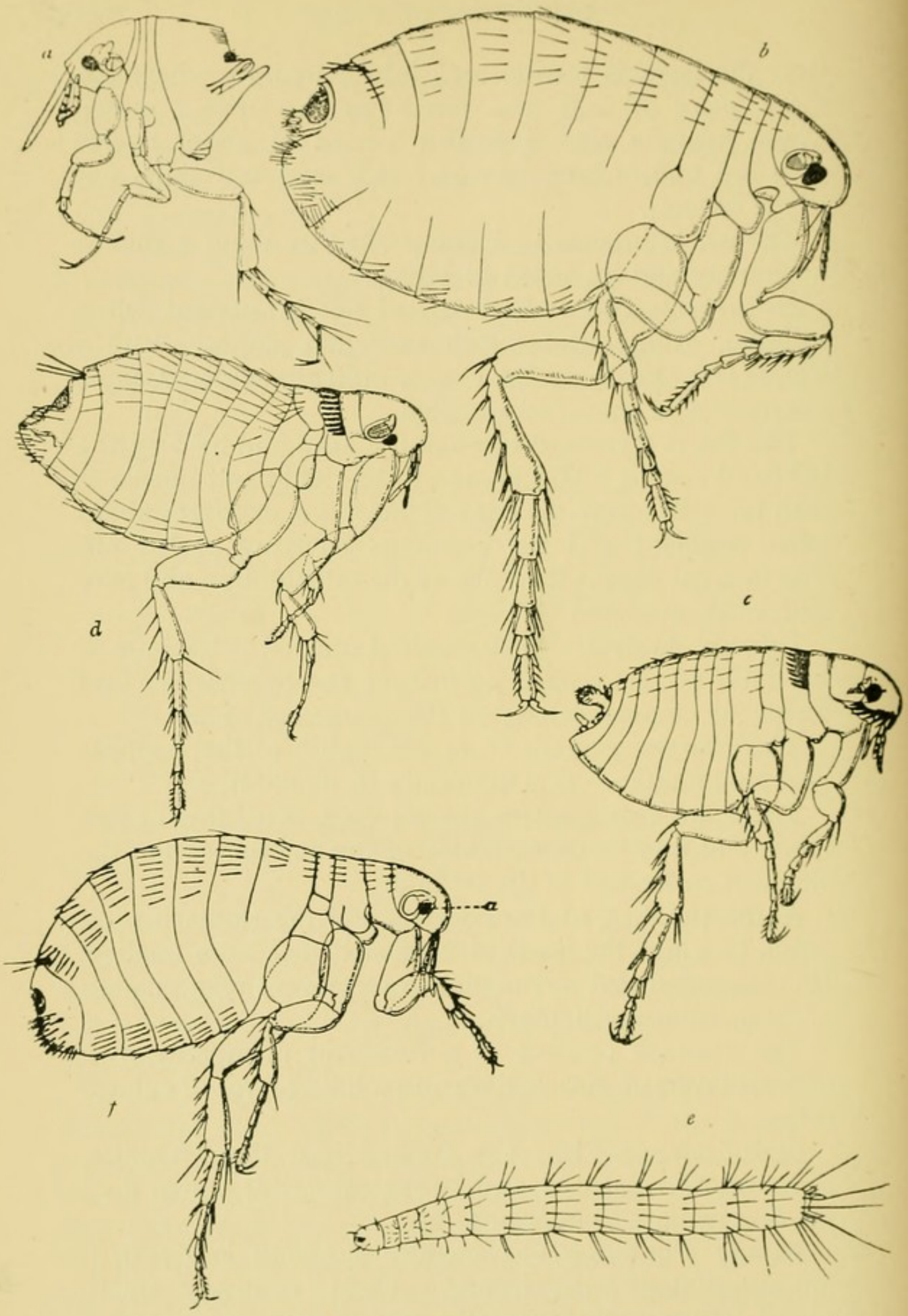


FIG. 125.—*a*, *Sarcopsylla penetrans*; *b*, *Pulex irritans*; *c*, *Ctenocephalus erraticeps*. *d*, *Ceratophyllus*; *e*, Larva of *Pulex*; *f*, *Pulex cheopis*.

the side, or along its inferior border. Labial palps always consisting of four segments. Thorax longer than in *Sarcopsyllidæ*, in some genera having a comb of spines at the posterior border of the prothorax, sometimes also at that of the metathorax. In others there are combs at the posterior border on one or more of the segments of the abdomen. The females never become fixed in the tissues of the host, nor does the abdomen become greatly distended as in *Sarcopsyllidæ*.

Genera of family *Pulicidæ* :—

A. Eyes well developed.	
Comb along inferior border of head and another along posterior border of prothorax	*Ctenocephalus.*
Comb along posterior border of prothorax. No comb on head. Third joint of antenna completely segmented	*Ceratophyllus.*
No combs. Third joint of antenna segmented dorsally only	*Pulex.*
B. Eyes rudimentary or absent.	
Comb on inferior border of head and another along posterior border of prothorax. Spines on posterior aspects of tibiæ arranged in pairs	*Ctenophthalmus.*
Comb on inferior border of head and another along posterior border of prothorax. Spines on posterior aspects of tibiæ arranged in a close set row	*Ctenopsylla.*
Comb on inferior border of head and another along posterior border of prothorax. Combs also on one or more abdominal segments. Body covered with hairs and small spines ...	*Hystrichopsylla.*

Pulex cheopis[1].—This flea has been described under various names, *P. pallidus*, *P. murinus*, &c. It is the commonest flea found on rats in warmer climates, and in some localities is the only flea. It has been found in Marseilles, Italy, Sydney, Philippines, India, Sudan, South Africa and South America.

It is a non-pectinated flea, *i.e.* without combs, resem-

[1] This flea has recently been removed from the genus *Pulex* and placed in the genus *Xenopsylla*. The latter genus is distinguished from the former by the character of the lines on the meso-sternum.

bling the human flea, but differs from it, among other characteristics, in the body being lighter in colour, yellowish instead of brownish, and in the position of the ocular hair, which is situated in front of and above the eye, while in *P. irritans* it is situated in front of and below the eye.

Other rat-fleas are *Ceratophyllus fasciatus*, *Ctenopsylla musculi*, *Ctenocephalus serraticeps*, *Hystrichopsylla talpæ*, as well as many less common species.

Family SARCOPSYLLIDÆ.—Body small, head relatively large, angular or rounded above, never armed with spines. Labial palps unsegmented. Thoracic segments small. Abdomen variable, more or less swollen in the fertilised female. Never any combs on thorax or abdomen. The fertilized female burrows into the skin of the host.

This family comprises two genera, *Sarcopsylla* and *Rhynchopsylla*.

The genus *Sarcopsylla* contains the important parasite, *Sarcopsylla penetrans*—the Jigger or Chigoe. It is a small flea, the male measuring about 1 mm. in length, and the unimpregnated female about the same size. The general colour of the body is brownish. The head relatively to the body is large ; its upper surface slopes obliquely in front, and joins the lower surface at an acute angle. The eye is situated in the front part of the head at the border of the fossa of the antenna.

The perforating and suctorial mouth-parts are well developed, but the maxillæ are very small quadrilateral structures, only seen with difficulty.

The three thoracic segments are very small.

The males and unimpregnated females are parasitic on man occasionally, for the purpose of sucking blood. The impregnated female, however, bores her way into the skin, particularly about the feet, and the abdomen of the insect undergoes great distension following the development of the eggs, the head and thorax remaining unchanged.

The eggs develop on the soil, and the metamorphosis is similar to that which obtains in other fleas.

The Jigger is widely distributed in the Tropics. Originally found only in Central and South America, it was introduced into West Africa about 1872, and in a few years had become disseminated throughout the greater part of Africa.

Another member of this genus, *S. gallinacea*, attacks fowls, particularly about the head, and causes great destruction among them. *S. gallinacea* does not bury itself in the skin so completely as does *S. penetrans*, nor does the abdomen become so greatly distended, as the eggs are laid one by one as they mature.

Order ANOPLEURA or SIPHUNCULATA.

These must be distinguished from *Melophaga* and bird lice. The former belong to the order *Diptera*, whilst the latter are degenerate *Neuroptera* and do not suck blood but live on epidermis.

The members of the order *Anopleura* suck blood by means of a sort of double tubular proboscis, armed with spines which are usually retracted under the head. Eyes simple. Antennæ of 3 to 5 joints. Thorax composed of three segments. Three pairs of legs terminated by strong claws.

There are six to nine segments in the abdomen terminated by two basal lobes in the female and a large triangular penis in the male.

Family PEDICULIDÆ.—The body is flattened. The rostrum is provided with barbed hooklets. The antennæ are inserted in a kind of sinus in the front of the head, and are composed of three, four, or five segments, the number of these being used for the separation of genera. The three thoracic segments are distinctly separated. The abdomen comprises six to nine segments. Legs are terminated by one or two long claws.

The eggs are pyriform in shape, fixed by their smaller end to the bases of the hairs of the host; the larger end is provided with an operculum which the young split off

in emerging. After the eggs are laid the young are hatched out. They resemble the adults and mature rapidly. There is no metamorphosis.

The family comprises several genera, of which we consider only *Pediculus* and *Phthirius*.

Antennæ with five segments. Legs with a single claw	Thorax narrower than abdomen	Distinct neck ...	*Pediculus.*
		No distinct neck	*Hæmatopinus.*
	Thorax broader than abdomen ...		*Phthirius.*

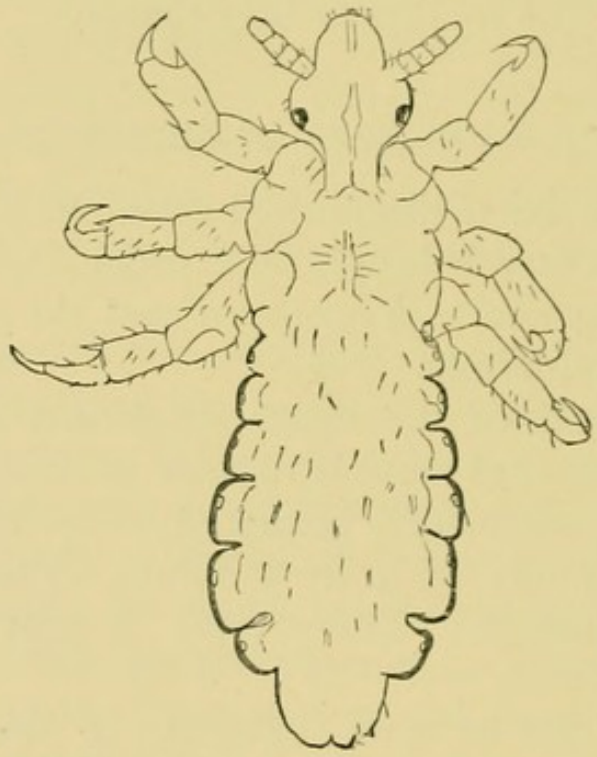


FIG. 126.—*Pediculus vestimenti.*

Pediculus capitis (Head-louse).—The male is 1 to 2 mm. in length, the female somewhat larger. The head is triangular in shape, the thorax narrower than the abdomen. The abdomen has eight segments, is pigmented all round and is hairy.

The female lays fifty to sixty eggs which hatch out in about seven days. The young are able to reproduce in seventeen to twenty days after birth.

Pediculus vestimenti.—(Body-louse).—Male 2 to 3 mm. in length, females larger. Head somewhat rounded.

Antennæ longer than in *P. capitis*. The thorax is as wide as the abdomen, which is not hairy or pigmented.

The female lays seventy to eighty eggs, which hatch out in three to eight days.

Phthirius inguinalis (Crab-louse).—The genus *Phthirius* includes a single species. This species is peculiar to man. It may be found in any hairy part except the scalp, but is most commonly found on the pubic hairs.

Male 0·8 to 1·0 mm. in length : female 1 to 1·5 mm. The head is large and provided with two long antennæ. Thorax is broader than the abdomen and shows no trace of separation from it. There are six segments in the abdomen.

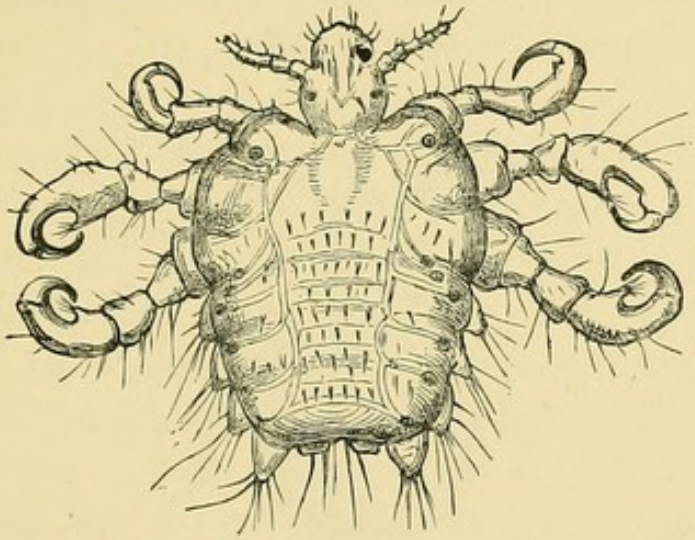


FIG. 127.—*Phthirius inguinalis.*

The female lays ten to fifteen eggs, which hatch out in six to seven days.

An interesting observation made by Murray, that the pediculi of different races differed in colour and even in certain anatomical details, has since been confirmed by many observers. This is regarded as a kind of protective mimicry. From observations on these parasites in the Federated Malay States, we believe that the pediculi of one race, for example the Chinese, do not pass readily, if at all, to another race, for example, the Tamils.

Order HEMIPTERA or RHYNCOTA.

This order includes insects of very dissimilar characteristics. The rostrum is the feature by which they are

most easily distinguished. This organ is a modification of the inferior lip, it is tubular in character, and in a state of repose is folded up under the head and thorax. The rostrum encloses the hair-like penetrating parts.

There are two important families belonging to this order—*Cimicidæ*, *Reduviidæ*.

Family CIMICIDÆ.—This family of the *Hemiptera* belongs to the division known as *Heteroptera*, as there is a marked difference between the two pairs of wings.

The family is characterized by the absence of ocelli, by the wings being rudimentary, very short and broad, so that the abdomen is left uncovered. The head is short and broad, and the rostrum is received in a groove beneath the head. Tarsi are three-jointed. Antennæ long and composed of four joints.

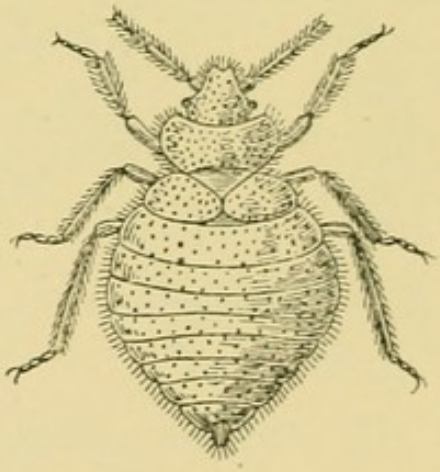


FIG. 128.—*Cimex lectuarius.*

Genus *Cimex* comprises at least two species parasitic on man, *C. lectuarius* and *C. rotundatus*. Considerable interest centres about these, in view of Patton's suggestion that they are the carriers of the parasites of kala-azar, and the older hypotheses that they may convey relapsing fever and leprosy. There is no proof in support of these hypotheses.

Cimex lectuarius.—This insect is oval in form, brownish-red in colour, with a much flattened body. It measures 4 to 5 mm. in length and 3 mm. in breadth. Eight abdominal segments.

The eyes are simple and there are no ocelli. The elytra are rudimentary and lie on the metathorax. The prothorax is semilunar in shape, its anterior angles being considerably developed and coming close up to the eyes.

The female lays about fifty whitish eggs, three or four times a year. The complete development takes about eleven months, during which time it casts its skin five times. The young larvæ are at first pale white.

Bed-bugs live in cracks in the walls, under carpets, behind pictures, wall papers, &c., and only come out at night to suck blood, which is their only food. They may live from five to ten weeks, or even longer, without food. The average duration of life is three to four months.

Cimex rotundatus.—This species was originally described from the island of Reunion in 1852. Patton has shown that it is distributed throughout India, Burmah and Assam.

It is rather smaller than *C. lectuarius*, and in general outline less rounded. The main feature, however, is the character of the prothorax, which has well-rounded borders and gives to the animal an appearance of rotundity. The anterior angles of the prothorax do not come up so close to the eyes as in *C. lectuarius*.

Other species of *Cimex* described are *C. ciliatus* from Kasan, Russia, which is smaller than the common bed-bug, yellowish-red in colour, and thickly covered with hairs, *C. columbarius*, *C. hirundinus*, and *C. inodorus*, which have been found attacking birds.

Family REDUVIIDÆ.—This family is a large one and of world-wide distribution. The members of this family are mostly of large size and are distinguished from the cimicidæ by the fact that the rostrum curves backwards under the head and does not lie closely applied to the under surface.

Some of the Reduviidæ are useful as they are predatory on other insects.

To the important genus Conorrhinus belongs *C. sanguisuga*, a large South American bug. This sucks blood and has been shown to be the host of a human trypanosome, *T. cruzi*.

CHAPTER XVI.

ARACHNOIDEA—TICKS, MITES, POROCEPHALUS. CRUSTACEA—CYCLOPS.

The ARACHNOIDEA are a class of the *Arthropoda* readily distinguished from insects, as they have four pairs of legs in the adult stage. The head and thorax are usually fused into a compact mass, the *cephalothorax*. The *abdomen* is generally without appendages; it may be segmented or unsegmented; it is generally distinct from, but may be fused to the cephalothorax. Compound eyes such as occur in insects are never present—the *eyes* when present are always simple. Respiration may be by means of tracheæ or cutaneous. The *genital orifice* is in the middle or in the anterior half of the ventral surface and is distinct from the alimentary canal. The sexes are distinct. In most cases the newly hatched young are essentially like the adults.

The class *Arachnoidea* is divided into a number of orders, including *Scorpionidæ* (scorpions), *Pseudoscorpionidæ* (book scorpions), *Pedipalpi* (whip-scorpions), *Araneidæ* (spiders), *Acarina* (mites and ticks), and certain aberrant orders, as *Linguatulida* or *Pentastomiidæ*.

Order ACARINA (Mites and Ticks).—These are small Arachnoids, usually parasitic. They occur in earth, in fresh or salt water, or on animals or plants. They feed on the organisms they infest or upon organic matter.

The abdomen and cephalothorax form a fused mass. The mouth-parts are formed for biting or for puncturing and sucking, according to the mode of life. Respiration may be cutaneous, but in most there are tracheæ with two

stigmata. Many of the young have only three pairs of legs when hatched, but after moulting have four pairs.

The *Acarina* which cause disease or act as carriers of disease are divided into families as follows :—

Non-vermiform acarina	(*a*) Legs inserted directly into integument	Legs with six joints	Tracheæ opening in the posterior part of body	Cheliceræ with hooklets	*Ixodidæ.*
				Cheliceræ didactylous or styliform	*Gamasidæ.*
	(*b*) Legs articulated on distinct epimeræ ...		Tracheæ opening in anterior part of body	Cheliceræ styliform or with hooklets; palps free	*Trombididæ.*
			No tracheæ ...	Cheliceræ didactylous; palps cylindrical and adherent to inferior lip	*Sarcoptidæ.*
Vermiform acarina	Legs articulated on distinct epimeræ	Legs with three joints	No tracheæ ...	Cheliceræ styliform; palps with hooklets	*Demodicidæ.*

IXODIDÆ.

The family *Ixodidæ* is composed of comparatively large Acarines with leathery skins. They have flattened bodies, but after sucking blood become much distended. The mouth-parts, which are characteristic, comprise a median penetrating organ, the hypostome, armed with recurved teeth, two rod-like organs surrounded at their bases by a sheath, the *cheliceræ*, each of which is terminated by a process bearing large hooked teeth. On either side of the cheliceræ and hypostome lies a four-jointed palp, which in some genera is closely applied to the piercing organs and appears to act as a sheath for them, but in other genera is quite free (fig. 132).

Each leg consists of six main segments known respectively as *coxa*, *trochanter*, *femur*, *patella*, *tibia* and *tarsus*. The trochanter may have spines or teeth. The tarsus bears on its terminal segment two claws (fig. 129). There are two sub-families, *Ixodinæ* and *Argasinæ*.

In the sub-family *Ixodinæ* the skin of the dorsal surface is thickened to form a hard, leathery, chitinous plate, the *scutum* or dorsal shield, which in the male covers almost the whole of the dorsum, while in the female it covers only the anterior portion of the dorsum. This arrangement serves to distinguish males from females. In the sub-family *Argasinæ* these shields are not present.

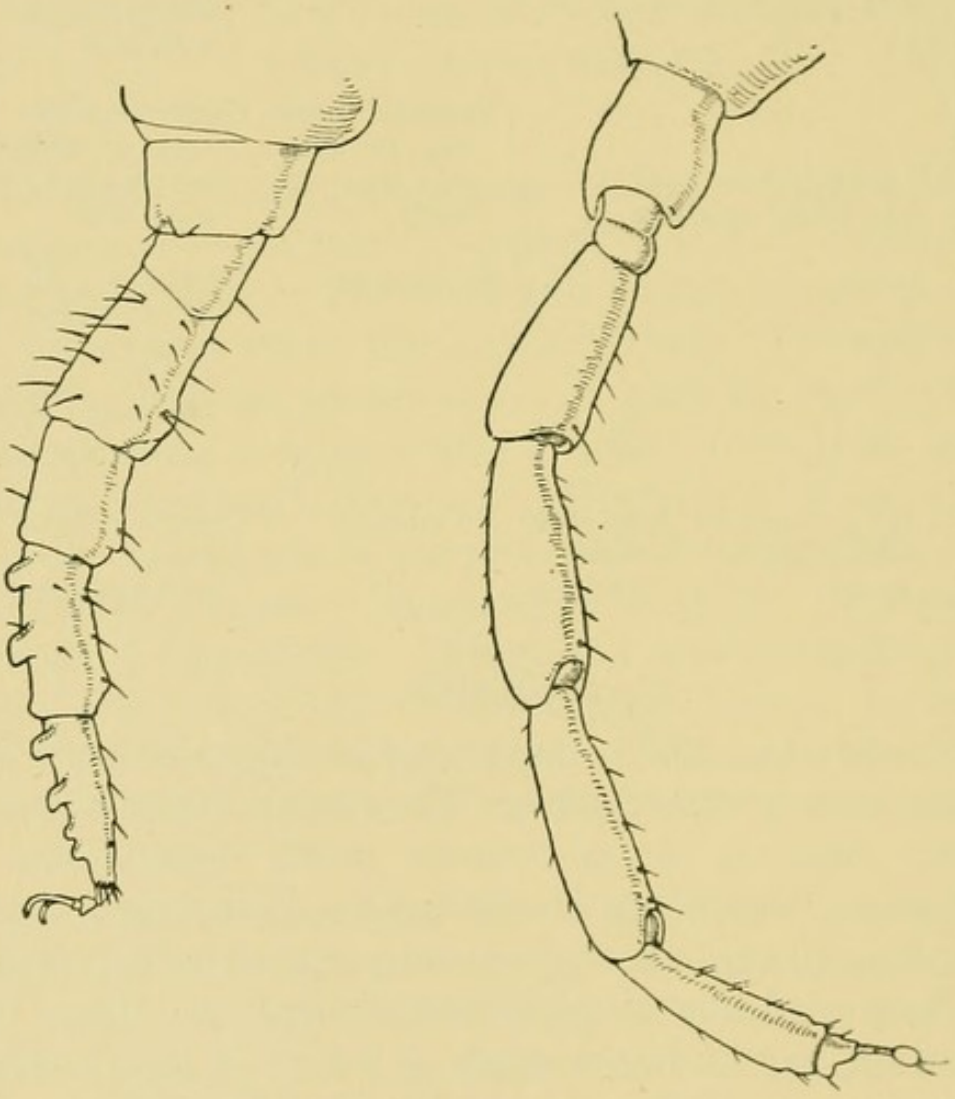
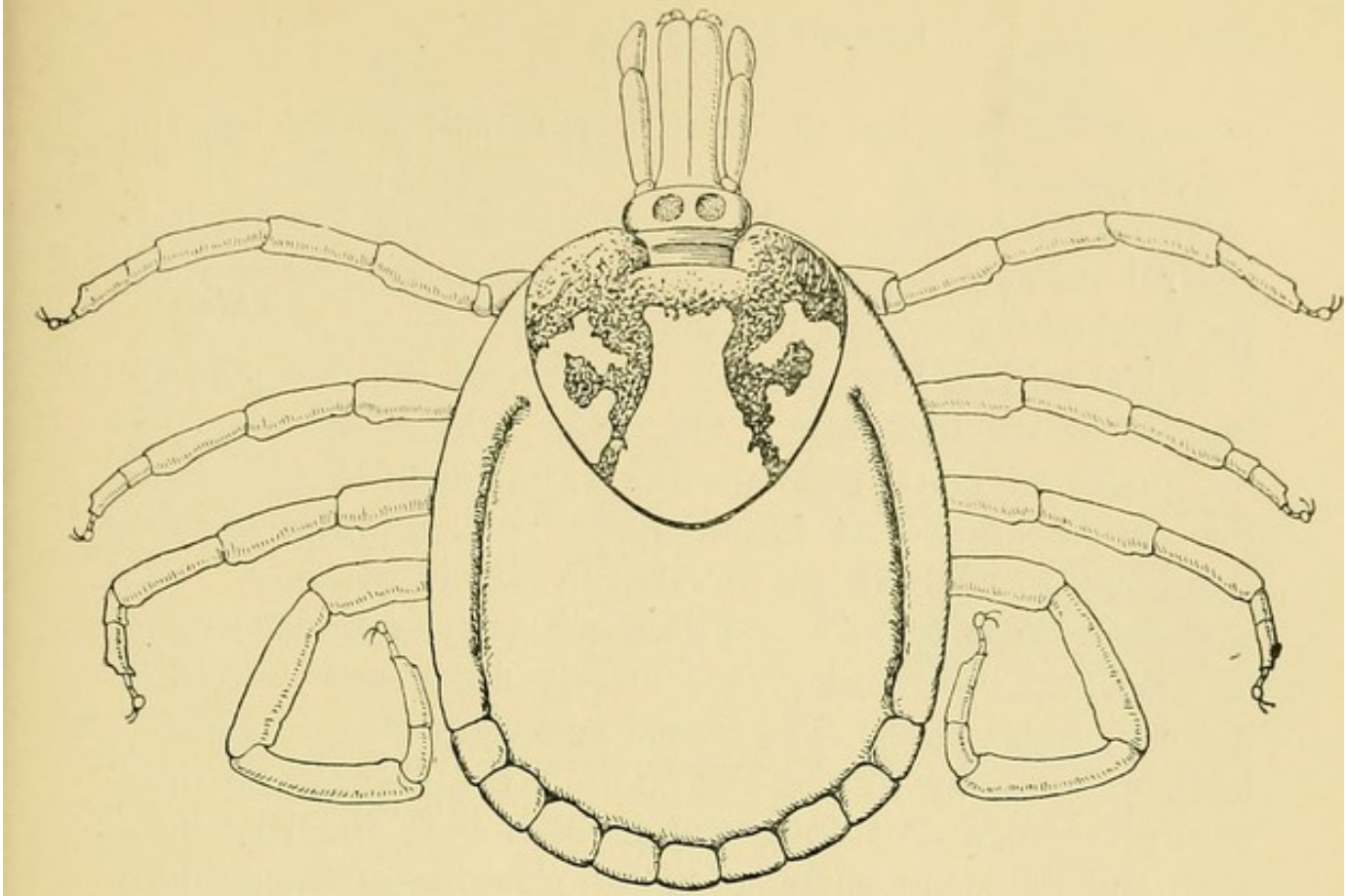


FIG. 129.

Eyes are not always present. They consist of a simple lens only and may be globular or flat. In the *Ixodinæ* they are situated at the margins of the dorsal shield; in the *Argasinæ* on a ridge above the coxæ.

The stigmata or openings of the tracheal system are situated behind the level of the fourth pair of legs in



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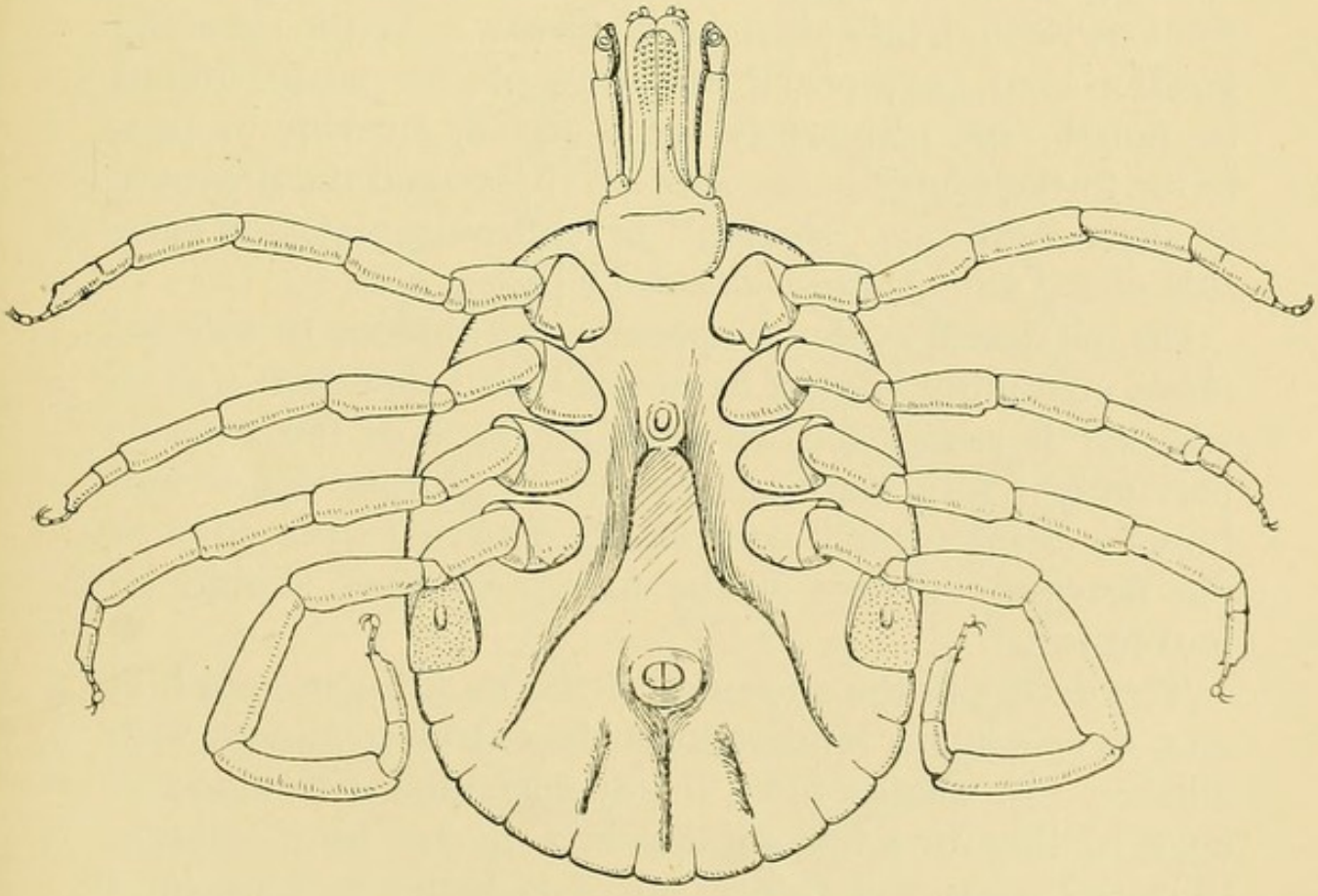


b

FIG. 130.—*Ixodina* (Female). *a*, Dorsal aspect, showing shield; *b*, ventral aspect.

Ixodinæ and between the third and fourth pair of legs in the *Argasinæ*.

The *genital orifice* is situated in the middle line on the ventral surface, a short distance behind the rostrum at the level of the second and third coxæ.

In the middle line, behind the level of the *last* pair of legs, is the valvular *anus*. On either side of the anus in the males of certain genera are situated thickenings of the cuticle known as *adanal plates:* the number, shape, and position of these are of value in classification.

Examination of Ticks.—Ticks can be examined living or dry, and the main points clearly made out with the aid of a hand lens. The dorsal and ventral aspects must be examined in turn. The points to observe on the ventral surface are along the median line, the rostrum, with the palps on each side; the opening of the genital organs in the anterior half of the body and the anal opening in the posterior half, and any deep furrow in front or behind this anus, *anal furrow*. At the sides the position and any special markings on the coxæ should be noted; the respiratory area, *stigma*, anterior or posterior to the fourth coxæ and its shape, and the presence or absence of any "shields" or chitinous plates on each side of the anus, *adanal shields* or plates.

On the dorsal aspect the presence or absence of a *dorsal shield* or scutum should be noticed, and its extent; as to whether it covers the whole of the back or the anterior part only. Any markings, bosses or protuberances, and how far these form a continuous pattern to the edge of the dorsum, or vary at the margins. Eyes should be looked for.

The details in the structure of the mouth-parts are best seen in specimens rendered translucent by treatment with alkalies. In smaller ticks, if not distended with blood or eggs, boiling for a few minutes in a 10 per cent. solution of caustic soda will suffice, but it is better to leave for a longer time in the solution. Many ticks contain chromatin and are not readily decolorised. After the treat-

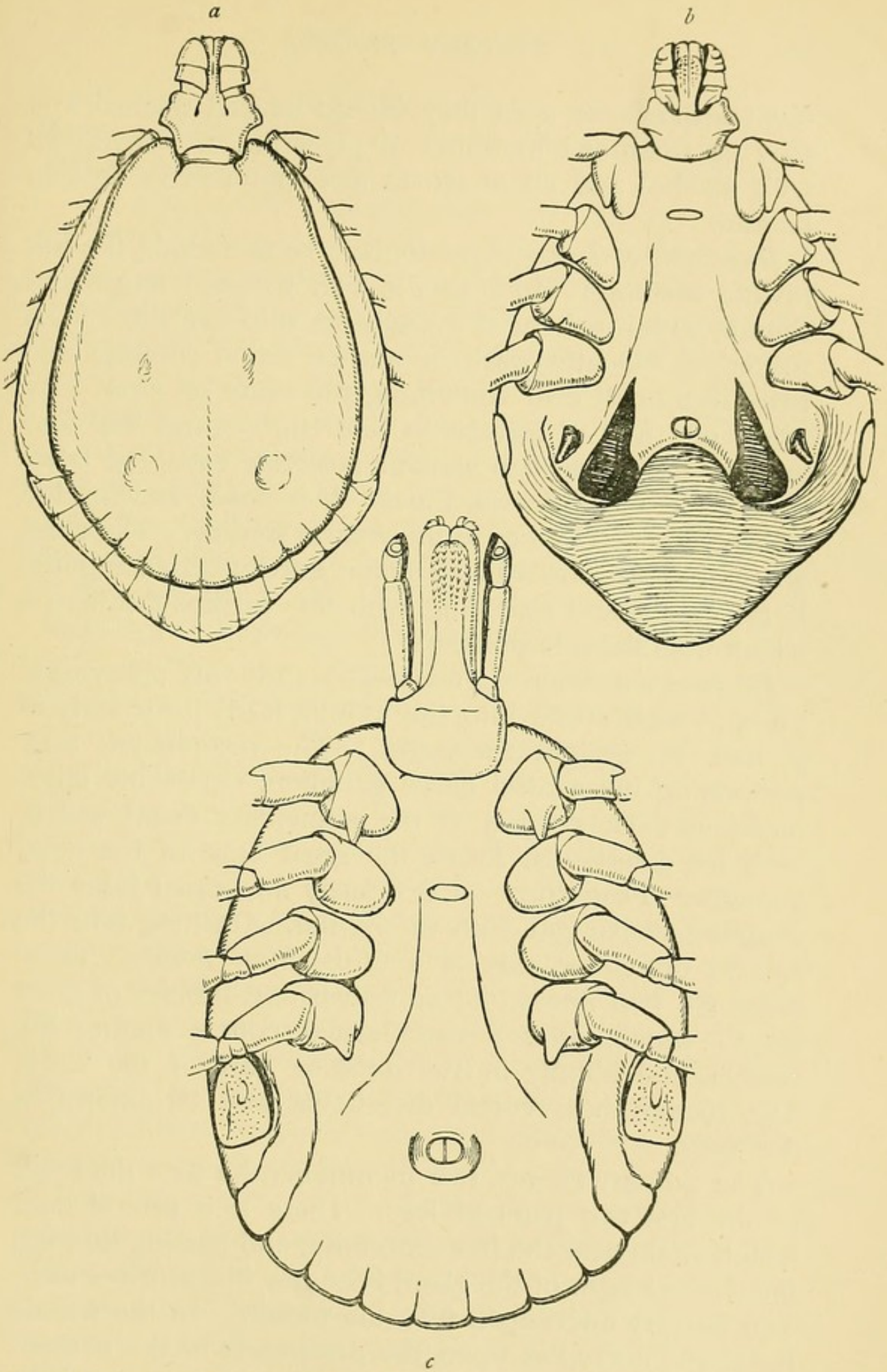


FIG. 131.—*Ixodina* (Males). *a*, Dorsal aspect, showing dorsal shield; *b*, ventral aspect in species with adanal plates; *c*, ventral aspect in species without adanal plates.

ment with caustic soda they should be well washed, and may be examined mounted in glycerine or dehydrated with alcohol and oil of cloves and mounted in Canada balsam.

Dissection of Ticks.—Christophers recommends the following method: Holding the tick between finger and thumb, with a pair of sharp scissors snip fine slices from the edges. Gently wash in normal saline solution and place in a small dish containing the same solution. By using one pair of forceps to seize the ventral flap and another the dorsal flap at their posterior ends, the latter may be dragged forward over the head, leaving the viscera attached *in situ* to the ventral surface. The chief anatomical features can be made out by displacing the larger organs and removing with fine forceps the tissues which hold these in position.

Internal Anatomy of Ticks.—The chitinous pharyngeal pump, which opens into the mouth, leads posteriorly to a narrow, straight *œsophagus.* The œsophagus, after perforating the central nerve ganglion, enters the enormous saccular portion of the alimentary canal which, with its diverticula, forms the great bulk of the body contents. Posteriorly an extremely fine canal joins the central saccular gut with the *rectum.* Opening into the rectum on either side is a *Malpighian tubule.* These latter are two fine white or transparent tubules of great length which, after a complicated course among the viscera, end blindly in the anterior part of the body. The rectum has several diverticula and terminates in the *anus.*

The *salivary glands,* two in number, lie over the bases of the first two pairs of legs. There is a central duct which arises near the free extremity, and passing through the whole length of the gland becomes the *salivary duct.* The salivary ducts open into the mouth. In the female the single *ovary* lies upon the diverticula of the alimentary canal in the posterior portion of the body. Leading from it on either side are the long coiled *oviducts.* In

front of the ovary is the *spermatheca*. In the male, in the same position as the ovary in the female, is a thin delicate tubule, the *testis*.

Life-history.—The ova of ticks are laid in recesses in the soil; in the *Ixodinæ* the number of these is 2,000 to 4,000, in the *Argasinæ* a few hundreds only. After a variable period the six-legged *larva* is hatched out. The larvæ of the *Ixodinæ* attach themselves at the first opportunity to some vertebrate, from which they suck blood. In some ticks the larva, having gorged itself with blood, drops off upon the ground, and after a few days casts its skin and emerges as the eight-legged *nymph*, which differs from the adult form mainly in the absence of a genital opening. The nymph in turn attacks a fresh host, and, having fed, drops off, and after moulting becomes the adult tick. *Hæmaphysalis leachi* undergoes its development in this way. In other ticks, such as *Boophilus annulatus*, the metamorphosis from larva to nymph takes place on the host. The nymph may or may not leave the host before reaching the adult stage. In *Ornithodoros moubata* and *O. savignyi* the larvæ do not suck blood.

Ticks as Carriers of Disease.—Ticks are known to be carriers of several species of hæmatozoal parasites belonging to the genera *Piroplasma* and *Spirochæta*. In man a spirochæte (*S. duttoni*) which is the causative agent in African relapsing fever is carried by a tick, *Ornithodoros moubata*, and an analogous disease of fowls, also due to a spirochæte, is transmitted by *Argas persicus*. In mammals a number of diseases conveniently grouped under the name Piroplasmoses are carried by ticks belonging to the sub-family *Ixodinæ*.

It is important to note the stage in its development at which a tick may transmit parasites, as this differs according to the species of parasite and the species of tick concerned. For example, larvæ of *Boophilus annulatus*, from an infected mother, are able to transmit Texas fever, while in the case of *Hæmaphysalis leachi*, one of the carriers of canine piroplasmosis, the larvæ and nymphs

are not infective, and only the adult can convey the disease; again, in the case of *Amblyomma hebræum*, which conveys the parasite of heartwater in sheep and goats, the nymph is infective if fed as a larva, and the adult if fed as a nymph.

Systematic Classification of Ticks.

Rostrum on ventral surface of body not visible from above in the adults. No dorsal shields or adanal plates. Stigma in front of fourth coxa. Palps free or not grooved.....Sub-family *Argasinæ*.

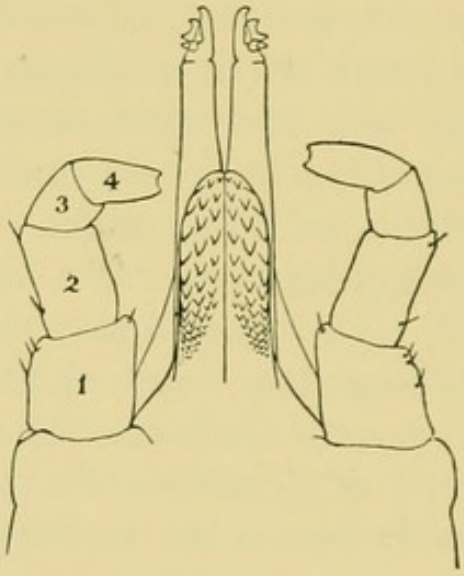


Fig. 132.—Mouth-parts of *Ornithodoros*.

Rostrum terminal. Dorsal shields present. Adanal plates in males of some genera only. Stigma posterior to fourth coxa. Palps grooved on internal aspect and usually closely applied to rostrum .....Sub-family *Ixodinæ*.

Sub-family *Argasinæ*.

Body with sharp edges. No eyes. No deep furrows on ventral surface. Skin wrinkled. Pattern of marking on the edge differs from that on the remainder of the dorsum ...Genus *Argas*.

Body with thick edges. Eyes present in some species. Often deep furrows on ventral surface. Skin covered with bosses and the pattern of these is continued to the edge...Genus *Ornithodoros*.

Sub-family *Ixodinæ*.

Palps long, the second joint much longer than broad .....Group I. *Ixodæ*.

Palps short, the second joint much broader than long .....Group II. *Rhipicephalæ*.

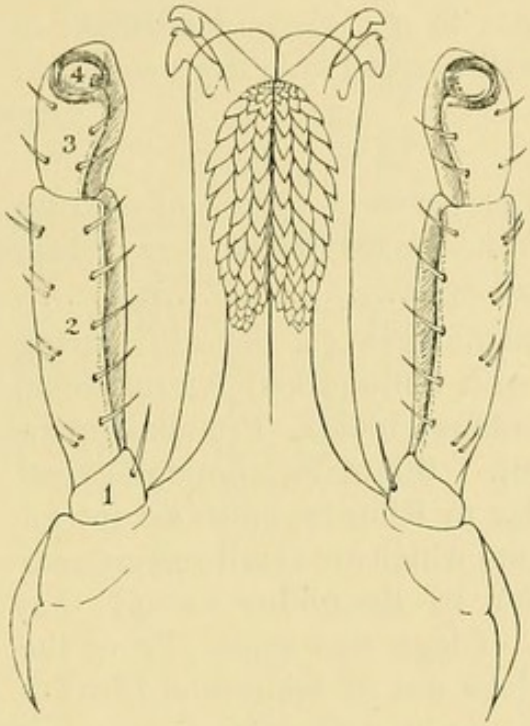


FIG. 133.—Mouth-parts of *Ixodes*.

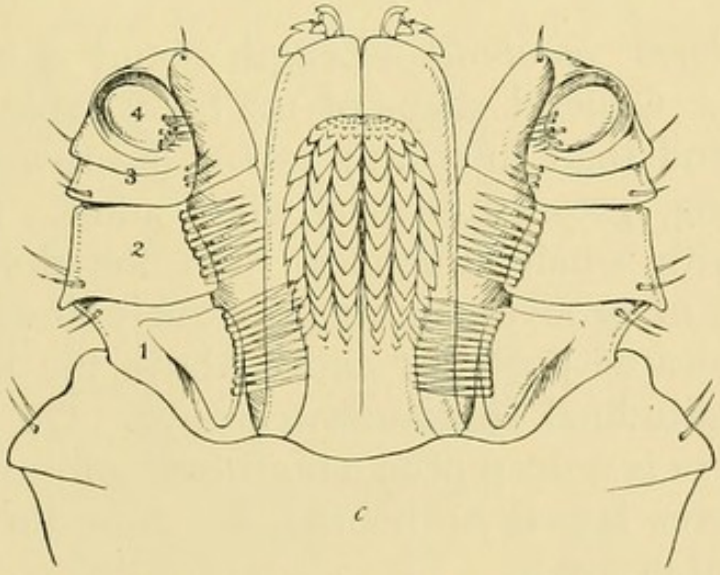


FIG. 134.—Mouth-parts of *Rhipicephalus*.

Group I. *Ixodinæ*.

1. Anal furrow in front of anus. No eyes ...Genus *Ixodes*.
2. Anal furrow behind anus, often continued laterally to genital furrow (fig. 130).
   (*a*) Adanal plates present in ♂. Eyes present Genus *Hyalomma*.

(*b*) No anal plates in ♂.
(*a*) With eyes.....Genus *Amblyomma.*
(*β*) Without eyes .....Genus *Aponomma.*

Group II. *Rhipicephalæ.*

1. No eyes present.
No adanal plates in ♂. Second segment of palp has a well-marked lateral projection...
Genus *Hæmaphysalis.*

2. Eyes present.
(*a*) No adanal plates in ♂. Base of rostrum quadrilateral. Coxæ of last pair of legs are always large..... *Dermatocentor.*
(*b*) With adanal plates in ♂. Base of rostrum hexagonal with well-marked lateral angle.
(1) Two adanal shields. Pre-anal furrow present. Stigmata, comma-shaped *Rhipicephalus.*
(2) Similar to Rhipicephalus except for stigmata which are small and circular *Boophilus.*
(3) Similar to Boophilus except that joints of legs and especially of the hind legs are of enormous breadth *Margaropus.*
(*c*) No adanal plates. Coxæ of last pair of legs produced into enormous spines: more marked in ♂ than in ♀ ..... *Rhipicentor.*

Ornithodoros moubata.—Length about 8 mm., width 6 to 7 mm. General shape of the body ovoid, somewhat wider behind than in front; yellowish-brown in colour when young, greenish-brown when adult. Integument studded with small tubercles. On the dorsal surface three pairs of grooves running obliquely downwards and inwards towards posterior end. Above the bases of the legs a longitudinal supra-coxal groove. On the ventral surface there is a deep pre-anal groove joining the supra-coxal grooves laterally; behind the anus three pairs of longitudinal grooves.

There are no eyes present. Stigma semilunar in front above the supra-coxal groove. Fourth leg one and a half times as long as the first; tibiæ and tarsi of first three pairs with three teeth on the upper side.

