

The bacteriology of every-day practice / [J. Odery Symes].

Contributors

Symes, J. Odery.

Publication/Creation

London : Baillière, Tindall and Cox, 1904.

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No. II

THE BACTERIOLOGY OF
EVERY-DAY PRACTICE

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THE BACTERIOLOGY OF EVERY-DAY PRACTICE

BY

J. ODERY SYMES

M.D. (STATE MEDICINE) LOND., D.P.H., ETC.

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SECOND



EDITION

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BAILLIÈRE, TINDALL AND COX
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PREFACE TO SECOND EDITION.

THE present edition has been largely rewritten, and sections have been added upon the following subjects: the preparation and staining of blood films; meningitis; trypanosomiasis; influenza, and diseases of the alimentary system. The new illustrations introduced are taken from my article on Surgical Bacteriology in Carwardine's 'Operative and Practical Surgery,' and I am indebted to Mr. Carwardine for the loan of the blocks.

J. O. S.

II, RICHMOND HILL,
CLIFTON, 1904.

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INTRODUCTORY.

THE increasing importance of the part played by bacteriology in practical medicine renders it absolutely essential that both practitioner and student should be acquainted with the elementary methods of bacteriological investigation. The notes on which the present monograph is based were originally compiled for the use of clinical clerks in their ward work, with a view (1) to point out in what cases bacteriological examination might help in clinical diagnosis; (2) to describe methods of securing and identifying microscopic specimens such as the student could himself prepare; (3) to give directions for taking cultures and for preserving tissues to be sent to the laboratory. Further experience has led to the belief that there exists amongst general practitioners a need for information of a similar kind, and it is to meet that want that the writer's notes have been embodied in book form.

The value of a bacteriological examination, both from the point of diagnosis and from that of treatment, is too obvious to need more than passing mention. That it is not more often employed is due partly to a lack of knowledge as to the cases in which it is likely to prove useful, and partly to the want of the appliances needful for the work. Of late years, however, there have sprung up in all parts of the country public and private laboratories to which morbid products may be sent for examination, and

in this way the facilities for such investigations have been greatly increased. Whilst there are many cases in which, by the aid of a few staining reagents and simple instruments, the medical man may establish the diagnosis for himself, yet there are others which necessitate his calling in expert aid. In the following pages, therefore, not only have the microscopic methods of identification been dealt with, but also the ways in which materials should be preserved for examination, tubes of nutrient media inoculated, and materials forwarded to the examining depot. No attempt will be made to give minute descriptions of bacteria or their methods of growth; for such the reader is referred to the ordinary text-books. Especial care has been taken throughout to emphasize the practical bearing of the bacteriological report upon the all-important questions of diagnosis, prognosis, and treatment. The increasing attention now being paid to serum therapeutics renders the necessity for a bacteriological confirmation of diagnosis daily more apparent.

Bacteria belong to the order of the splitting fungi or Schizomycetes. They are unicellular protoplasmic bodies averaging about $\frac{1}{25000}$ inch in breadth, but whose length may be seven or ten times greater. They show considerable variety of shape. Thus, they may be rounded (cocci), long, straight, rod-like bodies (bacilli), short rods (bacteria), or curved spiral filaments (spirilla). Many bacteria have long, cilia-like processes called flagella, and are capable of movement.

Multiplication takes place, as a rule, by simple fission, the organism dividing into two parts, each of which becomes a separate individual. Under certain circumstances some bacteria have the power of altering their condition, and of entering upon a quiescent and resting stage known as that of spore formation. Spores have

high resisting powers to heat and cold. Ultimately they may return to the form of the original organism, whose existence may thus be prolonged over a season of adverse circumstances.

The bacteria associated with disease derive their nourishment from organic materials, such as the fluids and tissues of the body. Artificially, they are generally cultivated on coagulated blood serum, or on peptone broth, to which, in order that a transparent, solid medium may be obtained, gelatine or agar has been added. As agar does not melt below 98° C., organisms may be grown in this substance at a higher temperature than can be used with gelatine. Organisms which require for their growth free oxygen are termed *aerobic*, and those which do not grow in the presence of free oxygen *anaerobic*. Microbes deriving their food-supply from living tissues are called *parasites*, whilst those having the power of living on dead matter are called *saprophytes*. An organism may be parasitic at one period of its existence and saprophytic at another. The terms *pathogenic* and *non-pathogenic* are applied to micro-organisms according as to whether they are or are not capable of exciting disease in man and the lower animals. The pathogenic properties of bacteria vary between wide limits, being at one time intense (in which case we speak of the organism as being virulent), and at another hardly perceptible (avirulent or attenuated).