This species is widely distributed in Central Africa from

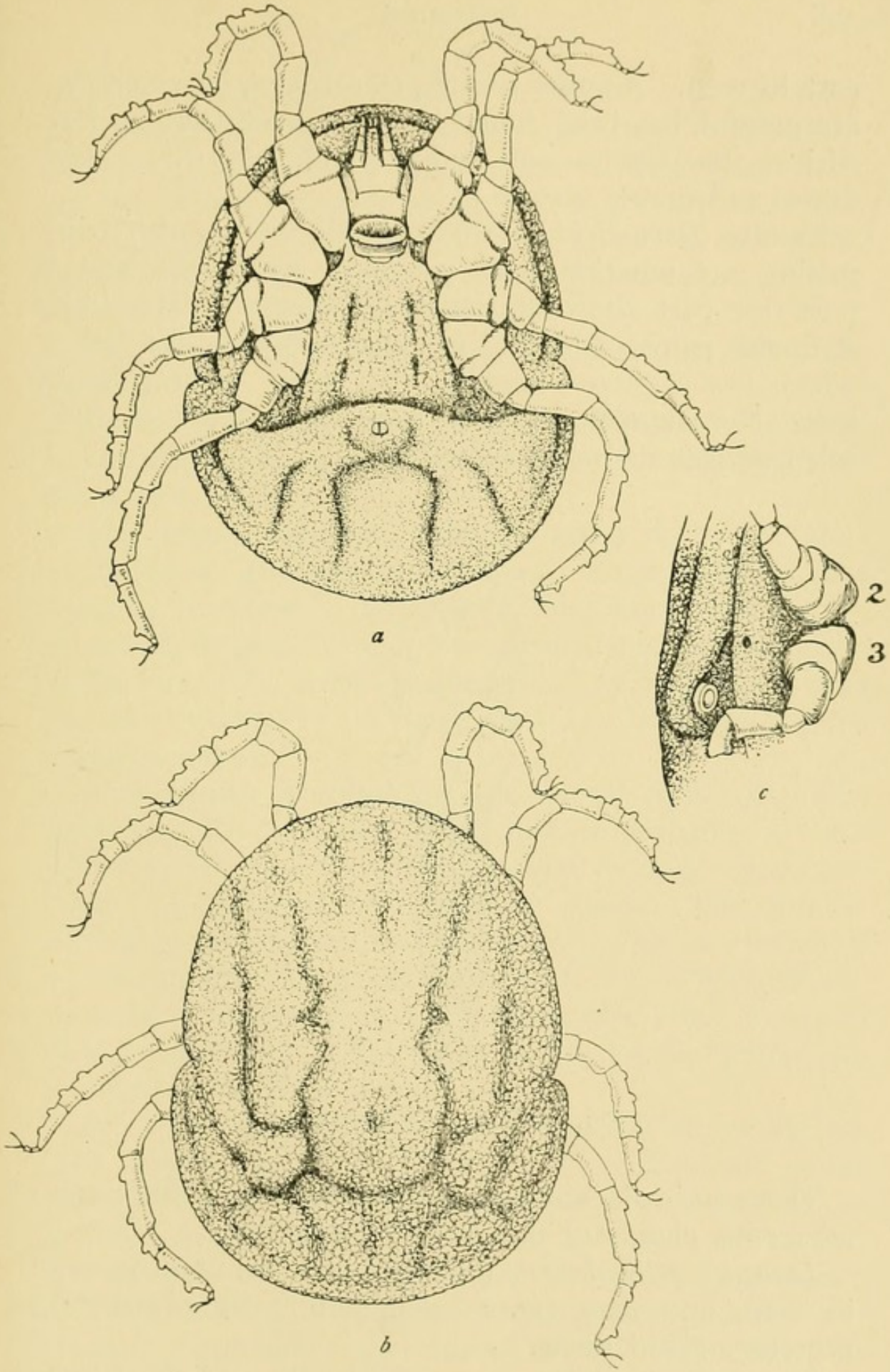


FIG. 135.—*Ornithodoros savignyi*. *a*, Ventral aspect ; *b*, dorsal aspect ; *c*, lateral aspect between second and third pair of legs.

east to west. Another species, *O. savignyi*, in which eyes are present, has been found in Somaliland, German East Africa, the Congo and India. (Fig. 135, *O. savignyi*, dorsal and ventral surface.)

Family *Demodicidæ*.—Body small, vermiform, comprising an anterior part provided with legs and a posterior part showing transverse striation. Mandibles styliform, palps consisting of three segments, the last of which has a curved hooklet. No eyes. Four pairs of legs. No stigmata. Larvæ have three pairs of rudimentary legs or may be footless.

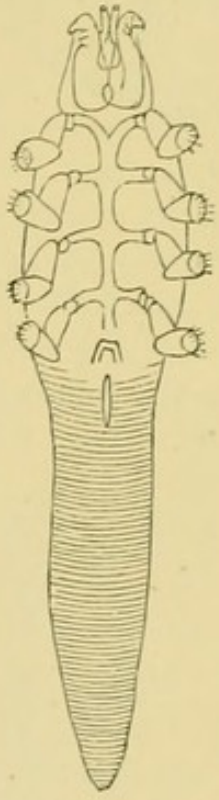


FIG. 136.—*Demodex follicularum*.

These animals are parasites of the hair follicles and sebaceous glands of the skin of mammals.

Demodex follicularum (fig. 136) is a common parasite in man, and may cause inflammation by obstructing excretory gland ducts.

The male is about 3 mm. in length and the female 4 mm. in length.

Order LINGUATULIDÆ.—Probably degenerate *Arach-*

noidea. They are worm-like in form and externally annulated; there is no distinction between head, thorax, and abdomen. No oral appendages are present, but there are two pairs of movable hooks near to the mouth, which are regarded as remains of the antennæ and palpi. Alimentary canal simple, no Malpighian tubes. There are no sense organs or tracheæ.

The sexes are distinct; the males smaller than the females.

Adults live in the nasal cavity, frontal sinus, or lungs of the dog, wolf, and other animals; occasionally they are found in man.

The females lay eggs, which if taken up by an intermediate host give rise to embryos. These embryos pass from the intestine to the liver or lung where they encyst, moult, and pass through a larval stage, in which the characters of the adult are developed. Finally they reach the nasal cavities of the same or a new host and become sexually mature.

Porocephalus armillatus. The larval form originally known as *Pentastomum constrictum* is probably a *Porocephalus*. It has been found on several occasions encysted in the livers of negroes in Africa. The number of rings is never more than twenty-two, which corresponds to the number of rings in the larval forms of *Porocephalus armillatus*. According to Sambon the adult forms of this group occur in the lungs of the Royal Python and in those of the Nose-horned Viper.

Class CRUSTACEA are not as a rule carriers of disease. The larger ones are edible, and occasionally some, especially prawns and shrimps, are believed to harbour typhoid bacilli. From the point of view of carriers of disease the Copepoda are of most importance. Order COPEPODA: Elongated crustaceans, usually with distinct segments. No dorsal shell. Five pairs of biramose thoracic appendages, the last of which may be rudimentary. Two pairs of antennæ. Abdomen without limbs. The females carry the eggs in external ovi-sacs (fig. 138).

To this order belongs the freshwater Cyclops ; a tropical species of this genus is the intermediate host for *Filaria medinensis*, the well-known Guinea-worm.

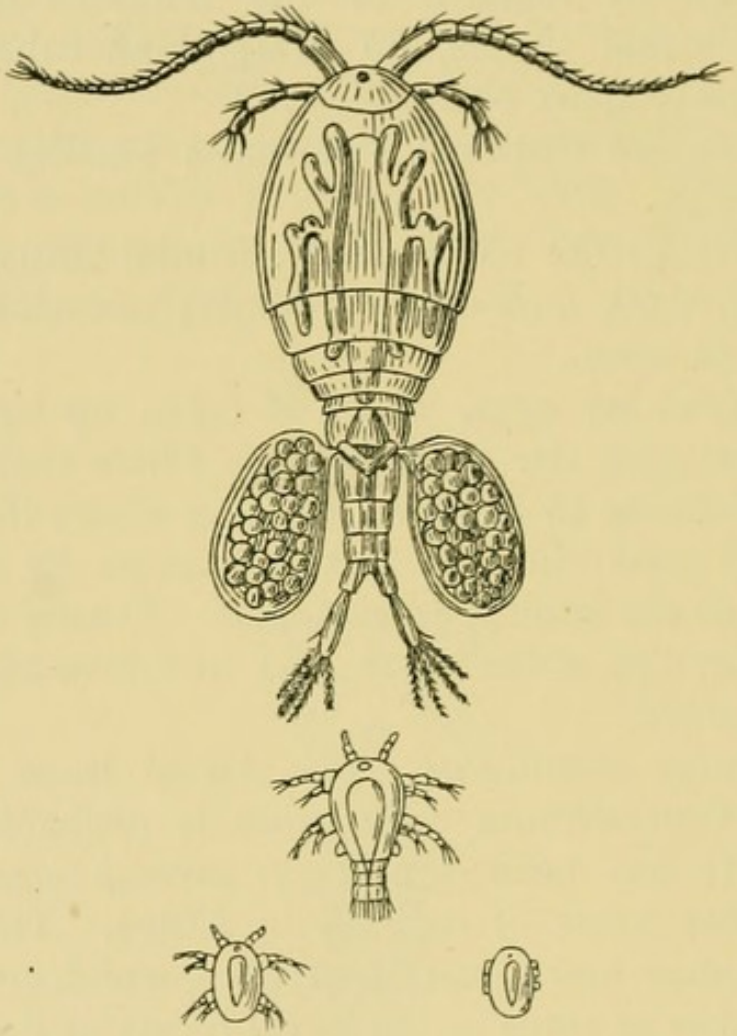


FIG. 137.—Cyclops in various stages.

CHAPTER XVII.

Pigment Deposits and Degenerations in Tissues.

The pigment deposits are in the main those derived from altered hæmoglobin. Of these the most important is melanin, the residue from the digestion of the red corpuscles by the parasites of malaria. This pigment is taken up by the leucocytes and other phagocytic cells and deposited in various parts of the body.

As seen in the interior of a parasite it is at first finely divided and varies in colour according to the species of parasite, later it may be aggregated in each parasite into a mass. In the large masses which are found in the tissues it is black with a slight greenish tinge, it is insoluble in acids, in alcohol and in ether, but is readily dissolved by alkalies. It is very stable and is not destroyed by putrefaction. In solution in alkalies it has a distinctly greenish tinge, but shows no characteristic bands when examined with the spectroscope. From this solution it can be precipitated by the addition of acids, and by repeated solution in alkaline fluids and precipitation with acids, can be isolated in an impure condition. Analysis shows that it is very rich in iron, more so than hæmoglobin or any of the other hæmoglobin derivatives. The iron is in firm organic combination, and does not give the inorganic iron reactions. After frequent precipitations it becomes brown in colour, and the same brownish tinge can be observed in pigment left in certain organs as a result of old malarial invasion.

Melanin in an acute attack of malaria is found (*a*)

in parasites, (*b*) in leucocytes, and (*c*) in certain cells of the connective tissue type in the liver, in the parenchymatous cells in the spleen, and occasionally in the nuclei of the endothelium of the capillaries in various parts of the body, such as the brain, liver, suprarenals, &c. In an invasion of short duration the only pigment found is in small granules, often no larger than those set free after the breaking up of a sporulating body. At this stage the cells containing the pigment stain normally and do not differ in appearance from other cells of the same type which do not contain pigment.

When death occurs some little time after the termination of an attack of malaria the distribution of the melanin deposits is much more limited. It will not be found in the red corpuscles as there are no parasites, nor in the leucocytes or endothelial cells, but will be restricted to the connective tissue cells in the liver and the parenchyma of the spleen. The pigment itself is now aggregated into larger masses, though these may be seen to be composed of separate granules. The cells stain faintly with ordinary stains and often appear to be shrunken or distorted. If examined still later the pigment will only be found in blocks or masses, and the cells containing these blocks will not take basic stains, and appear merely as an outline round the pigment masses, some of which appear to be free. The change to a brownish colour, particularly at the edges of such masses, is sometimes to be noted.

Later, even months or years after the malarial attack, pigment may be found. If present it will be imbedded in the fibrous stroma, and no trace of the cells will be seen. At this stage the spleen is usually the only organ in which the pigment will be found.

A consideration of these changes will show that the date of a malarial invasion or invasions can be inferred from the melanin deposits in the organs if the state of division of the pigment, the staining reactions of the cells containing the pigment, and the situation of the pigment be observed.

It cannot be concluded because no pigment is present that there has not been antecedent malaria. We have proof that pigment is rapidly removed from all organs except the liver and spleen (and occasionally the lymphatic glands), and also that it may be removed from either the liver or spleen. In all recent attacks both organs contain pigment; after a longer interval it may be present in the spleen only, or rarely in the liver only.

In the older cases when malarial infections have not occurred for a prolonged period, the amount of melanin found is usually small. In some cases, even a few weeks after an attack of malaria, the amount of melanin is so small that careful search is required to reveal it. It seems probable that as long as the pigment is contained in living cells it is fairly readily removed.

In many cases there is evidence that the pigment (melanin) deposited is the result of several distinct attacks, as in the same specimen finely divided pigment in cells which stain normally, coarse pigment in cells that stain poorly, blocks of pigment with no traces of a containing cell, and pigment in between strands of fibrous tissue, can all be seen.

Melanin is the only pigment which is characteristic of malaria.

Another pigmentary deposit of a bright yellow colour is often found in the organs in cases of malaria, but this is also found in pernicious anæmia, in the anæmia of ankylostomiasis, and in other cases where hæmolysis or blood destruction has taken place, such as trypanosomiasis, kala-azar, and blackwater fever, as well as in some cases of poisoning.

This yellow pigment differs from melanin not only in colour, but in that it is insoluble in alkali as well as in acid. It appears to be slightly soluble in alcohol, Whether or not it contains iron is difficult to ascertain. as it is frequently associated with other substances containing iron in inorganic combination. When found alone, it usually does not give the reactions for inorganic iron.

This yellow pigment is found in the true hepatic cells in the secreting cells of the kidney, particularly in the first part of the convoluted tubules, and in the spleen.

It is evidence of blood destruction from any cause, whether acute, as in blackwater fever, or chronic, as in pernicious anæmia or ankylostomiasis.

Both these pigment deposits can be observed without cutting sections by making "squash" preparations of pieces of the organs, but the arrangement is better shown in sections.

Merely to detect the pigment, no stain is needed, but to show the character of the cells containing the pigment, it is well to stain lightly. Hæmatoxylin gives good results, but a better stain is carmine, as both the melanin and the yellow pigment stand out better against the red background. Thionin should not be used, as it has an affinity for these pigments or the protoplasm surrounding them.

The method of staining with carmine is as follows :— Having removed the paraffin in the section with xylol, the xylol with spirit, and the spirit with water, stain for five minutes with borax or lithia carmine. Pour this off, and, without washing with water, treat with acid alcohol (1 per cent.) for two minutes. Remove this by rinsing several times with ordinary spirit, treat with oil of cloves, to clear the specimen, and afterwards remove the oil of cloves with xylol, and mount in Canada balsam.

In many cases granules that contain iron in a condition to react to the usual tests for inorganic iron are associated with the yellow pigment. Ammonia sulphide is sometimes used as the test for the demonstration of inorganic iron, but has the disadvantage that the brown sulphide of iron deposited can be confused with malarial pigment. A better reagent is ferrocyanide of potassium in an acid solution, as the blue ferrocyanide of iron is characteristic, and causes no confusion.

The section should be first treated with a $2\frac{1}{2}$ per cent. aqueous solution of potassium ferrocyanide for five

minutes, and then with a 1 per cent. solution of hydrochloric acid in glycerine, or with acid alcohol. The acid glycerine should be slightly warmed, and must be left on till the blue colour is quite distinct. If the blue colour only shows faintly, the specimen can be replaced in the ferrocyanide solution, and again treated with acid glycerine or acid alcohol. The specimen can then be washed in water, dehydrated in alcohol, cleared in xylol, and mounted in balsam.

It is important that the sections should not be touched with iron after they are cut, so that they must not be lifted with a needle, as in any place touched with iron there may be a deposit of the blue ferrocyanide of iron.

Loosely combined iron will be shown blue, whilst the melanin in which the iron is in firm combination will remain black. The yellow pigment may be in part turned blue or may be unaltered. The outlines of the cells can generally be seen and counter-staining is not necessary, but weak carmine solutions can be used if it is desired,

The iron may be diffused throughout the cells or may be found in granules either alone or mixed with yellow pigment.

The relationship of these ferruginous granules to the yellow pigment is not definitely known. In the most acute hæmolytic processes, such as in blackwater fever, both are present, and the iron-bearing granules are the most numerous. In the most chronic forms, such as some cases of ankylostomiasis, yellow pigment alone will be found.

The balance of evidence is in favour of the view that ferruginous granules are evidence of active and recent hæmolysis, the remainder of the hæmoglobin being discharged into the intestine as the iron-free product urobilin. The yellow pigment is a more permanent substance, and though formed as a result of acute hæmolysis is, when in considerable amount, evidence rather of a prolonged or chronic hæmolysis.

Some authorities hold other views, and consider that the yellow pigment when old gives the iron reaction.

The important point is that both these substances are proof of blood destruction, and are not evidence of malaria, although often found in cases of malaria. They are evidence of the general blood destruction that may be caused by the parasites of malaria as well as by other organisms. Further evidence of blood destruction is given by the increase of urobilin in the urine and the much greater amount passed with the fæces in acute hæmolysis such as blackwater fever.

In the vicinity of certain skin lesions there may be considerable disturbance in the normal arrangement of the pigment, so that instead of being deposited only in the deeper layers of the epidermis it is scattered not only in the superficial layers of the epidermis, but also in the subcuticular connective tissues.

This disturbance of the arrangement of pigment is most conspicuous in growths of the granulomatous group, including lichen hypertrophicus.

Pigment is normally present in the skin, and it is common to find pigment in the mucous membranes, particularly of the mouth in the coloured races. Such pigment is generally found in patches in the mucous membrane of the tongue, cheeks, or gums. It has no connection with malaria or other disease, but is more conspicuous in cases of advanced anæmia, as the pigmented patches then stand out more markedly against the general white background.

The normal pigmentation of the pia mater has been already mentioned. It is extravascular, and is not dissolved by alkalies.

The pigment in all these cases is much less soluble in alkaline solutions than the melanin of malaria. Pigmentations of the skin as a result of Addison's disease is also well known.

In melanotic sarcoma, black or brown pigment is also deposited in the growth.

Degeneration. — Cells exposed to various influences undergo degenerative processes. Death or necrosis of cells may take place, and in such cases the cell ceases to stain normally, so that instead of taking up basic stains it stains with acid stains, or feebly with both acid and basic stains. The nuclei break up and lose their characteristic staining reactions, and the whole cell may disintegrate and be converted into granular *débris*, or caseation may take place in which a mass of cells is replaced by granular fatty material. The mass may become calcified.

Where the morbid influences are insufficient to cause cellular death, changes occur in the protoplasm. Of these the more important are : (1) "Cloudy swelling," in which the protoplasm of the cell becomes swollen and the aspect of the cell changed so that its contents become obscured and very finely granular. This change is best seen in fresh, unfixed cells and is shown in stained specimens by an irregularity in the staining. This change occurs in the early stages of inflammatory action and may be general in any prolonged pyrexia. (2) Fatty degeneration may affect any cells, but more especially muscular fibres and the glandular cells of the liver, kidney, intestinal mucosa, &c.

In this latter form droplets of fat are found in the interior of the cells : these at first are small, but in advanced cases the whole contents of the cell appear to be replaced by fat and the nucleus is squeezed to one side. With fresh specimens the high refractive index of the fat renders the diagnosis easy. In specimens passed through alcohol, &c., the fat is dissolved out and the condition is then recognized by the meshwork of the protoplasm having clear, round, unstained spaces which were previously occupied by the fat globules.

Special methods show this form of degeneration more clearly. In specimens hardened in any of the osmic acid fixatives, such as Fleming's solution, or cut fresh and treated with weak osmic acid, the fat will be stained

a deep and intense black. Such sections can be counter-stained with safranin.

Soudan III. and Scharlach R. are also good stains for fat. Fresh tissues or tissues hardened in formalin must be used. The sections are treated with a saturated alcoholic solution of the stains (80 per cent. alcohol) for fifteen minutes, rapidly rinsed in 50 per cent. alcohol and washed in distilled water. They can be counter-stained with hæmatoxylin and mounted in any glycerine medium. The fat will be stained a deep red. A rough estimate of the amount and extent of the fatty degeneration may be made from such a section, but the main advantage of the method is to show the distribution of the degeneration and the class of cells mainly involved.

A promising method for the estimation of the extent of this degenerative process is the determination of the specific gravity of the organs. In some cases the fat is in sufficient amount to cause the entire liver to float in water, but more commonly it is short of this. To determine the specific gravity a large portion of an organ is weighed and the volume of this portion determined. This volume can be ascertained in the course of an ordinary *post-mortem* examination by the use of a vessel with an open tube fixed at the side.

The vessel is filled with water till the water escapes from the tube. When the water has ceased to escape a receiver is placed under the tube and the weighed portion of the organ is placed in the vessel. Water will again escape from the tube, is collected in the receiver and measured. The volume of this water is the same as that of the organ placed in the vessel, as it is the amount displaced by it.

We now know the volume of a given weight of the organ and therefore its specific gravity. This method is sufficiently exact for ordinary purposes if a sufficiently large piece of the organ is taken, but for comparative purposes more information is required than we at pre-

sent possess as to the normal variations in the specific gravities of organs.

Fatty degeneration is an important factor in many tropical diseases. It is marked in yellow fever almost as much as in poisoning by phosphorus. In the anæmia of ankylostomiasis it is constant and pronounced, and as it affects extensively the intestinal mucosa, it is, in the more chronic cases, largely responsible for the impairment of the digestive processes in these cases. It also occurs in the liver and kidneys in this disease, and as there is also a deposit of hæmosiderin these organs often appear to be of a dusky chrome-yellow colour. The occurrence of this degeneration in the cardiac muscles is more serious, as cardiac failure, either acute or chronic, frequently is due to this condition.

Amyloid degeneration is best shown in fresh sections. Macroscopically it can usually be determined by treating a cut surface of an organ with tincture of iodine; a deep brown colour is produced in such portions as contain this amyloid material.

Sections can be similarly treated and mounted in glycerine media.

Methyl violet stains amyloid material a deep red, standing out clearly from the surrounding violet.

Amyloid degeneration is not common in tropical diseases, with the exception of leprosy. In that disease, even when there has been no extensive suppuration, amyloid degeneration is sometimes found.

Fibrous Degeneration. — As a result of degenerative changes in many parts, and particularly in the nervous system, the nerve elements are replaced by fibrous tissue. This is well seen in spinal diseases in which degeneration of nerve tracts is followed by the formation of fibrous tissue in the tracts occupied by the degenerated nerves. This fibrous tissue stains with ordinary basic stains and is well shown by carmine. This change is sclerosis, and is the final result of the degenerative changes. The early nerve degenerations require special and com-

plicated methods and could not be satisfactorily studied without special knowledge and appliances.

The simplest method for demonstration of early nerve degeneration is that of Marchi. Small pieces of tissue are hardened in Müller's fluid for one week, taking care to avoid mechanical injury. The tissues are then transferred to freshly prepared Marchi's fluid (Müller's fluid two parts and 1 per cent. aqueous solution of osmic acid one part) in which they remain for one week at about 37° C. Brain tissues require a longer time. The tissues are then washed for twenty-four hours in running water, hardened in increasing strength of alcohol, imbedded and cut. Sections are then dehydrated, cleared and mounted in balsam. Nerves are best teased out and then mounted.

Degenerated nerve tissue (fat) is stained black ; all else brownish-grey. The earliest degeneration is shown by a flecking with black about the internodes ; later fat droplets staining black more or less completely replace the white substance of Schwann-Wallerian degeneration.

CHAPTER XVIII.

Parasites in the Tissues.

Bacteria, protozoa, trematoda, nematoda, and their eggs and larvæ,. may be found in the tissues in various diseased conditions in man and animals in various parts of the body. Bacteria are considered separately.

Protozoa.—The common parasites are coccidia, sarcosporidia, and flagellates in their resting stage. The first two are probably accidental parasites in man, but in other mammals are very common.

Coccidia are sporozoa which are encysted, and by their continuous asexual multiplication form visible white masses not unlike tubercles.

The rabbit is very commonly infected, and the large masses are found in the liver, though the intestinal mucosa may also be infected.

The young coccidia enter hepatic cells or those of the bile duct and speedily destroy the cell. They may develop asexually so that a cyst is formed in which the protoplasm divides into a number of young coccidia, which, on the rupture of the cyst, enter neighbouring cells, and in turn multiply. Massive tumours composed of these encysted sporozoa are thus found.

Some of the young coccidia develop into sexual forms, male and female.

In the male forms the cell protoplasm and nucleus divide, so that a number of motile bodies, *microgametes*, are formed. When the cyst ruptures, these microgametes enter a female and fertilize it. The female forms *macrogametes*, do not divide till the microgametes have entered

and fertilized them. After this fertilization, which takes place in the liver, the cyst wall of the fertilized macrogametes becomes impervious, and ultimately this cyst, now known as the *oöcyst*, becomes detached and passed with the bile into the fæces of the host, and is voided with the fæces. Lying on the ground the cell contents of

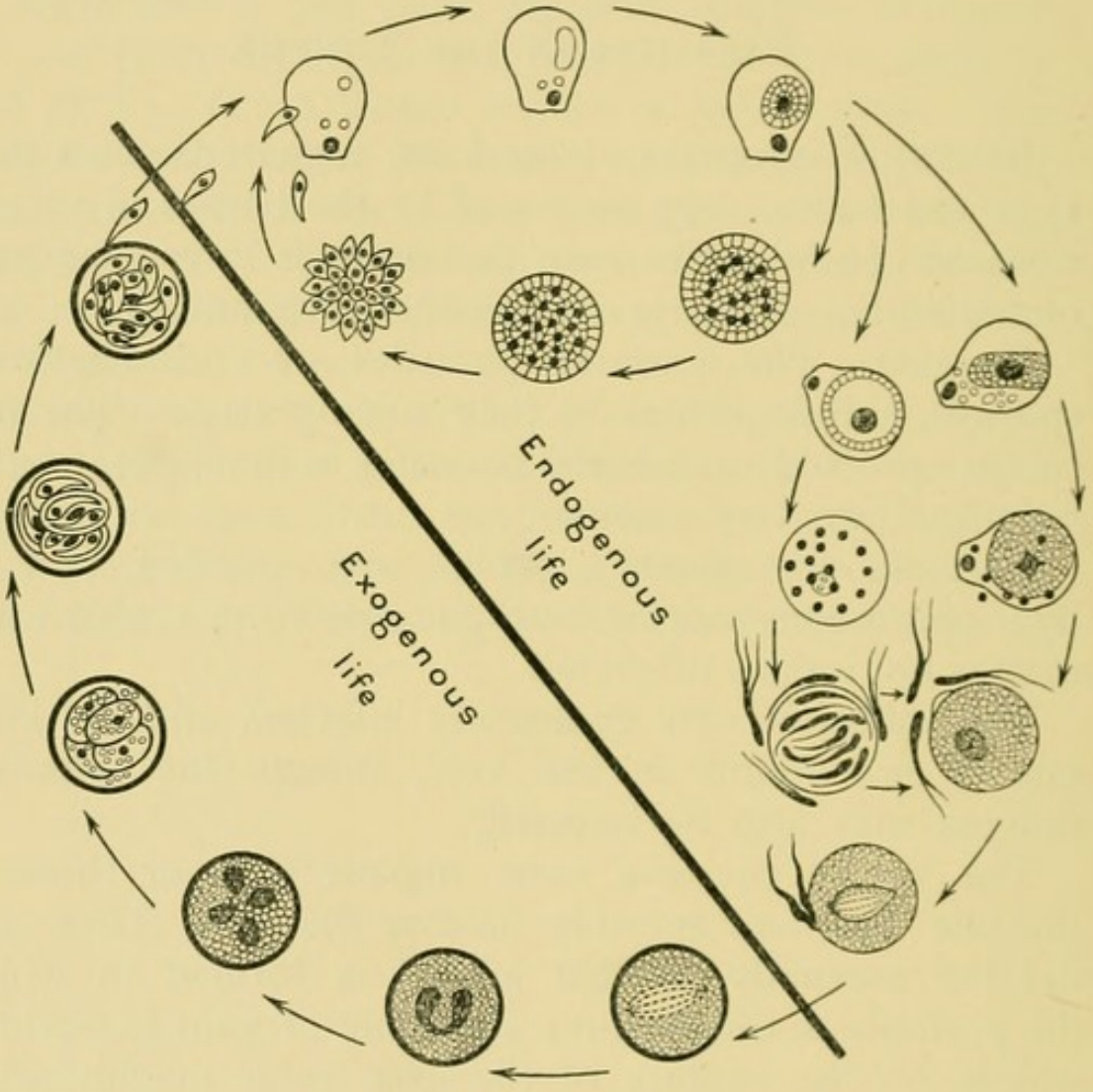


FIG. 138.—Coccidia life cycle.

this oöcyst or fertilized macrogamete divide so that eight spores are formed. When swallowed by a suitable host the cyst wall is dissolved off, and the spores enter cells and become young coccidia (fig. 138).

The different coccidia which have occurred in man are :—

Coccidium cuniculi (*C. oviforme*), in which the oöcysts

measure ·033 to ·049 mm. long by ·015 to ·028 mm. wide. This parasite has been found in the liver in man on three or four occasions.

Coccidium hominis, in which the oöcysts measure ·024 to ·035 mm. by ·012 to ·020 mm. This is a parasite of the intestinal epithelium of rabbits in which it causes death from severe diarrhœa. It has been described in the intestine of man on a few occasions. Some authorities hold that it is identical with *C. cuniculi*.

Coccidium bigeminum, in which the oöcysts measure ·012 to ·015 mm. by ·007 to ·010 mm. In this form the oöcyst divides into two parts, each of which encysts and forms sporoblasts. A small number of cases have been described in man.

Demonstrations.—The demonstration in tissues of the coccidia is not difficult, as even without staining the shape and general appearance can be readily made out. They are very liable to be mistaken for eggs of trematodes. To stain the cell contents is a difficult matter. The wall of the organism is not readily penetrated by stains, and when the stain does penetrate the contents are apt to be so deeply stained that details cannot be made out. Good results can be obtained by staining with iron alum—hæmatoxylin.

The method is as follows. The solutions required are :—

A.	2·5 per cent. solution of iron alum.	
B.	Hæmatoxylin crystals	1 grm.
	Absolute alcohol	10 c.c.
	Distilled water	90 c.c.

This solution should be kept for one month to "ripen." Then add water, 100 c.c.

Sections are first placed in the iron alum solution (A) for six to twelve hours, washed in water for one minute, then left in the hæmatoxylin solution (B) for twenty-four to thirty-six hours. Afterwards they are washed and differentiated in iron alum solution until the section becomes deep blue, and the nuclear structures stand out sharply. This stage is best controlled by watching the

process under a low power. When completed, wash in running water for fifteen minutes. Counterstain very lightly with eosin if desired. Dehydrate and mount as usual.

Sarcosporidia.—In the muscles of some animals, such as cattle, sheep, pigs, rats, &c., elongated bodies are found which are often visible to the naked eye. On microscopic examination these so-called "Miescher's tubes," or "Rainey's capsules" are seen to be composed of a dense fibrous envelope enclosing a vast number of spores.

The interior of the "Rainey's capsule" is divided up by extensions of the fibrous envelope into loculi, each of which at an early stage of growth contained a single large cell known as a pansporoblast. Each of these pansporoblasts is a single individual, only a part of the protoplasm is converted into spores, the remainder continues to live and form spores. This continuous formation of spores without the destruction of the original cell leads to the formation of large masses. This peculiarity in the asexual development separates the sarcosporidia and the allied forms which occur in fishes, *Myxosporidia*, from the other sporozoa, and they are known as *Neosporidia;* whilst the parasites in which the whole protoplasm divides and the parental cell is destroyed in the process are known as *Telosporidia*. The sarcosporidia stain readily with basic stains.

Little attention has been paid to these bodies. The sarcosporidium may be invisible to the naked eye, but as they usually produce some colour change, appear as light streaks or nodules; such streaks should be looked for and scrapings of them examined microscopically. In many cases they are easily visible and may be over an inch in length.

The nature of the bodies can be demonstrated in sections or, better, by isolating one of the bodies and rupturing it. A large mass of spores is set free and these can be readily stained after drying by Leishman's

or Giemsa's stain. They are oval, often sausage-shaped bodies, with a nucleus with diffuse chromatin staining. Often detached granules staining with the polychrome red are present in the protoplasm. At one end is a clear space which may be unstained or very deeply stained, and is known as the polar capsule. Nothing is known of the extra-corporeal life-history or sexual development of sarcosporidia. Similar bodies have been recorded in man on a few occasions.

The few species of the sarcosporidia known are included in the genus *Sarcocystis*.

Another protozoan parasite which has been described by Minchin and Fantham in man is the *Rhinosporidium kinealyi*. It occurs in vascular pedunculated growths on the septum nasi of natives of India.

The youngest parasites are of irregular form and consist of granular protoplasm enclosed by a hyaline membrane and containing numerous minute nuclei. As the parasite grows it becomes spherical and its hyaline envelope becomes greatly thickened, forming a definite cyst wall. Towards the centre of the body the protoplasm becomes segmented into spherical pansporoblasts, each at first with one nucleus. In these pansporoblasts the nuclei multiply and give rise to spores numbering two to twelve. Each pansporoblast thus becomes converted into a spore morula. Spore formation goes on continually at the expense of the peripheral zone of growing protoplasm. A cyst will then consist of three zones within the envelope; at the periphery a zone of uninucleate pansporoblasts; internal to this an intermediate zone of spore formation; and most centrally a great number of spore-morulæ (fig. 139). Nothing is known of the life-history of the parasite outside the body.

In dogs affected with rabies certain bodies found in the brain may be protozoa.

These, known as Negri bodies, occur mainly in the grey matter of the hippocampus major. They are of varying size, the smallest spherical and structureless,

larger ones with a central granule or nucleus, whilst the largest are round or ovoid and contain several (as many as eight) nuclei (fig. 140.) Nothing is known of their development or life-history.

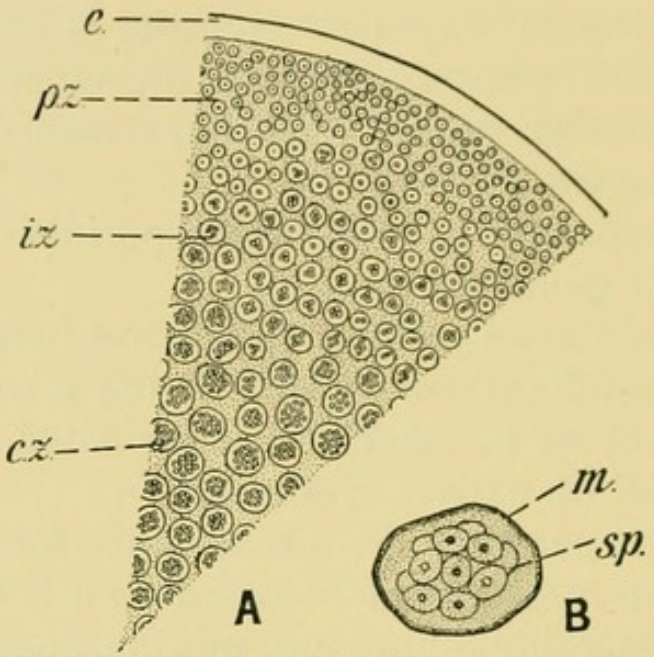


FIG. 139.—*Rhinosporidium kinealyi.* A, segment of a section through a cyst—*e*, hyaline envelope; *pz*, pansporoblasts in peripheral zone; *iz*, intermediate zone of pansporoblasts; *cz*, central zone of spore morulæ. B, a ripe spore morula—*sp*, spores; *m*, membrane.

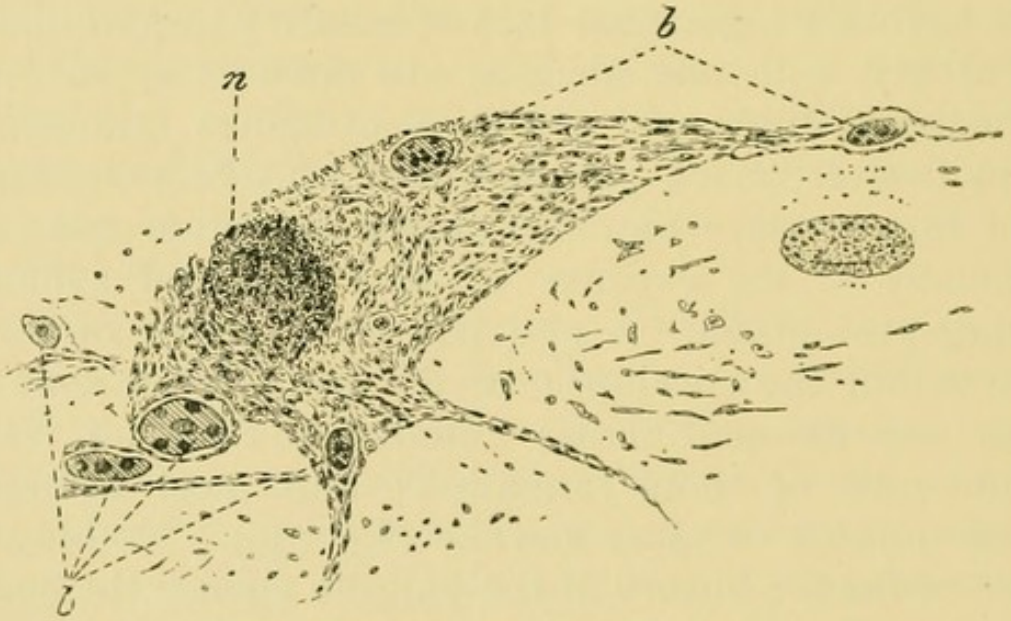


FIG. 140.—Negri bodies. *n*, nucleus of nerve cells; *b*, *b*, the Negri bodies.

The most important of the protozoa found in the tissues in man are the Leishman-Donovan bodies, now known to be the resting stage of a flagellate.

These bodies are found in the spleen, liver, mesenteric glands, sometimes as shown by Cochran in superficial glands, submucosa and in the lungs, and are present in enormous numbers. They are contained in endothelial cells, in the macrophages, and imbedded in a hyaline matrix, possibly the remnant of a broken-down tissue cell between the cells of the organ or tissue.

They are small, round, oval or oat-shaped bodies, characterized by having two unequal chromatin masses, one large and oval, staining moderately well with the red polychromed methylene blue, and the other smaller, more compact, usually rod-shaped, and staining very deeply with chromatin stains. They may be readily seen in smears made from an infected organ and can be recognized in sections. During life they may be obtained by puncture of the spleen or liver. They are more easily obtained from the spleen, but there is a certain amount of risk in puncturing that organ. They are not so numerous in fluid drawn from the liver, but the risk in puncturing that organ is slight, and therefore it is on the results of liver puncture that the diagnosis should be made. The puncture must be made with strict antiseptic precautions, and there must be no fluid in the syringe used, or the bodies will be broken up. Aspiration must be slow and gradual, or blood will be sucked up in too great a quantity.

In the peripheral blood at some stages of the disease, the bodies may be found in the leucocytes. They are said to be particularly numerous in this situation just before death.

Leishman's stain is an excellent one for smears of the organs, or smears of the fluid obtained by puncturing the liver, or to demonstrate the parasites in the leucocytes; dilute carbol fuchsin also gives satisfactory results.

In sections, carbol thionin or hæmalum used as described for malaria parasites in sections of the blood-vessels gives good results. Van Gieson's stain brings them out rather better (*vide* p. 86).

The bodies appear very small in sections and the two chromatin masses are close to each other, as in the hardening process there is great shrinking of the parasites. The disease caused by these parasites is that known as kala-azar. Similar bodies can be obtained by scraping the floor of certain ulcerated surfaces on the skin. These ulcers are the Oriental sores or Delhi boils. Probably the parasites are of a different species, as the topographical distribution of the two diseases is different.

The two unequal chromatin masses suggested to Leishman the similar appearances found in degenerate trypanosomes.

Rogers and others have shown that in a medium containing 2 per cent. citrate of soda changes occur in the Leishman-Donovan bodies and that ultimately they develop flagella. It is essential that the temperature of the culture should not exceed 25° C. Rogers states that by adding a little citric acid to his cultures, development occurs more rapidly and certainly; this addition is not, however, an essential.

HELMINTHS.—Trematodes may be present in various tissues. Some, such as the schistosoma, are found in blood-vessels; other trematodes may be found in bile-ducts or in the pulmonary alveoli.

Filaria in man and many animals are found in connective tissue, or in the muscles, or in the peritoneal cavity. Others, as in some cattle, are found beneath the endothelium of large vessels, such as the aorta, and others, again, partly beneath the endothelium and partly free in the lumen of the vessel.

Some nematodes penetrate the intestinal wall, as the *Trichinella spiralis*, *Strongyloides intestinalis* and *œsophagostoma* occur encysted in the submucosa commonly. The *Anchylostoma duodenale* is reported to so occur exceptionally. Eggs are not uncommon in the tissues. Those of *Schistosoma hæmatobium* are commonly present in the submucosa of the bladder or rectum, and of the *S. japonicum* in the submucosa of the small and large

intestines. Eggs of these worms may be found in other parts and those of *S. japonicum* appear to be constantly present in the liver and lymphatic glands, whilst the ova of *S. hæmatobium* are sometimes found in the lungs.

Embryos are frequently found in blood-vessels and exceptionally are extravascular, but in the natural life-history of *Trichinella spiralis* the larvæ are encysted in muscles of various parts of the body and in the diaphragm and intestinal walls. Nematode embryos, such as those of the *Anchylostoma duodenale*, penetrate the skin and can be found in the skin, subcutaneous tissues, lungs and various parts of the body.

Eggs and larvæ can be readily stained in section with any basic stain—hæmatoxylin and carmine perhaps give the best results. They can also be detected unstained. Larvæ of *Trichinella spiralis* can be well seen in teased-out specimens or in the intestinal walls of small animals, such as rats, spread out between two slides. If hardened whilst so spread out in alcohol, the intestine can then be rapidly treated with sodium hydrate solution till the intestinal wall is rendered transparent. The worms are then more readily seen.

CHAPTER XIX.

Fæces.

The examination of fæces is of the greatest importance, as most of the intestinal entozoa deposit their eggs whilst in the intestinal canal; others inhabiting the liver deposit their eggs in the biliary ducts, while others, again, pass their eggs or embryos through the tissues into the alimentary canal. The eggs of all these are passed with the fæces. In other cases the parasites themselves may be passed, or in the case of tape-worms the mature segments or proglottides only.

In dysentery, cholera, &c., the organisms found in these diseases are present and can be isolated from the stools.

Macroscopic examination of the stools is very necessary and much information can be gained. The stools can be examined as passed in any vessel, but are more conveniently examined if passed into transparent glass vessels; these can be covered with a larger glass cover fitting over the lower vessels like an enlarged Petri dish.

The points to observe are :—

(1) The presence or absence of blood, mucus, muco-pus or pus, and the arrangement relative to the stool of such a discharge.

(2) The colour of the stool and its consistence.

(3) The presence or absence of evidence of gaseous fermentation.

(4) The odour.

(5) The reaction, determined as soon as possible after the stool is passed.

(6) The bulk of the stools.

(7) Any visible signs of animal parasites, such as the worms themselves or the proglottides or segments of tape-worms.

(1) Mucus alone, or streaked or mixed with the blood, usually indicates inflammatory action in the lower bowel, not necessarily dysenteric. It may be caused by anything that sets up such inflammation, such as bilharzia, ulcerated hæmorrhoids, or chronic ulcerations of various kinds of the rectum. These latter include malignant growths, granulomatous growths, and the ulceration left as a sequela of dysentery. Rarely mucus derived from the small intestines is passed with the fæces. Such mucus is recognized easily as it is usually stained yellow with bile.

Clear mucus, whether streaked with bright blood or not, without any admixture of fæcal matter, is met with in early or acute dysenteric attacks. Turbid or purulent mucus, sometimes in large quantities, and passed either without any stool or with solid formed motions, is more indicative of a chronic ulceration of the rectum, from whatever cause.

Sometimes the mucus is passed in large masses and consolidated, and may include much *débris* and numerous epithelial cells. In the condition known as membranous colitis, complete casts, several inches in length, of the rectum may be passed. These are usually twisted up when passed, and may be mistaken for worms. They can sometimes be floated out in water, and in any case the microscopic structure should render any mistake impossible.

With ulceration limited to the rectum, stools are often coated with mucus. The more intimately the mucus and blood are mixed with the fæces, the higher up are the lesions from which the mucus or blood is derived. In some lesions the mucus is so intimately mixed with the fluid fæces that it is difficult to discern, but tilting the vessel from side to side will often indicate its presence by the manner in which the stool flows. In some

cases it is better shown by adding water to the fæces, when the flakes or masses of mucus can be more readily seen, especially if the diluted fæces are poured from one vessel to another. Amœba are killed, or have their motility destroyed by this addition of water, and therefore this method should only be adopted after microscopic examination.

Blood may be passed, bright red or in clots, in large quantities. This is no proof that it is passed from the rectum, as if in sufficient quantity and not mixed with the fæcal contents of the intestine it need undergo very little change in passing through the large intestine. Such blood is occasionally passed in ankylostomiasis. If intimately mixed with the fæces, it may have lost completely the red colour and appear black and tarry—melæna.

When in very small amount altered blood can be recognized by Weber's test. The fat is first extracted with ether, and the stools are then rubbed up with water and a third part of acetic acid added. They are now shaken up with ether and an ethereal solution of acid hæmatin is obtained if altered blood is present (*vide* table of spectra).

In other cases, though still red, the stool has a duller colour, more like anchovy sauce. Such stools are passed in some cases of dysentery where the small intestines are implicated, and may also be passed in cases of extensive enteritis secondary to malaria.

Microscopic as well as macroscopic examination of the mucus and blood, as well as of the stool, should be made.

(2) The colour of the stool is much modified by the diet, milk especially causing pale stools. Articles of diet taken by a patient have a marked effect, and amongst abnormal articles that may be met with are earths of various kinds, coal-dust, &c., which to the inexperienced may cause much confusion. The pipe-clay stools of obstructive jaundice may be simulated by those of some earth-eaters, and the black stool of the coal-dust eater has

been mistaken for melæna. The dark blue stools passed by patients taking methylene blue are easily recognized, and so are the bright yellow stools passed by patients when taking ipecacuanha.

Bile pigments and acids are not usually present in normal fæces, as they are absorbed in the small intestine or broken down in the large intestine. They may be present in diseased conditions or when the food is passed too rapidly through the intestinal canal. Urobilin, according to Ross, is a measure of the blood destruction taking place in the liver.

BILE ACIDS may be recognized by *Pettenkofer's reaction.* A small portion of the fæces is mixed with a little sugar and placed on a white porcelain dish. A little sulphuric acid is allowed to come in contact, and if bile acids are present a crimson colour appears.

BILE PIGMENT may occur either as *Bilirubin* or as *Biliverdin.*

(*a*) *Schmidt's Reaction.*—A saturated solution of perchloride of mercury is added to the fæces, and in the presence of bile pigments a bright green colour is produced.

(*b*) *Gmelin's Reaction.*—A drop of yellow nitric acid (*i.e.*, containing nitrous acid) is brought into contact with the fæces, and in the presence of bile pigment there is a play of colours, one of which must be green. This test is more decisive with a watery extract of the fæces.

(*c*) *Huppert's Test.*—The fæces are mixed with slaked lime suspended in water, and the precipitate is filtered and washed. An extract of the dried precipitate is made with hot alcohol and a little sulphuric acid. If bile pigments are present this extract is green.

Urobilin is present in small quantities, ·03—·06 gramme per diem in normal stools. In cases of any hæmolytic disease the amount is much increased, and is markedly so in malaria, the increase, according to G. C. E. Simpson, is greater in subtertian malaria, up to 1·73 grammes, than in benign tertian. In exceptional cases it may be much

greater. Urobilin, though it does not contain iron, is one of the final products of the destruction of hæmoglobin.

Simpson extracts the urobilin from the fæces by repeatedly shaking the fæces with large amounts of water acidulated with dilute sulphuric acid.

The filtrates are then freely exposed to daylight for some time and examined spectroscopically.

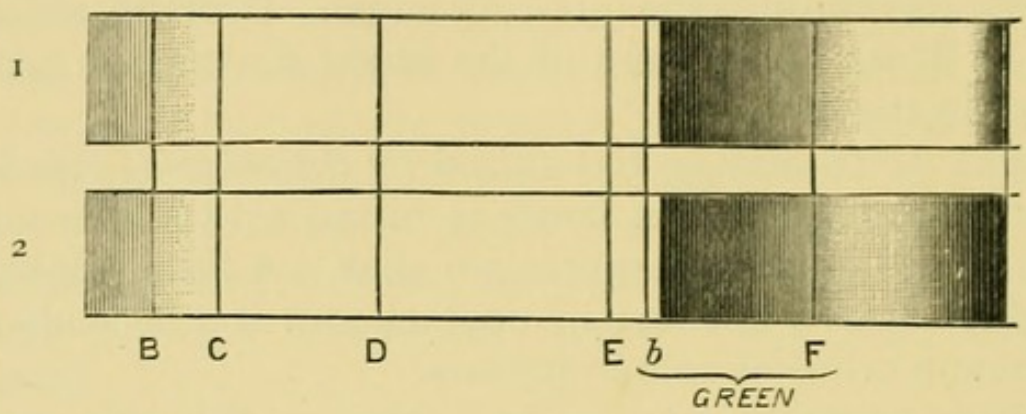


FIG. 141.—1, Spectrum of urobilin; 2, spectrum of urobilin, masked by other pigments.

The method of quantitative determination was by observing the amount of dilution required to render invisible the spectrum of urobilin in a layer 15 mm. thick.

The spectroscope was standardized with a solution of purified urobilin of known strength.

The consistence of the stool is of great importance, and it will be found that "looseness" of stools is of more importance in tropical practice than in England. In ulceration of the cæcum and upper part of the colon, even when this is acute and extensive, there need be neither visible mucus nor blood, nor even tenesmus. "Tropical diarrhœa" is frequently shown at *post-mortem* examinations to be dysenteric. It is very fatal. On the other hand, mucus and blood may be passed with formed or even hard stools when there are a few chronic ulcers high up in the large intestine.

(3) In some forms of tropical diarrhœa, particularly

that form known in the East as sprue, the stools passed are full of air-bubbles and are undergoing active gaseous fermentation.

(4) The odour varies so greatly with the diet that it is of minor importance. In the races subsisting mainly on a scanty vegetable diet the odour is singularly slight. The smell is mainly due to indol and skatol. In cases of dysentery associated with formation of sloughs the ordinary fæcal odour is replaced by the peculiar penetrating smell associated with that condition. Excessive decomposition of the stools may cause an increase in the intensity of the normal smell, or if the diet is mainly of carbohydrate foodstuff no increase but even a diminution.

Variations in the odour indicate changes in the decomposition of the contents of the intestine, often from variations in the food, but sometimes from variations in the "flora" of the intestinal contents, rarely from structural lesions of the intestinal wall.

The result of the administration of intestinal antiseptics is more often a diminution in the putrefactive changes in the contents of the bowel than any real improvement in the diseased condition of the intestinal wall.

(5) The normal reaction of the fæces as determined by litmus is nearly neutral; when fasting it is acid, with a milk diet faintly alkaline. It is usually acid to phenolphthalein. In many cases of diarrhœa and dysentery this is replaced by a decidedly alkaline reaction. To determine the reaction the fæces must be examined as soon as they are passed, as a change rapidly occurs in most fæces, particularly when fluid, rendering them alkaline. Solid motions must be rubbed up with water in a mortar.

(6) *Bulk of Fæces.*—The amount of fæces passed by a European on the average is about 130 grammes *per diem*. On a meat diet it is about half this. In vegetarians it is much greater. The amount passed varies with the amount of food taken, and inversely as the amount

digested. The excretions from the intestinal wall form a proportion of the fæces, as during prolonged fasts 22 grammes may still be passed. Most native races consume a large amount of crude carbohydrates in bulky vegetables. Much of this is indigestible, and is therefore passed with the fæces. The average weight of the excreta in native races is greater than in Europeans, and in India is about 233 grammes. These amounts are of importance in estimating the amount of excrementitious matter that has to be disposed of in a community. It is usually estimated per 1,000 of the population, and is given for a European community as half a ton, and for a native community as two-thirds of a ton (these figures including urine, &c.).

The bulk of the excreta is variously affected in disease. Discharges from the intestinal walls, usually watery in character, may form an important part or even, as in cholera, nearly the whole of the excreta.

In other cases where there is an extensive ulcerated surface muco-pus may be discharged in quantity and no fæces at all. Abscesses such as hepatic abscesses may open into the intestine, and then there may be a profuse discharge of pus, usually of an anchovy sauce colour.

Water is generally absorbed in the small intestines, but in many conditions where the intestines are irritated, food and even water are hurried so quickly through the alimentary canal that little absorption takes place.

In some diseases, as in pneumonia, and towards the crisis in relapsing fever, there is a great tendency to the occurrence of frequent large watery motions.

In dysentery, as a rule, though the motions are frequent, the amount of fæces passed is small, and in the acute and early stages no fæces at all may be passed, though mucus or blood, or both, are passed in quantity.

In most general diseases, as in malaria, digestion and absorption are fairly active, and the motions then are constipated, but the amount passed is not more diminished than might be anticipated from the diminution in the amount of food taken.

In the disease known as sprue or psilosis the motions are usually bulky. Digestion and absorption are both imperfect. As the guiding principle in the treatment of this disease is to give as complete rest to the alimentary canal as is consistent with a sufficient supply of nutriment to the tissues, it is of importance to know what forms of food are not digested and which are.

In other diseases, as in obstruction of the pancreatic duct, it is also important to know which of the digestive juices is wanting, as it may be possible to supply the deficiency or so modify the diet that little or no call for this agent is necessary.

An analysis of the fæces will show which of the important food elements have undergone little or no change, provided that the amount and composition of food taken, from which the fæces is derived, is accurately known.

Allowance has to be made for excretions from the intestinal wall, and for the secretions from the liver, pancreas, &c., which are poured into the intestine in variable amounts.

The investigation is difficult, as the amount of the main ingredients excreted in this way may be considerable, and is affected by the food taken. The amount of fats excreted, for instance, is greater when fat-free food is taken than in the same individual when he is fasting.

For any investigation food of known composition and amount must be taken. The simplest diet is a milk diet. Some inert, easily recognized substance, such as charcoal or carmine, should be taken at the commencement of the experiment. The first fæces containing this substance, usually twenty-four hours after the administration, but in cases of diarrhœa four hours, or even less, must be saved, as well as all subsequent excreta. A period of three days or five days should be taken, and at the completion of the experiment a second dose of charcoal given. As soon as the charcoal appears in the fæces the experiment is over.

The fæces passed during the period must be all collected, and should be analysed: (*a*) For water; (*b*) for nitrogen; (*c*) for fats; (*d*) for carbohydrates; (*e*) urobilin.

(*a*) The water can be determined by weighing before and after drying, but as volatile substances are present this must be conducted over sulphuric acid in a drying chamber not over 60° C. The average amount of water is 75 per cent.

(*b*) Nitrogen can be determined by taking a weighed portion of the fæces, to which is added 15 or 20 c.c. of $\frac{N}{10}$ sulphuric acid to prevent loss of ammonia.

This mixture is then dried in a water-bath till fairly hard, and the desiccation completed in a drying chamber at 10° C. over sulphuric acid.

A gramme of this dried powder is then mixed with 25 c.c. of strong sulphuric acid and 1 grm. of sodium pyrophosphate, and this is allowed to stand for some hours, and then cautiously boiled. The nitrogen will all now be in the form of ammonium sulphate.

This can be estimated after allowing to cool by adding 600 c.c. of water and sodium hydrate solution till strongly alkaline. Some granulated zinc to prevent bumping should also be added.

The mixture is then distilled and the distillate allowed to pass into a measured amount of $\frac{N}{10}$ sulphuric acid. The ammonia will neutralize a certain amount of this, so that by subsequent titration the amount of ammonia that has distilled over can be estimated.

Undigested proteids passed unchanged form a very small part of the nitrogen.

(*c*) *Fats* are usually determined as "total fats," consisting of fats, soaps and fatty acids.

The fæces should be thoroughly dried over sulphuric acid and treated with 1 per cent. hydrochloric acid to split up the soaps.

Ether is then added and the ethereal extract after drying is re-dissolved in water-free ether.

The residue left after the evaporation of the ether is considered as total fats.

In any question of the digestion of fats it must be remembered that fats differ greatly in digestibility, and that a healthy person will pass unchanged about 8 per cent. of mutton suet, which melts at 52° C., whilst he will only pass 2·5 per cent. of pork fat, which melts at 30° C. A healthy adult on a pure milk diet will digest about 95 per cent.

(*d*) Sugar and carbohydrates are usually digested completely unless enclosed in an impervious capsule, as in some vegetable foods.

The amount of gas formed from such fæces, kept at blood-heat for twenty-four hours, will give a fair indication of the amount of carbohydrates present.

(*e*) Urobilin to indicate the amount of blood destruction taking place.

Parasites.

(7) Parasites of various kinds may be seen by direct examination, but more often it is necessary to strain the stools. This is best done by placing the stool on a muslin or strong fine wire gauze strainer (fig. 142) and adding water and stirring well. By repeating this process all the smaller particles of the fæces will be carried through the muslin, and only the coarser particles and any entozoa present will be left on the strainer. Some of the smaller entozoa may be carried through the strainer. The fluids that have passed through can be strained again, or passed through a muslin bag. This is conveniently done with a bucket big enough to hold the sieve. The bucket is filled with water so that it covers the wire gauze. The fæces are then placed on the gauze and stirred well. From time to time the sieve is lifted so that the stirred fæces pass into the bucket. When the water becomes turbid the strainer is placed in a second bucket filled with clear water and the process repeated. On examining the strainer the greater number

of the worms will now be readily seen. After standing a little the superjacent fluid in both buckets should be poured off and the deposit again passed through the sieve. More worms will be found. Entozoa are damaged a little by this proceeding. When they are required for detailed examination they should be picked out of the undiluted fæces.

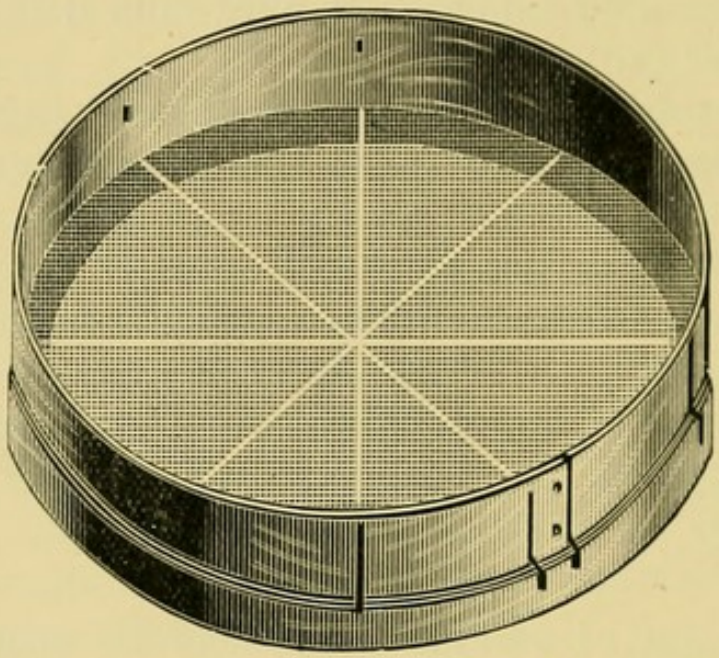


FIG. 142.—WIRE GAUZE STRAINER.

Microscopic Examination.—The most important objects of this are (1) the detection of ova of parasites, (2) the detection of animal micro-parasites, and (3) the investigation of the bacteria present.

Ova are readily seen with a low power, two-thirds inch objective, but for their identification at least half an inch or, better, quarter of an inch objectives are requisite.

The preparation of the stool is very simple. A small particle of the fæces is placed on a slide : it can be conveniently taken up with a splinter of wood such as a match stick. If not too hard it should then be compressed by a cover-glass into a thin layer; if too hard for this it can be mixed with a little water. If the stool be watery it should be allowed to stand, and with a pipette some of the fluid taken from the bottom, as the eggs are heavier than the fluid stool and sink to the bottom of the

vessel containing it. The eggs that may be met are those of the *Ascaris lumbricoides, Trichocephalus dispar, Ankylostomum duodenale, Oxyuris vermicularis,* several species of tape-worms, several species of *Fasciolidæ,* those of *Schistosomum hæmatobium* with the lateral spine sometimes, and those of *S. japonicum,* and the embryos of *Strongyloides intestinalis.* The attached diagram shows the appearance of the more important of the ova.

The eggs of *Ascaris lumbricoides* (round-worm) are enclosed in a thick, clear capsule usually coated with an albuminoid covering stained yellow or brown by the fæcal colouring matter. The protoplasmic contents are granular and do not as a rule completely fill the inner capsule (fig. 143, *a*).

If too much pressure has been used the albuminoid covering may have been ruptured and the egg is seen surrounded only by its thick transparent capsule. These Ascaris eggs can be readily distinguished from eggs with thin capsules, such as those of *A. duodenale,* not only by the thickness of the capsule, but by its more spherical shape, and by the granular and unsegmented character of the egg contents. Unfertilized eggs are larger, more oval, and the egg contents contain numerous refractile globules, which should not be confused with segmentation.

The eggs of *Trichocephalus dispar* (whip-worms) are easily distinguished, as they are small oval eggs contained in a thick, deeply stained outer capsule which has an opening at each end. Inside this capsule is a thinner, unstained capsule, and the egg contents are granular. In many instances the openings in the outer capsule are seen to be plugged by mucus (fig. 143, *b*).

The ovum of *Ankylostomum duodenale* is enclosed in a single, thin, transparent, unstained capsule. At the time the egg is passed segmentation usually into about four segments has taken place (fig. 143, c^1, c^2), but if the stool be kept a large number of segments will be present according to the time and temperature, and in twenty-four

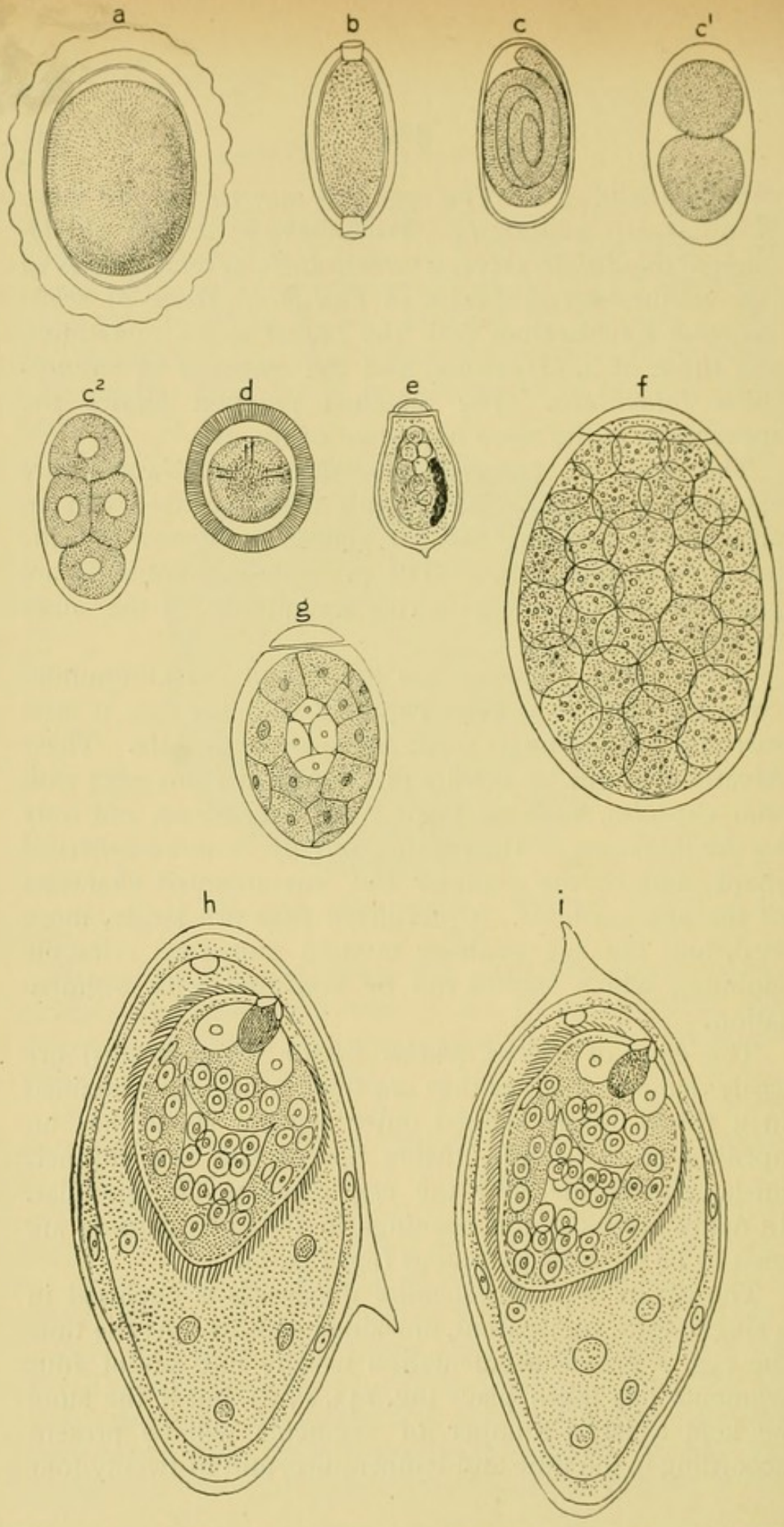


FIG. 143.—*a*, *Ascaris lumbricoides*; *b*, *Trichocephalus dispar*; *c*, *Oxyuris vermicularis*; c^1, c^2, *Ankylostomum duodenale*; *d*, Oncosphere of *Cestode*; *e*, *f*, *g*, various *Fasciolidæ*; *h*, *Schistosomum* (?) *hæmatobium* (from fæces); *i*, *Schistosomum hæmatobium* (from urine).

to forty-eight hours a fairly well formed embryo will be found in many of the egg capsules, and the egg then closely resembles that of *Oxyuris vermicularis*. The eggs of the closely related *Necator americanus* are slightly larger, but otherwise similar, to those of the Ankylostome.

Oxyuris vermicularis (thread-worm) has an egg that in size and general appearance is not unlike the ankylostome, but is usually flattened at one side. At the time the stool is passed, this egg contains a well-formed embryo (fig. 143, *c*).

The "eggs," or rather *oncospheres*, of the different species of tape-worms may only present slight differences from each other, but they are readily distinguished from all other ova by the radial striation of the thick capsule and the presence of a differentiation in the contents into an embryo; the six hooklets of this embryo can usually be made out (fig. 143, *d*). These oncospheres consist of the embryo and embryonic capsule only, the outer part of the egg having usually disappeared.

The eggs of the various *Fasciolidæ* (flukes) can be recognized by the presence of an operculum or lid (fig. 143, *e*, *f*, *g*), and distinguished from each other by their size. The eggs of the *Trematodes* found in man are usually yellow or brown.

Schistosomum hæmatobium has a highly characteristic egg, as it is armed with a sharp spike. In eggs passed with the fæces, with which they may be mixed, or contained only in mucus on the surface of the stool, this spike is at one side (fig. 143, *h*), in urine the spine is terminal (fig. 143, *i*), and by some it is believed that the eggs with a lateral spine are from different species of *Schistosoma*. If water be added to the fæces, it will be seen that the egg contains a ciliated embryo which soon becomes active and bursts through the egg capsule. The free-swimming embryo remains alive in water for some days, but undergoes little further change. An intermediate host, perhaps a fresh-water mollusc, is probably necessary for its further development.

Schistosomum japonicum.—The eggs are passed into the intestinal canal higher up than those of *S. hæmatobium*. They have a lateral knob as shown by Leiper, in place of a spine, no operculum, and are about the same size as the eggs of ankylostomes. They contain a formed miracidium when passed.

MEASUREMENTS OF OVA.

Tænia saginata	0·03 to 0·04 mm. by 0·02 to 0·03 mm.
Tænia solium	(Spherical) 0·03 mm.
Hymenolepis nana ...	,, 0·4 ,,
Dibothriocephalus latus ...	0·068 to 0·071 mm. by 0·045 mm. (operculated).
Fasciola hepatica	0·13 by 0·08 mm.
Fasciolopsis buski ...	0·125 ,, 0·77 ,,
Dicrocœlium lanceatum ...	0·04 ,, 0·03 ,,
Clonorchis sinensis ...	0·027 ,, 0·016 ,,
Heterophyes heterophyes ...	0·03 ,, 0·017 ,,
Paragonimus westermani...	0·08 to 0·1 mm. by 0·052 to 0·075 mm.
Gastrodiscus hominis ...	0·15 by 0·07 mm.
Cladorchis watsoni ...	0·12 ,, 0.075 ,,
Schistosomum hæmatobium	0·01 ,, 0.03 ,,
Schistosomum japonicum ...	0·06 to 0.09 ,, by 0.03 to 0.05 mm.
Ascaris lumbricoides ...	0·05 ,, 0·07 ,, ,, 0·04 ,, 0·05 mm.
Oxyuris vermicularis ...	0·05 by 0.016 ,, to 0·024 mm.
Ankylostomum duodenale...	0·056 to 0.61 ,, by 0·034 to 0·038 mm.
Necator americanus ...	0·064 ,, 0·072 ,, ,, 0·036 mm.
Trichocephalus dispar ...	0·05 ,, 0·054 ,, ,, 0·023 ,,

In measuring eggs no pressure must be used. Some, such as those of *S. japonicum*, are very readily distorted by pressure. Hence the variation in the measurements given.

The embryo of the *Strongyloides intestinalis* is frequently passed with the stools. The embryos of the *Trichina spiralis* are very rarely passed in the stools, as they normally penetrate the intestinal walls and pass into the surrounding tissues.

In the fæces, thread-worms, segments of tape-worms, and occasionally round-worms are passed naturally. After the administration of powerful anthelmintics, the whole tape-worm, round-worms, ankylostomes, flukes, and whip-worms may be passed. Some species are never found under any circumstances in the fæces.

CHAPTER XX.

Intestinal Parasites.

The worms met with in the human intestine and its appendages belong to the following classes :—

A. *Cestoda.*—These flattened worms have a segmented body, no digestive tube, and are hermaphroditic.

B. *Trematoda.*—In these the digestive tube is incomplete ; there is no anus, and the body is not segmented. They are hermaphroditic except the *Schistosoma.*

C. *Nematoda.*—These usually have a complete digestive tube. They are cylindrical worms, and they are not hermaphroditic.

A. Cestodes.

The human *Cestodes* are : *Tænia solium*, *T. saginata*, *T. confusa*, *T. africana*, *Dipylidium caninum*, *Hymeno lepis murina* (*T. nana*), and *H. diminuta*, *Davainea madagascariensis*, *Bothriocephalus latus*, *Diplogonoporus grandis.*

Cestodes or Tapeworms.—The embryonic or cystic forms of the *Tænia echinococcus* may be found in the liver, muscles of man, &c. The definitive host is the dog. These cysts, the hydatid cysts, can hardly be mistaken for non-parasitic cysts ; they can be readily distinguished if there is any doubt by the laminated cyst wall and the presence of hooklets in the cyst or discharges.

In the case of the *echinococcus* man is the intermediate host. A larval form of *Bothriocephalus* (*B. mansoni*) has been found in the connective tissues of men in Japan, and similar larval forms have been obtained from an aboriginal of British Guiana and in Central Africa. These larvæ may be the larval form of a *Dibothriocephalus*, but it is not certain. They are classed as a separate genus, *Sparganum* (Stiles).

The greater number of the tape-worms found in man attain sexual maturity in him. Man is therefore the definitive host of these worms.

The general structure of tape-worms should be known, and the differences indicated in the tabular statement of the well-known human tape-worm will then be understood.

Tape-worms consist of a head or fixed portion attached by hooks or suckers, or both, to the intestinal wall. This "head" is called the *scolex*. From this scolex growth takes place continuously in one direction; at first as a narrow neck which is not segmented, but which rapidly becomes segmented, and as growth continues each segment increases in size and becomes sexually mature. Each segment is known as a *proglottis*, and together these form the *strobila*. When sexually mature, the eggs are fertilized, and finally the genital organs atrophy and the proglottis is reduced to a muscular sac distended by a uterus filled with fertilized eggs. These proglottides become detached and are passed in the stool. Each proglottis is motile and may live for some time after it has been passed in the stool. It creeps about discharging its eggs. These eggs are taken up by the intermediate host, another mammal, a fish, or even an insect, and develop in that animal into the cystic or larval stage. In the case of some of the tape-worms, as in *Bothriocephalus*, a ciliated embryo is formed which swims freely in water, and in its intermediate host does not form a cyst, but an elongated, worm-like larva known as a "*plerocercoid*" larva.

If taken, with food or otherwise, into the intestinal tract of man, the cyst is set free and the head becomes the scolex of the mature tape-worm. This scolex fixes itself to the intestinal wall and gives rise to the proglottides by growth from it.

The tape-worm derives its nutriment by osmosis from the intestinal tract. There is no intestine and no trace of one. There are water vascular tubes, the water vascular system, running the whole length of the worm.

With this exception, and the nervous system, each segment or proglottis is a distinct individual jointed on to its predecessor and successor.

The points in the structure of a proglottis are best observed in a half-grown proglottis, as earlier the organs are not fully developed and the last segments are merely muscular egg-sacs with atrophied organs.

For permanent specimens the method to be adopted is as follows: Stain for twenty-four hours with very weak borax carmine; soak in glycerine for some months. Compress between two slides clamped together and place in methylated spirit. When partially hardened the pressure can be relaxed and the specimen dehydrated in alcohol. Clear with oil of cloves and mount in balsam. Pressure should be applied to the cover-glass till the balsam has hardened.

The proglottis is covered with a transparent cuticle, and has a powerful muscular wall with longitudinal and transverse or circular bands. In the interior of the segment are the organs of generation, male and female, as each segment is hermaphroditic. The arrangement of these organs varies greatly in different species, but they conform to a common type.

The space between the organs is occupied by parenchymatous tissue, in which are often included highly refractile calcareous masses, which must not be mistaken for eggs.

The male genital organs consist of a number of small testes. Minute *vasa efferentia* unite about the centre of the segment into a common vas deferens, this terminates in the copulatory organ or *cirrhus*, opening with the vagina into a genital cloaca.

The female genital organs consist of the vagina leading as a straight tube from the genital cloaca into an enlargement, the receptaculum seminis; from this the tube is continued to the *shell gland*, and near it the ovarian tube, or tubes, if, as is usual, the *ovary* is paired, open. There is a diverticulum running longitudinally in the

centre of the proglottis, which at first is simple, but later branched—the *uterus*. The continuation of the vagina is surrounded by the shell gland and the duct of the *vitellarium* or yolk gland opens into it.

The spermatozoa pass up the vagina and the eggs discharged from the ovaries are fertilized, receive their

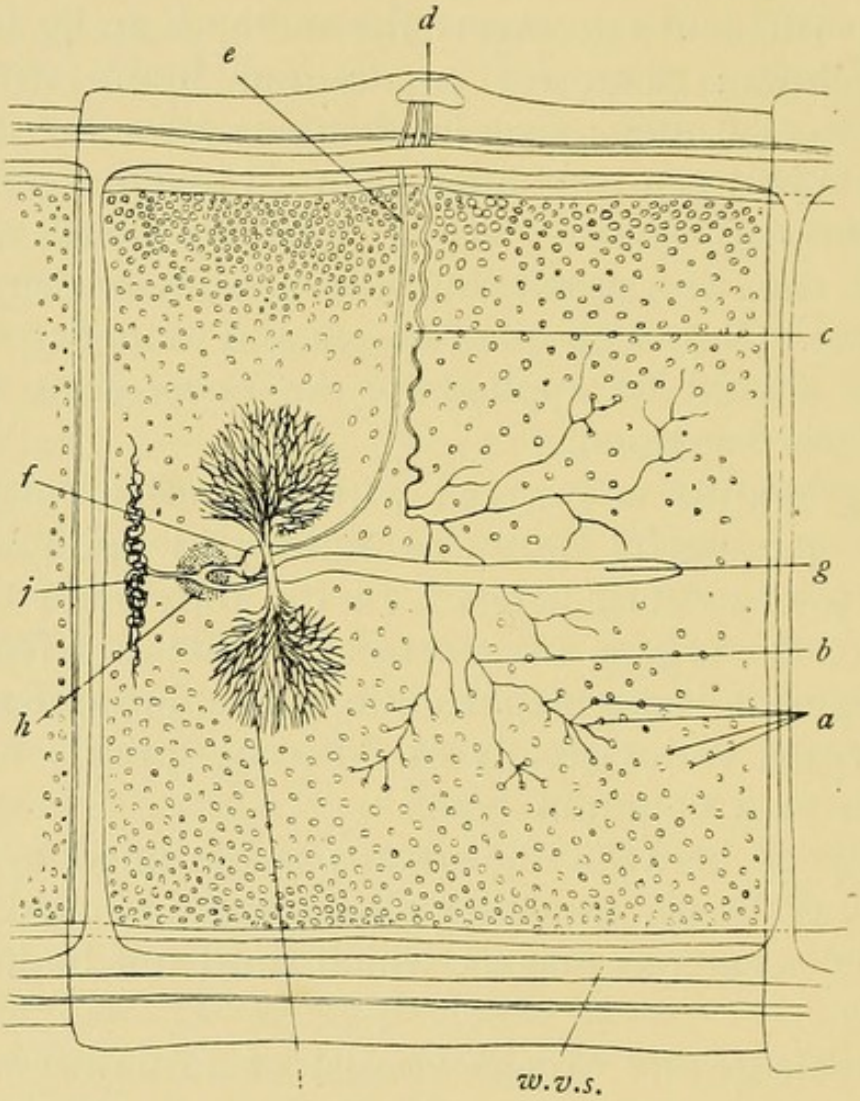


FIG. 144.—*a*, Testes; *b*, vasa efferentia; *c*, vas deferens; *d*, genital pore; *e*, vagina; *f*, receptaculum seminis; *g*, uterus; *h*, shell gland; *i*, ovary; *j*, vitellarium or yolk glands; *w.v.s.*, water vascular system.

yolk and shell, and are then forced into the longitudinal diverticulum or uterus. As more and more eggs pass into the uterus this tube becomes distended and the lateral diverticula enlarged, and ultimately the whole proglottis is occupied by the uterus distended with ova.

The projection marking the genital cloaca, into which both the male and female organs open, is known as the genital pore (fig. 144).

In examining a tape-worm the points to observe are :—

(1) The size, shape and number of proglottides in the worm.

(2) The size of the scolex and its armature, which may be suckers only, or suckers and hooks, and the number of these.

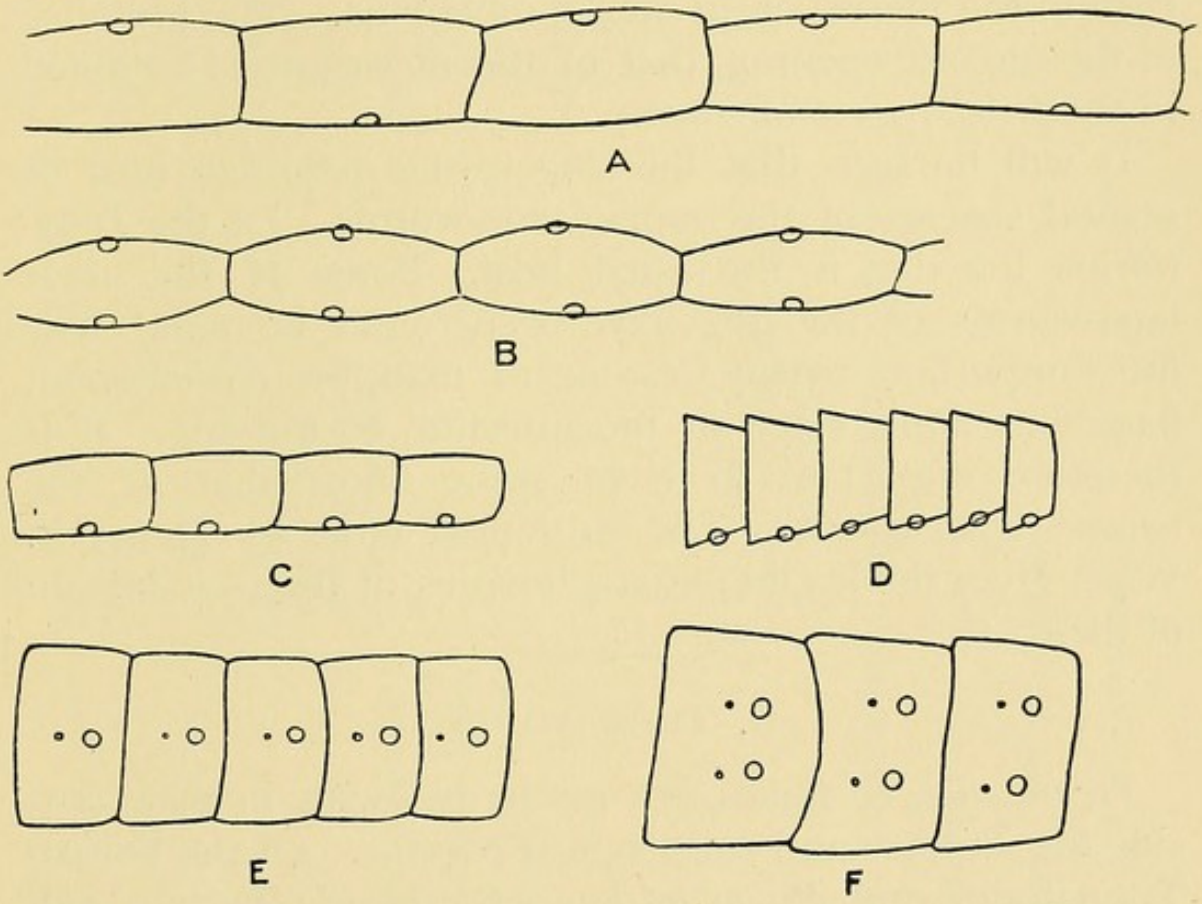


FIG. 145.—*a, Tænia saginata ; b, Dipylidium ; c, Davainea ; d, Hymenolepis ; e, Bothriocephalus ; f, Diplogonoporus grandis.*

(3) In the proglottides the relative length and breadth of the segments, particularly of the mature ones. The number of genital pores in each proglottis—two in *Dipylidium* and *Diplogonoporus*, one in most of the other genera. The position of the pore, which is marginal in most, but in the mid-ventral line in the middle of the broad surface of the proglottis in *Bothriocephalus* and on the flat surface in all the *Dibothriocephaloidea*. It must also be noted if the genital pores in the different

segments are all on the same side of the worm, as in the *Davainea*, or alternately (frequently irregularly so) on opposite sides of the worm, as in *Tænia saginata* (fig. 145, *a*). In the ripe proglottides the branching of the uterus should be noted and the arrangement of the eggs.

With proper attention to these points there is little difficulty in differentiating between the different species of the human Cestodes.

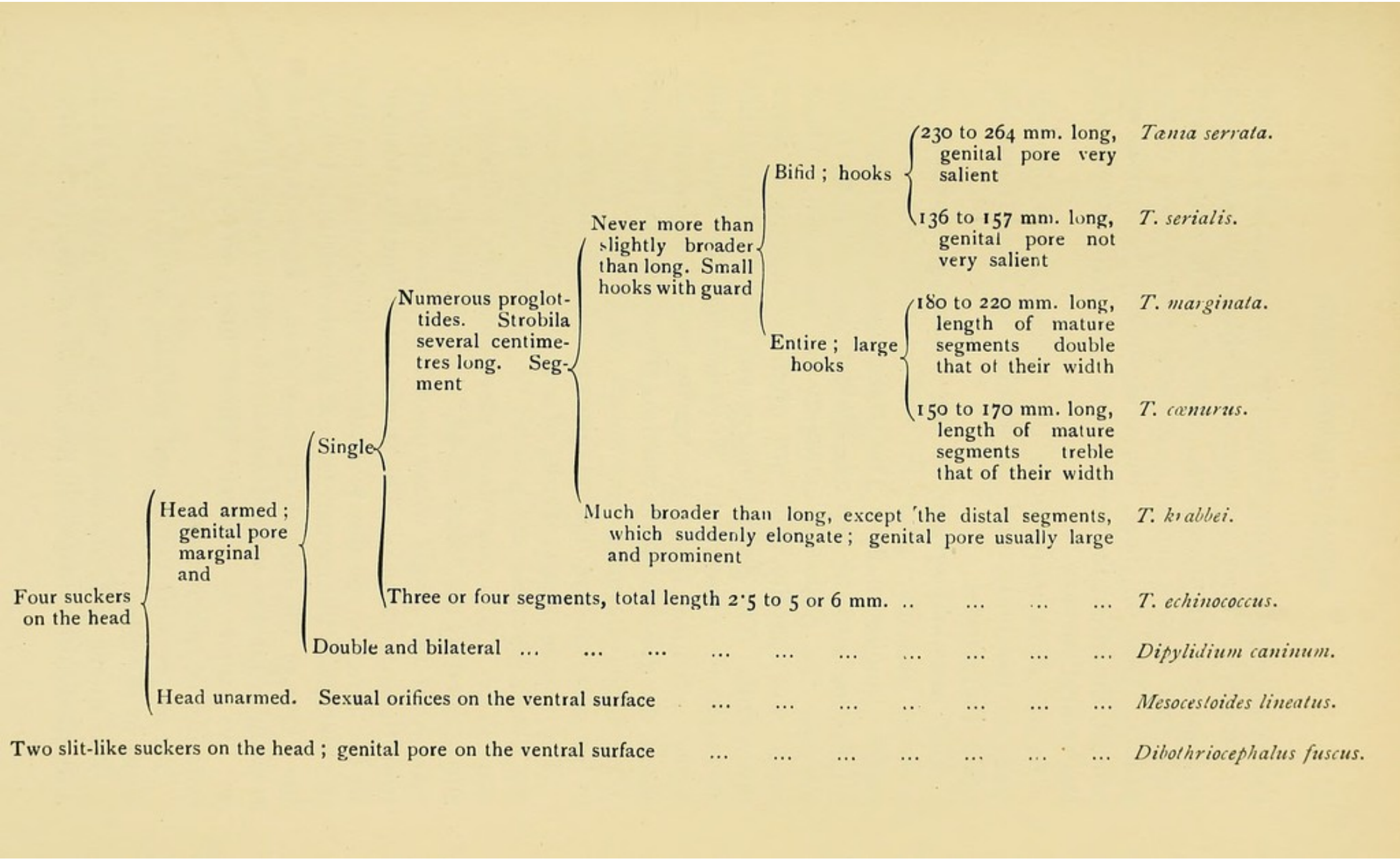
(4) The number of testes, and the arrangement of the eggs in the uterus or in the parenchyma. The presence of the second opening, that of the uterus, must be noted in the *Dibothriocephaloidea*.

It will be seen that the dog is the definitive host of several species of the human tape-worms. Of the *Dipylidium* the dog is the usual host. Some of the other tape-worms of the dog have been found in man. The most important cystic Cestode of man, the *echinococcus*, passes its adult stage in the intestine of the dog. It is therefore important to have some knowledge of the canine tape-worms. The subjoined table by Henry B. Ward gives the leading characteristics of the best known of these.

B. Trematodes.

Trematodes, or flukes, are rarely met with in man outside the Tropics and sub-tropical regions. Of the human Trematodes one, *Paragonimus westermani* (*Distoma pulmonale*), is found in the lungs ; others, the *Schistosomum hæmatobium* (*Bilharzia hæmatobium*) and *S. japonicum*, occur in the blood-vessels. Other trematodes are present in the liver, and the eggs only, which are passed down the bile ducts, are found in the fæces, and still others are found in the intestinal tract, so that ordinarily the eggs, and after the administration of powerful anthelmintics, like thymol, the adults also of these are passed by the rectum.

The Trematodes include the *Fasciolidæ*, which are flattened bodies, of oval shape with pointed ends ; from


Four suckers on the head
Head armed; genital pore marginal and
Single
Numerous proglottides. Strobila several centimetres long. Segment
Never more than slightly broader than long. Small hooks with guard
Bifid; hooks
230 to 264 mm. long, genital pore very salient
Tænia serrata.
136 to 157 mm. long, genital pore not very salient
T. serialis.
Entire; large hooks
180 to 220 mm. long, length of mature segments double that of their width
T. marginata.
150 to 170 mm. long, length of mature segments treble that of their width
T. cœnurus.
Much broader than long, except the distal segments, which suddenly elongate; genital pore usually large and prominent
T. krabbei.
Three or four segments, total length 2·5 to 5 or 6 mm. ... ...
T. echinococcus.
Double and bilateral ... ...
Dipylidium caninum.
Head unarmed. Sexual orifices on the ventral surface ... ...
Mesocestoides lineatus.
Two slit-like suckers on the head; genital pore on the ventral surface ... ...
Dibothriocephalus fuscus.

the peculiarity of their shape they are popularly known as "flukes." They are hermaphroditic, non-segmented, and possess an incomplete intestine. They are armed with two suckers placed near each other in most of the genera. One of these, the oral sucker, surrounds the mouth, the other, the ventral sucker, or *acetabulum*, is on the ventral surface.

In the *Paramphistomidæ* the suckers are at opposite ends of the body, and they differ in shape from the other Trematodes.

The intestinal system of the Trematodes consists of a short muscular pharynx leading from the anterior sucker longitudinally. The œsophagus terminates by bifurcating into the two cæca which pass round the body towards the posterior extremity of the worm. These cæca end blindly, but are often sacculated or have diverticula. The genital organs are complicated and the arrangement varies. In *Schistosomidæ* the male and female are distinct, and the female lives in an incomplete canal, the gynæcophoric canal in the male. In the other Trematodes the male and female organs are contained in the same animal; but the openings of each are distinct.

The female organs consist of a convoluted uterus opening externally near the second or ventral sucker in the *Fasciolidæ*. This convoluted uterus leads to a dilatation surrounded by the "shell gland," and into this the ovarian tube from the single ovary opens, and also the opening from the spermatheca. The common vitelline duct formed by the junction of the two vitelline ducts which receive the yolk from the numerous yolk glands distributed along the edges of the animals opens with it. There is a canal leading from the ventral surface to the oviduct known as the canal of Laurer, which may serve for the entrance of spermatozoa.

There are two compound testicles which lie one in front of the other.

The ducts, *vasa deferentia*, from these pass forwards and open into a dilatation, the *vesicula seminalis*, the duct

from which leads to the penis, which opens externally close to the female genital opening (fig. 146).

The details of the arrangement vary greatly. Fertilization is probably by a different worm. The fertilized eggs are passed with the fæces, sputum, urine, &c., of the definitive host.

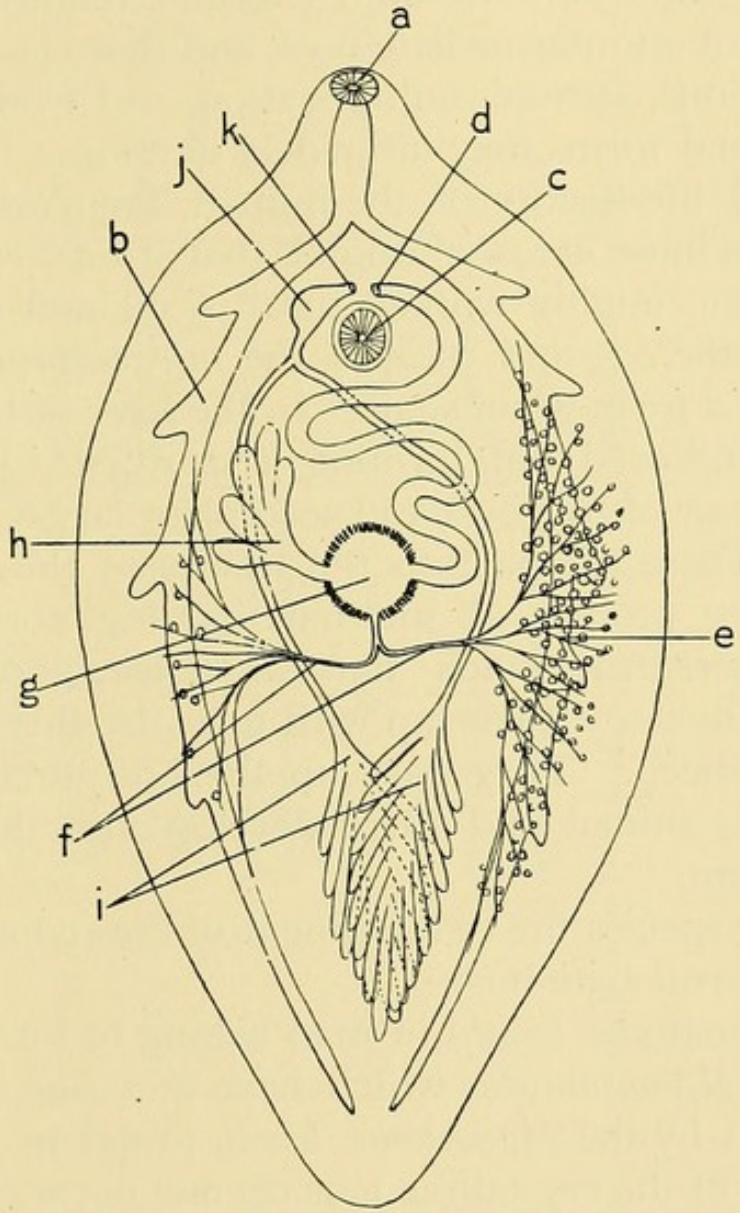


FIG. 146.—*a*, Anterior sucker; *b*, cæcum; *c*, ventral sucker or acetabulum; *d*, opening of uterus; *e*, yolk glands; *f*, vitelline ducts; *g*, ootype; *h*, ovary; *i*, compound testicles; *j*, vesicula seminalis; *k*, penis.

The structure of *Fasciolidæ* is best shown as in the case of the Cestodes by prolonged immersion in glycerine and then passing through alcohol and oil of cloves, after staining with weak borax carmine for some days.

In one division of the *Trematoda*, the *Monogenia*, the eggs develop into a condition suitable for the invasion of their definitive host without the intervention of an intermediate host, and there is no alternation of generations or asexual multiplication. From one egg, therefore, one sexually mature form is developed. The eggs of the other and more important division, *Digenia*, require for their development an intermediate host, and there is alternation of generations, asexual multiplication, and from one egg several sexual forms may ultimately develop.

The full life-history of the human Trematodes is not known. Of those in lower animals that of *Fasciola hepatica* has been thoroughly worked out. A ciliated embryo is formed in the egg and escapes, the *miracidium*. It then passes into a fresh-water snail. In the liver of the snail it becomes hollowed out, forming a *sporocyst*. Buds form in the interior of this cyst and secondary larvæ, *rediæ*, are formed. These escape into the tissues of the snail, and by a further process of internal budding form tertiary larvæ or *cercariæ*, which have a sucker, and escaping from their host are taken up with grass by their definitive host, the sheep. They pass up the bile ducts into the liver of this animal and there develop into the sexually mature form.

In other species the development of the rediæ require a second intermediate host.

The Trematodes found in man belong to four families :

(1) The *Monostomidæ*, which have only one sucker, are represented by the *Monostoma lentis*, found in the superficial layer of the crystalline lens on one occasion only.