Most pathogenic germs grow well at body temperature (37° C.); their growth is arrested by freezing, but they are not killed. Prolonged boiling, on the other hand, is fatal both to bacteria and their spores. Direct sunlight and lack of moisture are prejudicial to the life and growth of germs. Some pathogenic germs can exist and multiply in the soil for weeks, provided that there is a sufficient

quantity of organic matter and water present to provide the nourishment required. Thus typhoid bacilli may remain alive for months in organically polluted sites. Many varieties of micro-organisms have considerable resisting power to drying, and may thus be disseminated in the form of dust. Tubercle bacilli from dried sputa may be spread in this manner.

The causal relation that many micro-organisms bear to disease is now well established. The pathogenic microbe may be introduced by the alimentary tract with food or drink, as in enteric fever; by the respiratory tract, as in diphtheria or pneumonia; or by inoculation of the skin or mucous membranes, as in tetanus or anthrax. The immediate effect of the entrance of the micro-organism will depend on several factors, such as the number of organisms introduced, their virulence, and the power of resistance or susceptibility of the subject of infection. Either from paucity of numbers, from loss of virulence (attenuation), or from the natural resistance of the body tissues, the entrance of pathogenic bacteria into the body may be unattended with symptoms. The poisonous action of pathogenic bacteria is due to chemical bodies elaborated by them. The true nature of these bodies is not fully understood, and they have been variously styled toxins, enzymes, and proteoses. The growth and multiplication of the infecting bacteria may be to a great extent localized to the point of inoculation, as in diphtheria; and in this case the condition is one of *intoxication*, the toxins elaborated by the bacteria being absorbed into the general circulation, and exciting certain symptoms. On the other hand, the micro-organisms may at once enter the general circulation, lodge in distant organs, multiply and elaborate toxins in these organs, and set up a general *infection*, such as is seen in septicæmia.

THE BACTERIOLOGY OF EVERY-DAY PRACTICE.

CHAPTER I.

MATERIALS AND INSTRUMENTS.

UNDER the heading 'Materials and Instruments' we shall describe only such as are necessary for the preparation of cover-glass preparations or for the making of cultures. The practitioner has not, as a rule, either time or space to devote to more elaborate apparatus for the manufacture of media or the incubation of cultures. Particulars of microscopes, and simple directions for their proper working, will be found in the Appendix.

Cover-glasses should be of No. 1 quality, and are to be prepared by boiling for ten minutes in nitric acid; then washing in distilled water, and storing in absolute alcohol. Before use the cover-glass is either wiped dry with a clean piece of linen, or is passed once or twice through the flame to burn off the alcohol, and then polished. Square cover-slips are preferable to round ones. Glass slides should measure 3 inches by 1 inch.

They should be cleaned by washing in a little ammoniated water, and then rubbed with alcohol.

Platinum Wire Loop.

For inoculating tubes, spreading liquids on cover-glasses, or transferring small portions of material, platinum wires fused into glass rods are used. The wire of the most convenient size is No. 24 Birmingham

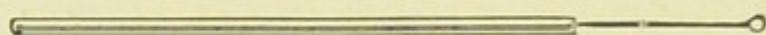


FIG. 1.

wire gauge. At least two such wires should be provided, about 3 inches in length, one having a loop turned at the end, and the other having the point beaten out into a flat spatula.

Cornet's Spring Forceps.

For holding cover-glasses it is convenient to have one or more pairs of spring forceps (Cornet's). The latter when placed upon the table hold the cover-glass in a

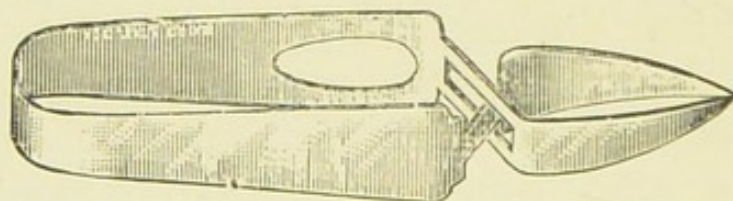


FIG. 2.

horizontal position, thus permitting the stain to be applied and to remain upon the surface of the film.

A syringe is often required for drawing off fluids. An exploring syringe or serum syringe may be used for this purpose, and should have a capacity of not less than 5 c.c. In order that every part may be capable of being boiled for sterilization, both the barrel and the piston rod should be made either of glass or metal.