(2) The *Fasciolidæ* have two suckers, one terminal and the other ventral. Of this family, representatives of eight genera are found in man. In six of these the ventral sucker is near the oral sucker. *Dicrocœlium*, in which the testicles are in front of the female genital organs; *Fasciola*, *Fasciolopsis*, *Fascioletta*, *Opisthorchis* and *Clonorchis*, in which the testicles are behind the female genital organs, and in these the ventral sucker is in the anterior part of the ventral

	Length	Breadth	Suckers	Situation	Hosts	Geographical distribution
Fasciola hepatica	20-30 mm.	8-13 mm.	The ventral sucker is much the larger	Biliary canals	Sheep and other animals. Man very rarely	Widely distributed.
Fasciolopsis buski (*Distomum crassum*)	35-75 ,,	14-20 ,,	Ventral sucker the larger. The two suckers are very near each other	Small intestine	Man	India, China.
Fascioletta ileocana ...	4-6 ,,	·75-1·35 ,,	Posterior sucker much the larger	Intestine ...	Man	Philippines.
Dicrocœlium lanceatum (*Distoma lanceolatum*)	8-10 ,,	1·5-2·5 ,,	Ventral sucker slightly larger	Biliary canals	Sheep. Very rarely man	Widely distributed.
Opisthorchis felineus ...	8-11 ,,	2-2·5 ,,	Ventral sucker slightly smaller than the terminal one	Biliary canals	Dog, cat. Found in man	Siberia.
Opisthorchis noverca (*Distoma conjunctum*)	9-12 ,,	2-5 ,,	Ventral sucker smaller ...	Biliary canals	Fox, dog, man	India.
Clonorchis sinensis ...	15-18 ,,	3-4 ,,	Anterior sucker the larger	Biliary canals	Man	China, Japan, India.
Heterophyes heterophyes ...	1-2 ,,	·7 ,,	Anterior sucker very small	Intestine ...	Man	Egypt.
Paragonimus westermani (*Distoma ringeri* or *pulmonale*)	8-10 ,,	4-6 ,,	Both small	Lungs, &c. ...	Man	China, Japan.
Gastrodiscus hominis ...	5-8 ,,	3-4 ,,	Posterior sucker much the larger. The genital pore is nearer the anterior sucker	Large intestine	Man	India.
Cladorchis watsoni ...	8-10 ,,	4-5 ,,	Posterior sucker large. No distinct sucker anteriorly but well-marked pharyngeal pouches	Small intestine	Man	Nigeria.

surface, not far away from the oral sucker. These three genera are separated from each other by the shape of the anterior extremity, which is conical in the *Fasciola*; by the character of the intestinal cæca, which are much branched in *Fasciola*, unbranched but sinuous in *Fasciolopsis*, and unbranched but nearly straight in *Clonorchis*. *Fasciolopsis* and *Fascioletta* are further distinguished by the great size and depth of the acetabulum or ventral sucker, and by the conspicuous and long cirrhus. In *Fasciola* and *Clonorchis*, as well as in *Dicrocœlium*, the ventral suckers are about the same size as the oral suckers. In the other two genera the ventral is nearly in the middle of the ventral surface. *Heterophyes*, in which the genital opening is behind the posterior sucker, which is very large, much larger than the oral sucker; and *Paragonimus*, in which the genital opening may be median, or right or left of the middle line. The ventral sucker is about the same size as the oral sucker.

(3) The *Paramphistomidæ* have two suckers, both terminal, one at the one end and the other at the other end of the animal. They include two divisions, species of each of which are parasitic in man—*Amphistomum*, or *Paramphistomum*, and *Gastrodiscus*. These genera are distinguished by their external appearance, as in the *Gastrodiscus* the anterior extremity is conical and appears to rise as a projection from the dorsal surface of a flat, rounded mass which contains the organs of reproduction. The human species is *Gastrodiscus hominis*. In the *Amphistomum* or *Paramphistomum* the shape is more or less conical, and there is no marked division between the anterior and posterior part of the body. The human representative is *Amphistomum watsoni*. This is more correctly a *Cladorchis*, as there are pharyngeal pouches which are not present in the *Paramphistoma*. In both *Paramphistomum* and *Gastrodiscus* the opening of the genital pore is in the middle of the body and not near the acetabulum.

(4) *Schistosomidæ* have two suckers, but the male and female organs are in separate animals.

NEMATODES.

The commoner Nematodes found in the human intestine are the *Ascaris lumbricoides*, rarely *Ascaris mystax*, *Gnathostoma siamense*, *Oxyuris vermicularis*, *Ankylostomum duodenale*, *Necator americanus*, *Strongylus subtilis*, *Trichocephalus dispar*, *Trichina spiralis*, *Strongyloides intestinalis* (*Anguillula intestinale*).

The larger worms are readily found, but some of the smaller ones are only to be seen by very careful inspection. The Nematodes are liable to shrink, rupture, or otherwise become distorted, and are not very easy to stain. As a general rule it is best to examine them unstained, and describe them as they appear in the fresh condition, mounted in normal saline solution. For permanent specimens fixation in 70 per cent. alcohol just on the boil gives good results. The 70 per cent. aqueous solution of alcohol should be heated till bubbles begin to form, and the living worms dropped into it. The lamp should be put out, and the worms left in the fluid. After this treatment they may be mounted in glycerine jelly without shrinking.

Ascaris lumbricoides.—These are large round worms. The males are 15 to 17 cm. in length and 2 or 3 mm. in breadth. The female is rather larger, 20 to 25 cm. long and 5·5 mm. in breadth. These worms are found in any part of the intestinal tract and occasionally pass through the common duct into the gall-bladder or even the biliary ducts. They have been found in hepatic abscesses. They may be numerous in the intestine. Rarely *Ascaris mystax*, commonly a parasite of the cat, a smaller ascaris, with lateral alar cuticular appendages on cephalic end of the body, is found in man.

Oxyuris vermicularis is a small cylindrical worm which tapers towards the tail to a sharp point. The male is 3 to 5 mm. in length and at the tail is coiled up in a spiral. The female is 9 to 12 mm. in length. This worm is found in the whole length of the large intestine and rectum and may escape through the anus (fig. 147, *a*, *b*). The males

are found higher up in the alimentary canal than the females.

Trichocephalus dispar (Whip-worm).—The characteristic of this worm is a long, thin, anterior portion somewhat resembling the lash of a whip. The male is 35 to 45 mm. in length, and the female 35 to 50 mm. These worms are found commonly in the cæcum and also in the ascending and transverse colon. They are very rarely found in the ileum (fig. 142).

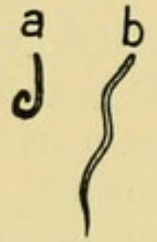


FIG. 147.—*Oxyuris vermicularis*. *a*, Male; *b*, female.

FIG. 148.—*Trichocephalus dispar*. *a*, Male; *b*, female.

The *Ankylostomum duodenale* is of the greatest importance. These worms are found in the small intestine and may be very numerous. Both males and females are found. They fix themselves to the intestinal wall and live on epithelium, *débris*, &c., but not blood. The female adult worms are 7 to 15 mm. in length and ·8 mm. in breadth. They have a mouth surrounded by a powerful armature consisting of two pairs of curved teeth on the posterior wall of the opening and of two triangular plates terminating in sharp points anterior to the mouth. The

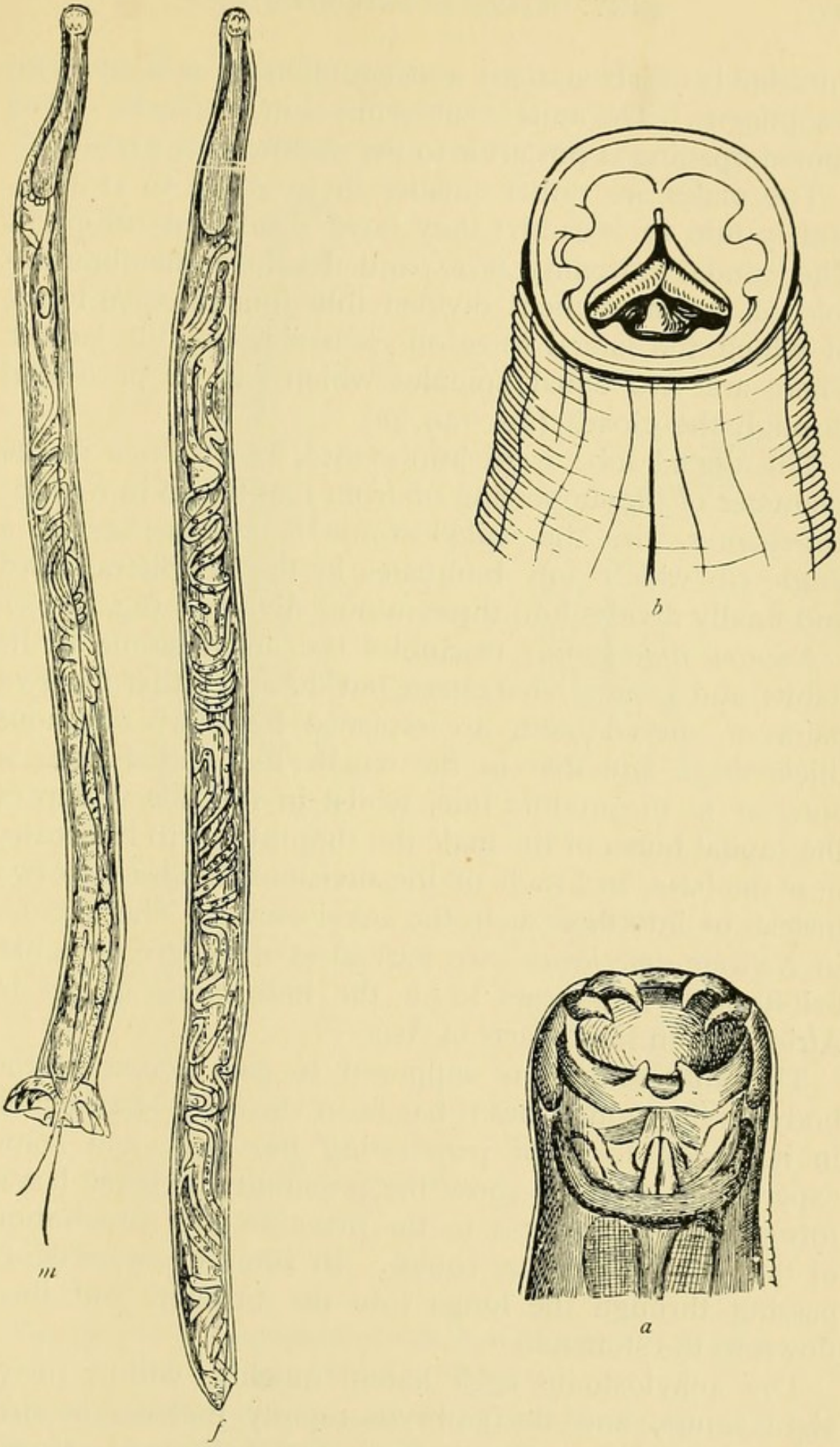


FIG. 149.—*m*,*f*, Male and female Ankylostomes; *a*, head of *A. duodenale*; *b*, head of *Necator americanus*.

intestine is nearly straight and commences as a powerful œsophagus. The anus is subterminal in the female. The genital opening is posterior to the middle of the body.

The males are rather smaller in length, 6 to 11 mm., and ·5 mm in breadth; they have similar mouth-parts. The caudal extremity is expanded into a membranous fold of the integument divided into four unequal lobes, of which the lateral ones on each side are the largest. There are two equal spicules which can be protruded through the cloaca (fig. 149, *m*).

The dorsal lobe is of importance, as it differs in the character of the supporting rib from that found in *Necator americanus*. In the ankylostome it commences as a single rib which only bifurcates in the peripheral third and finally divides into three minute divisions (fig. 150, *a*).

Necator americanus resembles the ankylostome in its habits and general characters, but differs in that the two pairs of curved teeth are replaced by sharp chitinous thickenings, and that in the female the genital pore is anterior to the middle line, whilst in the dorsal flap of the caudal bursa of the male the supporting rib bifurcates near the base, and each of the divisions divides into two instead of into three as in the ankylostome. The spicules in *Necator americanus* are barbed at their free ends like fish-hooks. It appears to be the indigenous species in Africa and in some parts of Asia.

The ankylostome is supposed to gain access to the body by the mouth, but it has been shown to be capable in its larval form of penetrating the skin, and some experiments seem to show the possibility of these larval forms obtaining access to the intestine after penetration of the skin by devious routes. In some cases certainly passing through the lungs into the bronchi and then down to the stomach.

The ankylostome eggs hatch quickly, within forty-eight hours, and the embryos rapidly increase in size. If kept in the fæces they soon die, but if allowed to escape into the earth they undergo further development, but do

not become sexually mature, or reproduce outside the body. In their final stages they are enclosed in their old larval skin as a sheath and are inactive for long periods.

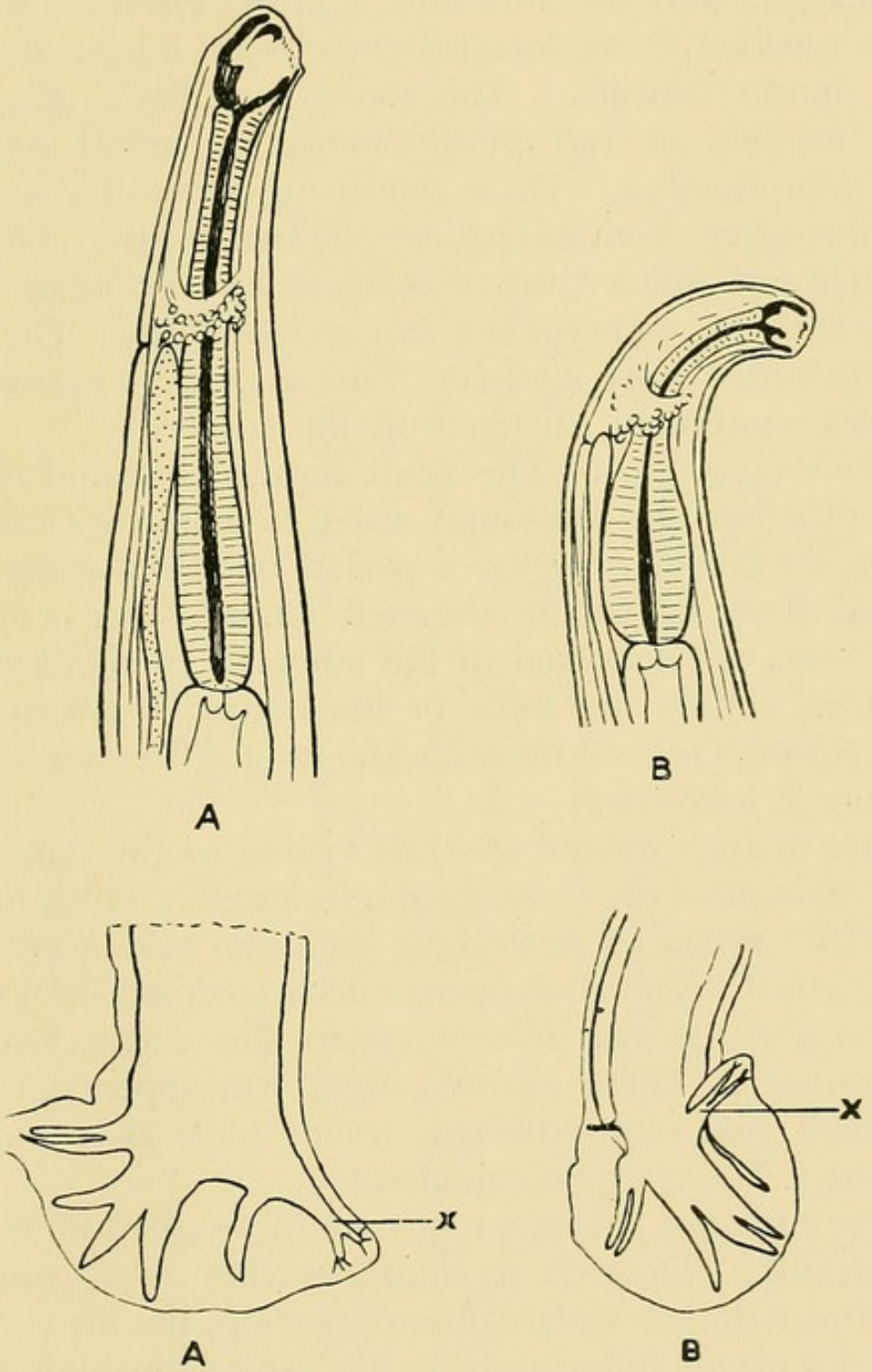


FIG. 150.—*a*, *a*, Head and tail of male *A. duodenale* ; *b*, *b*, head and tail of male *N. americanus*.

Strongyloides intestinalis, *Anguillula intestinalis*, or *Rhabdonema intestinale*. — This is a small worm only 1 mm. long and 50 μ in breadth. It is found in the small

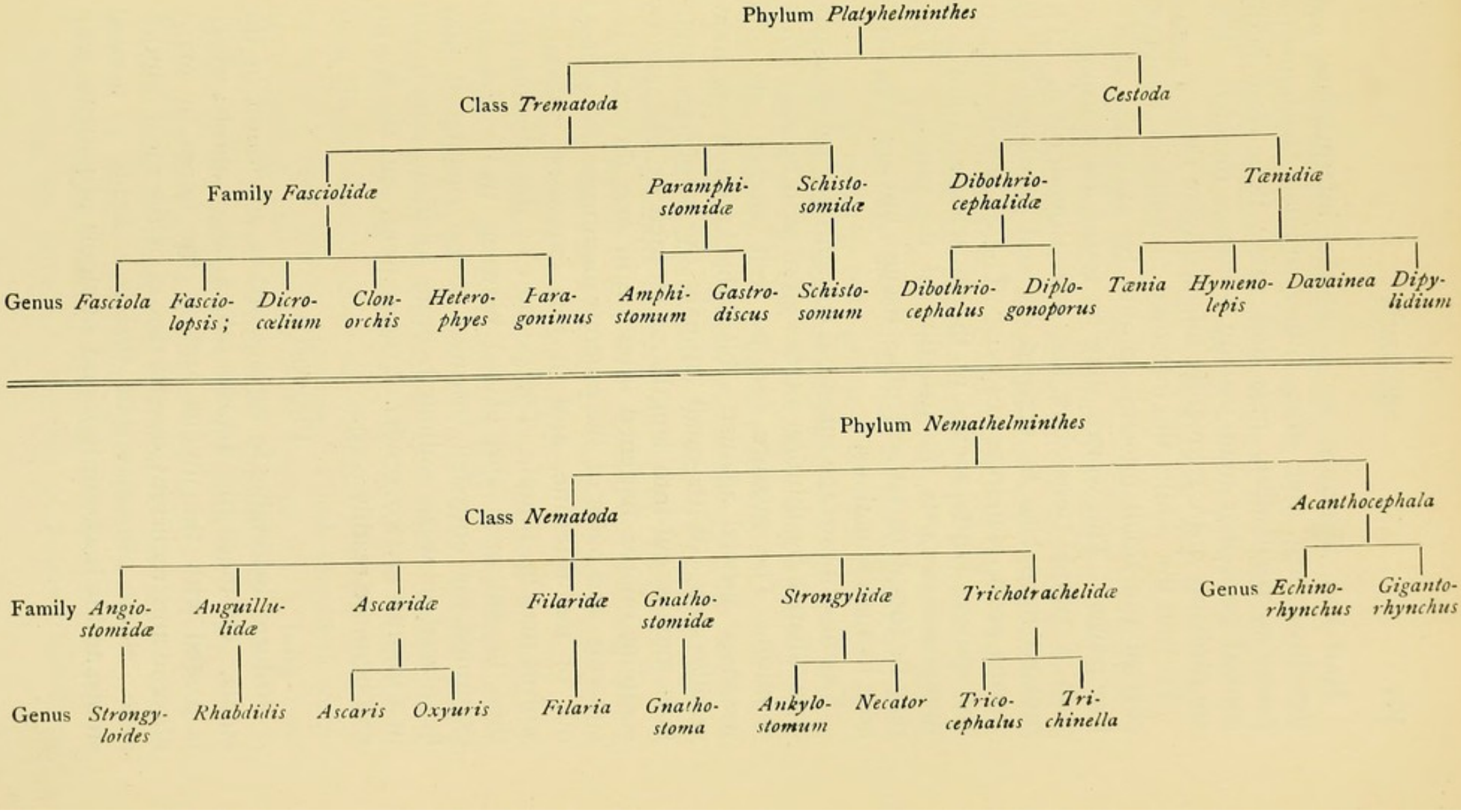
intestine and the male is not known. Only a small number of eggs are formed, four or five as a rule. The embryos hatch out either whilst still in the adult or when discharged into the intestine. These embryos can be distinguished from Ankylostome larvæ by the shape of the mouth capsule. The embryos outside the body are capable of full development to sexual maturity and reproduction. These free-living sexual forms differ in all respects from the parasitic sexual forms. They are shorter and broader, the œsophagus has a double dilatation and the embryos are more numerous. The free-living forms must alternate with a parasitic generation for the continued existence of the species.

Trichina spiralis.—The adult forms are found only in the intestines and intestinal walls of man, pigs, rats, &c. They are found only for a period of a few weeks after eating flesh in which encysted embryos were present. The cysts are dissolved in the stomach, the embryos are set free, and in the walls of the human small intestine pass through several metamorphoses and become sexually mature in a few days.

The mature worms are just visible to the naked eye. The male measures 1 to 5 mm. in length and ·04 mm. in breadth. There is a straight intestine with a powerful œsophagus. The anus opens into a terminal cloaca into which also the vas deferens from the single testicular tube opens. There are two digitiform appendages, one on each side of the cloaca, which serve as copulatory organs. There are no spicules.

The female is larger, 3 to 4 mm. in length and ·06 mm. in breadth. There is a single ovarian tube, which is continued into a widely dilated portion, the uterus, from which a narrow tube leads to the genital opening, which is situated about the junction of the anterior fourth with the rest of the body.

The embryos are passed alive, but do not appear in the fæces, as they pass through the intestinal wall, and finally reach the muscles and there become encysted. They may become encysted in the intestinal walls.


Phylum Platyhelminthes
Class Trematoda
Cestoda
Family Fasciolidæ
Paramphistomidæ
Schistosomidæ
Dibothriocephalidæ
Tænidiæ
Genus Fasciola
Fasciolopsis;
Dicrocœlium
Clonorchis
Heterophyes
Faragonimus
Amphistomum
Gastrodiscus
Schistosomum
Dibothriocephalus
Diplogonoporus
Tænia
Hymenolepis
Davainea
Dipylidium
Phylum Nemathelminthes
Class Nematoda
Acanthocephala
Family Angiostomidæ
Anguillulidæ
Ascaridæ
Filaridæ
Gnathostomidæ
Strongylidæ
Trichotrachelidæ
Genus Echinorhynchus
Gigantorhynchus
Genus Strongyloides
Rhabdidis
Ascaris
Oxyuris
Filaria
Gnathostoma
Ankylostomum
Necator
Trichocephalus
Trichinella

Man can therefore be both the intermediate and definitive host, but many other animals harbour the parasites, particularly pigs and rats. It is from badly cooked pork that man usually becomes infected. Pigs are probably more frequently infected from rats.

To find the adults the intestinal contents of the upper part of the small intestine should be examined with a low power. The worms may be found by examination with a simple lens, but are easily overlooked. The mucosa must also be scraped off and examined, as the worms soon penetrate into it. The encysted embryos are seen as white specks in the muscles, and are most numerous towards the insertion of the muscle. They may be found in fat and other tissues, but are less easily seen in such situations.

Examination of the intestinal contents for small worms is facilitated by dilution of the stool with normal saline solution. The mixture can be well shaken, and the worms if viewed against a black background are more readily seen. With small animals the unopened intestines may be cut into lengths of an inch or two, and by scraping on the flattened intestine in one direction the contents and parts of the mucous membrane are pressed out of the intestine, and in this expressed substance worms may be found. The portion of the intestine can then be slit open and placed between two slides and examined for encysted worms. If too opaque it may be placed in weak sodium hydrate solution, which will in a minute or two render it transparent, and the worms will be more readily seen.

PROTOZOA.

Protozoa belonging to various orders are found in the stools. Of these the *Amœba coli*, a large amœba which is passed with the mucus and in the fæces in many chronic and recurrent dysenteries, is of the most importance. Some observers state that it is found in normal stools, and it is certainly found in stools of patients who

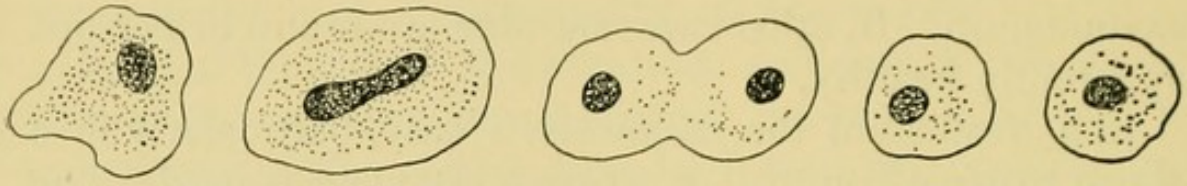
do not complain of either diarrhœa or dysentery. In a large proportion of these cases it will be found that mucus is passed with each stool, and in some ulceration of the colon has been present and found on *post-mortem* examination. In other cases amœbæ are found in persons apparently healthy.

In a fresh stool the diagnosis is easy, particularly in a warm tropical country, or where a hot stage is used. The large cells with active amœboid movement often containing in their interior red corpuscles and food vacuoles, permit of no mistake. If the stool has been some time passed, allowed to cool or treated with antiseptics, diagnosis is less easy, as the amœbæ, when they die, become globular, and are not then easily distinguished from other large cells in the mucus. If they contain blood corpuscles or other substances taken as food, they can be more readily recognized. They stain well with any basic stain, but there is no satisfactory differential stain. For the study of stained specimens smears of the fæces or mucus should be fixed whilst still wet, by placing them in a saturated solution of perchloride of mercury for ten minutes. They are then well washed in running water, and can be stained with borax carmine, or by the iron alum hæmatoxylin (see Appendix).

The life-history of the different amœbæ is not fully confirmed. In the commoner intestinal amœbæ asexual multiplication is by simple fission. This occurs in the intestine and in hepatic abscesses. Under certain conditions an amœba will become encysted, and the contents then divide after a series of changes partly outside the body into eight young amœbæ. These encysted forms are resistant, and retain their vitality for a long time outside the body. They are probably the important agents in the propagation of the parasite. The pathogenic properties are disputed by some. In the most severe cases of dysentery the *Amœba coli* is not found, and it is usually absent in epidemic dysentery. It is most frequently found in relapsing or recurrent dysentery.

Amœbæ are found in the pus of hepatic abscesses.

They are very difficult to find in the pus discharged at first. If the pus be examined three or four days after the abscess is opened they are usually readily found.



Asexual multiplication by simple division of nucleus and cytoplasm.

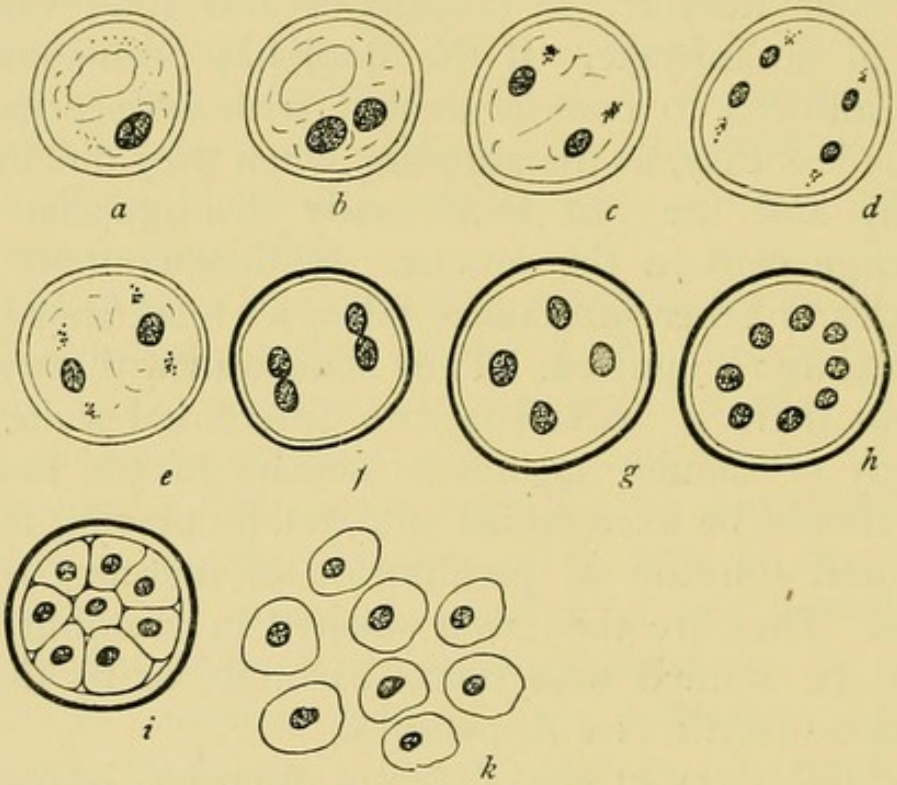


FIG. 151.—Scheme of Development of Amœba. Multiplication in encysted forms (autogamous). ? sexual multiplication. The early stages of division of the nucleus (*a*—*d*) and conjugation of the divided nuclei in pairs (*e*), followed by further division of these products of conjugation, first into two and then into four each (*f*—*h*). The thick wall of the cyst in the later stages indicates the hardening of the cyst wall during the stages when the cysts are outside the body. Stages *a*—*h* take place within the body. Stage *i* is only found outside the body, whilst stage *k* is believed to occur in the stomach of a second host.

Schaudinn differentiates two distinct species of Amœba in the human intestine. The one, the *Entamœba coli*, may be present in the intestines of healthy persons, and does not invade the tissues or pass into other organs in the body. The other, which he named *Entamœba histolytica*,

does invade the tissues of the alimentary canal, and may pass to various parts of the body, liver, &c. The morphological differences in the amœba as seen in the stool are slight. In the *Entamœba coli* the ectosarc is not clearly differentiated from the endosarc when the amœba is at rest. The pseudopodia are formed from both ectosarc and endosarc. The nucleus is large and rich in chromatin. Development is as described for the type in main essentials.

Entamœba histolytica has a small nucleus poor in chromatin and placed eccentrically. In the resting amœba the ectosarc is clearly defined and pseudopodia are formed from the ectosarc only. The development also differs. Multiplication may take place (1) by simple fission, as in *Entamœba coli ;* (2) by irregular gemmation, the number of young amœbæ formed being indefinite ; or (3) autogamy, in which the nucleus breaks up into a number of chromidia which are diffused through the protoplasm. Secondary nuclei then form at the periphery from which spores are produced. These are surrounded by a yellowish cyst wall and are the resistant forms.

In this amœba, therefore, the resistant encysted forms are young spores, whilst in *Entamœba coli* it is the mature form which is encysted and the spores are formed inside the cyst. Others view with suspicion all amœbæ and consider that some of the free-living forms may become parasitic and pathogenic.

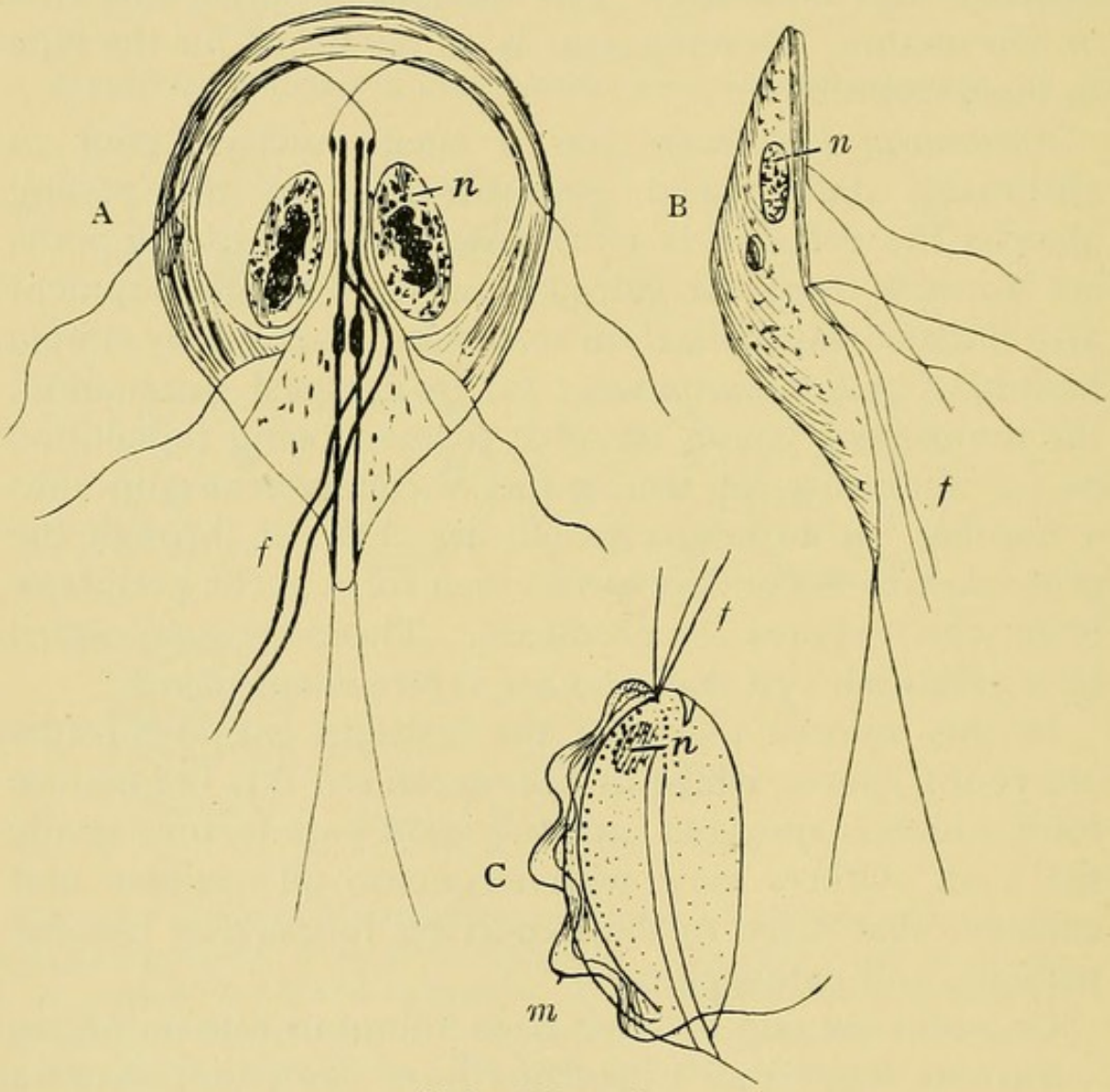
Coccidia are said to have been found in human fæces.

Various flagellated organisms have been described in the stools. One is *Cercomonas hominis.* It is a small round body with one or two long flagella. It is rarely found in healthy stools but may be common in some cases of diarrhœa. Recently doubt has been cast on the existence of Cercomonas.

Other intestinal flagellates which merit attention are Trichomonas and Lamblia.

Trichomonas (fig. 152) is pyriform in shape, the anterior end being rounded, whilst the posterior is pointed. At

the anterior end there are three flagellæ of equal length and fused together at their base. There is also an undulating membrane arising at the anterior extremity and running obliquely backwards. The nucleus is situated near the anterior extremity. This parasite seems to be quite harmless to its host.



(After Wenyon).

FIG. 152.—A, Lamblia; *n*, nucleus; *f*, flagellum. B, Lamblia, side view; *n*, nucleus; *f*, flagellum. C, Trichomonas; *n*, nucleus; *f*, flagellum; *m*, undulating membrane.

Lamblia (fig. 152) occurs usually in the duodenum or jejunum. It has a pear-shaped flattened body, with a large sucker-like depression on the ventral surface, by which it adheres to epithelial cells. It has four pairs of flagella, all directed backwards, and a double nucleus.

This parasite is probably pathogenic. The symptoms are of a chronic, recurrent diarrhœa, with abundant discharge of mucus, often bile-stained and frequently mixed with the fæces and sometimes with blood.

When there is diarrhœa the parasite may be found in abundance ; at other times only encysted forms, devoid of any flagella, will be found.

Flagellated organisms have also been found in the mouth and in abscesses in connection with the mouth cavity.

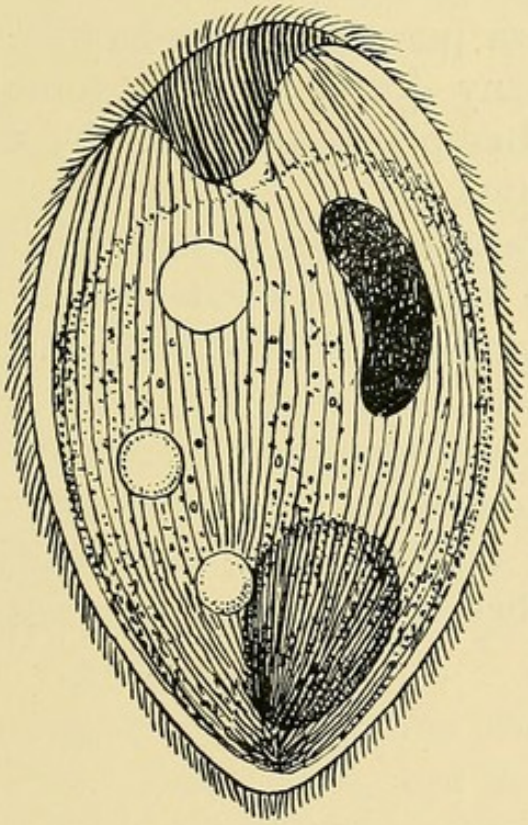


FIG. 153.

Spirochætæ are sometimes found in healthy stools, and are common in some cases of dysentery.

Infusoria are found in some cases of diarrhœa ; the best known resemble a large Paramœcium—*Balantidium coli*. It measures 65 to 85 μ in length. It may be found in very large numbers in the stools, and in such cases it may also be found in the intestinal walls and even in the blood-vessels ; it has been found in the pus of an abscess of the liver. It is probably pathogenic. It is a

parasite found very commonly in the intestines of pigs (fig. 153). It differs from Paramœcium in the characters and arrangement of the cilia guarding the peristome.

Vegetable micro-organisms abound. Many of these belong to the *coli* group, and include organisms which are harmless and others which are pathogenic. Many of the organisms, as, for instance, the *Bacillus coli communis*, though harmless to persons in good health as long as they are contained in the alimentary canal, can, under certain circumstances, invade the tissues and then become actively pathogenic and in some cases pyogenic. The intestinal mucosa possesses considerable power of resistance even to many decidedly pathogenic organisms, and consequently attempts at infection by the imbibition of cultures, &c., often fail.

Impaired resistance due to bad health, malnutrition, combined with enhanced virulence of an organism, is necessary in many cases for even pathogenic organisms to cause disease.

The isolation and identification of pathogenic and non-pathogenic organisms in the alimentary canal is a matter of considerable difficulty and complexity on account of the large number and species of organisms normally present.

CHAPTER XXI.

URINE.

IT is not proposed to consider the ordinary tests for the abnormal constituents of urine, such as albumin, sugar, and the like, but only a few special points in connection with the examination of urine in the Tropics.

Blood is found under special circumstances as a result of parasitic invasions by *Filaria nocturna* and *Schistosomum hæmatobium* (bilharzia) respectively, and there is at least one form of tropical hæmoglobinuria—black-water fever.

Hæmaturia can be easily distinguished from hæmoglobinuria by the presence of red corpuscles in the deposit. In many cases it is easily distinguished with the naked eye, as the superjacent fluid may not be coloured with blood in hæmaturia. If coloured with blood it is cloudy and not a clear and transparent red, as is the solution of hæmoglobin in hæmoglobinuria.

In hæmaturia from bilharzia infection the bulk of the urine is often free from blood, but if the patient, after apparently emptying the bladder, strains, the last few drops may be found to contain blood or mucus, and in this blood or mucus the characteristic ovum with its terminal spike and the contained ciliated embryo will be found. In all suspected cases it is therefore necessary for the patient to strain, and the few drops so passed are the most important for examination.

Schistosoma have been found not only throughout Africa, in Arabia and Cyprus, but also in some of the smaller West Indian Islands, and it is therefore possible that bilharziosis may become a more widely diffused disease than is at present the case.

In some of the places, as in the West Indies, it is reported that the *Schistosoma* eggs are found only in the fæces, and that hæmaturia does not occur. It is believed by some that the eggs passed *per rectum* belong to a different undescribed *Schistosomum* which has been named *S. mansoni*. The eggs of *Schistosomum japonicum* are not found in the urine nor is hæmaturia caused by them.

In most cases of bilharziosis there will be a history of occasional attacks of hæmaturia. In these cases, by finding the ova in the last few drops of urine expressed from the bladder, the causation of the disease can be determined.

Hæmaturia from filariasis is rarely an admixture of blood only. More often chyle is also present and usually chyle occurs without any admixture with blood (chyluria). Coagulation of the chyle and blood frequently takes place, so that clots of blood-stained substance, or of pure blood, are formed, or the whole mass may set as a pinkish jelly.

The embryos of the *Filaria bancrofti* may be found in the urine, but are more abundant in the blood. If scanty any small masses of blood or filaments of thread should be examined as the filariæ often adhere to them. Some authors advise filtering the urine, and in the last few drops left in the filter the embryos will be found. There is no periodicity in the appearance of the filarial embryos in the urine. If filariæ are not found in the urine they may be found in the peripheral blood of the patient if the examination be made at night.

Hæmaturia may also result from other causes, such as calculus, malignant disease, &c., but those are not limited to the Tropics.

Hæmoglobinuria, or the passage of urine coloured with dissolved hæmoglobin, is the characteristic of "blackwater fever." Cases of paroxysmal hæmoglobinuria would, no doubt, if they occurred in an endemic area, be mistaken for blackwater fever. Hæmoglobinuria is met with in Africa as a common disease, in some places 10 per cent. of the most susceptible portion of the popu-

lation (European) are attacked annually, or one-quarter of that proportion of the less susceptible Asiatics. In some parts of India a fair number of cases are met with, but only in small proportions as compared with Africa. Cases are reported from other malarial countries, South America, West Indies, Soloman Islands, Malaya, South of Europe, &c., but the disease is less common in those countries.

The urine when first passed is clear and, when diluted sufficiently, is transparent, any deposit present is mainly of casts and epithelium. As it cools, and particularly when it becomes alkaline, a thick amorphous albuminoid and brown deposit is thrown down. The greater the dilution required to render the urine transparent the more concentrated is the hæmoglobin solution in the urine, and the larger the amount of the hæmoglobin the more severe and protracted will be the attack.

Rate of Secretion.—In this and also in yellow fever the rate of secretion of the urine is a matter of great importance, as if the urine is much diminished the prognosis is grave and active measures are urgently required. The times of micturition and the amount passed each time must be observed, and the amount of urine passed at each micturition, divided by the number of hours that have elapsed since the previous micturition, will give the rate of excretion per hour during that period. Any fall in this rate is an important warning. If suppression is once established recovery will not take place in either disease.

Bile in the urine may occur in some cases of malaria as a transient phenomenon. The persistent presence of bile in an acute attack of malaria is a rare but a serious and frequently fatal complication.

Bile is met with in the urine, frequently in relapsing fever, and is not very rare in lobar pneumonia.

There are cases of jaundice occurring in the Tropics, associated with high fever, which are neither yellow fever nor malaria. These require investigation. Nothing is known of the true nature of these diseases. Possibly some of them are cases of Weil's disease.

Hæmoglobinuric urine can be distinguished from bilious urine by dilution, when the red colour of the hæmoglobin is seen. By shaking the urine and noting the pink tinge of the froth as compared to the yellow tinge of the froth of bilious urine, the distinction is readily made.

The most satisfactory method for diagnostic purposes is the use of a spectroscope, when the hæmoglobin bands will be clearly seen (*vide* Table of Spectra). In some of the cases all through, and in others at onset and end of an attack, methæmoglobin is passed alone. Such urine is of a brownish colour and can only be distinguished by the spectroscope (spectra 4 and 5). There is reason to believe that many mild cases of blackwater fever are overlooked, as the urine contains only this methæmoglobin. In this disease casts are present often in large numbers; the casts are granular, do not often include epithelial cells, but generally contain granules of bright yellow pigment derived from the hæmoglobin. Such casts are found for weeks after an attack of blackwater fever, though the urine is free from albumin.

It is important to be able, in watching a case, to form an estimate of the variations in the amount of hæmoglobin present. This is readily done if the first urine be diluted in the test tube to a convenient known extent. This is the standard, and the other urines found are similarly diluted if necessary till they match the standard.

Indican is very commonly present in the urine of patients in the Tropics, usually in cases of intestinal disorder. It is best detected by conversion into indigo blue. The simplest method is to place a crystal of potassium chlorate at the bottom of a tube and cover this crystal with the urine. Strong hydrochloric acid is allowed to run down to the crystal without mixing with the urine. A blue ring forms at the point of junction of the two fluids if indican be present.

Nitric Acid Test.—Strong nitric acid added so as to form a layer below the urine will lead to the formation

of a red colour at the junction of the fluids. If little indican is present this colour will appear in five to ten minutes; if in considerable amount it will appear at once, and if greatly in excess will be almost black.

Bacteria are frequently met with in the urine. Of the pathogenic organisms, the warnings which will be given as to the danger of confusing the smegma bacillus with tubercle must be borne in mind. The typhoid bacillus may be found in the urine for prolonged periods after recovery from the disease; and so may the micrococcus of Malta fever. In systemic infections with *B. coli communis* the organism is frequently present in the urine. In examinations for such organisms it is important that the urine should be drawn off by a sterilized catheter and received into a sterilized vessel.

Flakes of pus or muco-pus require careful bacteriological examination. Often they are the remnants of a gonorrhœal infection and the gonococcus may be found abundantly. Sometimes they are due to tuberculosis. In all such examinations the urine first passed in the morning should be examined, as such discharges accumulate during the night. The urine should be divided into three parts: that first passed contains the washings of the urethra. The great mass of the urine which will contain, with little admixture, substances derived from the kidneys and ureters; and lastly that passed after forcible expression of the last few drops, which will contain discharges from the wall of the bladder and from prostatic crypts.

The bacteriology of the urine in the Tropics has received little attention, but there are many cases of systemic infection with *B. coli communis* which originate in the Tropics, and in which the organisms are found in the urine, and probably there are cases in which a similar infection with other organisms takes place.

It is well to remember that urine can be used as a medium for the growth of organisms. That passed about two hours after a meal is the best, as it is not too acid.

B. typhosus and many others grow fairly well. It requires boiling, filtering and sterilization, and can be used either as a fluid medium, or by the addition of gelatine or agar made into a solid medium.

Some of the diseases in the Tropics are attended with deviations from normal in the metabolic and catabolic processes indicated to some extent by changes in the urine. The diminution in the amount of urea in beri-beri cases is an instance in point.

Other changes have been noticed in the urine of beri-beri patients and deserve close investigation. Hewlett has noted that casts, usually hyaline, but sometimes granular, can always be found in centrifugalized urine, and in addition that peculiar refractile bodies are present. These he describes as of three classes : (1) Small forms one-third to one-half the diameter of a red corpuscle, very refractile and apparently having a thick capsule with hyaline contents ; (2) larger forms, 12 to 20 μ, spherical in shape, and containing refractile granules, and appearing to contain also what may or may not be a nuclear body ; (3) occasional very large bodies, 30 μ in diameter, containing fine granules and a distinct nucleus with nucleolus.

These cannot be dried, fixed and stained, but in normal saline, in hanging drop preparations, they stain with neutral red, methylene blue and methyl green, but much less deeply than epithelial cells or leucocytes.

None of these bodies stain with osmic acid or with Sudan III., and therefore they cannot be fat. They may be myelin bodies. They are not found in normal urine or in that of persons with nephritis.

Hewlett suggests that they may be the result of peculiar degenerative changes in cells, or possibly are due to protozoa.

Diazo-reaction is a reaction which is constant in typhoid fever. The reaction may occur in other diseases, notably miliary tuberculosis, measles, scarlet fever and erysipelas, usually in severe cases of these diseases. The reaction is usually to be obtained about the fourth to seventh day in enteric fever, and though not conclusive as

a test as it occurs in other diseases, it is an important aid in the exclusion of enteric in obscure cases of continued fever.

Two solutions are required for the test :—

Solution 1.

Sulphanilic acid	2 grm.
Hydrochloric acid	50 c.c.
Distilled water	1,000 c.c.

Solution 2.

Sodium nitrite	0·5 grm.
Distilled water	100 c.c.

One part of solution No. 2 is added to fifty parts of solution No. 1, and mixed with an equal quantity of urine in a test tube. This mixture is then rendered strongly alkaline by ammonia.

If the reaction is positive, the mixture becomes carmine-red in colour, and if the test tube is shaken, this colour is seen in the foam. If the colour does not appear in the foam, the reaction is negative.

Estimation of Quinine excreted in the Urine.—It is occasionally necessary in treating patients with quinine to estimate the amount of that drug excreted by the urine, and for that purpose the method recommended by Christophers and Stephens may be employed.

Two hundred cubic centimetres of urine are acidified with a few 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 filtrate 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 albumin, and again filter. The residue is now digested in an Ehrlenmeyer flask with 50 cc. of 3 per cent. soda solution for half an hour on the water bath. Now add 60 cc. of chloroform, and shake for two hours in a shaking machine. 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 on a water bath, and dry at 120° C. The residue is quinine.

CHAPTER XXII.

Bacteriology.

The pathogenic micro-organisms with vegetable characteristics are less generally studied in the Tropics than elsewhere. Much of the easier work could be done without complicated apparatus or any great difficulty.

The methods now employed in British laboratories require too much apparatus, and are too complicated to be used by a private worker in the Tropics, and only the simpler methods which are at his disposal are considered here. This account of the methods which can be used is therefore intended only for those obliged to use primitive methods and makeshifts.

For the isolation and cultivation of vegetable micro-organisms artificial media are necessary, and the basis of the standard media is nutrient broth. There is much difficulty attending the making of nutrient broth from meat in the Tropics, but meat extracts, particularly Bovril or Liebig's, make an efficient substitute. In broth prepared from either of these, the organisms that will grow in nutrient broth made from meat will grow fairly well.

An iron enamelled jug, measures, scales, and weights, a glass rod, a funnel, and ordinary filter paper or white blotting paper is all the apparatus required. Bovril peptone and common salt and water are the substances needed, and litmus paper, or better, phenolphthalein, which is required for the neutralization of the broth when made, as well as a carbonate of soda or sodium hydrate solution.

Nutrient Broth.—To make the broth: Take 1,000

c.c. or 1 litre of water : then take 5 grm. each of Bovril (or Liebig) and salt, and 10 grm. of peptone (Wittes' is usually used). Mix the peptone with about 25 c.c. of the water, and stir it well so as to form a kind of emulsion ; then to this add the remainder of the water and the salt and Bovril. The Bovril can be conveniently weighed in a watch-glass, or if Liebig is preferred, this can be spread with a spatula on a piece of filter paper, and the watch-glass with the Bovril in it, or the filter paper with the Liebig's Extract on it, can be placed in the water with the other ingredients. The whole should now be boiled for a quarter of an hour, and well stirred to ensure thorough solution. It is now ready for neutralization. When made with Liebig, the broth will be much too acid to get good growths, and with Bovril, though much less acid, may be too acid to be quite satisfactory. Moreover, the degree of acidity of different specimens varies.

Neutralization.—Litmus paper can be used in an emergency to determine the reaction of the broth, but is unsatisfactory, as many of the organic acids do not affect litmus paper, and the dibasic sodium phosphates act on litmus paper as an alkali. Many specimens of broth also have a double reaction, turning red litmus paper blue and blue litmus paper red, so as to leave the point of neutralization uncertain.

Where possible phenolphthalein should be used. A ·5 per cent. solution of phenolphthalein in spirit is the indicator. This solution is colourless when acid or neutral, but turns a deep magenta colour with any free alkali.

Carbonic acid should be expelled from a measured quantity of the broth, say 25 c.c. by boiling ; to this broth a few drops of the phenolphthalein should be added, and then drop by drop the alkaline solution, till the broth turns a flesh or faint pink colour, indicating that the acid is completely neutralized. The amount of alkaline solution has been measured, and as there are 975 c.c. of

broth left the amount required for the neutralization of the 25 c.c. multiplied by $\frac{97.5}{25} = 39$ will give the amount of the alkaline solution required for the neutralization of the remainder of the broth.

It is to be noted that to exactly neutralize the broth it is of no importance what the strength of the alkaline solution may be.

A neutral broth so prepared will serve for the growth of most organisms, but the best growths are obtained with a broth slightly acid to phenolphthalein. If it be desired to use a less or more alkaline broth it is necessary to have an alkaline solution of known strength.

The solutions used are the so-called "normal solutions." A normal solution is a solution of the "equivalent" weight in grammes of the substance dissolved in a litre of distilled water. If the metal of the salt be monovalent, *i.e.*, if it is replaceable in a compound by one atom of hydrogen, the "equivalent" weight is the molecular weight in grammes. If the metal be bivalent, *i.e.*, if it requires two atoms of hydrogen to replace it in a compound, the equivalent weight is half the molecular weight in grammes. A decinormal solution is one-tenth of that strength or one-tenth of the equivalent weight in grammes dissolved in a litre of water. A centinormal solution is one-hundredth of the same weight dissolved in a litre; whilst a dekanormal solution is ten times as strong as the normal, or ten times the weight dissolved in a litre. For instance, the equivalent weight of sodium hydrate NaOH is $23 + 16 + 1 = 40$, of sulphuric acid H_2SO_4, as it neutralizes two molecules of sodium hydrate, is $\frac{1}{2}(2 + 32 + 64)$ or $\frac{98}{2} = 49$. A normal solution is represented by $\frac{N}{1}$, a decinormal by $\frac{N}{10}$ a centinormal by $\frac{N}{100}$. A normal solution of sodium hydrate therefore is 40 grammes dissolved in water and diluted to 1,000 cc., whilst a normal solution of sulphuric acid will be 49 grammes diluted to 1,000 cc.

A neutral broth is one which is neutral when tested hot with phenolphthalein; such a broth is usually alkaline

when tested by that uncertain standard, litmus paper. The degree of alkalinity of a broth is measured by the number of c.c. of normal alkaline solution added per 1,000 cc. of broth over and above that required for neutralization. The minus sign — is used to indicate the alkalinity, so that — 4 would indicate that 4 c.c. of a solution $\frac{N}{1}$ of alkali had been added to 1,000 litres of the broth in excess of the amount required for neutralization.

If the broth used is still acid as tested by phenolphthalein that is indicated by the plus sign +. A broth described as + 10 would still require the addition of 10 c.c. of $\frac{N}{1}$ solution of alkali per litre for neutralization. Many specimens of Bovril broth, without neutralization, are not more acid than this, and + 10 is a favourite reaction for the growths of many organisms.

This question of neutralization and of uniformity of reaction is a simple but important matter. The degree of alkalinity or otherwise of the media affects the properties of growths so materially that it is necessary to be particular on the point, but for mere growth of most organisms a broth neutral to phenolphthalein will suffice.

After neutralization or procuring the required degree of alkalinity or acidity to phenolphthalein, the broth should be boiled and kept at the temperature of boiling water for half an hour. It should then be allowed to cool, as it is not till it is cold that the mass of the phosphates will be precipitated. It is then, whilst cold, to be filtered through ordinary white filter paper. The broth is now prepared, but in the course of the preparation many organisms will have gained access to it from air, vessels, &c., and if left as it is these would multiply. Sterilization is therefore necessary. This can either be done in bulk or the broth can be decanted into a series of test tubes in quantities suitable for use.

The procedure differs little in the two cases. If it be desired to keep the broth in bulk it should be poured into a clean narrow-necked vessel (Erlenmeyer flask,

fig. 154), which will stand heat, and the mouth of this vessel plugged tightly with non-absorbent cotton-wool. If it is to be divided, some 10 c.c. should be poured into each of a series of clean test tubes and the mouth of each should be plugged with cotton-wool. It is better to sterilize, by dry heat, the flask or the tubes and wool before pouring in the broth. This is not absolutely essential, as the tubes, wool, and broth contained in the tubes can all be sterilized together, but is advisable.

For sterilization a single boiling does not suffice, as

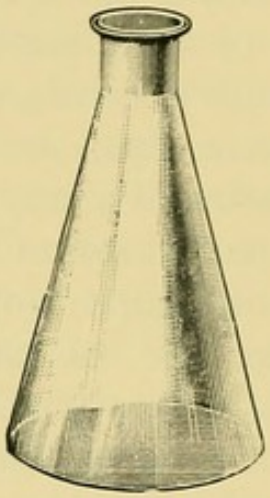


FIG. 154.

spores are only slowly killed at the temperature of boiling water.

Sterilization.—To sterilize, the broth and the vessels containing it should be maintained at the temperature of boiling water for at least half an hour on three consecutive days and allowed to cool in between. This intermittent method allows the spores which have escaped the first sterilization to develop into the less resistant organisms before the second heating, which then destroys them. The third sterilization, which is not always absolutely necessary, is a precaution in case any spores or organisms have escaped from the two previous sterilizations.

Storing Media.—The broth when cool is ready for use and can be kept till required. The tubes, wool, and broth are all sterile and remain so for a considerable

period. Organisms can only gain access to the broth by growing through the wool. This does not take place through dry wool, but in moist, warm climates, such as are met with in the Tropics, the wool gets damp and growth through it takes place.

In such climates it is advisable as a routine every week to heat the end of the test tube containing the cotton-wool so as to ensure the wool being dry and to kill any organisms that have grown in it. Unless this precaution is taken the tubes soon become contaminated. In a moist climate tubes pept in a cool incubator become contaminated so rapidly that the cotton wool should be heated daily.

The number of organisms falling on to the cotton-wool can be greatly reduced by covering the top of the tube with an inverted paper cone, such as a folded filter paper. This measure would delay the contamination of wool even in the Tropics.

Glycerine Broth, &c.—For many purposes additions are made to the nutrient broth. These additions must be made before neutralization and sterilization; if made after, the sterilizations will require to be repeated and the neutralization readjusted.

Glycerine broth is made by the addition of 6 per cent. of glycerine. Glucose, lactose, maltose or saccharose, added in the proportion of 2 per cent. to the broth, make glucose broth, lactose broth, &c., respectively.

Solid Media.—The broth is mixed with gelatine or agar-agar in sufficient proportion for the solution to set when cooled to the temperatures at which it is desired to study the growths.

Nutrient Gelatine.—The gelatine medium is made by the addition of 9 to 12 per cent. of the best French gelatine to the crude broth. Broth that has been neutralized and filtered can be used, but it is waste of time, as neutralization will have to be repeated. Gelatine is always acid.

After the gelatine has been added in the required proportion keep in the steamer for half an hour; neu-

tralize, render alkaline, or leave acid to the required extent. Allow to cool to 45° C.

Whip up the white of an egg for each 500 cc. of the gelatine broth and mix well with the medium. Steam for half an hour. The white of the egg diffused through the medium will coagulate, and in its coagulation will carry down many of the impurities of the gelatine. Filter whilst hot through a coarse filter paper—Chardin's—which should be moistened with hot water. Store in flasks or decant into test tubes as required. Sterilize as with nutrient broth for half an hour on three consecutive days. This medium is known as nutrient gelatine, or simply "gelatine."

Glucose, &c., &c., can be added to it if required, the addition being made preferably before neutralization and always before sterilization. Too prolonged heating causes hydrolytic changes in the gelatine, so that it will not set. Extra sterilizations must be avoided where possible on this account.

Nutrient Agar.—Agar or nutrient agar is made in a similar manner; 1·5 to 2 per cent. of the powered agar is added instead of the gelatine. It is much more difficult to filter, and where the necessary time cannot be given, a passable substitute is to allow it to cool slowly so as to permit the coagulated egg albumin and other precipitates to settle to the bottom. When cold the mass can be removed from the vessel and the lower part containing the great mass of the impurities cut off. The residue, though much inferior in appearance to the filtered product, is sufficiently clear to be translucent and can be satisfactorily used without filtration for cultures.

The clearer filtered product is better. To filter it is necessary that the filter paper should be kept hot. This is best effected by placing the funnel, filter paper, and receptacle in the steam sterilizer and allowing the filtration to take place in the steam sterilizer.

Filtration of these media is facilitated by folding the

filter paper so as to have a large number of angles and very little of the paper in contact with the glass. These papers can be bought ready folded or can be folded before use.

Addition of glucose, &c., can be made as in the case of other media before neutralisation and filtration.

The solid media are essential for the separation of the various organisms usually present in the animal tissues, discharges, or other substances to be examined.

Separation of Organisms.—The method of procedure is based on the principle that by successive dilutions of a minute quantity of the substance to be examined the individual organisms will be so scantily distributed through the medium that they will be separated from each other by an interval appreciable to the eye. If this medium be then allowed to become solid the organisms will remain well separated from each other, and if kept under conditions favourable for their growth will multiply and form in the course of a few days "colonies" which will be visible to the naked eye. From these colonies sub-cultures can be made, and the colonies may also be examined directly.

A piece of platinum wire, 3 to 4 in. in length, is inserted into the fused end of a glass rod, and a small loop is made at the other, the free end, of the platinum wire. This wire is sterilized by heating in the flame, and a loopful of the substance to be examined is taken up by this loop.

A tube containing the gelatine medium melted by placing in hot water, of a temperature not exceeding 28° C., is then inoculated with this loopful, and the tube is rolled between the hands to secure uniform admixture.

The amount of the substance is thus diluted by the amount of the fluid gelatine.

After sterilizing the needle a loopful from this tube is inoculated into a second tube and will again be diluted to the same extent. A third tube is treated in the same manner and the dilution will now be extreme.

In other words, provided the mixing is thorough the organisms will be so much diluted by these successive dilutions that they will be separated from each other by appreciable intervals. A fourth or fifth dilution may be made, but it is not usually required, as the third dilution is in most instances sufficient.

The end of each of these tubes, with the plug withdrawn, is heated in turn to destroy any organisms which may be present at the end of the tube and the gelatine is poured into a flat sterilized glass dish (fig. 155) —a Petri dish—which is quickly covered with another similar but larger sterilized dish. The melted gelatine

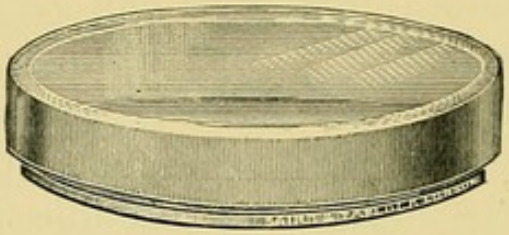


FIG. 155.

solidifies as a thin sheet of nutrient gelatine, and is allowed to remain at a temperature of about 20° to 22° C.

Some organisms will grow quickly and others slowly, and by colour, size, shape of colonies and effect on the gelatine it is usually possible to distinguish that several organisms are present. In the plate from the first tube the colonies are so numerous that they are separated from each other by too small a distance to isolate. In the plate from the second the organisms may be sufficiently far apart, and in the third and subsequent dilutions the colonies resulting from the growth of the widely separated organisms are usually far enough apart to be easily distinguished from each other. From these cultures can be made.

As the first dilution is too little diluted for practical work and the second is usually so, it is unnecessary to make these dilutions in the solid medium or to make

plates of them. The two first dilutions may be done in sterile broth or even in a weak sterile salt solution, 5 grm. to a litre, and only the third in the solid medium. This economizes the solid medium, which is the most troublesome to prepare.

Plating may be done with agar, but a thermometer must be used to make sure that the agar is cool enough, as if too hot the organisms may be killed. The agar will have to be heated to nearly the boiling point of water to become thoroughly fluid, and allowed to cool to about 44° C. before inoculation. It is not so easy a proceeding as plating with gelatine, but agar is the only solid medium that can be used in many parts of the Tropics, as above 22° C. the gelatine will not set. Stronger solutions, as 20 per cent. gelatine, will remain solid at 37.5° C., but these stronger gelatines are not easy to work with and frequently undergo changes during sterilization that cause liquefaction or acid production. Unless ice and a cold incubator are available we are restricted to the use of agar for plating.

A convenient method of plating on agar is to make the agar plates and inoculate when the agar has set either from the second or third broth solution, by making a series of parallel strokes with a platinum loop on the solidified surface, or, and better, by using a sterilized brush—camel's hair—and brushing lightly over the surface of the medium after dipping this brush in the second or third broth dilution. Excess of fluid is to be avoided by draining off from the brush against the inner side of the tube. The brush should be sterilized in a dry tube, plugged with wool, by three successive sterilizations. The platinum wire is sterilized as usual by heating in the flame.

Some important organisms will not grow on any known artificial medium, and others only on special media or under special conditions. Separation of such organisms is either impossible or difficult. Standard books on bacteriology should be consulted for methods,

but these will not usually be practicable for the solitary practitioner under the conditions of tropical life and work.

Description of Organisms.—Having obtained a pure culture of an organism the more important points to determine are as follows :—

(1) Size, shape and arrangement. Morphological appearance.

(2) Motility.

(3) Spore formation.

(4) Structure. Flagella, capsule, &c.

(5) Staining reactions : (*a*) Simple stains ; (*b*) Gram's method ; (*c*) Ziehl-Neelson.

(6) Growths on artificial media : (*a*) In broth ; (*b*) on gelatine ; (*c*) on agar.

(7) Conditions : (*a*) Essential to growth ; (*b*) favourable to growth ; (*c*) inimical to growth.

(8) Chemical products : Gas formation and curdling of milk ; acid or alkali formation ; indol formation.

(9) Reaction with blood sera, particularly with the blood sera of patients suffering from definite diseases.

(10) Pathogenic properties.

In organisms such as *B. lepræ*, which are cultivated with great difficulty, only a few of these points can be determined ; and the pathogenicity has not been proved experimentally, as with doubtful exceptions, lower animals are insusceptible. The causal relation is *inferred* from the constant association of the organisms, identified by their staining reactions and appearances in the lesions resulting from the disease, and from the observation that they are not found in man except in this disease.

We propose to take briefly the methods of observing these various points. The descriptive terms used with reference to them, the relative value and the limitations to the value of each point for diagnostic purposes will also be considered.

(1) The size and shape of an organism is best observed in stained specimens, and any simple stain combined

with a mordant will suffice. Films should be made on a slide or cover-glass. For this purpose with a culture in broth all that is required is to spread a drop with the platinum wire. If the culture is on agar or gelatine it should be rubbed up with a little water (sterile), and of this emulsion a portion should be spread out with a needle.

Preparation of Films.—If it be desired to make a film or smear from any natural fluid or excretion it may require the addition of a little water to make a satisfactory smear. From blood thick films may be used and decolorized by removing the hæmoglobin with sterile water. Tissues should be cut with a sterile knife and the cut surface rubbed on the slide.

The most difficult films to make are those such as sputum containing much mucus. The most satisfactory method is to use two slides and warm both. A portion of the mucus is transferred to one slide and this is warmed over a Bunsen flame or spirit lamp, and a second slide is warmed at the same time. The second warmed slide is used to rub the mucus on the first and is placed with the long axis at right angles to the other, and the surfaces parallel and in contact. The slides are then separated and both are again warmed in the flame with the smeared side of each uppermost. They are again, whilst still hot, rubbed together with the smeared surface of the two in contact. This process is repeated till the films are nearly dry, when they are finally rubbed together harder. Good thin, dry films are easily and quickly obtained by this method.

However the films are made they require fixation. This is best done by heat and is usually accomplished by passing through a smokeless flame three times, the smeared side always uppermost. Do not char the film.

As many films are so thin that they are difficult to see when dry, it is well to mark the smeared side of the slide with a grease pencil. The staining fluid is simply placed over the film for the requisite time and then

washed off. Stains used are Löffler's blue, five to ten minutes; carbol thionin, five minutes; carbol fuchsin (1—4) or gentian violet for organisms that do not take other stains deeply, one to ten minutes.

SCHIZOMYCETES.

Bacteria or *Schizomycetes* are unicellular vegetable organisms. Reproduction is by fission. Resistant forms called "spores" may be produced.

They are differentiated by these characters from *Hyphomycetes* or moulds, in which spores form in specially differentiated cells, and from *Blastomycetes* or yeasts, which are oval or rounded bodies, and in which reproduction is accomplished by budding.

(1) *Morphology.*—The various shapes of bacteria usually described are *Cocci* or rounded or oval bodies, with the greatest diameter not more than twice the least. The term *Micrococci* is used for the smaller forms. If division takes place only in one direction the organisms may remain attached in pairs, *Diplococci*, or in chains, *Streptococci*, if a series remain attached.

In other cases division takes place in two directions, and we then find the organisms arranged in squares of four or multiples of four. Such growths are called *Tetrads*. Others divide in three directions at right angles to each other and form cubical masses, these are known as *Sarcinæ*.

A common arrangement is irregular growth in all directions, leading to an irregular mass or cluster of cocci, *Staphylococci*.

Bacilli are cells that are rod-shaped. They are longer, at least twice as long as they are broad, and the shorter forms are distinguished from oval cocci by having the two sides parallel. By fission they may grow into long jointed rods—streptobacilli.

Curved organisms are, when short, known as *Vibrios*, when long and more twisted as *Spirilla* or *Spirobacteria*.

Leptothrix.—Rod-shaped, filamentous forms showing differentiation between base and apex, but not branching.

Streptothrix.—Filamentous forms showing true branching. These form the connecting link between the *Schizomycetes* and the *Hyphomycetes.*

Measurements are made as for other minute bodies. Some organisms readily change their form with variations in the condition under which growth has taken place. If the variations are great the organism is described as "pleomorphic." Slight variations occur in all organisms, so that morphological characters alone are not to be relied on.

(2) *Motility.*—This can only be observed in living cultures, though it can be inferred for organisms which are shown to have flagella. The motion of motile organisms must be clearly distinguished from the oscillatory movement—Brownian movement—common to all minute particles suspended in fluid.

True motility is best observed in a "hanging drop" preparation. This is made by making a thick ring with vaseline on a slide and taking a clean cover-glass, rather larger than this ring, and placing near the centre a small drop of the culture of living organisms to be examined. The slide is then taken up and turned so that the vaseline ring is directed downwards, and is gently brought into contact with the cover-glass so that the drop of culture on the cover is in the centre of the ring of vaseline. The cover will adhere to the vaseline ring and form a sealed chamber, and when the slide is turned over again the drop will hang from the lid of this chamber, the cover-glass, and can then be examined. If the temperature is so high that the vaseline runs, lard can be substituted for it.

The organisms are colourless and transparent and difficult to focus, so that the light must be reduced by nearly closing the iris diaphragm. Either $\frac{1}{6}$ or $\frac{1}{12}$ oil immersion objective may be used. It is well to focus first on to the edge of the vaseline ring and then move

the slide towards the drop, keeping the droplets of water of condensation which usually form on the under surface of the cover-glass in focus till the edge of the drop is reached. With a little practice and a dim light the organisms can then be brought into focus and the presence or absence of automatic motility determined.

This property, though an important point of difference between some organisms that closely resemble each other morphologically, is subject to considerable variation, and the degree of motility in motile organisms varies from slight causes, such as slight difference in temperature, reaction of the medium, &c.

(3) *Spore Formation.*—All the micro-organisms reproduce by fission, but some of them also enter into a resting stage—spores. The resting form is much more resistent to agencies, chemical, heat, &c., which destroy organisms, so that spores will withstand for some time the temperature of boiling water, though the active phase of the organism is at once destroyed.

Some organisms form spores very readily, others only under certain circumstances not thoroughly understood, and many pathogenic and other organisms do not form spores under any known circumstances.

The spores can often be recognized in the living culture, as they are usually more highly refractile as well as rounder. For the demonstration in dried films advantage is taken of the fact that spores stain with greater difficulty, but when stained, retain their stain better than the organisms from which they were derived. A simple method is to stain with warm carbol fuchsin for five minutes. This much overstains both spores and bacilli. Treat rapidly with 2 per cent. sulphuric acid; this, if done *rapidly*, will leave the spores stained, but remove the stain entirely from the bacilli. Wash well to remove the last traces of acid and counter-stain with Löffler's blue for ten minutes, or carbol thionin. The spores will be stained by the fuchsin and the bacilli blue by the methylene blue or thionin.

This method is successful for most spore-forming organisms.

(4) *Structures of Organisms.*—Certain points in the structure of organisms are sufficiently definite to be of use in diagnosis.

Capsules.—Some organisms have a thick capsule which does not stain deeply with basic stains, or may not stain at all. The simplest method of demonstration is to stain the film with carbol fuchsin and to examine *in water*, not in Canada balsam. The organism is surrounded by a clear space, and the capsule with a defined edge can usually be seen.

Welch treats the film with 2 per cent. acetic acid, which causes the capsule to swell and enables it to take the stain, and then after removal of the acid he stains with aniline gentian violet for five to thirty seconds. Capsulated organisms often lose their capsules in culture, but the presence or absence of a capsule, as seen, for instance, in sputum, is of value.

A cell wall is probably present in all the organisms, but it is difficult to demonstrate. In some, however, it is fairly well marked. It is best shown after the cell contents have been caused to shrink by salt solutions or iodine solution (plasmolysis).

Flagella.—Motile organisms have been shown to have flagella. They are variable in number, and whilst the vibrios have usually only one or two the motile bacilli may have large numbers. The number of flagella is of some value in the differentiation of species, and the presence, absence, or plan of arrangement is of differential value in grouping organisms.

The methods of demonstration cannot be considered as satisfactory or easy, and there is considerable uncertainty in the results; they are all troublesome. The two common methods successfully employed are Muir's modified Pitfield and MacCrorie's.

By Muir's method the mordant employed is composed of :—

Tannic acid 10 per cent. aqueous solution	10 c.c.
Corrosive sublimate saturated aqueous solution	5 c.c.
Alum saturated aqueous solution ...	5 c.c.
Carbol fuchsin	5 c.c.

This is well mixed, allowed to settle, and the clear fluid decanted off and centrifugalized. This mordant keeps for about a fortnight, but must be centrifugalized each time before use.

The stain employed is composed of a saturated solution of alum, 25 c.c., with 5 c.c. of alcoholic gentian violet saturated solution. This must be prepared immediately before use.

FIG. 156.

The smears should be made from agar cultures, twelve to eighteen hours old, emulsified with a little distilled water. Spread very gently on a cover-glass, freshly flamed to free from grease. Hold in Cornet's forceps (fig. 156), being careful that the film side corresponds to the fenestrated side of the forceps, otherwise mistakes as to which is the film side may occur.

Allow the film to dry in air and fix by passing through the flame. Cover with the mordant and heat till it steams for two minutes. Wash well in water and dry carefully. Pour on the gentian violet stain and heat till the staining fluid steams for two minutes. Wash in water, dry and mount in Canada balsam.

This method requires the use of a centrifuge, but gives a large proportion of successful results.

MacCrorie's method is simpler. A single stain combined with a mordant is used for staining the flagella and the bacilli are counter-stained with carbol fuchsin.

The stain is composed of :—

Night blue saturated alcoholic solution	10 c.c.
Potash alum saturated aqueous solution	10 c.c.
Tannin 10 per cent. aqueous solution...	10 c.c.

Gallic acid 1 or 2 grm. improves the solution.

Films from young agar cultures are prepared as above and the stain is placed on the film for five minutes and slightly warmed. It must be flushed off with running water or a thick, dirty deposit will be left. Counter-stain with carbol fuchsin, dry and mount.

(5) *Differentiation by Methods of Staining.*—There are three main methods of diagnostic value :—

(*a*) SIMPLE STAINS.—There is great variation in the ease with which different organisms take up stains, and this difference is sometimes of value. Some organisms do not stain uniformly, and such differences as preferential affinity of stains for the ends of a bacillus, bipolar staining, is one of the characteristics of the plague bacillus. In cultures organisms frequently lose their characteristic staining reactions.

Of greater value are two special methods.

(*b*) GRAM'S METHOD is based on the fact that some organisms will retain their stain when treated with alcohol if, after staining, they are treated with a solution of iodine. A freshly prepared solution of gentian violet in aniline water is made by shaking up a few drops of aniline oil with water and filtering. To this is added drop by drop a saturated alcoholic solution of gentian violet till a metallic film begins to form on the surface. With this stain the fixed film is stained for five minutes. Carbolic acid 1 in 20 can be used instead of the aniline water. If now treated with alcohol the stain would be completely removed from all the organisms.

In some organisms the addition to the film of Gram's iodine solution, composed of iodine 1 part, potassium iodide 2 parts, and water 300 parts, for two minutes, will fix the stain in these organisms so that when the film has been treated with alcohol they still retain the

purple colour, whilst it is removed from everything else. The organisms which retain their stains are those which are described as "staining by Gram."

The alcohol is kept on till it ceases to remove any more colour *and not longer*, as in time it will remove the stain even from the organisms which stain by Gram.

It is convenient instead of using a plain alcohol to use an alcoholic solution of eosin ½ per cent., as then organisms which do not stain by Gram will be stained faintly by the eosin. This is of most use when working with a mixture of organisms, such as is met with in many secretions, &c.

For sections it has the additional advantage that it does not require such prolonged treatment with alcohol as is required if alcohol is first used to decolourize, and then again for dehydrating after counter-staining.

As the action of alcohol is so rapid when working with organisms that retain the stain less firmly, another agent that decolourizes more slowly is better. The agent used is aniline oil. This decolourizes the non-Gram staining organisms as effectually as alcohol. It is used in the same way but can be left on longer. If counter-staining is desired the counter-stain must be used before staining by Gram.

The use of aniline oil instead of alcohol is better for sections. Counter-staining with eosin or Bismarck brown should be done first. After staining with Gram and adding the iodine, blot and treat with aniline oil. This will dehydrate as well as remove the stain from non-Gram staining organisms. Then wash off the aniline oil with xylol and mount in xylol balsam.

Most of the pyogenic cocci, the organisms usually found in suppuration, stain by Gram. Many of the organisms associated with intestinal and other diseases do not stain by Gram.

(*c*) ZIEHL-NEELSON'S METHOD.—A comparatively small number of groups of organisms are described as *acid fast*, because when once stained they retain the stain even after

treatment with fairly strong, 25 per cent., solutions of the mineral acids. Hydrochloric, nitric or sulphuric acids are those used.

The method employed is to use a strong basic stain such as fuchsin in a 1 in 20 aqueous solution of carbolic acid, and either to stain in the cold for some hours, or more conveniently to warm until the carbol fuchsin steams, and then keep warm for five minutes.

This is conveniently done on the slide. The film is fixed as usual and covered with the carbol fuchsin. The slide is placed on a copper which has been warmed in the flame and left there, fresh stain being added if evaporation is too rapid or the stain shows signs of boiling.

The stain is flushed off and replaced by a 25 per cent. solution of sulphuric acid. The pink colour disappears and is replaced by a yellow. The film is again washed and if still pink again treated with sulphuric acid. This is repeated till on washing at the most a faint pink colour returns.

The specimen is well washed in water to completely remove the acid and counter-stained with Löffler's blue for five minutes. Wash, dry, and either examine directly by placing a drop of oil on the film or mount in Canada balsam. The acid-fast organisms retain the red colour of the fuchsin, whilst other organisms are stained blue by the methylene blue which is used as the counter-stain.

In tropical work it is important only to use fresh carbol fuchsin. The solution keeps well in England, but in the Tropics it deteriorates, so that sometimes in a week or so, and at others in some months, it ceases to stain well. Colour-blind people will find it well to use gentian violet instead of fuchsin. A saturated alcoholic solution of gentian violet is added to the 1 in 20 carbolic acid to make the stain, and Bismarck brown is used as the counter-stain.

The more important members of the acid-fast group cannot be cultivated on the simple media. It will therefore be convenient to consider these organisms here.

There are four main groups of the acid-fast organisms, which will be considered under the heading of the best known member of the group :—

Tubercle ; lepra ; smegma ; Timothy grass.

Some forms of the *Streptothrix* group are also "acid-fast."

The tubercle group includes the organisms found in tuberculosis, in mammals, birds and reptiles.

The organisms can be cultivated on blood serum and nutrient glycerine agar, or in glycerine veal broth. Growth is slow and much affected by the temperature. The preferential temperature is that of the animal from which the organisms were obtained.

The mammalian, avian and reptilian tubercle bacilli therefore grow at different temperatures and are pathogenic to mammals, birds and reptiles respectively. Some authorities hold that they are modifications of one and the same organism and that they can, by suitable methods, have their characters altered so that the differences disappear. By most authorities the three are considered to be specifically distinct, and some go further and do not admit the specific unity of the tubercle organisms in different mammals. Koch holds that bovine and human tuberculosis are distinct, on the ground that their pathogenicity varies.

Tubercle bacilli in man are found in the secretions or excretions from an infected organ and therefore may be found in sputum, urine, &c. They usually set up a granulomatous new growth which has a marked tendency to caseate and break down, The organisms may be present in large numbers, but in some situations, such as the skin, bones, pleural effusions, &c., they are usually found only in small numbers. They are found but rarely in the blood.

The Lepra Bacillus is the only representative known of this group. It can be cultivated on artificial media

only under very special conditions, and experiments at inoculation of lower animals have usually failed, except with these doubtful cultures. The organisms are found in extraordinary numbers in leprous tubercles in the skin, and when these ulcerate, in the discharges from these ulcers. Before ulceration the bacilli can be readily demonstrated, by compression with a clamp of a tubercle or portion of infiltrated skin. On pricking this, serum loaded with the bacilli will exude. The bacilli are not found in ulcers or sores in purely nerve leprosy, as in that form they are present in the nerve sheaths and the ulcer or necrosis is not due to the breaking down of a leprous granuloma, or of tissues infiltrated with the organisms.

One of the most constant natural discharges to contain the bacilli is the mucus discharged from the nose. It is also one of the earliest manifestations in many cases, including some of nerve or anæsthetic leprosy. By some it is believed that the earliest and most constant lesion of leprosy of all forms is a deposit in or below the nasal mucous membrane.

Various differences in size, staining reaction, &c., between lepra and tubercle bacilli have been described, but they are not sufficiently marked or constant to be of diagnostic value. The most important diagnostic point in films of mucus is the aggregation of the bacilli into small, dense clumps in leprosy, in many cases still retaining the outline of the cell in which they grew. In sections of skin the extraordinary profusion of the organisms, as well as the aggregation into compact masses, is characteristic of lepra bacillus as opposed to human tubercle. In some of the lesions of avian tubercle a similar grouping may be found, whilst in internal organs in man there may be diffuse growth of the lepra bacilli.

Smegma Bacilli.—This group probably includes several species. In most specimens of smegma the organisms, though truly acid fast, are decolourized by alcohol, that is, they are not alcohol fast. Some varieties, however, do not lose their stain in alcohol, and are like the tubercle, both acid and alcohol fast. This organism has been the cause of frequent mistakes in diagnosis, as urine easily becomes contaminated with this bacillus, and it may readily be mistaken for that of tubercle and a diagnosis of urinary or renal tuberculosis given. In the majority of cases the use of alcohol, as well as of acid, will prevent this mistake, but as some specimens of the smegma, including Lustgarten's so-called syphilis bacillus, are also alcohol fast, the possibility of the confusion should be avoided by using the catheter.

The fourth group, of which the Timothy grass bacillus, or *B. phlei*, is taken as the type, are the only organisms of this group which grow readily on almost any medium. They are found on several species of grass used as fodder, and may be found in enormous numbers in the fæces of cattle. As a consequence they are often found in milk and products, such as butter and cheese, derived from milk. Several varieties or species have been described. This group is of economic importance, as cattle have been condemned as tuberculous, on the grounds that acid-fast bacilli were found in the stools and milk; butter and cheese have also been condemned on this insufficient reason. These organisms are not pathogenic.

(6) *Growths on Artificial Media.*—With organisms that can be cultivated, the growths on artificial media (nutrient broth, gelatine, or agar), differ in some cases sufficiently to be of diagnostic value, and in any case the character of the growth is one of the properties of an organism that requires description.

Cultures may be made on plates as in the separation

of different organisms, or, and more conveniently, in tubes. The growths in fluid media are made by taking on a sterilized platinum loop a portion of the culture and inoculating the tube. The nature and character of the naked-eye appearance in the broth at varying periods should be described. The temperature at which the cultivation is made must also be noted where incubators are available. Blood heat, 37° C. and 21° C. are the most convenient, and terms like "room temperature" should be avoided. In many tropical countries the temperature of the air will range from 25° to 30° C., and satisfactory growths of the more important organisms can be obtained. In a description of the growths at these temperatures a result intermediate between those at incubator temperatures will be obtained. All cultivations must be carried on in the dark.

The points to be observed in a broth culture are the surface, whether covered with or free from a film or pellicle. In the body of the fluid note if the fluid is turbid and the degree of turbidity, if not turbid whether quite clear or with floating particles; the presence or absence of a precipitate and, if one be present, whether it is composed of a uniform fine deposit or if in separate masses. Any change in colour, and bubbles from formation of gas, must be further noted.

On solid media the growths may be observed on plates or in tubes. In tubes the growths can be seen on sloped cultures made by drawing the inoculated platinum loop over the surface of the medium. This sloped medium is obtained by placing the tube, whilst the medium is still liquid, in a sloped position, and allowing it to set, or stab cultures may be used. In these the medium is allowed to set with the tubes vertical. An inoculated wire, not a loop, is plunged steadily into the depths of the medium and withdrawn without splitting the medium.

The appearance of the separate colonies is most important. There are great diversities in the appearance of growths on solid media, and an accurate series of

defined terms for descriptive purposes is much needed. Such a series of descriptive terms has been drawn up by Chester, but many of the terms will probably not be generally accepted and they are used at present by few bacteriologists.

The observer should note and describe the size of the individual colonies, their shape, the character of the edge, their elevation, whether raised or depressed, and give a detailed account of the character of the surface. The macroscopic appearances of the colony: if transparent, whether highly refractile or not, and if not clear whether opalescent, finely or coarsely granular, or irregularly blotchy. Any colouration, either of the colony itself or the surrounding medium, must be noted. In some organisms the different colonies remain distinct even when in contact, whilst with other organisms adjoining colonies readily grow together or become confluent. In growths on gelatine the presence or absence of signs of liquefaction in the surrounding medium is a point of the first importance.

In stab cultures any surface growth must be noted, as well as the growth in the line of puncture. It may be uniform, finely or coarsely granular, composed of numerous fine or coarse colonies which remain discrete and are not confluent, or of large masses. Extension into the gelatine in the neighbourhood of the puncture may take place, and the character of these extensions, whether as knots or as fine filaments, or in an irregular, arborescent manner, is worthy of attention.

If liquefaction, in a gelatine medium, has taken place it will be well shown. It is most abundant in the upper part of the line of puncture when the organism requires oxygen, but with organisms that grow best in the absence of air will be more conspicuous in the depths.

Air bubbles along the line of puncture, indicating formation of gas and any colouration of the growth or of the medium, must be noted.

The amount of growth that takes place in a given time

as compared with other organisms, or similarly the relative amount of liquefaction, gas formation, &c., in the time, is an aid in distinguishing allied or similar organisms, though liable to be modified with different strains of the same organism.

There is no cultural characteristic that cannot be modified by frequent subculture, culture under different conditions of temperature, reaction of medium, and other influences. The cultures of some organisms vary more than those of others. The information gained as to the character of growths, though of considerable value, has to be considered with other properties of the organisms.

Cultures on milk, potatoes, &c., are often of more diagnostic value for special organisms.

(7) *Conditions Affecting the Growth of Organisms.*—One of these, the effect of oxygen, is of special practical value.

Some organisms will only grow in presence of oxygen ; such organisms are strictly *aerobic*. Others will not grow at all in the presence of oxygen, these are said to be strictly *anaerobic*. The largest number of bacteria are intermediate between the two and are termed *facultative anaerobes*.

Aerobic organisms grow readily under the ordinary conditions, as even in stab cultures there is usually sufficient oxygen present for the commencement of growth.

Anaerobic organisms are most easily grown in stab cultures of glucose-agar or gelatine, or in glucose formate agar made by adding ·02 per cent. sod. formate to glucose agar (Kitisato). The tubes must have been freshly boiled to expel air from the medium, and the stab should be made with a fine needle so as to carry down as little air as possible. After the needle is withdrawn the upper part of the medium should be heated so as to melt it and seal the opening made by the needle.

It must be remembered that though the growth of anaerobic organisms does not take place in presence of air, the organisms, and particularly the spores, may

retain their vitality and grow if transplanted into more favourable conditions.

(8) *Chemical Products of Organisms.*—These vary both with the nature of the organism and the character of the medium. Gas formation is one of the easiest to determine, but it is also necessary to have in the medium some substance from which the gas can be formed. The sugars are valuable for this purpose, and it will be found that whilst one organism will form gas from either glucose or lactose another will form gas only from glucose. Another manifestation of chemical activity is the formation of acid or alkali. Formation of acids and gases are of particular importance, as so many of the

FIG. 157.

organisms found in the intestine either form gas and acid from glucose or form acid only.

FORMATION OF GAS.—The production of gas can be shown in most stab preparations as bubbles of gas form along the needle track. It is better shown by melting the gelatine, or, better, glucose gelatine, and rotating the tube alternately in opposite directions after inoculation. Such a preparation is known as a "shake culture," and after it has set and grown, bubbles of gas will be formed all through the medium.

A better method that can be used with fluid media is to place in the medium a small inverted tube, Durham's tube. During sterilization of the medium the gas will be expelled from this small tube, which will be completely filled with the fluid medium. If the tube be inoculated

with an organism that forms gas from the medium the gas formed in the small inverted tube will accumulate in it and cause it to float (fig. 157.)

Gas formation from glucose is one of the characteristics of some of the commoner intestinal bacteria.

FORMATION OF ACID.—Acid formation can be shown by using a neutral or slightly alkaline medium coloured with litmus; the formation of acid is shown by the change in colour of the litmus.

Most of the intestinal bacteria form acid readily. An ingenious application of these properties of the intestinal organisms is that of MacConkey for the detection of fæcal contamination of water, milk, and other substances. He uses Durham's tubes and employs a medium containing bile salts. Bile salts inhibit the growth of many organisms, but are favourable to the growth of intestinal bacteria.

The medium he employs is composed of: Peptone, 2; salt, ·5; sodium taurocholate, ·5; water, 100; to which is added glucose or lactose in the proportion of ·5 per cent. The medium is neutral and is coloured with neutral litmus. A Durham's tube is placed in the test tube containing the medium and during the three sterilizations required will be filled with the medium.

A measured amount of the water, &c., to be tested is added and the tube incubated, preferably at 42° C., for twenty-four hours. Organisms which in this medium produce acid and gas may be suspected to be possible inhabitants of the intestinal tract.

Other pathogenic and non-pathogenic intestinal organisms form acid only. Of organisms other than these many will not grow in the medium at all, or if they do grow form neither acid nor gas.

If, therefore, neither acid nor gas is formed, the evidence is strong that there is no living fæcal contamination, and no contamination with the commonest of the intestinal organisms, *B. coli communis*. If acid alone is formed it is doubtful whether there is such contamination,

as *B. coli communis* must be absent. If acid and gas are both formed there is strong probability that the water, &c., is contaminated with organisms that are inhabitants of the intestinal tract.

INDOL FORMATION is another important chemical product of some bacteria. A simple medium is required, and plain peptone water made by boiling 10 grm. of peptone and 5 grm. of salt in a litre of water is usually employed. This should be filtered and sterilized as usual.

The tube of this medium should be inoculated with the organism to be examined and incubated for at least twenty-four hours. Other tubes inoculated at the same time are incubated for longer periods.

To this culture a little pure sulphuric acid is added; a red colour develops in a few minutes if *indol* and a *nitrite* have been formed. If the colour remains unaltered, three or four drops of a ·05 solution of sodium nitrite should be added to the mixture, and if a red colour now develops *indol alone* has been formed. Yellow-fuming nitric acid, which contains traces of nitrous acid, may be satisfactorily used instead of sod. nitrite.

Amongst other chemical products are ammonia, alcohol, phenol, sulphuretted hydrogen, and the substances which cause curdling of milk.

EFFECTS ON CERTAIN ANILINE COLOURING MATTERS.

Neutral-red Agar.—This medium consists of ordinary agar, to each 100 c.c. of which ·3 grm. of glucose and 1 c.c. of a saturated aqueous solution of neutral-red have been added before the medium is poured into tubes.

In this medium *B. typhosus* and *B. dysenteriæ* grow without changing it, while *B. coli communis* and the paratyphoid bacilli, in twenty-four to forty-eight hours decolourise the medium and produce a greenish fluorescence, forming gas at the same time. Stab cultures or shake cultures may be employed.

Drigalski-Conradi's Medium.—To prepare this medium, 2,000 c.c. of 3 per cent. nutrient agar are treated

with 20 grm. nutrose, then with a solution of 30 grm. of lactose in 260 c.c. litmus solution. The procedure is as follows: The litmus solution is boiled for ten minutes in the steam sterilizer, the lactose added, and the mixture again boiled for ten minutes. The litmus lactose solution is cooled to 40° to 50° C., and the nutrose agar cooled to 70° C. is added to it. The mixture is rendered alkaline with hot 10 per cent. soda solution to the extent that, on shaking, the froth formed is distinctly blue after a few minutes' standing. Finally, 20 c.c. of 0·1 per cent. freshly prepared solution of crystal violet are added, and the medium sterilized in the usual way—it should be bluish violet when solidified.

On this medium *B. typhosus* produces small transparent colonies, while *B. coli communis* produces larger colonies, brilliant red and non-transparent.

(9) *Reaction of Organisms with various Blood Sera.*—Certain pathogenic organisms effect a change in the blood serum of persons infected with these organisms, so that the serum contains substances which, when mixed with a living culture of the organism, cause loss of motility of the bacteria, and also cause them to aggregate in little clumps or masses. This aggregation is called agglutination, and the serum which causes this *agglutination* is said to contain *agglutinins.*

The application of the test is simple. The blood serum, free from red corpuscles, is obtained, as has been already described, and diluted with sterile broth to a known extent, ten, twenty, thirty, or more times. Sometimes the blood is sent in capillary tubes or the serum has not well separated. In such cases, to obtain clear serum it is necessary to use the centrifuge (fig. 158). This diluted serum is mixed with an equal volume of a living active culture of the organism to be tested and the mixture examined as a hanging-drop preparation. Loss of motion of the organisms and agglutination should take place within a certain time limit and a control made by using the same dilution of normal blood serum with

more of the same culture. This action of the serum on the organisms is specific and affords a means of proving the correlation of the organism and disease.

It is true that strong undiluted normal serum will cause in some cases a similar agglutination, but not with the great dilutions which will act in serum from a person with disease.

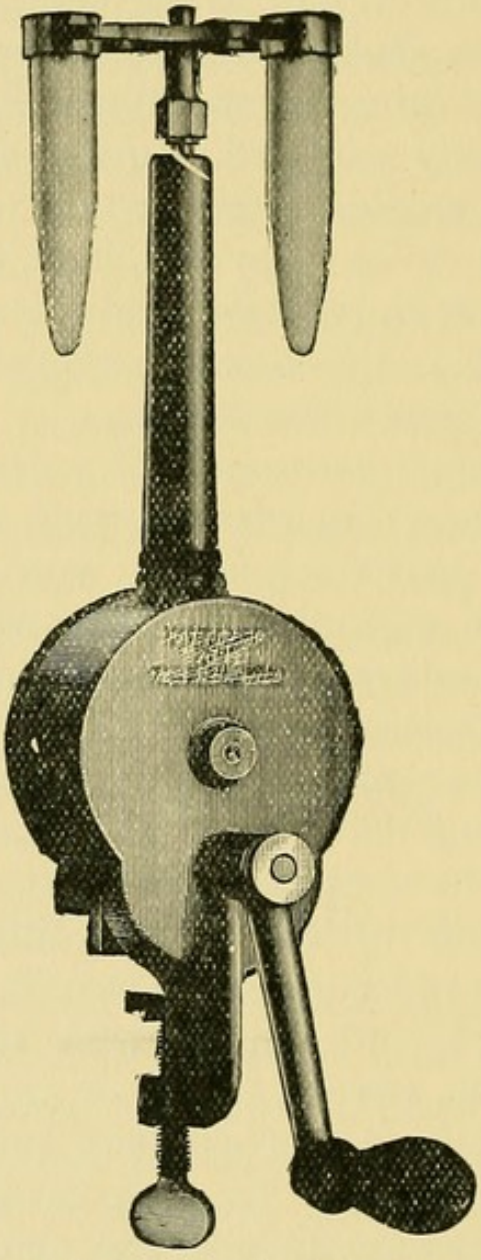


FIG. 158.—Centrifuge.