Capillary Glass Pipette.

Glass pipettes can be manufactured from quill glass tubing. A piece of tubing 6 to 8 inches long is constricted in the centre by heating and drawing out. One end is then plugged with a little cotton-wool, and the other drawn out into a capillary point. The whole is sterilized by boiling or by heating in the flame of a spirit-lamp. The capillary point is inserted into the fluid to be examined, some of which is sucked up into the tube, when the cotton-wool prevents contamination of the mouth. The constriction in the centre is then broken through, and the ends sealed by heating in the flame.



FIG. 3.

Ordinary vaccine lymph tubes may be utilized as pipettes. They are first sterilized by heating in the flame, and when cool the fluid may be sucked up into them. If, however, there be a danger of infection in sucking up the fluid, the following procedure may be adopted. The open end of the capillary tube is placed in the fluid, and a certain amount rises into the tube. If this end of the tube be then brought near to the flame, care being taken not to heat the glass, the liquid is driven still further up the tube, and a second portion may be taken. This is repeated until the tube is nearly full. It is then sealed by fusing the ends, the fluid being preserved from excessive heating by covering it as far as possible with the fingers.

Diphtheria outfits are sent out by many of the research laboratories, or may be obtained from the larger chemists and dealers. The 'outfits' consist of a throat swab, a piece of cotton-wool twisted around the end of a metal

rod, the whole contained in a plugged test-tube which has been carefully sterilized.

Tubes of culture medium, serum, agar, or gelatine, can be purchased from firms dealing in bacteriological apparatus.

The most useful culture tubes to purchase are those of sterile, solidified blood serum. They should be bought in small quantities of not more than half a dozen at a time, as they are apt if kept for a lengthened period to become dry and useless. This, however, may be obviated by covering the mouth of the tubes with rubber caps or gutta-percha tissue.

The following are useful formulæ for stains :

1. Saturated watery solution of methylene blue.

2. Löffler's methylene blue—

Saturated alcoholic solution of methylene blue	...	...	30 c.c.
Solution of potassium hydrate (1 in 10,000)	...	...	100 c.c.

3. Carbol-gentian violet—

Saturated alcoholic solution of gentian violet	...	...	1 part.
5 per cent. solution of carbolic acid	...	...	10 parts.

4. Gram's solution—

Iodine	...	...	1 part.
Potassium iodide	...	...	2 parts.
Distilled water	...	...	300 parts.

5. Carbol-fuchsin (Ziehl-Neelsen)—

Basic fuchsin	...	...	1 part.
Absolute alcohol	...	...	10 parts.
Carbolic acid (1 in 20)	...	...	100 parts.

6. Jenner's combined eosin and methylene blue stain.

7. A 20 per cent. solution of nitric acid in water.

Grübler's stains are the best. They are conveniently kept as saturated alcoholic solutions.

All the above-mentioned stains are made in 'soloid' form by Burroughs Wellcome and Co. The 'soloid' stains enable solutions to be prepared in small quantities when required, and the risk of decomposition is thus avoided. Full directions for dissolving the stains are supplied with the 'soloids.'

Stains should be filtered before use. As they are not very stable bodies, it is better to make them up in small quantities from time to time. Watery solutions, it should be mentioned, may permit the growth of micro-organisms, and, as this might lead to errors in diagnosis, they should be examined occasionally by inoculating tubes from them. Stains are best kept in 1 oz. glass bottles fitted with droppers, such as are used for ophthalmic work. For the purpose of mounting specimens xylol balsam (Canada balsam dissolved in xylol) is preferable to Canada balsam, as it dissolves out the stains far less readily.

A spirit-lamp, watch-glasses, Swedish filter-papers, and small labels are other materials which may be required from time to time.

STERILIZATION AND DISINFECTION.

The two methods commonly employed are disinfection by heat, and by the use of chemical bodies. Of these, the first is by far the more trustworthy. It is now a well-established fact that the only efficient method of dealing with clothing, and with fabrics generally, is by treating them with steam under high or low pressure in apparatus especially provided for the purpose. Dry heat, having only slight penetrative power, is of little use for this purpose. Whilst bacilli and spores are killed by exposure

to steam for five minutes at 212° F., dry air only accomplishes the same in four hours at a temperature of 220° F.