The converse of this test is to use a culture of an organism to test the serum reaction of a patient suspected to be suffering from the disease which the organism can cause. Typhoid and Malta fever are the diseases in which the reaction is most decisive. This application of the

principle is known as the agglutination test, or the Grünbaum-Widal or Widal reaction.

In the application of the hanging-drop method the serum may be diluted in Wright's tubes or by a number of loopfuls of broth being added to a loopful of serum and well mixed. To obtain a dilution of 1 in 20, one loopful of serum is mixed with nine loopfuls of broth, and of this mixture one or two loopfuls are mixed with one or two loopfuls of an active culture.

If higher dilutions of the serum only, say 1 in 100, are required, it would be inconvenient and tedious to mix one loopful with ninety-nine of broth. It is easier to make a dilution of 1 in 10 and dilute one loopful of this dilution with nine of broth, which gives the same dilution more quickly.

The gradual loss of motility and aggregation of the organisms can be watched under the microscope in the hanging-drop preparation.

Many observers prefer the macroscopic demonstrations of the same effect. This is done by aspirating into a tube a mixture of serum diluted to the required extent with broth mixed with an equal quantity of active broth culture. The mixture of culture and diluted serum is drawn up into a narrow tube and placed vertically in the incubator. The organisms will lose their motility and aggregate into a mass and fall to the bottom of the fluid, leaving the superjacent fluid clear and free from turbidity, in marked contrast to the control, which will still remain turbid. This method is known as the "sedimentation test."

There are many fallacies which may occur in connection with these tests. The culture must be an active one and recently made. A control with serum of normal blood must be made and the dilution must be sufficient.

Some strains of the organisms agglutinate more readily than others, and even with diluted serum of normal blood, agglutination may take place if the organisms are grown on unsuitable media, or if the cultures are too old.

The change in the serum may be a persistent one, so that a positive reaction in the case of a person who has had a previous attack of typhoid or Malta fever gives no information as to his present condition.

(10) *Pathogenic Properties.*—The pathogenic properties of an organism are shown by the effect of inoculating a susceptible animal with the organism in pure culture if possible. Where that is not possible, with fluid containing as few other organisms as possible.

In some instances, as in *tuberculosis*, the similarity of the lesions produced by a similar organism in animals naturally indicated that cultures of tubercle bacilli should be tried on other animals. In others a series of animals had to be tried before a susceptible host was found. Rats, guinea-pigs and rabbits are the animals most commonly used, but in other cases monkeys, dogs, cattle and horses have had to be employed. No such experiments can be made under the Vivisection Acts without a licence, and in any case there are so many difficulties and fallacies that without a thorough study of these and of the methods employed the results obtained would be valueless.

Material used for injection may be :—

(1) Pure cultures of an organism.

(2) Products of bacteria in solution such as toxins.

(3) Fluid excretions, secretions and portions of diseased tissues.

(4) Blood.

The injections are usually made with strict antiseptic precautions into the subcutaneous cellular tissues. With fluid cultures there is no special difficulty. Cultures on solid media require to be emulsified with sterile saline solution. Solid tissues, portions of spleen, &c., should be rubbed up in a sterile glass mortar with a little sterile broth and then injected. Occasionally a small mass of solid tissue is inserted into an aseptic pocket made under the skin for the purpose, and the wound closed by a sealed dressing such as gauze and collodion.

In other cases the injection is intramuscular or intraperitoneal. In intraperitoneal injections of small animals a big fold should be taken up between the finger and thumb, care being taken to include the whole thickness of the abdominal wall and to see that no intestine is included. Whilst still held this fold should be transfixed with the needle of the hypodermic syringe containing the substance to be injected so that the point just protrudes. The finger and thumb are now removed, the abdominal wall will flatten out and the point of the needle will be in the abdominal cavity. In this way the injection can be made without any risk of injuring the intestines.

The results of inoculations are not always conclusive. The resulting disease, even in a susceptible animal, may show very little resemblance to the disease caused by the same organism in man or other animals.

An organism that is pathogenic may by successive cultures lose its virulence, and become with some animals non-pathogenic, whilst on the other hand, if passed through a series of animals, the virulence may be increased.

Koch's postulates are :—

(1) The organisms must be found in the tissues, fluids or organs of the animal affected with the disease.

(2) The organism must be isolated and cultivated outside the body on suitable media for successive generations.

(3) The isolated and cultivated organism on inoculation into a suitable animal should reproduce the disease.

(4) The same organism must be recoverable from the inoculated animal.

These in the main are still considered sound, though not practicable for all organisms, as some cannot be cultivated : for others no susceptible animal is yet known, and in some of the lower animals, though a disease is produced by the injections, it bears little or no resemblance to the human disease under investigation.

Streptothrix maduræ.—This organism is the cause of a disease of special importance in the Tropics—madura foot. It occurs in India, Straits Settlements, East Africa, British Guiana, Cyprus, and Cuba, and is probably to be met with all through the Tropics.

Clinically it is a chronic disease which causes much swelling of a firm fibrous nature and destruction of the tissues, with the formation of sinuses discharging watery or oily-looking fluid. In some cases in this discharge, white, black, or pink granules, visible to the naked eye, are found, and these granules are masses of branching filaments with mycelial arrangement. In other cases the granules are very rare, and only the branching filaments of the mycelium are found; in still other cases these may also be absent, though in sections of the tissue the mycelial clumps are to be seen.

The organism in tissue is characterized by the dense clumps of mycelium formed of the branching filaments of the streptothrix. The ends of the filaments at the edges of these masses degenerate and become swollen, forming the so-called clubs.

The organism will grow on any of the ordinary media, forming limpet-shaped masses.

In general character the mycetoma resembles the streptothrix of actinomycosis, but it does not stain by Gram in sections, does not liquefy gelatine, and the "clubs" are rounder. The clumps take any of the ordinary basic stains, including hæmatoxylin, and either this stain or carbolthionin is to be recommended to show the growth in sections.

This streptothrix sets up changes in the tissue, so that the growths are surrounded by a mass of newly formed tissue of the granulomatous type. At the periphery of the granulomatous mass is much badly formed fibrous tissue, and the centre is often broken down It is in the breaking down of this granulomatous tissue that the mycelium clumps are liberated and are discharged with the fluids from the sinuses.

Bacteriological Examination of Water.

In the bacteriological analysis of water we determine (1) the total number of organisms of all kinds in a given quantity of the sample ; (2) the presence or absence of *B. coli communis;* (3) the detection of definite pathogenic organisms, such as the *B. typhosus*, Koch's comma bacillus, &c.

The enumeration of the number of organisms irrespective of the kind is of value when we wish to determine the efficiency of filter beds, effects of sedimentation, storage, &c.

The detection of *B. coli communis* is the main object of any bacteriological examination. Not that *B. coli per se* is to be regarded as a definite pathogenic organism, but, occurring as it does in all dejecta, its presence is regarded as an indicator of sewage contamination. Where sewage contamination is shown to have occurred, it is quite possible that other and more deadly organisms occurring in the intestinal tract, such as *B. typhosus* and Koch's comma bacillus, may also have gained access to the water under consideration. The *B. typhosus* has but rarely been isolated from a water supply, though Koch's comma bacillus can readily be isolated from water containing these organisms.

Perhaps the simplest and best method of proceeding to make a bacteriological examination of any given water is that recommended by Savage, which, with slight modifications, is as follows :—

Collection.—Not less than 2 oz. to be collected in a sterile glass-stoppered bottle. When taking a sample from a tap, allow the water to run to waste for some five minutes before collecting the specimen ; when from a pond or river, the sample should be collected well away from the bank. If the water of a well is to be examined, the sample should not be taken from the surface of the water, but from a point about a foot deeper. It is best to pack all samples in ice, and transmit at once to the laboratory.

Inoculations.—Everything must be in readiness before the examination is started, *i.e.*, gelatine and agar tubes melted, and at a suitable temperature, other media tubes ready, sterile pipettes and Petri dishes at hand. Use a 1 c.c. pipette graduated in $\frac{1}{10}$ c.c. Mix the sample thoroughly. Add 0·2, 0·3, and 0·5 c.c. respectively to three gelatine tubes, and label with a grease pencil. Add 0·1 and 1·0 c.c. respectively to two agar tubes; label and replace at once in the hot-water bath. Add 1·0 cc. to a tube of bile salt broth. The effect of this medium is to inhibit the growth of organisms other than those which flourish in the intestinal tract. Add 10 c.c. to a tube of double-strength bile salt broth. All tubes of bile salt broth are provided with Durham's tubes. Add ·1 c.c. to a tube of glucose neutral red broth provided with a Durham's tube. Add 1 c.c. to a tube of glucose neutral red broth provided with a Durham's tube. Add 10 c.c. to a tube of glucose neutral red broth double strength, and provided with a Durham's tube. To the water remaining in the bottle, add the contents (about 10 c.c.) of a tube of four times strength neutral red broth. Replace the stopper.

The gelatine and agar tubes are now poured into Petri dishes after thorough admixture of water and medium has been made. The Petri dishes are labelled, and their contents rapidly solidified.

The agar plates are incubated at 37° C., upside down, and the gelatine plates at 22° C, but not reversed.

The bile salt and neutral red broth tubes are labelled and incubated at 37° C.

Examination of Plates and Tubes.

Plates.—Gelatine and agar plates should be counted at the end of twenty-four, forty-eight, and seventy-two hours; but in all cases the plates should be inspected earlier, in order that the count may be made at once should liquefaction render this necessary. To count the colonies, it is best to count against a dark background, and with a brush place a dot of Indian ink over each counted colony. In this way, as the older, already

counted colonies are marked, the number of new colonies that become visible each day can be noted.

To facilitate counting, divide up the area of the plate with lines on the back made with a grease pencil. All the colonies on the plate should be counted; but if they are very numerous, and an approximate estimate only is possible, then some mechanical aid such as Pake's disc may be used, a few segments being counted, and the total number deduced.

Tubes.—For *B. coli.*—If the 1 c.c. and 10 cc. bile salt and neutral red broth tubes show no gas or reaction after forty-eight hours, it can be assumed that *B. coli* is absent in these amounts. Then in every case plate out from the broth and water in the sample water.

If gas is formed in any of these tubes, use the one showing gas in the tube with the least quantity of added water for inoculating plates of solid media. For the actual isolation, it is sufficient to add one platinum loopful of the medium to a wide tube, containing sterile water, and to distribute a little of this over plates of solid media suitable for the purpose. Such media are lactose litmus agar, Conradi medium, and neutral red bile salt agar.

For spreading the diluted broth over the plates of solid media, a common method is to employ a glass rod bent at right angles near one end. The diluted broth is placed on the solid medium in the plate, and distributed by means of the sterilized glass rod.

By the next day, if the plates have been incubated at 37° C., the colonies will have developed sufficiently for examination and subcultivations, of which at least three should be made.

Having now isolated the coli-like organism in pure culture, it should be further tested on various media to see that it conforms absolutely to the reactions produced by an undoubted *B. coli.*

A coined word, "flaginac," is often used to express the results of subcultural tests of coli-like organisms. The word is made up as follows :—

fl. indicates greenish fluorescence in neutral red-broth cultures.

ag. indicates acid and gas in lactose peptone cultures.

in. indicates indol formation in broth cultures.

ac. indicates acidity and clotting of litmus milk.

Examination for Koch's Comma Bacillus.—About a litre of the water is placed in twelve large sterile Erlenmeyer flasks, 90 c.c. in each. To each is added 10 c.c. of a sterile solution, consisting of 10 per cent. peptone and 5 per cent. sodium chloride. The flasks are then incubated at 37° C. After eighteen hours' incubation microscopic preparations and examinations in hanging drop are made from the surface of each flask. The medium is one in which the cholera spirillum grows very rapidly, and, if present, it shows itself in the very thin pellicle on the surface of the liquid, often before the other organisms have had time to develop to any great extent. The flasks which show the presence of vibrios are used to inoculate agar and gelatine plates, a loopful of the fluid being withdrawn from the surface for this purpose. Suspicious colonies on the agar and gelatine plates are subcultivated upon agar slopes, and their characters studied in pure culture.

Care must be taken by employing all available tests such as Pfeiffer's test, hæmolysis test, &c., to determine that the vibrio isolated is a true cholera organism, and not one of the closely allied vibrios.

Interpretation of Results.—In the interpretation of the results of the bacteriological examination of a water, it is usual to have some sort of a rough standard of purity, and for that purpose the following table for temperate climates may be quoted; but it must be remembered that in the Tropics the ordinary water organisms grow more readily at the higher temperatures than in England, so that the counts on gelatine cultivated at 22° C. differ little from those on agar grown at 37° C.

(*a*) DEEP WATERS.

(*Springs and deep wells.*)

Gelatine count	...	...	Not over 50 organisms per c.c.
Agar count ...	...	...	„ 10 „ „
B. coli communis	...	...	Should be absent from 100 c.c.

(*b*) SURFACE WATERS.

(e.g., *rivers for drinking purposes, shallow wells, upland surface waters.*)

Gelatine count	...	...	Not over 500 organisms per c.c.
Agar count ...	...	...	„ 50 „ „
B. coli communis	...	...	Should be absent from 10 c.c.

It is hardly necessary to add that should the examination reveal the presence of either the *B. typhosus* or Koch's comma bacillus, the water should be condemned forthwith.

HYPHOMYCETES (MOULDS).

Of the various fungi which attack the skin and hair, some are widely distributed, though more common and luxuriant in their growth in the Tropics. *Pityriasis versicolor* comes under this head. Others, like *Favus*, may be common in some places, but as in temperate climates, are of limited distribution. The commoner fungi attacking the hair of the head are unknown in many tropical countries. The fungus attacking man at present recognized as peculiar to the Tropics is a cutaneous ringworm, *Tinea imbricata*, characterized clinically by the large size of the epidermal scales, which are partially detached, and the tendency to form geometrical patterns.

For the demonstration of the fungi causing these various affections, the older method consisted in soaking the hair or scales in a 7 per cent. solution of caustic potash, which rendered the keratin clear and transparent, whilst the fungus was less affected, and could be clearly seen. This method causes swelling of the fungus and

spores, and therefore is not suited for the differentiation of the varieties or species of fungi.

A modification of Gram's method of staining gives more useful results, but is slow. The hair or scale is stained in aniline gentian violet for five minutes, and dried with blotting paper. It is then treated with Gram's iodine solution for two minutes, and again dried with blotting paper. It is then covered with aniline oil, to which a little iodine has been added, and left till the fungus can be seen. It should be examined from time to time under the microscope, as, though the process is slow, ultimately even the fungus will be decolourised. Do not wait till all the tissue is clear, but when nearly so, treat with aniline oil, and clear in xylol. Mount in xylol balsam.

The points to observe are the arrangement of the growth, whether inside or outside the hair, scale, &c., the presence and the size of the spores. The nomenclature of these fungi is based on these points. According to the seat of growth of the fungus it is an ecto- or endothrix, and microsporon or megalosporon according to the size of the spores.

These ringworms are true *fungi*. The fungi are multicellular organisms composed of filaments, either simple or branched, or jointed or unjointed. These filaments are called *hyphæ*, and if they project into the air are *aerial hyphæ*, or down into the substance of the medium they are known as *sub-aerial hyphæ*.

They frequently form a compact mass—a mycelium—and if this form a hard, dense mass it is known as a sclerotium. Sexual reproduction as well as reproduction by fission has been proved to occur in most members of the group.

These fungi include the ordinary moulds, and some, such as ergot, form compounds which, when eaten, are poisonous.

In addition to the cutaneous fungi which cause the true ringworms (*Tinea*), fungi may be found in mouth,

ear or nose, as well as in pulmonary cavities. These are secondary growths. In bird-rearers, who take uncooked grain in their mouths, a true pneumono-mycosis occurs, and in the Tropics a similar pulmonary disease simulating tuberculosis occurs.

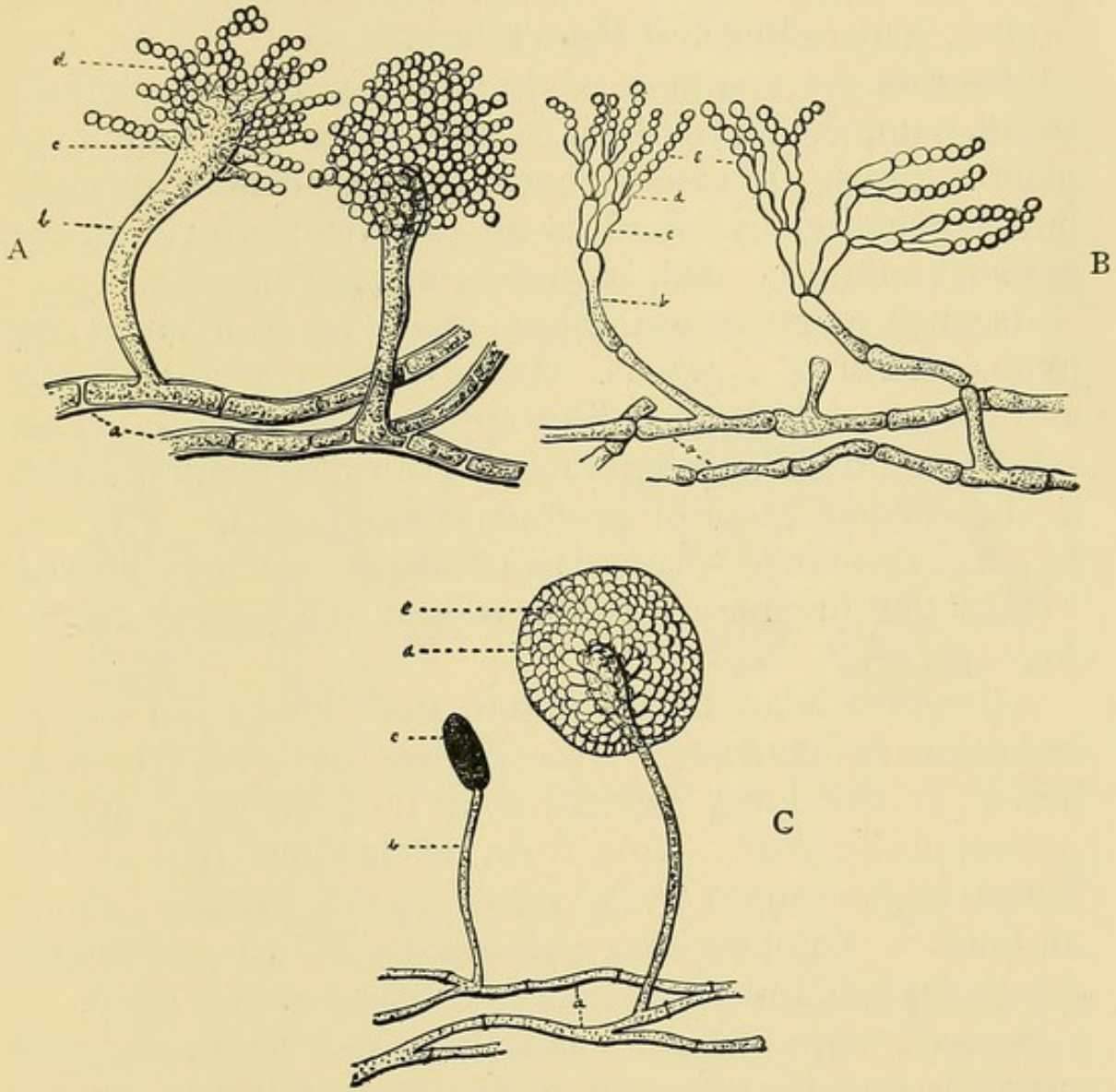


FIG. 159 (after Eyre).—A, Aspergillus. *a*, mycelium; *b*, hypha; *c*, sterigmata; *d*, spore. B, Penicillium. *a*, mycelium; *b*, hypha; *c*, basidia; *d*, sterigma; *e*, spore. C, Mucor. *a*, mycelium; *b*, hypha; *c*, columella; *d*, sporagium; *e*, spores.

It is possible that some cases of madura foot, the black variety, are due to a fungus and not to a streptothrix.

The tropical fungi, aerial and otherwise, have so far received little attention and should offer a fruitful field

for research. Most of the fungi grow readily on nutrient media, but best if a sugar be added. Maltose is the most suitable for the fungi of the ringworms.

Many of the aerial fungi will not grow at high temperatures, such as blood heat, though they flourish at lower temperatures.

Of the commoner moulds, mention may be made of Mucor, Aspergillus and Penicillium.

Mucor is the common white mould frequently seen on bread, jam, &c., and is a common contamination of plate cultures. It is not known to cause disease in man, but is occasionally found as an epiphytic growth in the external auditory canal, bronchiectatic cavities, &c.

Asexual reproduction takes place by a filament or hypha growing upwards. At its apex a septum forms and then a globular swelling appears—sporagium. This possesses a definite membrane. From the septum grows a club-shaped mass of protoplasm-columella. The rest of the contained protoplasm breaks up into spores. Finally the membrane ruptures and the spores escape (fig. 159, C).

Aspergillus is another common free-living form and is occasionally associated with disease in man. It may occur in the lung, especially in bird-fanciers, causing pneumono-mycosis. One form of madura foot is believed to be caused by a variety of this fungus. Some authorities maintain that pellagra is due to *Aspergillus fumigatus* contaminating damaged maize used as food.

Asexual reproduction is as follows:—A filament grows upwards and its termination becomes clubbed: on the clubbed extremity flask-shaped cells appear—sterigmata. At the free end of each sterigma are formed oval bodies—the spores—which when ripe are thrown off from the sterigma (fig. 159, A).

Penicillium is a common green mould found growing on damp bread or jam and is not known to be associated with any human disease.

Asexual reproduction takes place by a filament growing

upwards—goniodophore—and its apex dividing into several branches called basidia. At the apex of each branch a flask-shaped cell or sterigma appears. At the apex of each sterigma appears a row of oval cells forming the spores. These when ripe are cast off from the sterigmata (fig. 159, B).

Blastomycetes (Yeasts).

Yeasts, or blastomycetes, are frequently found in the mucous cavities and occasionally in ulcers or other skin lesions. They are distinguished from bacteria not only by the method of reproduction but also by their greater size. In some species endospores are formed, but these are multiple in each cell and not single as in bacteria.

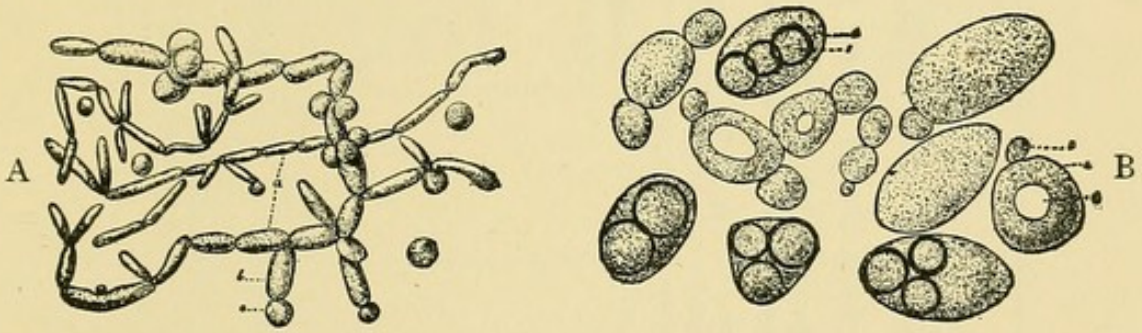


Fig. 160 (after Eyre).—A, Torula ; *a*, mother eell ; *b*, bud ; *c*, secondary bud. B, Saccharomycetes ; *a*, mother cell ; *b*, bud ; *c*, vacuole ; *d*, cell-forming spores ; *e*, spores.

The yeasts may be divided up into two groups according as they are able to produce spores or not.

These two groups are known as saccharomycetes and torulæ. Both reproduce by budding, but in the saccharomycetes or true yeasts there is an alternative method of reproduction, namely, by the formation of spores, each cell giving rise to four spores arranged like a pyramid of billiard balls (fig. 160, B). The torulæ never form spores.

Old cultures of yeasts frequently form films in which the individual cells become much elongated, like those in the mycelium of a mould.

The yeasts are of considerable interest, as alcoholic and other forms of fermentation are due to their agency.

TABLE OF ORGANISMS OF SPECIAL INTEREST.

Organisms which Retain their Stain when treated by Gram's Method.

A.—Organisms which are also acid fast.

Organism	Chief types	Artificial medium	Spore forming	Motility	Pathogenic to	Discharged with	Enters by
B. tuberculosis ...	Mammalian, avian, amphibian	Special blood serum and glycerine media	—	—	Most animals; guinea-pigs	Sputum, sometimes fæces and urine	Mouth; inhaled into lungs or swallowed; by tonsils; rarely inoculated.
B. leprœ... ...	Human only ...	(?)	—	—	Man only ...	Nasal mucus, ulcerating tubercles, absorbed with blood by blood-sucking insects when they feed on a tubercle	Nose (?)
B. smegmatis ...	Alcohol fast or not alcohol fast	Media containing urine	—	—	—	Urine and vaginal discharges	(?)
B. phlei ...	Various grasses, milk, butter, &c.	Any medium	—	—	—	Fæces in cattle; saprophytic...	Mouth in cattle.

B.—Not acid fast.

Organism	Spore forming	Motility	Gelatine	Pathogenic to	Discharged with	Enters by
B. tetani	+ Terminal	+	Liquefies slowly	Most animals; mice, guinea-pigs, rabbits, cattle, &c.	Anaerobic; saprophyte in earth	Inoculation; skin or mucous membranes.
B. anthracis	+	—	Liquefies	Most animals	Discharges from ulcers and throughout bodies of infected animals	Handling skins of infected animals, or inhaling dust from them.
Pneumococcus (Frankel)	—	—	No growth ... ... Not grow well except in media, such as blood serum	Many mammals; mice	Sputum	Normally often present in the mouth; inhaled.
Pyogenic staphylococci and streptococci	—	—	Streptococci do not liquefy; staphylococci vary, usually liquefy	Many animals	Pus from abscesses, ulcers, &c.; often found on the skin	Breach in skin or mucous membrane.
Streptothrices of actinomycosis	—	—	Liquefies	Cattle; rabbits	Abrasions of mucous surfaces	Breaches in mouth.
Madura foot	—	—	—	Man only ...	Discharges from sinuses ..	Feet or hands.
B. diphtheriæ	—	—	—	Many of the larger mammals; guinea-pigs, pigeons	Sputum; breath and discharges	Inoculation and inhalation, and inoculation of mucous membranes.

Organisms which do not Retain their Stain when treated by Gram's Method.

None of these are acid fast and none form spores.

Bacteria	Motility	Gelatine	Glucose	Lactose	Pathogenic to	Discharged with	Enters by
B. coli communis	+	—	Acid and gas	Acid and gas	Many animals; mice	Fæces	Mouth.
B. typhosus	+	—	Acid ...	—	Anthropoid apes	Fæces, urine ...	,,
B. paratyphosus	+	—	Acid and gas	—	Many animals; injected into the peritoneum	,, ,, ...	,,
B. enteritidis (Gärtner)	+	—	,, ,,	—		Fæces	,,
B. dysenteriæ, all varieties, Shiga, Flexner, &c.	+ (?)	—	Acid ...	—	—	,, ...	,,
B. pyocyaneus	+	+	,, ...	Acid ...	Many animals; guinea-pigs, rabbits	Fæces; occurs in nature	,,
Vibrios, such as *V. choleræ asiaticæ*	+	+	,, ...	,, ...	Guinea-pigs, rabbits, mice	Fæces and vomit ...	,,
And those described by Metchnikoff, Finkler Prior and others, in various intestinal diseases	+	+	,, ...	,, ...	Various animals	Fæces ,, ,, ...	,,
B. pestis	—	—	,, ..	—	Many animals; rats, guinea-pigs	Sputum; fæces; in blood abstracted by fleas and other blood-sucking insects	Inhaled, inoculated by food and through breaches in the skin.
B. septicæmiæ, *B. hæmorrhagicæ* and those of the numerous blood infections in many animals	—	+	—	—	Many animals	Various discharges ...	—
Micrococcus melitensis	+ (?)	—	Acid ...	—	Monkeys and goats	Urine, fæces, milk ...	By mouth, usually in goat's milk.
Gonococcus	—	Serum media	,, ...	—	Young guinea-pigs	Urethral, vaginal and occasional conjunctival discharges	Mucous membranes.

Note.—For the further cultural characteristics of the Coli group, see separate table.

CHIEF CULTURAL CHARACTERISTICS OF THE COLI GROUP.

—	Motility	Liquefaction of gelatine	Litmus-milk	Glucose	Lactose	Sucrose.	Mannitol	Indol production	Growth on potato
B coli communis	+	–	Acid and clotting	Acid and gas	Acid and gas	Acid and gas	Acid and gas	Plenty ...	Thick brown slimy growth.
B. typhosus ...	+	–	Acid	Acid	–	–	Acid	*Nil*	White invisible growth.
B. paratyphosus (Schötmuller)	+	–	Acid and then alkaline	Acid and gas	–	–	Acid and gas	Occasional ...	As coli.
B. paratyphosus (Brion and Kayser)	+	–	Acid	,, ,,	–	–	,, ,,	*Nil*	As typhosus.
B. gaertner ...	+	–	Acid and then alkaline	,, ,,	–	–	,, ,,	Occasional ..	As coli.
B. dysenteriæ (Shiga)	—	–	Acid and then alkaline	Acid	–	–	–	,, ...	,,
B. dysenteriæ (Flexner)	—	–	Acid and then alkaline	,, ...	–	–	Acid	,, ...	,,

The Chemical Analysis of Water.

The methods employed for the examination of a water to determine its suitability or otherwise for drinking and domestic purposes are usually four in number, and it is only by the employment of all these methods that a true and accurate opinion can be expressed as to the potability and fitness for domestic use of any water under consideration.

These four methods are:—

(1) Physical.
(2) Biological.
(3) Bacteriological.
(4) Chemical.

By a *Physical examination* we determine the turbidity, colour, odour and taste.

The *Biological examination* enables one to determine the presence or absence of many lowly organisms, bacterial and protozoal, and the ova and larvæ of intestinal worms and other parasites.

The *Bacteriological examination* is of the utmost importance. By this means pathogenic bacteria, if present, may usually be detected, or bacteria may be found, the presence of which points conclusively to sewage pollution.

The *Chemical examination* of water will frequently give sufficient data on which to condemn a water for potability or domestic usage.

In any chemical analysis of water the principal chemical substances which have to be sought for, and if found, quantitatively estimated, are as follows :—

(1) Poisonous metals; (2) Free ammonia; (3) Albuminoid ammonia; (4) Nitrates; (5) Nitrites; (6) Chlorides; (7) Hardness.

Metals in the form of salts are occasionally found in water. Chief among these are iron and lead, and more rarely copper, zinc, tin, and arsenic.

The usual tests for these metals are as follows :—

Iron (Qualitative).—Place 50 c.c. of the water in a white porcelain dish. Next dip a clean glass rod into

ammonium sulphide and draw the rod through the water in the dish. If iron is present a dark colouration or streak will be formed along the track of the rod, and will be more or less intense in colour, according to the amount of iron present in the sample. A few drops of hydrochloric acid, if added, will discharge this colouration.

Iron (Quantitative).—Place 50 c.c. of the sample water in a Nessler glass : next add one or two drops of ammonium sulphide. This will produce a brownish-black colouration. Into a second Nessler glass measure 50 c.c. of distilled water, adding one or two drops of ammonium sulphide, and carefully run into this mixture from a burette, drop by drop, a standard solution of ferric chloride, of such a strength that 1 c.c. equals ·1 mgm. of Fe, until the colour matches that of the water sample. Note the quantity of standard solution used, and by a simple calculation the number of milligrammes of Fe in the 50 c.c. of sample water may easily be determined.

The result should be expressed in parts per 100,000.

The standard iron solution is prepared by dissolving 1·0004 grm. of iron wire in nitro-hydrochloric acid, precipitate with ammonia, wash and re-dissolve the ferric oxide in a little pure HCl and dilute to ten litres. 1 c.c. of this solution equals ·1 mgm. of Fe.

Iron, although hardly a poisonous metal, if in considerable quantities in a water, renders it unsuitable for drinking purposes owing to its nauseous taste. Any water containing more than 1 gr. of Fe per gallon is unfit for use.

Lead (Qualitative).—This test is performed in the same way as the one described for the detection of iron. The brownish-black colouration produced is, however, unaltered by the addition of a few drops of HCl and of KCN.

Lead (Quantitative).—The estimation of the amount of lead present is made in the same manner as that described for iron, by substituting a standard solution of lead acetate for the standard solution of ferric chloride used in the estimation of that metal.

Standard lead solution is made by dissolving with the aid of acetic acid 1·83 grm. of lead acetate in ten litres of distilled water. 1 c.c. of this solution equals ·1 mgm. of Pb.

Copper (Qualitative).—The presence of this metal is detected by the same means as have been described for the detection of iron and lead. The colouration produced is unchanged on the addition of HCl, but is destroyed on adding KCN.

Copper (Quantitative).—Place 50 c.c. of the sample water in a Nessler glass and add a few drops of HCl, and sufficient of a solution of potassium ferrocyanide to produce the maximum colouration. Into a second Nessler glass measure 50 c.c. of distilled water, a few drops of HCl and about 1 c.c. of the potassium ferrocyanide solution, and add from a burette, drop by drop, a sufficient amount of a standard solution of copper sulphate to match the colour in the first glass. By noting the amount of standard solution used to produce this result, it is easy to determine the amount of copper present in the sample.

Standard solution of copper sulphate is made by dissolving 3·95 grm. of copper sulphate in ten litres of distilled water. 1 c.c. of this solution equals ·1 mgm. of Cu.

Zinc (Qualitative).—Place 10 c.c. of the sample water in a test tube and add a few drops of ammonium hydrate to render it slightly ammoniacal. Boil and filter. A few drops of potassium ferrocyanide added to the filtrate will give a white gelatinous precipitate of zinc ferrocyanide if zinc be present.

Zinc (Quantitative).—A measured quantity of the water, concentrated if necessary, is treated with a few drops of ammonium sulphide, causing a precipitate of sulphide of zinc. This precipitate is collected by filtration, well washed with dilute ammonium sulphide, dried, ignited at a bright red heat, cooled and weighed as zinc oxide ($ZnO \times 0{\cdot}8 = Zn$).

Tin (Qualitative).—Evaporate one litre of the water to

a small bulk, acidulate with HCl and saturate with H_2S in a white porcelain dish. A yellow precipitate of stannic sulphide, in the absence of other heavy metals, would indicate the presence of tin.

As confirmatory evidence the brucine test as here described may be used :—

A solution of brucine prepared as follows is required : ·5 grm. of brucine are dissolved in 5 c.c. of pure HNO_3 in the cold, 250 c.c. of water is added and the whole boiled for fifteen minutes. Sufficient water is afterwards added to make the total bulk up to 250 c.c. 100 c.c. of the water to be tested is taken and evaporated to dryness. The residue is dissolved in a few drops of distilled water and 1 c.c. of the brucine solution is added. If tin be present, a reddish-violet colour will be produced.

Tin (Quantitative).—One litre of the water is taken and slowly evaporated on a water bath to a small bulk (say 50 c.c.).

This is then acidulated with HCl and finally saturated with H_2S. Tin, if present, will then be precipitated as yellow stannic sulphide.

This precipitate is collected and treated with strong nitric acid, forming meta-stannic acid. This product is then ignited, producing stannic oxide.

By weighing this oxide the amount of tin present in the water used can be estimated ($SnO_2 \times 0{\cdot}785 = Sn$).

Tin is more frequently found in meat essences and food contained in tins. The contents must be evaporated to dryness and ignited. An excess of HCl should then be added and again evaporated to dryness. The residue is dissolved in water acidified with HCl and saturated with H_2S.

Arsenic.—To detect arsenic in water a litre of the water is rendered alkaline by solid sodium carbonate, evaporated nearly to dryness and then introduced into Marsh's apparatus.

Any water in which any of these metals, except iron, are detected, should be unhesitatingly condemned.

The determination of free ammonia, albuminoid ammonia, nitrates, nitrites and chlorides, is of importance, as these substances, if in considerable amount, usually indicate contamination with organic matter, possibly sewage. In moderate quantities they are not in themselves injurious.

Free Ammonia.—The method used for the estimation of this substance is a colorimetric one, and is known as Wanklyn's process.

Into a glass distilling flask, of about a litre capacity, connected with a condenser, pour 500 c.c. of the water and add a pinch of sodium carbonate and a small piece of pumice-stone. The flask is then heated over a Bunsen flame, and three Nessler glasses, of 50 c.c. each of the distillate, are collected. This amount of distillate, viz., 150 c.c., is found to be sufficient to obtain all the free ammonia in any sample of water.

Into the first of these distillates 2 c.c. of Nessler's solution are introduced from a burette, when a yellow colour will be produced of a greater or lesser intensity, according to the amount of free ammonia present. This colouration is then matched in another Nessler glass, using distilled water, 2 c.c. of Nessler's solution, and to this a sufficient measured quantity of a standard solution of ammonium chloride is added till the colouration matches. This standard solution is made by dissolving 3·14 grm. of anhydrous ammonium chloride in a litre of distilled water, again diluted one hundred times, so that 1 c.c. of the resultant fluid = ·01 mgm. of ammonia.

The second and third distillates are treated in the same way, and the total amount of standard solution of NH_4Cl used, is noted.

As each cc. of standard solution equals ·01 mgm. of NH_3, it is a simple matter to calculate the amount of free ammonia present in the original 500 c.c. of water taken, and from that to express the result in parts per 100,000.

Nessler's solution is prepared as follows :—

To 800 c.c. of distilled water are added 35 grm.

of potassium iodide and 13 grm. of perchloride of mercury. This is then boiled and well stirred until the salts are dissolved. Cold, saturated solution of $HgCl_2$ is next added, until a permanent red precipitate appears. 120 grm. of sodium hydrate are now added and the whole made up to 1 litre with water. The solution is then rendered sensitive by the addition of a little more of the solution of $HgCl_2$.

Albuminoid Ammonia.—To the residue left in the distilling flask after the last process add 50 c.c. of alkaline permanganate solution (K_2MnO_4 8 grm., NaOH 200 grm., to a litre of distilled water), and proceed to distil over as before, continuing the operation until no more ammonia comes over.

The determination of the amount of ammonia in this case is conducted in precisely the same manner as for the free ammonia.

Nitrites (Qualitative).—Make, with distilled water, a 5 per cent. solution of meta-phenylene diamine.

Decolourise by shaking up with animal charcoal and filter. The solution should now be colourless. If this is not so, repeat the treatment with animal charcoal until such result is obtained.

To 100 c.c. of the sample water in a Nessler glass add a few drops of dilute sulphuric acid and then about 1 c.c. of the meta-phenylene diamine solution, and place the glass in a warm place for half an hour. By the end of that time a yellow colour will be produced if the water contains nitrites.

Nitrites (Quantitative).—A standard solution of potassium nitrite is used of such a strength that 1 c.c. equals ·01 mgm. N. This is made by dissolving 1·1 grm. of pure silver nitrite in boiling distilled water. To this is added KCl, which precipitates the silver as AgCl. The whole is then made up to one litre and the silver allowed to settle. From the clear supernatant liquid 100 c.c. are taken and made up to a litre with distilled water. This solution will be of the required strength.

By using this standard solution the colour produced in the 100 c.c. of sample water with the meta-phenylene diamine may be exactly matched in the same quantity of distilled water. Knowing the amount of standard solution required to produce this result, a simple calculation is then all that is needed to determine the amount of nitrites in the water subjected to analysis.

Nitrates (Qualitative).—Take 10 c.c. of the sample water in a test tube and to this add about 1 c.c. of a saturated solution of brucine and well mix by shaking. Now carefully introduce down the side of the tube with a pipette about 2 c.c. of pure sulphuric acid, so that the acid forms a distinct layer beneath the mixture of water and brucine. If nitrates are present in the water under examination, a pink ring changing to one of a brownish-yellow colour will be seen at the junction of the two liquids.

Nitrates (Quantitative).—The most convenient way of estimating the amount of nitrates in a water is by the process known as the phenol sulphonic acid method.

For this the following solutions are required :—

(1) *Phenol Sulphonic Acid.*—This is made by mixing 12 grm. of pure phenol with 6 c.c. of distilled water and 74 c.c. of pure sulphuric acid, and digesting the mixture for two hours at 100° C.

(2) *Standard solution of Potassium Nitrate.*—Made by dissolving ·722 grm. of dried potassium nitrate in one litre of distilled water. 1 c.c. of this equals ·1 mgm. N.

The process is thus carried out : 10 c.c. of the sample water and 10 c.c. of the standard solution of potassium nitrate are evaporated separately to dryness in two porcelain dishes over a water bath.

To each of the residues 1 c.c. of the phenol sulphonic acid is added, and the dishes allowed to remain on the bath for a few minutes.

The contents of the dishes are now washed out successively with about 20 c.c. of distilled water into two Nessler glasses, and 20 c.c. of liquor ammoniæ added to each, the

whole amount of liquid in each glass being made up to 100 c.c. with distilled water.

Any nitrates in the solutions act on the phenol sulphonic acid, converting it into picric acid, which is again turned by the ammonia into ammonium picrate. This gives a yellow colour to the solution, the intensity of the same being proportional to the amount of nitrate present. Now take a third Nessler glass and pipette into it a sufficient quantity of the darker solution, which when diluted with distilled water up to 100 c.c. will exactly match in colour the more lightly coloured solution. Assuming that 5 c.c. of the contents of the Nessler glass containing the standard solution when diluted up to 100 c.c. exactly match the water sample, then the latter must contain $\frac{5}{100}$ of 10 c.c. of standard solution.

That is to say, 10 c.c. of the sample water contains an amount of nitrates equivalent to that contained in ·5 c.c. of the standard solution. The exact strength of the latter being known, it is easy to work out the parts per 100,000 of nitrates in the water under consideration.

Chlorides (Qualitative).—To 50 c.c. of the water in a Nessler glass add a few drops of nitric acid and then a little silver nitrate solution. If chlorides are present this gives a white haze, or a precipitate if they are abundant.

Chlorides (Quantitative).—Two reagents are used in the estimation of chlorides.

(1) A 5 per cent. solution of potassium chromate.

(2) A standard solution of silver nitrate, made by dissolving 4·8 grm. of $AgNO_3$ in a litre of distilled water. 1 c.c. of this solution = 1 mgm. Cl.

Place 100 c.c. of the water under examination in a white porcelain dish, and add 1 c.c. of the $KCrO_4$ solution and stir. Whilst constantly stirring with a glass rod, run in from a burette, drop by drop, the silver nitrate solution, until the yellow colour becomes permanently orange. Now read off on the burette the amount of silver nitrate solution used, and calculate from this the amount of chlorides present in the sample water.

Hardness in the water is either temporary or permanent. The temporary hardness, which may be got rid of by boiling, is produced by calcium carbonate and magnesium carbonate, held in solution by the action of CO_2. The permanent hardness consists principally of some sulphates, chlorides and nitrates of calcium and magnesium.

The total hardness is the sum-total of both the temporary and permanent hardness.

The usual way of estimating the amount of total hardness is by the application of what is known as Clark's process.

A standard soap solution, made with equal parts of spirit and water, is used, of such a strength that 1 c.c. exactly neutralizes 1 mgm. of calcium carbonate.

Take 100 c.c. of the sample water in a 200 c.c. stoppered bottle, and run in the soap solution 1 c.c. at a time, shaking well after each addition, until a lather $\frac{1}{4}$ in. thick remains unbroken for five minutes. Read off the number of cubic centimetres of soap solution used and deduct 1 c.c., as being necessary for the production of a lather in 100 c.c. of distilled water. The remainder will then give the amount of hardness present, expressed in parts per 100,000.

In expressing an opinion as to the suitability or otherwise of any water for drinking or domestic purposes, a careful survey of all the factors revealed by the chemical analysis must be made, combined with a critical examination of the source of the supply and of all vessels used in the storage of the same.

It is impossible to lay down any hard and fast standard of purity for a water, and every case must be judged on its merits.

Briefly, it may be stated that any water containing any nitrites, indicating recent pollution, should be condemned forthwith. The same applies to any water containing any poisonous metal.

The amount of hardness in water varies between wide

limits. A figure representing thirty or more degrees of total hardness would condemn a water as unfit for drinking or domestic purposes, unless that figure could be considerably reduced by a suitable process of softening.

The amount of both free and albuminoid ammonia, unless in exceptional abundance, does not necessarily condemn a water. Some waters, *e.g.*, rain water, may give a high figure for free ammonia, but the albuminoid ammonia will be small. On the other hand, peaty water may give a figure as high as ·01 per 100,000 for the albuminoid ammonia, but the free ammonia present will, in the absence of pollution, be practically a negligible quantity.

In all cases where the figures for either or both of the ammonias are high, these must be considered, in conjunction with the figures of the nitrates and chlorides, before giving an opinion. A water containing, say, ·005 parts per 100,000 or more free ammonia, and ·01 parts per 100,000 or more of albuminoid ammonia, should excite grave suspicion. If, at the same time, the figures obtained for chlorides and nitrates were also high, *e.g.*, Cl 5 parts per 100,000, and nitrates ·4 parts per 100,000, such water should be unhesitatingly condemned.

A high chloride figure must always be carefully investigated as it may indicate contamination with sewage or urine. Many waters contain considerable quantities of chlorides, as in waters derived from the lower greensands, and deep down in the chalk, which are harmless. An exceptionally high figure for chlorides in a well near the sea indicates that the same is polluted by the sea water, and the water therefrom is unpalatable.

REFERENCES.

NOTTER: "Theory and Practice of Hygiene."
SOMERVILLE: "Practical Sanitary Science."
WHITELEGGE: "Hygiene and Public Health."
FRESENIUS: "Quantitative Analysis."
SUTTON: "Volumetric Analysis."

CHAPTER XXIII.

Measurements.

Measurements of the various eggs, parasites and normal and abnormal cells, are of considerable importance and are easily made.

The simplest and most satisfactory method of microscopic measurement is by drawing to scale, which can be readily done by the use of a camera lucida or drawing camera. A micromillimetre scale is used as an object, and with the microscope vertical or inclined at an appropriate angle, depending on the form of camera used, the scale as it appears through the camera lucida is drawn on a piece of paper. Gower's hæmocytometer slide, which is divided into $\frac{1}{10}$ mm., or a Thoma-Zeiss, which is divided into $\frac{1}{20}$ mm., may be used instead of the micromillimetre scale, or any other will suffice. This drawing of $\frac{1}{10}$ mm. must be further subdivided by compasses. This gives the scale, and it must be determined for each objective. Provided that the distance of the paper from the camera lucida is constant, which is best ensured by working with the microscope vertical and the paper on the table, a scale once drawn can always be used.

The draw tube of the microscope must always be the same length.

To measure an object all that is needed is an outline drawing through the same camera lucida, and the application of the scale to this drawing will give the measurements.

Another simple method is by the use of a micrometer eye-piece, which consists of a glass disc on which a

scale is drawn, and this placed in the eye-piece so as to be accurately in focus by the anterior lens. The disc rests on the diaphragm, which can be moved so that the scale is sharply focussed. A measured scale is then placed on the stage. As before, Gowers' or a Thoma-Zeiss hæmocytometer scale may be used instead of the micromillimetre scale, and the number of the divisions in the micrometer eye-piece, which corresponds to $\frac{1}{10}$ or $\frac{1}{20}$ millimetre, or a multiple of these, with the different objectives is noted. With the tube at constant length the value of the divisions in the micrometer eye-piece so determined is constant for each objective. In measuring, the object to be examined is placed under the microscope and the measurements in terms of the micrometer scale determined, and from these the real measurements calculated.

For simple diameters, the micrometer eye-piece is perhaps the most convenient, but for irregularly-shaped bodies, and particularly for such objects as filaria, the use of the camera lucida is easier, quicker and more accurate.

By either of these methods all that is required to measure an object, once the scales are made, or the equivalent in micromillimetres of the eye-piece scale determined, is to change the ordinary eye-piece either for the camera lucida or for the eye-piece containing the micrometer scale.

Measurements may be represented as decimals or fractions of a millimetre, but in many ways it is more convenient to take as the standard $\frac{1}{1000}$ of a millimetre —a micromillimetre—usually indicated by the Greek μ.

If no scale be available to standardize the micrometer eye-piece or drawings, results by relative measurements can be taken and subsequently standardized. A convenient rough standard is the average diameter of a red corpuscle, which is about 7 to 8 μ.

Estimation of the Number of Corpuscles.—For the determination of the number of elements in a given volume of fluid, as for instance the number of red or white cor-

puscles in blood, it is usually necessary to dilute such a fluid to a known extent so that the number of elements in any given volume can be counted.

Such a dilution may be made in a graduated pipette by drawing up a given volume of fluid and as many more volumes of a diluting fluid as is necessary, and mixing well. Such a mixture can also be conveniently made in Wright's tubes, as the absolute volume is immaterial; all that is required is any volume and definite multiplications of that volume in order to get the degree of dilution.

Common instruments used for the purpose are the pipettes of the Thoma-Zeiss hæmocytometer (fig. 161). These are so graduated as to give a dilution of 1 in 10 or 1 in 100, but can be used to give dilutions at intervals of 10 from 1 in 10 to 1 in 100, and in intervals of 100 from 1 in 100 to 1 in 1,000; as the fluid last drawn up into the tube when the mark 101 or 11 is reached is not mixed with the blood but simply blown out again, it does not count in the dilution.

These tubes are convenient, and the glass bead in the mixing chamber facilitates mixing and prevents the aggregation of corpuscles into masses. The diluting fluid, when working with blood, must be carefully selected according to the object to be attained. If red corpuscles are to be counted the fluid must be isotonic or hypertonic, so as to prevent the red corpuscles being broken up. Such fluids as 10 per cent. solution of sodium sulphate are suitable.

In many cases it is convenient to count the white corpuscles at the same time, and in that case stains are mixed with the diluting fluid, which stain the leucocytes and enable them to be readily distinguished. Toisson's fluid, viz., glycerine 30 c.c., sodium sulphate 8 grm., sodium chloride 1 grm. methyl violet ·025 grm., and water 160 c.c., is very convenient for this purpose, but must be filtered each time before use. In other cases where it is not desired to count the red corpuscles

and where these may render the enumeration of other elements more difficult, it is better to destroy them. To ensure their destruction it is advisable to use a more powerfully destructive agent than distilled water, and weak acetic acid is the one generally employed. A 1 per cent. solution of acetic acid is suitable, and to this a little methyl violet may be added so as to stain the leucocytes faintly, thus rendering them more easily observable.

Leucocytes, &c., can be readily counted in blood only slightly diluted when treated in this manner.

However the dilution is made, the next essential is to obtain a definite measured volume of the diluted fluid.

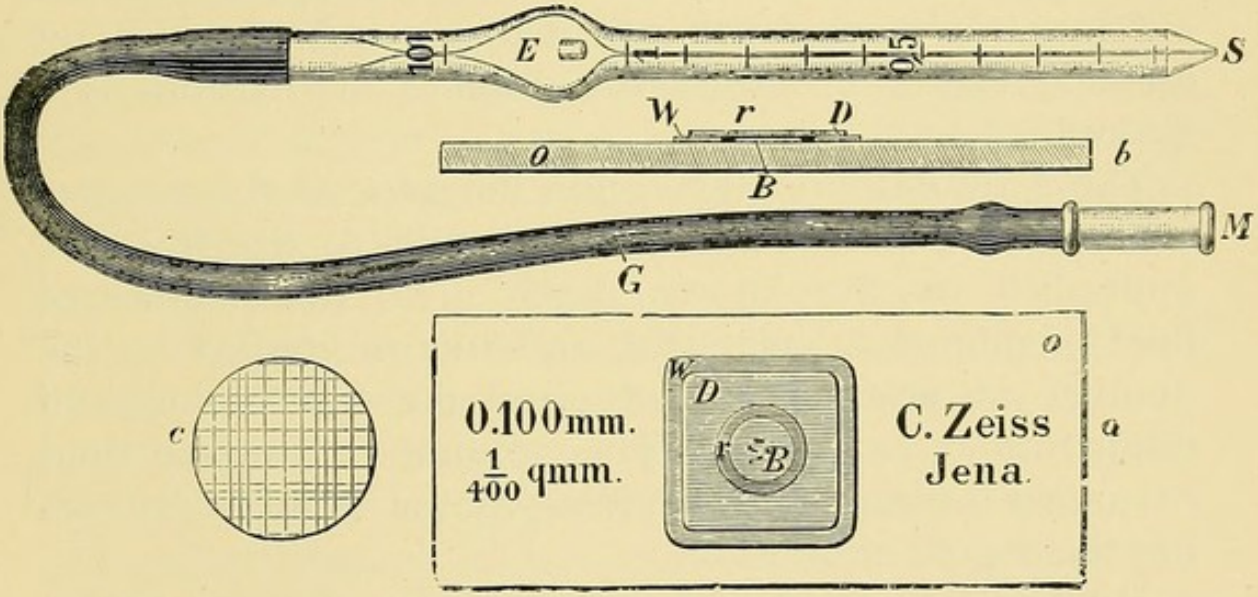


FIG. 161.—THOMA'S HÆMOCYTOMETER, BY ZEISS.

This is done by having a cell which, when covered with a cover-glass, has a definite known depth. It is also further necessary to be able to estimate the area of the base of this cell or of the portion of it examined. In Gower's and in Thoma-Zeiss' hæmocytometer (fig. 161) this area is determined by having the side ruled in squares with sides $\frac{1}{10}$ and $\frac{1}{20}$ of a millimetre respectively, so that the area is obtained by multiplying the sides of the squares by each other, and this is multiplied by the known depth of the cell, *i.e.*, the space between the cover-glass and slide, gives the volume of the fluid examined.

Instead of these squares others use a micrometer eye-piece ruled in squares. The size of these squares is determined by comparison with a scale under the microscope once for all.

In examining fluid for elements that are scanty it is often a saving of time to take the whole field as the area to be examined.

This area is most conveniently determined by obtaining the diameter of the field by observing the number of divisions of a scale—a hæmocytometer scale again will do—that form the diameter. To avoid fractions of a division the tube should be drawn out till the diameter is exactly a certain number of divisions, and, as pointed out by Grünbaum, much calculation can be avoided by drawing the tube out till the number of divisions is a multiple of 10.

The formula, πr^2, then gives the area of the circular field. The depth of the cell is known, so that this area multiplied by the known depth gives the volume of fluid examined in one field, and this multiplied by the number of fields examined gives the total volume of diluted fluid examined. The volume of original fluid examined is obtained by dividing by the number expressing the degree of dilution.

Dilution is merely for convenience and to render counting practicable. In an undiluted fluid, such as blood, the number of corpuscles in a small area, say $\frac{1}{100}$ mm. square, would be some hundreds, and therefore difficult to count, but by diluting 100 times the number will be reduced in that area to a dozen or less, a convenient number for counting.

In these calculations it is well to avoid the exclusive use of formulæ. A formula is easily forgotten, or only in part remembered, and confusion and error result. If the calculations are made on general principles there is a little waste of time, but the possibility of error is avoided. The dilution is made to a known extent—ten times, a hundred times, or so on, as is judged to be

convenient. A known volume of the diluted fluid is examined, represented by either the area of the field multiplied by the depth of the cell, or the area of the marked squares on the slide multiplied by the depth of the cell or the area of the square as seen in the micrometer eye-piece (previously determined) multiplied by the depth of the cell.

The number of elements which it is wished to have counted is determined in a certain number of these volumes of fluids, and the average is taken by dividing the total number by the number of volumes examined. The larger the number of volumes taken the smaller is the probable error.

All the factors necessary for the calculation are thus determined, and all that is necessary is to reduce them to comparable terms, so that the results obtained can be compared with other results. The number of elements is usually recorded as so many per cubic millimetre of undiluted fluid.

For example, suppose the blood has been diluted two hundred times, and in the area of 400 squares of a Thoma-Zeiss hæmocytometer there are counted 2,500 red blood corpuscles, or an average of $\frac{2500}{400} = 6{\cdot}25$ per square.

Now each square is $\frac{1}{20}$ mm., $\times \frac{1}{20}$ mm., and the depth of the cell is $\frac{1}{10}$ mm., therefore one square represents $\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10}$ c.mm. of the diluted fluid.

If in $\frac{1}{4000}$ c.mm. of the diluted fluid there are 6·25 corpuscles, then in 1 c.mm. of the diluted fluid there are $6{\cdot}25 \times 4{,}000$ corpuscles. And as the blood has been diluted two hundred times, in 1 c.mm. of the blood there are $6{\cdot}25 \times 4{,}000 \times 200 = 5{,}000{,}000$ red blood corpuscles.

The number of blood corpuscles in a cubic millimetre of blood may be calculated from the formula

$$x = \frac{\text{M. N. } 4{,}000}{q}$$

in which M. = the number of corpuscles counted, N. = the dilution of the blood, and q = the number of small squares counted.

This assumes that the elements are uniformly diffused through the fluid, and every effort must be made to ensure this being the case. For blood there must be no delay, and the blood must be mixed with the diluent as quickly and thoroughly as possible. Many of the elements in the blood and other animal fluids are adhesive and tend to adhere in clumps. If these clumps are present any result obtained will be unreliable.

Counts of red blood corpuscles are necessary in cases of anæmia. In some forms of anæmia the number of corpuscles is not reduced and may even be above normal. In these cases the hæmoglobin is much reduced, so that on the average each corpuscle is poor in hæmoglobin. *Chlorosis* is the type of this form of anæmia.

In others and the more common forms of anæmia, in the tropics, the number of red corpuscles is greatly reduced, sometimes to $\frac{1}{10}$ of the normal 5,000,000, and commonly to between 1,000,000 and 2,000,000. In these forms of anæmia the hæmoglobin is reduced only to about the same extent as the number of the corpuscles, so that, on the average, each red corpuscle contains about the normal amount of hæmoglobin, so that they are more allied to *pernicious anæmia*, though in this form the average amount of hæmoglobin per red corpuscle is greater than normal.

In the anæmia resulting from hæmoglobinuric fever the number of the red corpuscles is very rapidly reduced, so that in the course of three days the red corpuscles will fall from 5,000,000 to 1,000,000, or even less, per c.mm. of blood. In malaria it is unusual to find any great diminution in the number of red corpuscles as the result of an acute attack of malaria, though repeated attacks may cause a considerable degree of anæmia of this type, and even in slight attacks the red corpuscles show more variation than is normal in size, colour, and shape. Ross has shown that even in single paroxysms there is an increase in urobilin in the fæces, indicating blood destruction.

In *anchylostomiasis* in most cases there is a great reduction in the number of red corpuscles, so great in chronic progressive cases that only 500,000 red corpuscles may be found in a cubic millimetre. There is often, however, an even greater fall in the hæmoglobin ; so that though in the main this form of anæmia is of the type of pernicious anæmia, in some cases it is of a mixed nature.

The forms of anæmia of the pernicious anæmia type are usually due to blood destruction or *hæmolysis*. In such forms of anæmia yellow pigment and iron deposits are found in the cells of the liver, kidneys, &c. In many cases of ankylostomiasis these deposits are as extensive as in cases of pernicious anæmia.

Various chemical agents can cause hæmolysis, and it occurs as a result of the action of various organisms and animal parasites.

The total leucocyte counts should be supplemented by a differential count of the leucocytes in a well-stained film (*vide* p. 58). By the combination of these methods the number of leucocytes of each variety in a cubic millimetre of blood can be determined.

Increase in the total number of the leucocytes occurs in many diseases ; it is known as *leucocytosis*. Diminution in the number of leucocytes—*leucopenia*—is of less importance unless marked, but occurs in some stages of malaria, in kala-azar and other diseases. Leucocytosis is marked in most cases of pneumonia, and the amount of leucocytosis increases with the severity of the case to a certain extent. In the most severe attacks, however, there may be no leucocytosis, and therefore the absence of this condition is of even more unfavourable prognosis than a most marked manifestation of it.

In septic conditions, appendicitis with suppuration, hepatic abscess, septic endocarditis, &c., there is well-marked leucocytosis, though not to the same extent as in some cases of pneumonia. An increase also occurs in scurvy.

Even in health there is a considerable variation—7,000 to 10,000—in the number of leucocytes, and daily a variation occurs owing to the increase in the lymphocytes during active digestion.

It is not usual for the number of the different forms of leucocytes to be increased uniformly. Usually some forms are increased and others either increased to a much smaller extent or even diminished.

In pneumonia the increase is mainly that of the polymorphonuclear leucocytes, whilst the eosinophile leucocytes are not only in smaller proportion but in smaller numbers than in normal blood. After an attack of malaria the leucocytes are usually in normal number, as the larger mononuclear leucocytes are increased and the polymorphonuclear leucocytes diminished.

If in addition to the normal white corpuscles other cellular elements are present in considerable number in the blood, such as myelocytes, this indicates a definite blood disease.

If two diseases, such as pneumonia and malaria, coexist in the patient, the influence of the one disease appears to overpower the other, so that the leucocytic variation of pneumonia only will be present.

A variation in the total amount of blood in the body no doubt occurs in certain diseases, but there is no simple practical method of determining such variations.

To enumerate the larger parasites, such as filarial embryos, no dilution is required. A measured quantity of blood is taken up in a pipette and blown out on to a slide. This blood is spread out and allowed to dry and decolourized by placing in water. The total number of filarial embryos can then be counted and reduced to the proportion per c.mm. This is a matter of no difficulty and the only method to be relied on. Substitutes that are often employed are to make a thick dry blood film a certain size, or to use cover-glasses of a definite size in making fluid films, or to make a certain number of drops of blood in making the film. These methods as substitutes

for direct measurement are inferior, and results arrived at by these methods have little value. In the absence of a measured pipette any fine marked tube may be used. The volume may be estimated subsequently.

The use of measured amounts of blood, decolourized and stained, for the estimation of malarial parasites is practised by Ross. The results are reliable with special training. The parasites are distorted during the process, but with practice most of them can be recognized. An approximation to the number present can be obtained by first estimating the number of leucocytes present per c.mm., and then taking a fluid fresh film of the blood and determining the relative number of parasites to leucocytes in the film. If, for instance, ten parasites are found in this film and 100 leucocytes, there will be one parasite to ten leucocytes, and if the number of leucocytes determined separately is found to be 8,000 per c.mm., then the number of parasites should be one-tenth of this, or 800 per c.mm.

The results are approximate only, as leucocytes are not uniformly distributed in the fluid film. In Ross' method very small quantities of blood are taken in a fine tube such as that of a clinical thermometer which is marked. The volume is determined by calculation, but as these fine tubes are often not truly cylindrical it might be better to graduate by the weight of the mercury contained in each division. Rejecting the first drop the measured amount taken is blown out on to a glass slide and spread out and decolourized and stained. Every parasite is counted.

By this method Ross has been able to demonstrate a periodicity in the increases and decreases in the number of trypanosomes in a case of trypanosomiasis and has studied the variations in the numbers of malaria parasites with special reference to the number required to produce pyrexia and the effect of drugs. He finds that in infections with *P. vivax* (benign tertian) an average of 125 per c.mm. did not produce

fever, whilst with *P. falciparum* (subtertian) an average of 461 per c.mm. did not cause pyrexia. He estimates the usual pyogenic limit in *P. vivax* as between 200 and 500, whilst with *P. falciparum* 600 to 1,500 might be adopted. For determination of the total parasites he estimates that a man of 10 stone will have 3,000,000 c.mm. of blood. (For full particulars *vide* "*Annals of Tropical Medicine and Parasitology*," vol. iv., No. 1, December 1910).

It is of some interest to compare results obtained by counting the leucocytes and parasites in a fresh fluid film with Ross's more accurate method. Douglas Gray in 1901 and Sims in 1902, "*Journal of Tropical Medicine*," estimated from their observations made by counting against leucocytes in fluid films that the pyogenic limit in a case of quartan malaria (*P. malariæ*) was between 250 and 446, and that in benign tertian (*P. vivax*) it was 400 or more.

The relative numerical proportion of the parasites to leucocytes, if determined in a dry film, is far more inaccurate, as the distribution of the leucocytes is so unequal in such a film, and many of the leucocytes adhere to the needle, slide or paper used in making the film. The leucocytes in a thin part of the field, such as is used for the observation of parasites, will be from one-half to about one-tenth of the proper amount, and the error from this cause in counting the parasites will therefore vary to the same extent. An approximation can also be obtained by determining the average number of parasites in a field. If the average number of red corpuscles in the same field is also determined the method is of value, but it is tedious.

In fluids other than blood, where parasites, including bacteria, are numerous and minute, Wright suggests mixing this fluid with an equal quantity of blood diluted so that the number of corpuscles per c.mm. is known. The relative proportions of the parasites to the blood corpuscles, as determined by making a dried film of the

mixture and staining it, will then enable us to estimate the number of organisms present in any given quantity of the fluid.

The more usual method of estimating the number of living bacilli in a fluid is to take a measured volume of the fluid and add to it a measured quantity of liquefied gelatine and to plate it. The number of colonies found in this plate will give the number of organisms in the volume of fluid taken. If the colonies are too numerous the number found in any measured area may be counted, and this result reduced to terms of the total area of the plate. If the number of organisms is very great a second or third dilution, always with measured amounts of the fluid and gelatine, may be necessary.

For determination of the number of bacteria in air, a measured amount of air is driven through liquefied gelatine, which is plated, and the number of colonies estimated as before.

For earth or solids these must be finely divided and weighed amounts taken.

It is essential that the plates should be accurately levelled, and as much of the plate as possible should be counted. Agar plates may be used, and for complete investigation incubated at various temperatures. Other plates should also be made and incubated aerobically and anaerobically.

The numerical estimation of eggs in fæces hardly admits of practical application, as the amount of water, &c., in the fæces varies so greatly and eggs are not uniformly distributed. Here loose terms, such as numerous, very numerous, moderate number, or few, are more in accordance with the amount of information available than a numerical estimate could be, though that may have a superficial appearance of exactness.

Colorimetric Estimations are not very accurate. They are, however, the only simple methods that can be used with rapidity and ease. They are all based on the comparison of the diluted fluid with a substance standardized

as regards colour. The principle adopted is to so arrange matters that the equality in tint, or "matching," of the two objects compared is obtained, as equality in tint can be determined more exactly than degrees of difference.

In such colorimetric examinations the same source of light must invariably be used, and for general purposes artificial, particularly candle light, is to be preferred, as colours seen by one light will not match when viewed with another light.

The methods most used for blood work are Gower's hæmoglobinometer and its modifications, in which gelatine coloured with picro-carmine is used as the standard

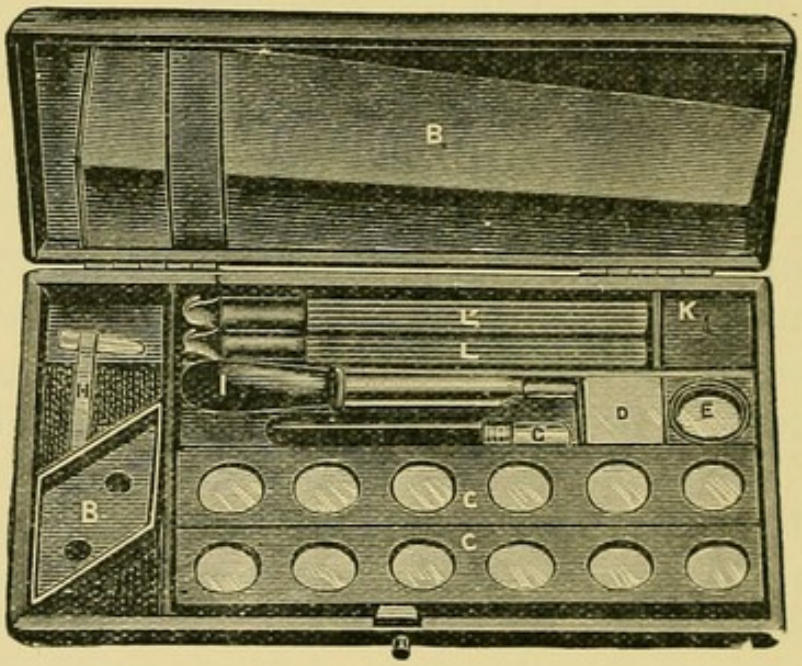


FIG. 162.—OLIVER'S TINTOMETER.

colour and standardized so as to represent a certain percentage — 1 per cent. — of hæmoglobin, and von Fleischl's hæmometer and its modifications, in which a wedge of tinted glass is used as a standard, this being moved till the thickness of the wedge is such that the depth of colour equalizes that of a definite depth of the fluid to be examined. In Oliver's method (fig. 162), a series of graded depths of coloured discs is used as the standard. The colour produced is compared with these standard colours by mixing with water a definite fixed

quantity of blood in a glass cell of fixed depth and capacity placed on a white plaster disc.

For all these methods it is necessary that the blood corpuscles should be broken up and the hæmoglobin be in solution. This can be done by dilution with distilled water, or, better, by dilution with a 1 per cent. aqueous solution of carbonate of soda.

In Gower's method (fig. 163) the standard colour is equivalent to that of normal blood diluted so as to represent 1 per cent. of hæmoglobin dissolved in water.

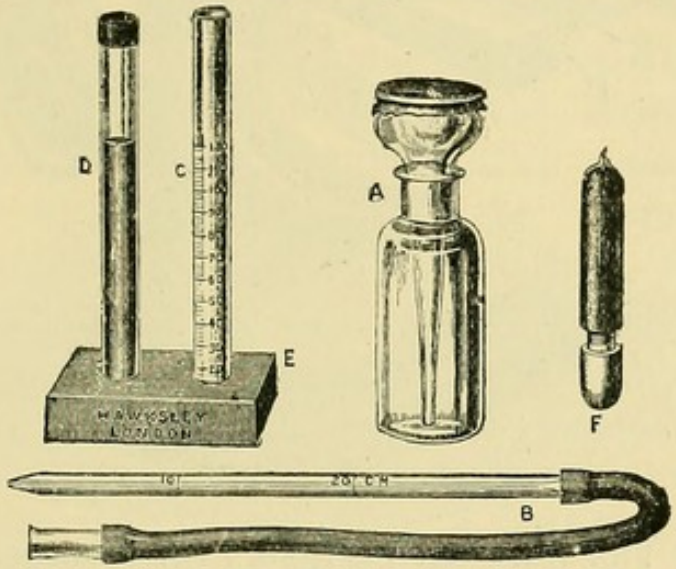


FIG. 163.—GOWER'S HÆMOGLOBINOMETER.

The fluid to be estimated is placed in a cylinder, or, better, in a flattened tube exactly similar to that containing the standard, and is diluted drop by drop till the two colours are matched. The tube is graduated so that the degree of dilution can be read, and the amount of dilution necessary to produce the same depth of colour indicates the relative amount of hæmoglobin as compared with the standard.

In von Fleischl's method (fig. 164) the wedge of coloured glass is arranged on a stand and illuminated from below by a plaster of Paris disc. Fitting into the circular opening of the stage is a metal cylinder with a glass bottom, and this cylinder is divided longitudinally into two compartments. One is filled with diluted and laked blood and the other with water. Under this second compartment is the wedge of coloured glass, and this

wedge is moved horizontally by a rack and pinion till the colour corresponds to or matches that of the diluted blood. The movement of the wedge is indicated on a scale graduated by comparison with hæmoglobin solu-

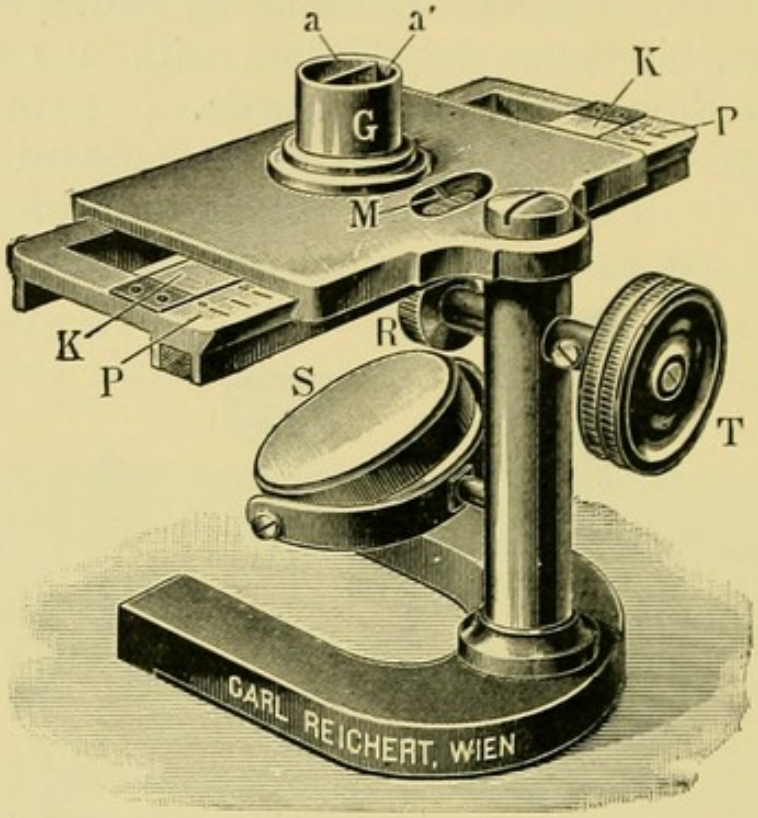


FIG. 164.—VON FLEISCHL'S HÆMOMETER.

tions of varying strength, so that the hæmoglobin equivalent of the portion of the glass wedge in the field can be read at once.

Tallquist's Hæmoglobin Scale.—This is a scale of colours representing the shades of red corresponding to definite percentages of hæmoglobin. A book of filter papers accompanies the scale.

To estimate the hæmoglobin a drop of blood is absorbed by the filter paper, and when the gloss has passed off this is compared with the scale—the number opposite the shade of colour which most nearly corresponds to it gives the percentage of hæmoglobin. The estimation should be done by daylight. It is not very accurate, but the results are about as reliable with this method as with any other in common use. It has the great advantages of simplicity and quickness of performance.

CHAPTER XXIV.

Statistics.

Statistics.—Statistics as reliable as those obtainable in England can rarely be obtained in the Tropics, and an important source of information is thus wanting. Even statistics of births, deaths, and the more important diseases, both of Europeans and natives, have to be admitted with great caution, and local knowledge of the manner in which they are compiled is essential before giving them even the slightest consideration. With local knowledge the statistics may be very valuable, but even then the errors due to selection of statistics, a personal equation, greatly reduces their value.

Statistics for tropical work therefore must, to a great extent, be the result of individual work, so that the large numbers that European statisticians are accustomed to work with are not obtainable.

It is therefore of the greatest importance at the outset that the probable error due to working with small figures should be clearly grasped.

The best method of arriving at the probable error in cases where a series of facts is divided into two groups is by Poisson's formula :—

If g = the total number of cases,
m = the number in one group,
And n = the number in the other group, so that m + n = g,

the proportion of each group to the whole series will be respectively $\frac{m}{g}$ and $\frac{n}{g}$; but these proportions will vary in succeeding series, and the extent of the variation

will be within the limits represented by the following expression :—

$$\frac{m}{g} \pm 2\sqrt{\frac{2mn}{g^3}}$$

Obviously, the larger the value of g the less will be the value of $2\sqrt{\frac{2mn}{g^3}}$, and consequently the less will be the limits of error in the simple proportion, $\frac{m}{g}$, and conversely the smaller the value of g the greater will be the limits of error.

For example, if in 100 cases of beri-beri ten have been fatal, and it is required to determine the limits of error in assuming this proportion to hold for the next 10,000 cases :—

Applying the formula. Here g = 100, m = 10, n = 90.

$$\frac{10}{100} \pm 2\sqrt{\frac{2 \times 10 \times 90}{100 \times 100 \times 100}}$$

$$= \frac{1}{10} \pm 2 \times \cdot0428 = \frac{1}{10} \pm \cdot0856$$

·0856 represents the variations per unit.

So that in the next 10,000 cases—

There may be 1,000 + 856 = 1,856 deaths ;

Or 1,000 − 856 = 144 deaths.

This formula only deals with the mathematical relationship of the figures. Further allowance has to be made for errors of observations and the numerous uncertain factors met with in any statistics.

A mere consideration of the effects of the extent of the possible mathematical error in dealing with small figures suffices to indicate the care that is necessary and the multitude of observations that are requisite before formulating a definite conclusion. In spite of the magnitude of this error, of the numerous possibilities of errors of observation, and even of the fact that mere increase in the number of observations may only multiply the same error or doubtful point, as the same source of error may be included in each observation, the acquisition and use of statistics is of high value, and often indicates a correct conclusion. The liability to error diminishes but does not destroy this value.

It is customary to indicate all results in percentages, and no doubt this method renders comparison easy; but a consideration of the formula of probable error shows that 1 in 4 is by no means the same thing as 25 in 100. The number of observations made must be included in any account, and whenever possible these observations should greatly exceed the 100. The consideration of the magnitude of the probable mathematical error under the most favourable circumstances should lead to as great an exactitude as possible and avoidance of other and avoidable sources of error.

Value of Evidence. — Considerable judgment as well as caution is requisite in obtaining information other than that derived from personal observation.

As regards occurrence of diseases, parasites, &c., much of the information received must be taken with great caution, as it is often from laymen and untrained observers. Even more in the Tropics than in England such persons hold theories either of their own or derived from others, and are anxious to bring forward only facts which are in support of these theories.

It is well in making enquiries to be careful to limit the enquiry to points that are within the power of any ordinary observer. It is not well to discard altogether such evidence, as on many important points information can be derived, and in some of these the liability to error is no greater than with a professional observer.

Various points in connection with malaria might be well taken as illustrations, both of the value of such information and the errors that are likely to occur as a result of too much confidence in such information, as well as of the general methods which have been adopted determining etiological and other factors.

These points comprise: (1) *As regards Individuals.*— Their susceptibility to the disease and the effects of the disease, including liability to relapses, length of period of intermission between relapses, and any evidence of the acquirement of immunity.

(2) *As regards the Population in General.*—Susceptibility, and any factors, age, race, or habits influencing it. Mortality per 1,000 of the population at various age periods; and case mortality in treated and untreated cases; liability to any special, immediate or remote complications; effect on general health; any evidence of acquisition of immunity.

(3) *As regards the Place.*—This should include enquiries as to any special house, village or district, as well as the country in general, where the disease is more or less prevalent than the average. Seasonal variations and their effects, particularly rainfall, temperature, and any cause affecting level of subsoil water. Any facts known as to the prevalence of the known main factor—in the case of malaria, prevalence of *Anophelinæ*—in the spread of the disease. Some numerical estimate, *endemic index*, of the liability to infection.

Most of these points can be determined to some extent by careful enquiries, though the results must be confirmed by observation, or where possible by the adoption, as a check, of other methods.

The results obtained in this way, though not to be implicitly relied upon, will be a valuable guide to the direction of researches required in a district or country.

Liability to Infection. — Enquiries as to individuals necessitates a selection of cases, and information of a reliable nature can only be obtained on every point from few persons. In the case of newcomers the date of arrival in a country and the subsequent movements, with approximate dates, are usually to be trusted. The date of the first attack of malaria can generally be obtained. Sufficient information about the attack, such as the character and duration of the "fever"; the effect of quinine, and absence of any other cause of pyrexia, such as septic infection or pneumonia, must be ascertained to render it probable that the attack was malarial.

Any form of indisposition in the older residents is

so frequently called malaria that less reliance is to be placed on these than on the history of the first attack.

In malaria it must always be remembered that relapses are so common that a second *attack*, even at an interval of several months, does not prove a second *infection*.

The Liability to Relapses is more difficult to determine, but with a fair number of individuals it can be ascertained, and great individual variations will be found. In newcomers three weeks to a month is a common interval, whilst in others the period may be as long as four or six months. In this connection careful enquiries as to the habits as regards quinine are of great importance, as if quinine is taken constantly, even in small doses, the relapse is often postponed till the quinine is discontinued.

Increase in the Interval between Relapses.—Any observations as to increase in the interval between the relapses with increased length of residence, or diminution in the severity of the attacks, may indicate that a degree of immunity has been acquired, and the length of residence required for this is, to a great extent, an individual peculiarity, though shorter in all in the more malarial districts.

As regards the population in general, it is essential that the actual numbers of the different races represented be known before any use can be made of totals, such as number of deaths, admission to hospital, &c. This warning may appear superfluous, but it is not. In published reports one of the commonest errors is to speak of a disease as being more or less prevalent in a district on the ground of the number of cases seen, not as it should be, on the proportion of the susceptible population attacked.

It is in connection with blackwater fever and yellow fever, in its diagnosable form, that such errors are most common.

Age Incidence.—Personal observations should be made on unselected cases and the number of cases examined

mentioned in the table, with the percentages. Ages cannot be ascertained with certainty, especially in countries where the differences in season are not very marked. With children age has to be estimated from the size, teeth * and development. In adults knowledge of local history and notable events, the dates of which can be fixed, are of considerable value. Age periods of five years are usually taken, but it is of the utmost importance in malarial investigation to subdivide the first quinquennial period and further subdivide the first year into quarters. The first quarter should be subdivided into months. Malaria is rare till the end of the first month.

As an age period the first ten years should never be taken as a whole, as such different results are obtained in a village, or in a series of observations, if a large proportion are, say, under four, or only a small proportion. Conclusions drawn from the incidence of malaria in the first ten years of life, taken as a whole, are often misleading.

Mortality is best estimated at the rate of so many

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* Ages at which teeth are cut in *Europeans*. The differences in native races have not yet been worked out. Table kindly supplied to us by Mr. K. W. Goadby.

TEMPORARY DENTITION.

Central incisors	5th to 8th month.
Lateral incisors	7th to 10th ,,
First molars	12th to 14th ,,
Canines	14th to 20th ,,
Second molars	20th to 30th ,,

PERMANENT DENTITION.

	Upper Jaw.	Lower Jaw.
Central incisors	7·7 years	... 7 years.
Lateral incisors	8 ,,	... 8 ,,
Canines	11 ,,	... 10 ,,
Premolar I.	10 ,,	... 10 ,,
Premolar II.	11 ,,	... 11 ,,
Molar I....	6½ ,,	... 7 ,,
Molar II.	12 ,,	... 12 ,,
Molar III.	24 ,,	... 24 ,,

deaths per 1,000 per year, as then the results can be compared. If dealing with short periods, as, for instance, one week, the death-rate would be the proportion of deaths per 1,000 of the population in that period, multiplied by 52. If a long period, say ten years, is taken, the death-rate would then be represented by the number of deaths per 1,000 divided by 10.

The factors necessary are the number of persons of the required class alive at the commencement of the period, the number of deaths of this class who died from the disease which it is desired to investigate in the period, and the length of the period.

Case Mortality is the percentage representing the proportion of cases terminating fatally. The number of cases of the disease and the number of deaths from the disease are the only two factors requisite. If it is desired to compare the "case mortality" in different years or other periods of time, cases occurring in those periods only must be included. In malaria untreated and treated cases must be considered separately, and the treatment mentioned as the case mortality is so much reduced by effective treatment. In yellow fever not only must the races be kept distinct, but the period when they were last exposed to a definite epidemic must be clearly indicated.

Remote or Indirect Mortality is the mortality due to remote complications, visceral changes and increased liability to other diseases, or to the tendency which malaria appears to have to aggravate some diseases. Our knowledge of this branch of the subject is most inaccurate and requires complete revision.

The effect on general health varies greatly in different conditions, and under circumstances little understood. Splenic enlargement, anæmia and diminished rate of growth are the most definite. Susceptibility to tuberculosis appears to be induced by chronic malaria in countries where tuberculosis is prevalent. The effect on the general health, apart from the actual attacks,

whether mild or pernicious, varies according to race. Some races, as children, have an uncertain degree of "tolerance," at least as regards toxic effects.

Period of natural *incubation* and its variations can be determined from the histories of patients, and then inquiry must be limited either to first attacks or to other attacks in which a long interval has elapsed. The most common history given is of some immediate antecedent. Exposure to chill, constipation, change of residence, particularly from a warmer to a cooler place, and even cessation of travelling, are given as the causes of the attack. These causes are not to be taken as those of the infection, though they may determine or accelerate the manifestation of the disease.

The time of actual onset of symptoms can usually be told with certainty, but the time of infection is difficult to determine. The frequency with which travelling in one form or other enters into the causation is usually to be ascribed to passing through a highly malarial district, or even to spending some hours in a house where infected mosquitoes are to be found. With a sufficient number of cases it is sometimes easy, as in the case of a steamer, or in persons travelling over known routes, to fix on the date of infection as the date on which a halt was made at a notoriously malarial place. Such cases show the wide limits of the period of natural incubation, often longer than those which have been determined experimentally by feeding infected mosquitoes on susceptible persons.

The *evidence of immunity* is to be considered under two heads: (1) Age incidence of the disease in natives and cessation of attacks with advancing years. (2) In newcomers the residential period during which attacks occur, and any evidence, by the diminishing frequency or severity of attacks, that some immunity is acquired. Immunity from a disease must be clearly distinguished from "tolerance" or immunity from the effects of the invasion by the parasites.

With malaria it is important to consider whether there are periods in which from climatic conditions infections do not take place. In the case of individuals, if there are periods during which they are not resident in places where malarial infection is possible. Immunity is destroyed or diminished by such periods, so that if they are long immunity is not acquired at so early a period, or at all. There is evidence that immunity is not of long duration in malaria, but more exact observations are required on this point.

In any consideration of immunity the liability to infection—endemic index—must be taken into account, as with a low endemic index individuals only, not a class, will acquire immunity.

(3) *As regards Place.*—In considering any place it is important to bear in mind that malaria is a local disease, and that even in houses close together one will be more malarial than another. Still more so are different quarters of the same town or district, and the localities where the disease is most prevalent vary from year to year. These differences, and the causation of the variation in the differences, require local investigation in all cases.

Seasonal variation may act in two ways, first by rendering the conditions more favourable for the multiplication of *Anophelinæ*, and secondly by presenting conditions more favourable for the development of the malaria parasites in the mosquitoes. Rainfall, both the amount and distribution, *i.e.*, whether in frequent light showers with short intervals, or heavy downpours with long intervals, is of great importance, but the effect may vary with the same monthly rainfall, as occasional heavy showers flush out and destroy mosquito larvæ, whilst the same amount of rain falling slowly will merely increase the size and maintain the same breeding places. The level of the subsoil water may be more affected by distant rain than by the local rainfall, and thus distant rainfall may be a cause of the unhealthiness of a place. Rain on mountains or hills behind a station is an example of this.

Where there are snow-covered mountains, as in Equatorial Africa, the water supply is dependent on the melting of this snow, and therefore a high temperature increases the water supply of a large district. A high temperature within certain limits causes more rapid breeding of mosquitoes, causes them to require food and therefore to attack men more frequently, and is favourable to the rapid development of the malaria parasites, and so in all these ways will favour the spread of malaria.

The *species of Anophelinæ* present and of those most numerous in the district should be determined, and these species of mosquitoes should be tested as to the readiness with which they may become infected by the malaria parasite. Different species vary greatly in this respect, and even with the same species infection seems to occur with varying difficulty under different circumstances. As a rule mosquitoes reared from larvæ are not as easily infected as those of the same species caught in the adult stage. The possible or known circumstances affecting the development of the parasites are the temperature, the age of the mosquito, whether impregnated or not, and the nature of the food previously taken by the mosquito, and probably other conditions, which all require local investigation.

Endemic Index.—A numerical estimate of the liability to malarial infection is an important factor to determine. The number of malarial attacks, or of hospital admissions for malaria, from a known number of persons, is of little value, even if the diagnosis is confirmed in every case by blood examinations, as these admissions will include recurrences and relapses, which will vitiate the figures, *e.g.*, a man infected once with malaria may have a dozen attacks of malaria as a result of this single infection in a year, or he may have only one. In the first instance he would appear in returns as 12, in the second as 1, though in both instances for our purpose his infection should be represented as 1.

If first attacks only are included this difficulty does

not occur, and first attacks have the further advantage of being usually severe, and in persons who have not yet acquired the habit of treating themselves. These therefore usually come under medical observation.

For an estimate of the liability to infection, or *endemic index*, by this method, the factors to ascertain are the dates of first attacks of malaria occurring during the course of the observations, verified by blood examinations or in other ways, effect of quinine, &c., and the length of residence previous to the attack, and the number of newcomers who have escaped infection during the period. As a separate estimate a statement by as large a proportion as possible of the resident population as to the length of time they had each resided in the country before their first attack of malaria. These figures usually lead to much the same result. Reliance has to be placed on histories only, and errors may occur, though each factor is one which most of the residents are capable of observing.

By this method the length of residence in weeks or months that is ordinarily required for an attack of malaria is determined. The period of incubation we know varies, but is commonly from ten days to three weeks, and this period should be subtracted from the length of residence required for an attack of malaria to develop in order to obtain the period of residence required for infection.

Where bodies of men are working together and are under medical observation, as in regiments, gangs of workmen, &c., this method is, we believe, the best and simplest, and includes no sources of error that are not common to other methods.

In such an estimate all persons who were born and have lived in malarial countries for prolonged periods should be excluded; also those who have contracted malaria in other malarial countries. For these exclusions there are two reasons—(1) to avoid including relapses, and (2) to avoid including persons who may be immune.

A somewhat similar method is to determine the proportion of untreated natives who harbour the parasites of malaria. In this method the ages must be known, and unselected children, including those apparently in good health, must be examined. Children should form a large proportion of the cases.

This method has been extensively used, and an arbitrary standard, ten years, has been selected; the proportion of children under 10 years of age with malarial parasites is then taken as the index.

A more satisfactory method is to determine the proportion at different ages. Thus in one district, whilst 86 per cent. of the children under 2 years of age were infected with parasites of malaria, only 28 per cent. of those from 5 to 10 harboured them. If, therefore, in such a place most of the examinations were made in young children, a much higher index would be obtained than if most of the children were over 5.

In many of the determinations no further information than "children under 10 years of age" has been given and in some of them the number of children examined is very small.

It is not very easy in some places to get a sufficient number of children for examination, but with patience it can generally be done. As these cases are untreated, many of them, if not most, will have had the parasites for considerable periods, and therefore the figures only indicate antecedent, perhaps remote, *infection*. If young children were examined monthly till parasites were found, the liability to infection under native conditions would be determined more accurately. In making any series of blood examinations for such purposes the time selected should be during a period of settled weather. If examinations are made during a change, particularly from hot to cold, the parasites will be more easily found, as the effect of chill is to favour the development of the parasites. Examinations made at such times will therefore show a higher index than those made in settled weather.

The Spleen Test, or the proportion of persons with enlarged spleens, is useful if age and race are taken into account. It is of more value amongst negroes than amongst other races, as the negro spleen does not continue to enlarge after immunity has been acquired in the same way that the spleens of many individuals of other races do. The test can be used easily, as there is nothing in the examination to excite alarm or frighten the children, and can be made more quickly than any other examination.

It indicates only antecedent, probably remote, infection, and is less certain proof of antecedent infection than the presence of parasites.

A large proportion with enlarged spleens, between 2 and 5 years of age, is an indication of a high endemic index. If the presence of malaria in a district is proved, the absence of enlarged spleens in negro adults, or a low proportion between 10 and 15, is equally a proof of a *high* endemic index, whilst if the proportion of enlarged spleens in adult negroes is appreciable or large in those between 10 and 15 the endemic index is *low*.*

The determinations obtained by the spleen test are less liable to be influenced by meteorological conditions than the test by blood examinations ; they are easier, and can be made in a larger number of cases, but otherwise are less accurate, as the conditions that lead to splenic enlargement after malarial infection vary and are not thoroughly understood, and splenic enlargement in a varying proportion is due to other causes.

Another proposed method of estimation of the index is by determining the proportion of the *Anophelinæ* that are found to be infected with the parasites of malaria. For this method to be of value the mosquitoes must be selected from different houses and places in equal proportion, as it will be found that there are great variations

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* With no other race but the Negro can such conclusions be drawn with certainty. (*Vide* page 23.)

in this proportion in adjoining houses and at different times. One good "crescent case" will infect almost every Anopheline of certain species that bites the patient, whilst only a small proportion of those that bite the more numerous poor crescent cases will be infected. *Anophelinæ* in European houses are rarely found to be infected, whilst in an overcrowded native house, where there is no protection of the inmates from mosquitoes, or in a hospital, a large proportion of infected Anophelines may be found.

The *proportion* of infected mosquitoes is not the real test so much as the *number* of infected mosquitoes, so that in these estimates the number of *Anophelinæ* that bite a man per hour or each day is also required.

A high endemic index, as determined by the other methods, will be found in places were *Anophelinæ* are very numerous, even when the proportion infected is very small.

It must always be remembered that a place with a large number of *Anophelinæ* of species known to be efficient carriers of the parasite, even if free from malaria at any one time, has the potentialities of a high "endemic index" if the place be occupied by newcomers or other persons susceptible to malarial infection. This is the reason that railway and engineering works are so often attended with outbreaks of malaria, even when conducted in places that previously were not considered to be very malarious. *Anophelinæ* are present and perhaps numerous; in any case many new breeding-places are formed during excavations, and the mosquitoes become numerous. Gangs of workmen are crowded together in temporary huts, and they are not protected from mosquito bites. The workmen will include susceptible newcomers, and frequently some persons harbouring parasites. A single good "crescent case," often a man with no symptoms of malaria, will infect a number of mosquitoes, and in the course of some ten days or so these mosquitoes will infect any susceptible persons who

sleep in the hut, and these persons will in about twelve days develop an attack of malarial fever.

The earliest numerical estimate was arrived at by determining the proportion of persons at different ages whose organs contained malaria pigment. This method can only be adopted under circumstances where *post-mortem* examinations can be obtained in both children and adults. The results indicate antecedent malaria infection. The method, though fairly good, is only of limited value, as the large number of *post-mortem* examinations required can be obtained in few places.

These, then, are the main methods for the determination of the endemic index :—

(1) By determining the length of residence required to render malarial infection probable in susceptible newcomers.

(2) The ages at which the largest proportion of natives harbour the parasites of malaria.

(3) Ages at which splenic enlargement is common.

(4) Percentage of persons dying from all causes with malarial pigmentation of the organs.

(5) Number of infected *Anophelinæ*.

Other evidences, though too complicated by other factors to be used numerically, are a high infantile death-rate amongst the natives, particularly a high death-rate from convulsions in infants over six months ; a high European death-rate not due to other endemic diseases, such as yellow fever or cholera ; and, we are inclined to add, the occurrence of blackwater fever.

Graphic representations in the form of "charts" are useful as indicating the main results of any enquiry, as they are easier to follow with the eye than columns of figures or rows of statistics.

The essential of a good chart is that it should be capable of translation back into figures, *i.e.*, a chart should be such that it can be read.

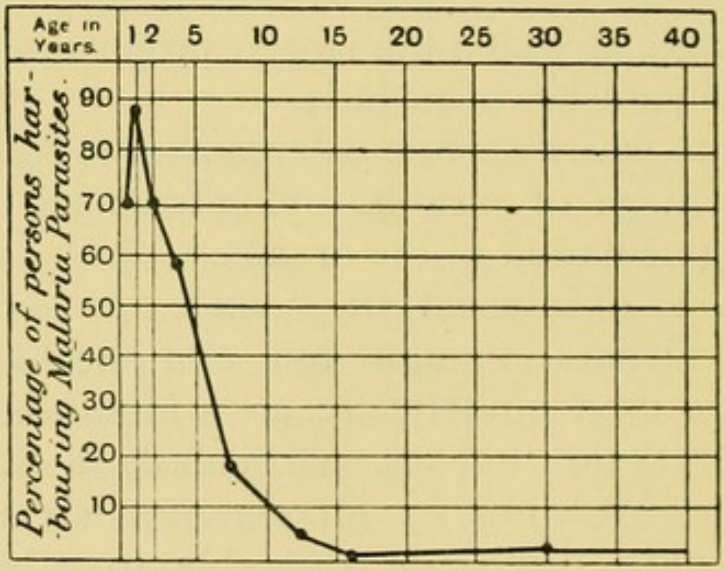
The principle of charting on a plane surface in two dimensions is that the horizontal line represents one

factor, usually time or periods of time, whilst the vertical represents the other factor.

Each of the factors should be represented according to scale in order that it can be read. This point is often overlooked, even in the familiar temperature charts, in that whilst the height of the temperature is recorded correctly in the vertical columns, the distances measured horizontally between the points representing the different observations are equally spaced, so as to look neat, whilst the real intervals of time are irregular, *e.g.*, temperatures taken at 2, 10, 12, 3 should not be recorded on a four-

CHART I.