The common practice of fumigating by sulphurous acid or formalin vapour, as applied to the disinfection of rooms, is, as a rule, a futile proceeding, for even if the gas be present in sufficient proportion to have a bactericidal action, it is found that the slightest protection is sufficient to preserve the organisms from the action of the fumigant. Rooms are best disinfected by spraying the floor, ceiling, and walls with some antiseptic solution, such as perchloride of mercury (1 in 1,000), the spray being applied by means of a special apparatus, such as the 'Equifex' spray.

It is not purposed here to discuss disinfection generally, but rather to indicate the necessary measures which must be taken to secure antisepsis in the course of bacteriological work for the purposes of clinical diagnosis.

The wires and forceps may be sterilized by heating in the flame to a dull red heat. Test-tubes, bottles, syringes, scalpels, all glass and earthenware materials, should be boiled for half an hour in water to which a little hydrochloric acid has been added. This is better than steeping them in antiseptic solutions, as traces of these chemical bodies may remain and vitiate future results.

All fragments and rubbish, pieces of morbid tissues, old swabs, cotton-wool plugs, used blotting-paper, etc., should be burnt. Used coverslips, slides, and glass apparatus should be immediately transferred to a bath of some antiseptic fluid, such as perchloride of mercury 1 in 500, and similar solutions should be used for disinfecting the hands after bacteriological work.

There is much misunderstanding as to what substances are true disinfectants, having definite bactericidal powers. Many of the solutions in popular favour are

from their nature or degree of dilution simply antiseptics which arrest the multiplication of micro-organisms, or deodorants which serve to mask effluvia. The following are useful solutions for working purposes, and if brought into intimate contact with the materials and allowed to act for some hours, may be trusted as disinfectants:

Carbolic acid, 5 per cent. solution in water.

Perchloride of mercury, 1 part in 1,000 of water, with 0.5 per cent. of hydrochloric acid.

Formalin, 1 per cent. solution in water.

Of these, formalin is to be preferred on account of its greater penetrative power.

In using disinfectants, the points to which attention must be paid are quantity, concentration, and duration of exposure. It is useless to attempt the disinfection of drains and sewers, for instance, by the addition of disinfecting powders or solutions, as these speedily become diluted and inert in the presence of the large quantity of fluid. Similarly, the administration of disinfectants, such as creosote, salicylic acid, carbolic acid, or the sulphocarbolates, by the mouth medicinally, as is frequently done in enteric fever, in gastro-intestinal or other disorders, can in no way render the tissues and tract aseptic, although to a limited extent they may act as antiseptics and check the undue multiplication of micro-organisms exciting fermentative changes. Certain pathogenic organisms, such as the *B. typhosus* and *B. coli*, can flourish in media containing as much as 0.05 per cent. of carbolic acid.

Disinfection of the Patient's Skin.

A portion of the skin may require to be sterilized before taking a specimen of the blood, preparatory to making an

incision to open an abscess, or before inserting an aspirating needle. It is first to be scrubbed with a nail-brush and soap and water. Potash soap is the best for this purpose. The clean surface is further defatted by rubbing with a piece of cotton-wool, soaked in ether or turpentine. It is then disinfected by the application of carbolic acid (1 in 20) or perchloride of mercury (1 in 500). If time permit, a pad, soaked in one or other of these solutions, is fixed over the spot and allowed to remain for three or four hours; but if this cannot be done, care should be taken that the solution is well rubbed in. Finally, the excess of disinfectant is washed off with absolute alcohol. Each stage of the process should be gone through thoroughly and methodically, with the knowledge that any neglect may lead to serious error in diagnosis.

Disinfection of the Operator's Hands.

In making films and cultures from the blood it is important that the operator's hands should be aseptic, as much so, in fact, as if he were about to perform a surgical operation. The method of preparing the hands for operation recommended by Lockwood is that generally adopted.

The nails should be short and trimmed. Scrub the hands with soap and hot water for three minutes. Soak them for two minutes in a solution of 1 in 500 biniodide of mercury, and then in 75 per cent. alcohol, to which a little water has been added. Wash them finally in biniodide of mercury lotion, 1 in 2,000.

CHAPTER II.

THE PREPARATION AND STAINING OF FILMS. INOCULATION OF CULTURE MEDIA.