—— Negroes (Native Africans).—Hausa and Yomba Children, 320; Hausa Adults, 100. Compiled from Official Report, Lagos, of W. H. G. H. Best.



hour chart, as if the time intervals were equal, but should be so recorded that the distances measured on the horizontal line are unequal in the proper proportion.

In that case the chart can be correctly translated back into figures, otherwise, if represented as equi-distant, the translation would read 4, 8, 12, 4.

The limit to the translation of the chart is the scale of the chart; where the intervals allowed for time are small, translation can only be approximate.

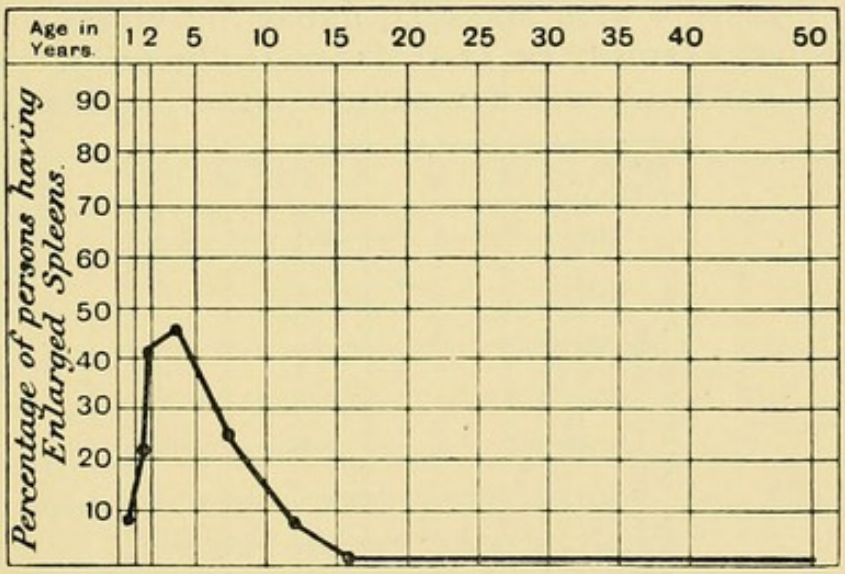
In blood charts it is usual to represent the heights as percentages of normal, as real figures would require such

enormous charts if it were desired to represent graphically on the same scale both red and white corpuscles. In such cases it is better to keep to the figures or to use different vertical scales for the more numerous and more scanty elements. It must be clearly indicated what each line on the chart represents.

Too many lines on one chart are difficult to follow, and the only cases in which it is advisable to have two or more lines is when it is desired to compare two or more results.

CHART II.

——-- NEGROES (NATIVE AFRICANS), CENTRAL AFRICA.—714 NATIVE CHILDREN UNDER 15, AND NUMEROUS ADULTS.



The different methods of determining the endemic index of malaria are conveniently rendered graphically and serve as illustrations of the method. Chart I. is compiled from an official report of W. H. G. H. Best, of the Lagos Medical Service, and formerly of the London School of Tropical Medicine, which is the earliest report published that gives sufficient details for the determination of the age incidence.

No cases are given under 3 months of age, and those under 6 months are very few. The chart shows clearly that under the conditions of a native life a large proportion of children are infected in less than six months, and practically all in less than a year, whilst the number

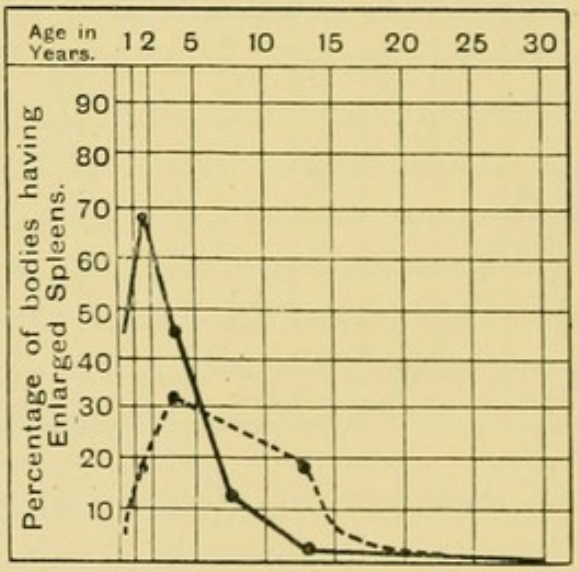
of infected children after five years is so small that the majority must have acquired immunity.

Chart II. shows the age incidence of enlarged spleens in Central Africa, and on Chart III. are shown the same cases subdivided into two widely different groups; in the one district Europeans often pass their first year without getting malaria, whilst in the other few escape for more than a few weeks. The earliest age incidence of enlarged spleen and the earlier period at which it ceases to be common in the more malarial districts are well shown.

CHART III.

——— Negroes (Native Africans), in a most Malarial District in Central Africa. Residence required for Probable Infection with Malaria, under Six Weeks.

------ Native African, in less Malarial District. Residence for One Year does not render Infection Certain.



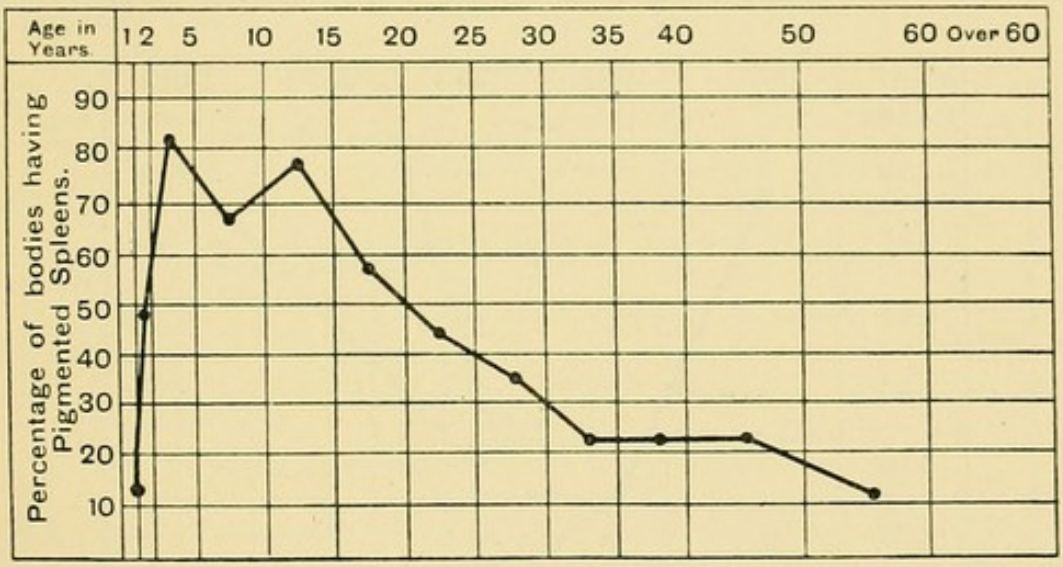
The weakness of the spleen test is that a considerable proportion even of untreated cases of malaria do not show marked splenic enlargement, and it is probable that 68 per cent. of enlarged spleens indicates universal infection as much as 90 per cent. harbouring parasites would do.

Chart IV. indicates the proportion of persons at different ages with malarial pigment in the spleen in a moderately malarial country. Two years' exposure was required for probable infection. It shows malarial infection later, and less complete immunity.

The liability to malarial infection as determined by the first method, the length of residence required for probable infection, would be simply charted for different districts by representing for each place in the vertical lines the number of months requisite for the infection of three-fourths of susceptible newcomers, or the percentage of persons who would be infected in a period of six months or a year, as is considered to be most convenient.

CHART IV.

—— NEGROES (NATIVE AFRICANS). COMPILED FROM POST-MORTEM EXAMINATIONS IN BRITISH GUIANA.



The line commences at one month, no pigmentation being found earlier. The next point is "under six months."

In some charts the distances on the horizontal line have no meaning, and it is simply for convenience that the horizontal spacings are made; a series of vertical columns packed together or widely and irregularly separated would have the same meaning but cause confusion.

The convenience of such charts is that various points can be indicated on the same chart and compared.

Graphic Representations of Effect of Prophylactic Measures. —The success that has attended prophylactic measures in many places has been marked. Some of the observations are imperfect, and in many of them several methods have been employed and have been associated with

improved treatment of the disease, and therefore the actual results do not depend entirely on a reduction of the endemic index.

Two illustrations of the results obtained are selected for charting and criticism.

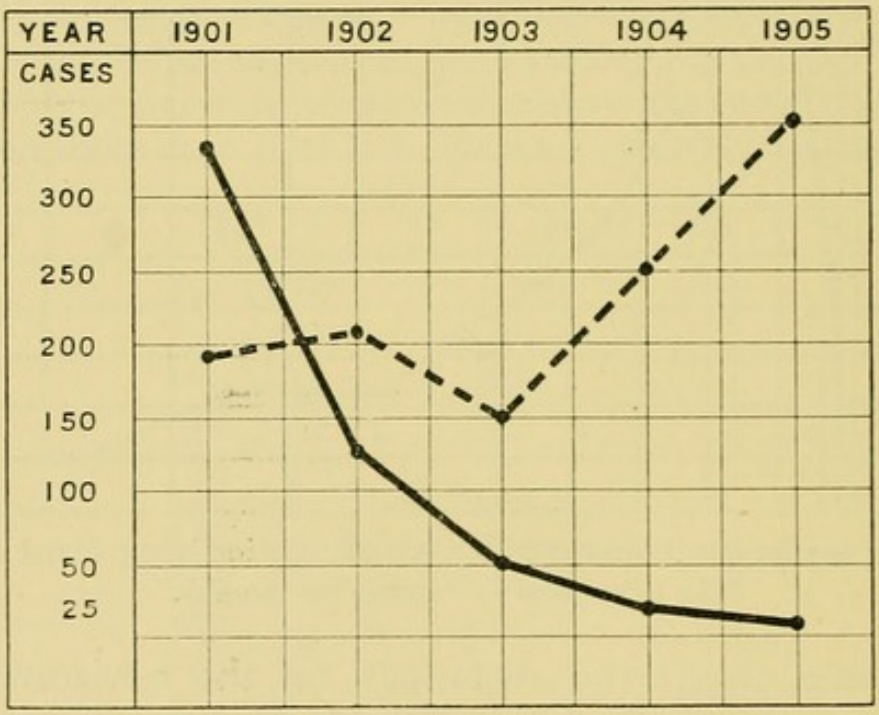
One is that recorded by Travers and Watson for the town of Klang in the Malay Peninsula. The figures

CHART V.

COMPILED FROM REPORTS BY TRAVERS AND WATSON.—*Journal of Tropical Medicine*, JULY 3, 1906.

—— Indicates number of cases admitted with malaria from Klang.

- - - - - - Ditto from surrounding districts, where no measures had been taken.



In 1901, 176 Government officials had a total of sick leave amounting to 1,026 days, whilst in 1904, 281 had only 71 days' sick leave.

reported are the admissions to hospital for malaria verified by blood examination, and the deaths attributed to malaria, in both, for a series of years.

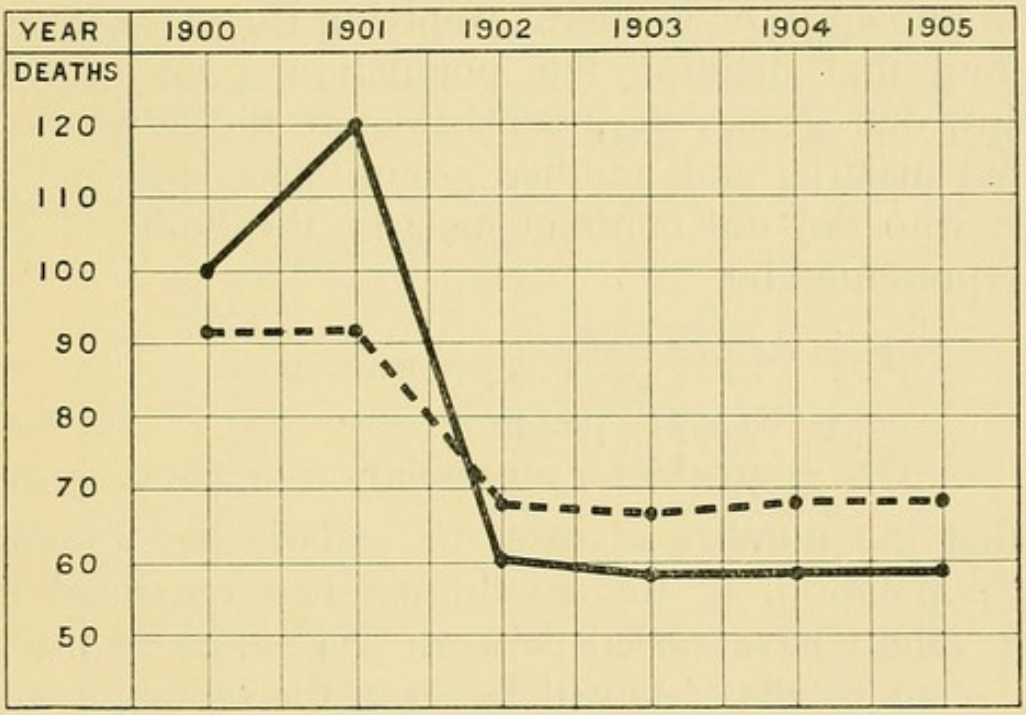
The population is known to have increased, but as the amount of increase is not known it is taken as stationary, and to that extent the results appear rather less striking than they really are.

The measures adopted were to intercept by drains, running across the base of a hill behind the town, the

water that would otherwise have joined the town subsoil water, and by the provision of numerous deep drains to lower the level of the subsoil water in the town. Later hollows were filled in. The effect has been to diminish the number of certain species of *Anophelinæ;* species such as *Myzomyia rossi* are still very abundant, but these are not efficient carriers of malaria.

CHART VI.

—— Deaths from malaria in the drained area.
- - - - - - Deaths certified as from other diseases.



During the same period in the surrounding undrained districts the death-rate certified as from fever increased from 173 in 1900, to 351 in 1905, and from other diseases from 133, to 271 in 1905.

These works were commenced in 1901 and continued since, the greater part being supplied early in 1902, and were carried out by the Government in accordance with recommendations made by a Committee composed of three medical men and three engineers.

The main results are indicated in the charts (V. and VI.). As a control the surrounding district where no anti-malarial measures were adopted is used. It will be seen that whilst in the towns both the number of cases of malaria and of the deaths was greatly reduced, though the

population had increased, in the surrounding districts there was an increase in both these items. The increase probably was due to the increase in the population.

One striking feature is that the deaths from other causes are diminished as well as those from malaria, indicating the indirect influence of malaria on the prevalence and severity of other diseases. This is shown in Chart VI.

The numbers are considerable, as the population is over 4,000, and the cases of malaria were 334 in 1901, and average twenty-nine from 1903 to 1904 with a population known to be greater. Applying Poisson's formula, we find that dividing the population 4,000 into two groups, one group, 334, consisting of those who contracted malaria, and another group, 3,666 consisting of those who did not contract malaria, the limits of error are represented by

$$\pm 2 \sqrt{\frac{2 \times 334 \times 3{,}666}{4{,}000 \times 4{,}000 \times 400}} \text{ per unit.}$$

$$= \pm \cdot 0123715 \text{ per unit;}$$

Or, $\pm$ 49·486 for a population of 4,000.

So that the number of cases of malaria per annum in this population, if the conditions had remained constant, might have varied between $334 + 49 = 383$, and $334 - 49 = 285$. As will be seen, the reduction to 29 in 1903 is manifestly outside the limits of mathematical error, and indicates a marked improvement in the conditions by which malaria was spread in this district.

Any error therefore, if it existed, is one due to errors of observations, and the "control" is a sufficient check on this.

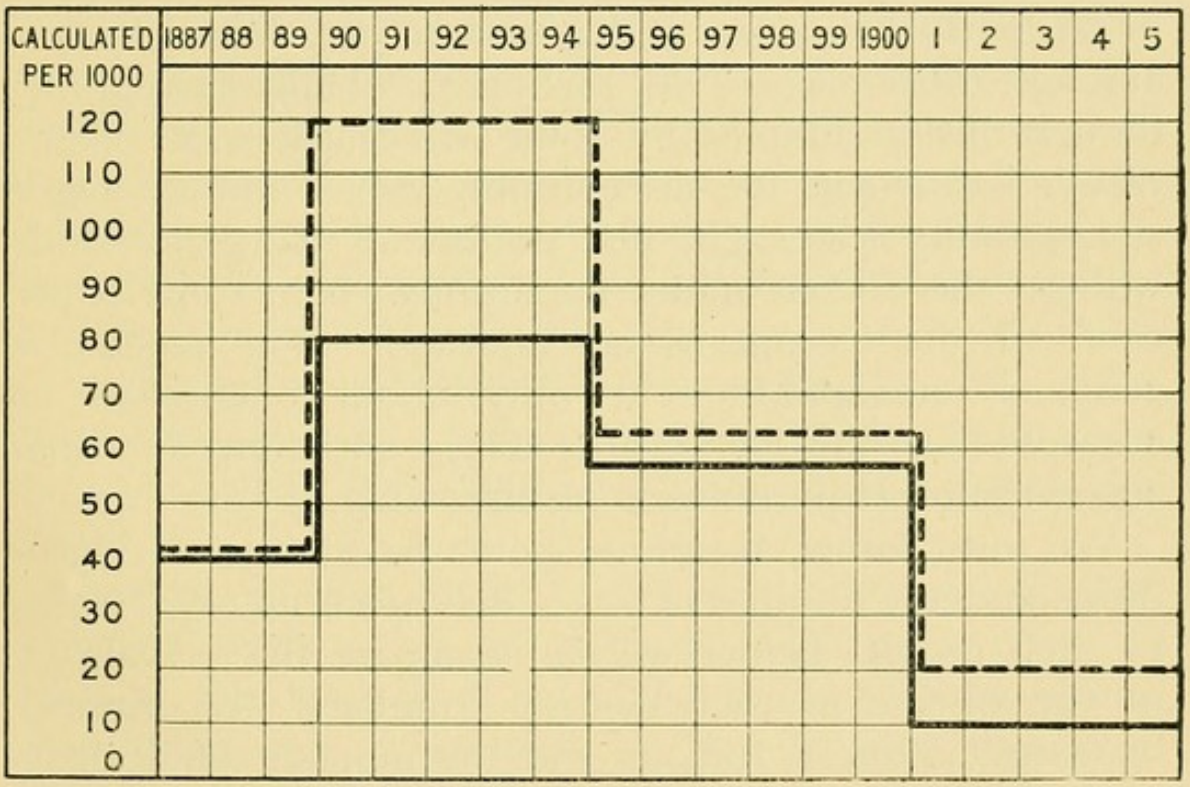
Chart VII. indicates the incidence of disease in a series of Mission stations in Central Africa for a period of years. From 1900 onward various anti-malarial measures have been taken. These measures varied in different stations, and were carefully planned by the medical officer, Dr. Howard, in accordance with the local conditions. As charted the effects are most striking, as the proportion invalided or who died during the five

years when these measures were practised was less than quarter of that in the preceding five years. The actual numbers are very small, as in the second period there were only an average of 24·4 workers and a total of 122 in the five years. Of these 7 died and 8 were invalided = a loss of 15; in 1901 to 1905 there were 182, of these 2 died and 4 were invalided = a loss of 6.

CHART VII.

COMPILED FROM REPORT BY HOWARD, PUBLISHED LONDON SCHOOL OF TROPICAL MEDICINE, FEBRUARY, 1907.

—— Deaths per 1,000. ------ Number invalided per 1,000.



In this chart the figures are so small that fluctuations from year to year are considerable, and the result is better shown by averaging for periods of five years. The value of the evidence is considerable, but the small figures available reduce this value to a great extent.

If we apply Poisson's formula to these figures we find that the ratio in the first period involved a probable error of ·059552 per unit; in a population of 182 in a similar period the number of deaths might have been 21, or a minus quantity. The reduction to two therefore falls within the limits of mathematical error.

This result, therefore, though striking, must be taken

rather as an illustration than as a proof of the value of such methods.

Many statistics require correction before charting. Of these corrections some are obvious and easily made. The number of cases is useless unless the total number of the population that are susceptible to the disease is also known. In dealing with inhabitants of different races any difference in the susceptibility has to be noted and allowed for.

Blackwater fever is a good illustration in point, and so many erroneous statements are made in connection with it that it well serves as an illustration. It occurs in Tropical Africa, in India, and in the West Indies, amongst other places. All races are probably susceptible, though this is doubted by some as regards some negro races : in any case the susceptibility varies, and the negro susceptibility is so slight that not one in many thousands will get the disease under conditions where some 8 per cent. of the Europeans are attacked. The Indian is certainly susceptible, but only about one-fourth as susceptible as the European. These variations in racial susceptibility require much further study.

We must know, therefore, both the number of Europeans and the number of cases that occur amongst them in each district before we can compare the prevalence of the disease in each district. Similarly the number of cases amongst Indians and the number of Indians must be known, and the proportion in the two races must be kept distinct.

If this is done it will be found as a general rule that in the most malarial district in Africa the prevalence of blackwater fever is the greatest, though the actual number of cases seen may be no more than in a more thickly populated but less malarial district. It will also be found that it is only in Africa that *a large proportion* of susceptible persons are attacked. In other countries, where perhaps as many *cases* may be seen, the number of susceptible persons is much larger.

Another correction, an important one, has to be made. Unfortunately the amount of the correction is dependent on a variable factor—the period of incubation of the disease. More cases of blackwater fever probably *occur* in England than in any one district in Africa, but these cases are all in people who have returned from Africa and acquired the infection there. In these cases there is no doubt that the infection should be attributed to the part of Africa from which they came. Here the matter is easy, but in Africa itself it is so often found that persons develop the disease who have been travelling, that it is a matter of great difficulty to attribute the disease to the correct place of origin. In many cases the place where the disease develops is certainly not the place where it was acquired. The correction to be applied here is essential, but can only be an approximate and arbitrary one in the present state of our knowledge, as the period of "incubation" is unknown, and probably variable. It is better to take the place of residence a fortnight before the attack as the more probable place to be implicated in a large proportion of the cases than the place where the symptoms of the disease manifested themselves.

Charting is often useful to represent the secretion or excretion rates either of definite substances, such as urea, or the volume of a mixed fluid, such as urine. Here times are represented by the distance measured horizontally, and amounts, weights, or volumes by the height measured vertically.

The only difficulty is that however it may be secreted urine as well as other fluids are only passed at intervals, and it is the rate at which urine is being formed, not that at which it is being passed, that is of importance. The only available method is to divide the number of ounces of urine passed, or, if necessary, drawn off by catheter, by the intervals measured in hours between the successive micturitions; the result will give the average rate per hour, assuming that the bladder is equally empty after each micturition.

Such charts are of special value in diseases like blackwater fever and yellow fever, in which there is a tendency to suppression of urine, and may indicate the periods of greatest danger.

The geographical and topographical distribution of disease, of parasites and of certain insects is of considerable importance. Maps should be drawn to the required scale and the places where an examination shows that the condition to be charted is present marked with a plus, +, and where absent negative, —. Places where no observations have been made should be clearly indicated, for if, as is sometimes done, they are represented as negative, most misleading conclusions as to the distribution of the disease will be drawn.

It is usual to represent the incidence of a condition by shading, and the depth of the shading indicates also the prevalance of the condition.

In determining the incidence of a disease in a town or village a plan must be drawn up and the houses or groups of houses infected indicated as above.

Extraneous conditions, such as wells, streams or other sources of water, must be shown, and when dealing with a question such as malaria, known to be carried in a certain way, other conditions favouring the prevalence of such carriers as *Anophelinæ* must also be indicated.

These maps and charts enable the conditions to be quickly understood, and are therefore of considerable value if accurate and carefully drawn up.

With a little ingenuity almost anything can be represented in a graphic manner, or charted. The value of a chart is the ease with which relations are shown and from which conclusions can be deduced. They show no more and prove no more than the figures or facts they represent, but are more easily followed by many. If, therefore, a chart does not represent matters more clearly than the figures the chart is useless.

APPENDIX.

Average Weights of Organs in Ounces.

	Brain	Heart	Lungs	Spleen	Liver	Kidney
Europeans (Quain) ...	49·5	11	45	6	53	11
Negroes (British Guiana)	42·04	10·8	23·7	6·9	47·7	9·9
Indians	41·9	8·7	27·2	18·4	48·4	8·9
Chinese	47·3	9	26·1	14·8	43·4	8·7

Variation in the Weights of Lungs with the Time after Death.

Examination made after death	Average weights	No. of cases in which the lungs weighed 20 ozs. or less	21 ozs. to 30 ozs.	31 ozs. to 40 ozs.	41 ozs. and over
Within 3 hours ...	18	71	21	1	—
,, 4 ,, ...	23	9	25	4	1
,, 6 ,, ...	27	11	32	26	3
,, 12 ,, ...	29·1	4	37	37	6
,, 18 ,, ...	34·8	3	47	52	35
Over 18 ,, ...	44·1	—	3	9	33

Average Weights of Brain in Negroes and Indians at Different Ages.

Ages:	16 to 20	21 to 30	31 to 40	41 to 50	51 and over
Negroes...	44·3	46	45·4	43	41·5
Indians ...	40	41·2	41·3	40·8	40·5

Proportion of the Spleens in Indians and Negroes Weighing 15 ozs. and Over in British Guiana.

Ages	Indians	Negroes
20 to 25 ...	30 per cent.	32 per cent.
26 to 45 ...	52 ,,	16 ,,
Over 45 ...	39 ,,	15 ,,

Preservation and Examination of Worms.

Small Nematodes (up to the size of and including Ankylostomes).

Preservation.

(1) Shake up the live worms in a 1 per cent. salt solution, to remove mucus, &c.

(2) Kill by dropping into boiling 70 per cent. spirit and allow to cool.

(3) Transfer to fresh 70 per cent. spirit for storage.

Clearing and mounting same.

(1) After treating with stages 1 and 2 as above, transfer to a

mixture composed of 70 per cent. spirit 95 parts with 5 parts of pure glycerine.

(2) Evaporate on a water or paraffin bath until all the alcohol has gone.

(3) Mount in glycerine jelly.

(4) Ring with gold size.

Examination without mounting.

(1) Take from the 70 per cent. spirit and place in methylated spirit.

(2) Transfer to colourless coal tar creasote, allow to clear, and examine in that fluid.

After the examination is concluded pass through methylated spirit back to 70 per cent. spirit to store.

Large Nematodes are treated in the same way as small ones, except that they cannot be cleared and mounted by the glycerine method.

Small Trematodes.

Preservation.

(1) Place alive in a test tube one-third full of 1 per cent. saline, and shake vigorously.

(2) Add to the test tube rapidly an equal quantity of saturated solution of sublimate.

(3) Shake vigorously for three minutes.

(4) Transfer to 70 per cent. spirit to store.

Examination without mounting. As for small nematodes.

To make stained and mounted specimens.

(1) Transfer from 70 per cent. spirit to a 1 per cent. solution of alum to which is added a little hæmatoxylin, until the whole is of a light claret colour. The hæmalum solution (page 52) diluted with distilled water gives good results, and so does a weak solution of carmine, but in that case longer staining is required. Leave in this one to four days.

(2) Decolourise slightly with $\frac{1}{2}$ per cent. acid water.

(3) Wash well.

(4) Dehydrate with spirit and oil of cloves.

(5) Pass through xylol and mount in balsam.

Large Trematodes.

Preservation.

(1) Drop alive into 1 per cent. saline and shake vigorously

(2) Add formalin (commercial) to this to make about a 10 per cent. solution, and shake vigorously till death occurs.

The specimens can be kept in 10 per cent. formalin.

Examination.

This is best done by embedding and cutting sections.

To mount specimens whole.

(1) Press between two slides whilst alive, and drop into 70 per cent. spirit.

(2) Stain and clear as in small specimens.

Cestodes.

Preservation.

(1) Shake gently in 1 per cent. saline.

(2) Add formalin to this to make a 10 per cent. solution and shake gently till they die.

The specimens can then be stored in 10 per cent. formalin.

To stain and mount segments.

(1) Place alive in weak glycerine faintly coloured with carmine and leave till stained, or dilute hæmalum may be used.

(2) Press between two slides and drop into methylated spirit. They should remain in this for twenty-four hours.

(3) Remove the pressure and place the segments in fresh methylated spirit for an hour or so.

(4) Clear in oil of cloves.

(5) Pass through xylol and mount in balsam.

(Creasote may be used to clear instead of oil of cloves.)

VARIOUS STAINING METHODS.

A.—*A method for Staining Gregarines.*

(1) Make a film on a coverslip and keep it moist.

(2) Whilst still wet drop the coverslip face downwards on to the fixing solution consisting of two parts of saturated sublimate solution and one part of absolute alcohol. Leave it to fix for fifteen minutes.

(3) Wash in 70 per cent alcohol.

(4) Place in 70 per cent. alcohol to which a few drops of Gram's iodine solution have been added, so that the colour of the mixture is like that of weak tea. Leave in this ten minutes.

(5) Place in 70 per cent. alcohol to which have been added a few drops of Delafield's hæmatoxylin and leave for twenty-four hours or longer as required.

(6) Wash in 70 per cent. alcohol.

(7) Wash in methylated spirit.

(8) Remove spirit with xylol and mount.

B.—*Levaditi's Method of Staining Spirochætes in Tissues.*

(1) Pieces of the tissue 1 mm. thick are fixed in 10 per cent. formalin for twenty-four hours.

(2) Wash and harden in 96 per cent. alcohol for twenty-four hours.

(3) Wash in distilled water till tissue sinks.

(4) Place in silver nitrate solution (1·5 per cent.) for three to five days at 37° C.

(5) Wash rapidly and place at room temperature for twenty-four to forty-eight hours in :—

Ac. pyrogallic	2 to 4 gr.
Formol	5 c.c.
Aq. destillata	100 c.c.

(6) Wash in distilled water and pass through absolute alcohol and xylol.

(7) Embed in paraffin and cut sections.

C.—*Staining of Amœbæ Cysts.*

(1) Make a film on a coverslip and do not let it dry.

(2) Whilst still wet drop it face downwards on to the fixing solution :—

Saturated solution of sublimate	2 parts.
Absolute alcohol	1 part.

Leave it to fix for fifteen minutes.

(3) Take out of the fixing solution and place in 40 per cent. alcohol for ten minutes.

(4) Place in 70 per cent. alcohol to which a few drops of Gram's iodine solution have been added. Leave in this ten minutes.

(5) Place in methylated spirit for ten minutes.

(6) Place in 70 per cent. alcohol for five minutes.

(7) Place in 40 per cent. alcohol for five minutes.

(8) Place in water for an indefinite time.

(9) Place in iron-alum solution ($2\frac{1}{2}$ per cent.) for two to three hours.

(10) Rinse lightly in water.

(11) Stain for two to three hours in hæmatoxylin solution made as follows :—

Hæmatoxylin crystals	1 grm.
Absolute alcohol	10 c.c.
Distilled water	90 ,,

This solution should be kept for a month to ripen. Then add another 100 c.c. of water.

(12) Wash well in water.

(13) Differentiate in iron-alum solution (·25 per cent.). This is best done in a watch glass with just enough solution to cover the underneath surface of the film. The process can be watched under the microscope with a $\frac{1}{6}$ objective.

(14) Dehydrate in spirit of gradually increasing strength : 40, 70, 90 per cent., and absolute.

(15) Pass through xylol and mount in Canada balsam.

D.—*Antiformin Method for Tubercle.*

Solution A.—Liquor sodæ chlorinatæ (Sodæ carbonas, 600 parts ; bleaching powder, 400 parts ; water, 1,000 parts).

Solution B.—Caustic soda, 15 per cent. solution.

To make antiformin take equal parts of each.

Method.

(1) Take 5 c.c. of sputum and 5 c.c. of a 25 per cent. solution of antiformin and mix in a test tube. Allow to stand for twelve hours.

(2) Pour off the supernatant liquid and wash with saline. Collect deposit by centrifuging.

(3) Wash again in saline, pour off excess of fluid after centrifuging and place deposit on a slide previously albuminised or having some of the original sputum on it.

(4) Allow to dry in the air, fix with heat and stain with Ziehl-Neelson and methylene-blue as for tubercle.

The antiformin destroys all bacteria except the acid-fast ones, and the advantage of the method is that it allows a considerable amount of the sputum to be examined.

Spinal Puncture.

Place the patient on his right side with the knees well drawn up. The tips of the fingers of the left hand are then placed upon the left iliac crest, when the thumb will indicate the site of puncture. This is at a point between the fourth and fifth lumbar vertebræ and lies half an inch to the left of the middle of a line joining the two iliac crests. Insert a stout hypodermic needle for one or two inches until it is felt free in the canal. No syringe should be used to effect suction, but the fluid should be allowed to escape naturally through the needle.

In health the cerebro-spinal fluid is of a pale clear colour and contains very few or no cells. It escapes from the end of the needle drop by drop, whereas in cerebro-spinal meningitis and cerebral tumours it usually spurts out.

If meningitis is present the fluid is turbid and contains a large number of leucocytes, lymphocytes predominating if it is tuberculous, whilst the polymorphonuclears are in excess in other forms of meningitis.

In normal cerebro-spinal fluid there is a considerable amount of sugar, whereas in meningitis this is lost or reduced to a mere trace.

Arrow Poisons.

(1) *Tetanus.*—The carcase of a dead animal is lightly buried under alternate layers of earth and leaves. After some time has elapsed the carcase is uncovered and the points of the arrows are dipped in the decomposing body. This poison is the chief one in use among the natives of the South Pacific Islands.

(2) *Antiaris toxicaria*, or Upas tree.—The inspissated juice is used often in combination with strychnos, chiefly in the Malay Archipelago. The poison is harmless if taken by the mouth, but when injected causes violent intestinal peristalsis and early death.

(3) *Curare.*—A decoction of *Malonetia nitida* used chiefly in South America. It paralyses the peripheral ends of the motor nerves in the voluntary muscles.

(4) *Strophanthus.*—Is occasionally used by natives in Africa.

(5) *Strychnos Tieute.*—A decoction of the bark mixed with *Antiaris toxicaria* is used by natives in the Malay States. The toxic agent is brucia.

Instruments and Reagents.

Microscope, with two eye-pieces, 2 and 4; three objectives, $\frac{2}{3}$ in., $\frac{1}{6}$ in., and $\frac{1}{12}$ in. oil immersion lens; substage condenser, and iris diaphragm and mechanical stage, micrometer eye-piece with scales or with squares, micromillimetre scale, camera lucida.

Watchmaker's glass.

Portable microscope.

Direct vision spectroscope.

Slides, No. 2 quality.

Cover-glasses, No. 1 quality, to be packed in oil.

Needles in handles. Cork felt. Entomological pins, No. 20. Forceps. Cornet's forceps. Mounted platinum wires.

Test tubes, thick and best quality. Durham's tubes. Watch glasses. Petri dishes. Photographic trays, half- and full-plate. Erlenmeyer's flasks. Funnels. Glass tubing. Glass rod. Beakers. Burette, 50 c.c. Evaporating dishes and copper dish for boiling slides. Spirit Bunsen. Primus kerosene lamp.

Glass measures, 500 c.c., 100 c.c., and 10 c.c.

Scales. Gramme weights.

Paraffin oven. Paraffin moulding dish and blocks.

Microtome.

Steam steriliser. Hot-air steriliser and incubator. Iron enamelled jugs.

Mounting and Embedding Reagents.—Alcohol, cotton wool, methylated spirits, oil of cloves, xylol, Canada balsam, glycerine, Farrant's solution, glycerine jelly, ether, chloroform, celloidin, paraffin wax, Hollis's glue, or shellac, acetone, aniline oil, creasote.

Stains.—Hæmatoxylin crystals, hæmatein, methylene-blue (Höchst's pure medicinal), thionin, gentian violet, fuchsin, carmine, picrocarmine, toluidine blue, night blue, Bismarck brown, methyl violet, acid fuchsine, eosine, both soluble in alcohol and soluble in water. Gram's stain made up. Leishman's stain. Eosin azur (Burroughs Wellcome's 'tabloids'). N.B.—Grübler's stains are the best.

Other Reagents.—Acids: Hydrochloric, nitric, sulphuric, picric, osmic, tannic, carbolic (pure), gallic, sulphanilic, pyrogallic. Agar-agar, alum, bleaching powder, ammonia, alcohol, methyl alcohol (pure for analysis), borax, creasote, iodine, filter paper, filter paper (Chardin), formalin, gelatine, glucose, sucrose, mannitol, lactose, lithium carbonate, lysol, mercuric chloride, naphthaline, peptone, platinum chloride, potassium ferrocyanide, potassium iodide, potassium bichromate, phenolphthalein, silver nitrate, sodium carbonate, sodium citrate, sodium hydrate, sodium sulphate, sodium taurocholate, sodium nitrite. Bovril.

PLATE IV.

STAINED WITH HÆMATOXYLIN OR EOSINE AND HÆMATOXYLIN

Figs.

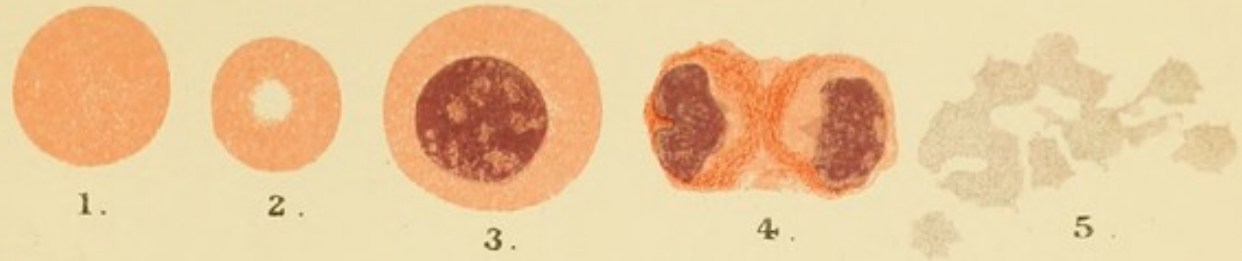
1, 2. Normal variations in red blood corpuscles.
3, 4. Nucleated red blood corpuscles.
5. Blood plates.
6, 7. Abnormal variation in size and colour.
8. Abnormal shapes, poikilocytes.
9. Basophilic granules.

MALIGNANT TERTIAN PARASITES (SUB-TERTIAN) STAINED WITH CARBOL THIONIN.

Figs.

10. Young form, rings.
11. Half-grown parasite.
12. Full-grown parasite.
14. Sporulating parasite.
13 & 15. Are full and sporulating parasites, as seen in sections of organs shrunk by the spirit and other processes.
16 to 19. Development of the gametes of malignant tertian.
20, 21. Benign tertian parasites, half-grown and sporulating.
22, 23. Quartan parasites, half-grown and sporulating.
24 to 27. Sporozoa of cattle and horses.

Plate IV.



1. 2. 3. 4. 5.

6. 7. 8. 9.

10. 11. 12. 13. 14. 15.

16. 17. 18. 19.

20. 21. 22. 23.

24. 25. 26. 27.

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PLATE V.

STAINED WITH LEISHMAN'S STAIN.

Figs.

1. Normal red corpuscle.
2. Blood plates.
3. Lymphocyte.
4. Large mononuclear leucocyte.
5. Polymorphonuclear leucocyte.
6. Eosinophile leucocyte.
7. Mast cell.
8. Transitional form.
9. Abnormal mononuclear cell found in certain diseases, including *trypanosomiasis*.

10 to 13. Myelocytes showing various types of granules.

14, 15. Halteridium.

16. Small drepanidium in red corpuscle.
17. Same drepanidium in plasma.

18, 19, 20. Large drepanidium in various stages.

21. Degeneration of red corpuscle caused by drepanidium (Schüffner's dots).

Plate V.

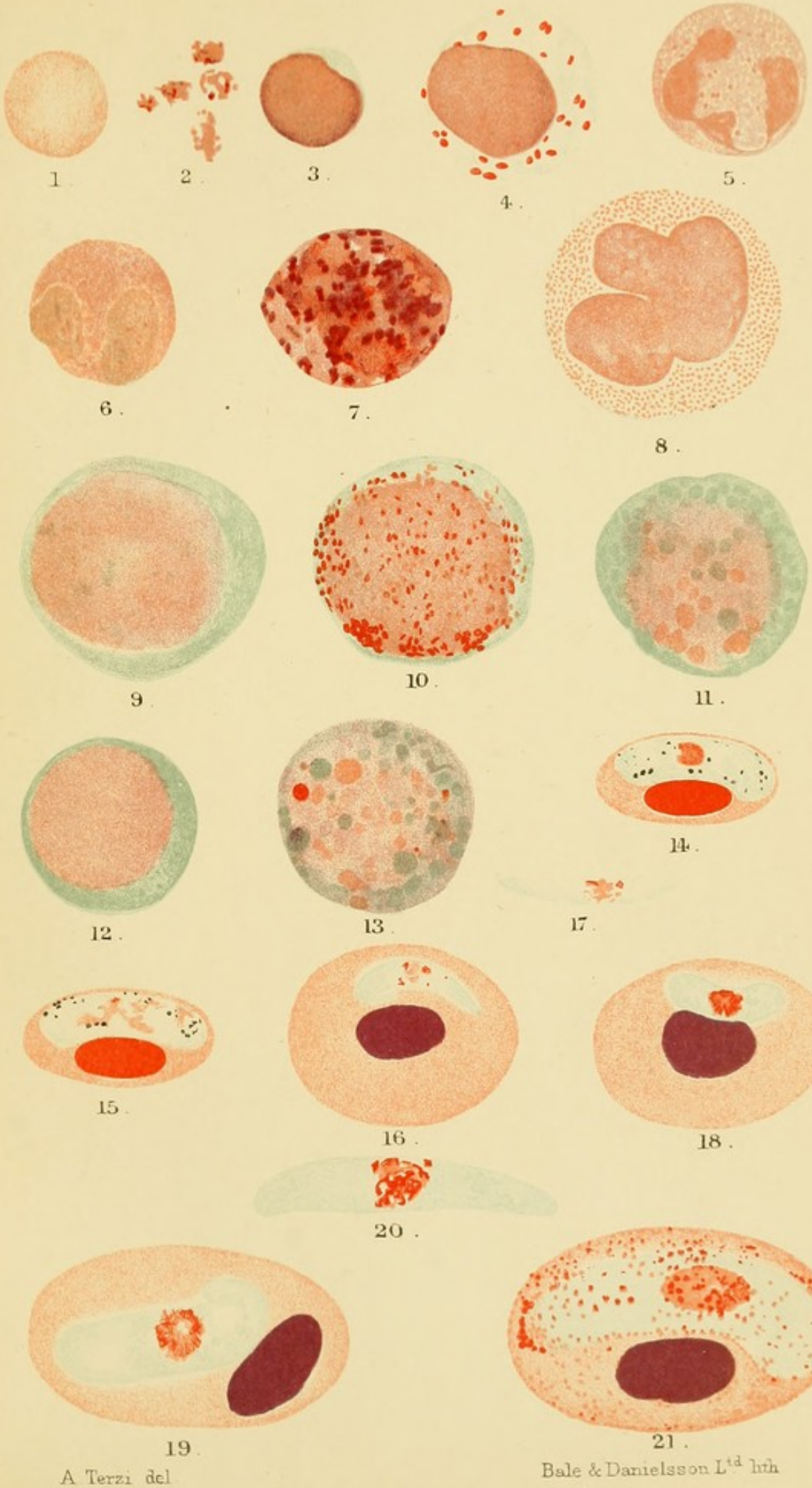

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PLATE VI.

STAINED WITH LEISHMAN'S STAIN.

Figs.

1 to 5. Stages of benign tertian parasite.

6. Gamete benign tertian.

7, 8, 9. Characteristic degeneration of red corpuscles containing benign tertian parasites (Schüffner's dots).

10 to 15. Stages of quartan parasite.

16, 17. Stages of malignant tertian (sub-tertian) which are seen in peripheral blood.

18. Male gamete, malignant tertian (sub-tertian).

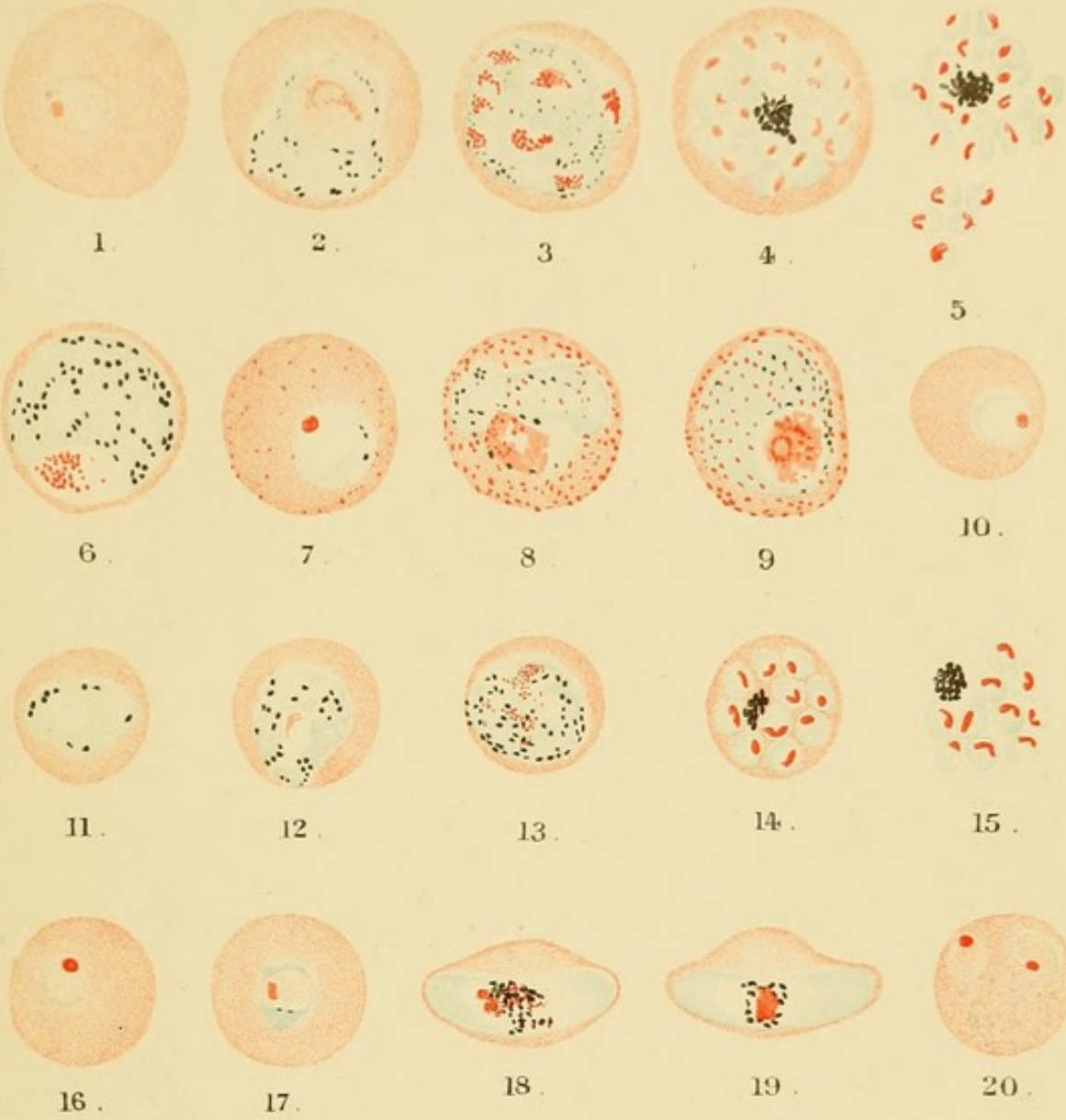
19. Female gamete, malignant tertian (sub-tertian).

20. Double infection with malignant tertian (sub-tertian) parasites of a red corpuscle ; Maurer's granules in red corpuscle.

21. Spirillum of relapsing fever (stained with carbol fuchsin).

22, 23. *Amœba coli.*

Plate VI.



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