COVER-GLASS films are used for the examination of all kinds of material—blood and other body fluids, pus, scrapings of tissues and portions of growth from nutrient media. Their mode of preparation is as follows: The cover-glass, held in forceps, is taken out of alcohol, dried, and passed through the flame of a spirit lamp or bunsen burner to burn off any particles of dirt. The platinum wire loop having been sterilized in the flame, a loopful of the fluid to be examined is transferred to the cover-slip and spread evenly over its surface, where it is allowed to dry, this process being hastened either by waving it to and fro, or by carefully exposing it in the fingers to the heat of the lamp at some distance from the flame. The film is then passed three or four times quickly through the flame of the lamp, in order to fix the micro-organisms to the surface of the glass and prevent their being removed in subsequent staining and washing operations. The film is now fixed and ready for staining.

If the material from which the film is to be made be solid or semi-solid, it is best to place a drop of sterilized distilled water on the cover-glass, to rub up a little of the material with this, and then spread over the surface with the platinum wire.

If the film obtained by spreading with the platinum loop be too thick, some of the material should be placed between two cover-glasses, which are then gently pressed together and drawn apart with a sliding movement. A

thin film is thus obtained, and is dried and fixed by passing through the flame.

In preparing *blood films* in which it is desired to preserve the details of histological structure, one or other of the following procedures may be adopted :

A small drop of blood obtained by puncturing the lobe of the ear is received direct on to a cover-glass. This is gently dropped upon another cover-glass, and as the drop of blood spreads out the two cover-glasses are separated by a lateral sliding motion of one on the other.

Or the drop of blood as it escapes from the puncture is received on a microscopic slide near one end, and the edge of a second slide at an angle of 45° is applied to it, and the blood spread by drawing the second slide over the first one.

A third method of spreading blood films is described in the paragraph devoted to malaria (see p. 70).

Blood films should not be fixed by passing through a flame, as this alters the shape of the corpuscles. They should be immersed when dry for from five to fifteen minutes in a mixture of equal parts of absolute alcohol and ether, and are then ready for staining. If Jenner's blood stain be used, no special method of fixing is necessary ; the dried film is covered with the solution, and the fixing and staining proceed simultaneously.

Staining.—The cover-slip is held in Cornet's forceps with the film side uppermost, and as much of the dye as will cover the surface is applied by means of a dropper. This is allowed to remain for a varying period of time according to the nature of the stain, and is then washed off by passing through a basin of distilled water. The film is then dried either by waving in the air, resting over a heated metal plate, or by pressing between blotting- or filter-paper. It may be examined, mounted either in

water, or if a permanent specimen be desired, in xylol balsam.

With **methylene blue** there is little risk of over-staining the specimen, however long it be left exposed to the action of the dye. Its action is very slow, but may be hastened by heating. If the film covered with the stain be gently heated until it begins to steam, and then left for ten minutes, it will generally be found that this is sufficient.

Carbol gentian violet and **carbol-fuchsin** are far more intense in their action, and it is sufficient to expose films to their action for a minute only. If the dye is allowed to remain too long, the specimen may be over-stained and useless.

Gram's Method is of service as distinguishing between various forms of micro-organisms, some of which are stained and some decolourized by the process. The following list may be of service :

Stained by Gram.	{	<i>Staphylococcus pyogenes aureus and albus.</i>
		<i>Streptococcus.</i>
		<i>Pneumococcus.</i>
		<i>Micrococcus tetragonus.</i>
		<i>B. diphtheriæ.</i>
		<i>B. tuberculosis.</i>
		<i>B. anthracis.</i>
		<i>B. tetani.</i>
		<i>B. aerogenes capsulatus.</i>
		<i>B. lepræ.</i>

Decolourized by Gram.	<i>Gonococcus.</i>
	<i>Diplococcus meningitidis.</i>
	<i>Pneumo-bacillus.</i>
	<i>B. typhosus.</i>
	<i>B. coli.</i>
	<i>B. pyocyaneus.</i>
	<i>B. influenzae.</i>
	<i>B. mallei.</i>
	<i>B. proteus vulgaris.</i>
	<i>B. enteritidis.</i>
	<i>B. pestis.</i>
	<i>B. œdema maligni.</i>

The method of using **Gram's** stain is as follows :

1. Stain in carbol gentian violet for three minutes, and wash with water.
2. Treat the film with Gram's solution for about a minute until it becomes of a dark-brown colour.
3. Decolourize by treating with absolute alcohol until only a light violet colouration is left ; wash in water, dry and mount.

Ziehl-Neelsen's method of staining is used for certain organisms which resist simple methods of staining, but which when once they have taken the dye are not easily decolourized by acids. They are sometimes spoken of as 'acid-fast' bacilli. The method is as follows :

1. Cover the film with carbol-fuchsin stain, and heat until steam arises. Allow it to remain for five minutes.
2. Wash in 25 per cent. solution of sulphuric acid until the colour has faded to a pale yellow. Complete the decolouration by washing in 60 to 70 per cent. spirit.
3. Wash in water.
4. Counter-stain with watery solution of methylene blue for one minute.

5. Wash in water. Dry and mount in xylol balsam. The bacilli are stained red on a blue ground.

Although this is the method most commonly adopted for the detection of the tubercle bacillus, it should be remembered that the leprosy and smegma bacilli, and the bacillus of syphilis described by Lustgarten, have very similar staining reactions. Where a number of films are to be stained at one time by Ziehl-Neelsen's method, or where it is required to expose films for a lengthened period to the action of a dye, it is often convenient to float the cover-slips, film downwards, upon a small quantity of the stain poured out into a watch-glass or other receptacle, which may at the same time be heated if desired.

Jenner's Blood Stain.—In using this stain, which is a mixture of eosin and methyl blue in alcohol, the procedure is as follows: The blood film having been dried by exposure to the air, is covered with the stain, and left for from three to five minutes protected by a watch-glass, so as to prevent evaporation. It is then quickly washed in distilled water, dried, and mounted in xylol balsam.

The micro-organisms in a film are not necessarily killed by the processes of fixing, staining, etc., and, as a precautionary measure, specimens which are no longer required should be kept immersed for some hours in a bath of antiseptic solution, such as formalin 1 in 100, or perchloride of mercury 1 in 500.

Inoculation of Culture Tubes.

To inoculate sloped agar or serum tubes the following procedure is recommended: The wire having been sterilized in the flame, a loopful of the fluid or a small portion of the material to be examined is taken up. The

tube is held mouth downwards in the left hand, and the cotton-plug extracted with the thumb and index finger of the right, and held between the fourth and fifth fingers of the left hand. The wire loop is then passed over the surface of the nutrient medium from the bottom of the tube upwards, care being taken not to touch the sides of the tube, and the plug, after having been singed in the flame, is quickly replaced. The wire is then sterilized by heating in the flame.

The cotton-wool plug must be twisted out so as to leave no strands adhering to the sides of the tube, otherwise these might become contaminated. The culture should be at once labelled so as to indicate the hour and day, and some further mark of identification added. It should be despatched for incubation at the very earliest opportunity.

When it is proposed to make films or cultivations at *post-mortem examinations*, it is necessary that the examination should be made as soon as possible after death, for, after the lapse of twenty-four or thirty-six hours, the tissues are frequently found to be invaded by the *Bacillus coli*, which may multiply so rapidly as to completely mask the existence of the organism for which search is being made. This is especially the case, we find, if death has ensued from disease of any of the abdominal viscera.

Films and cultures may require to be made from the blood and organs, or from localized, inflammatory, or suppurative lesions. In cases of suspected general infection, cultures are best made from the heart's blood and from the spleen.

The surface of the organ is seared over a small area by a heated instrument, such as the blade of an old scalpel, or the glass handle of the platinum loop. A cut is then made through this burned area into the substance

of the organ by means of a knife previously sterilized in the flame. Into the opening thus made a sterilized platinum loop is thrust, and from the material contained on the wire tubes are inoculated and films made.

Another method, suitable for such organs as can be removed, consists in rendering their capsule aseptic by soaking the viscus for half an hour in 1 in 1,000 perchloride of mercury, drying, cutting into its substance with a sterilized knife, and then taking scrapings with the platinum needle. In localized suppurations, such as abscesses, meningitis, or peritonitis, care is taken to disturb the parts as little as possible, and to prevent contamination from blood. The cavity is opened by a small incision with a sterilized knife, and a drop of pus taken up in the loop of a platinum wire, and inoculated upon culture media. The wire is then again sterilized, a second drop of pus collected and spread as films on cover-glasses. The films should be fixed at once, and stained by the method most likely to show the organism which is suspected to be present.

The Packing and Transmission of Specimens.

Much care is required in the collection, packing, and despatch of materials, such as diphtheritic membrane, serous fluids, urine, etc., which are destined for bacteriological examination in the laboratory.

In no case should any antiseptic or preservative be added. It is no uncommon thing for such specimens to be sent to the laboratory packed in antiseptic gauze, or contained in bottles filled with carbolic acid or spirit. Such materials are, of course, useless for cultural purposes.

Both fluids and solids are best sent in small wide-mouthed bottles which have been sterilized by boiling for half an hour or longer. The bottles should be tightly

corked, the surface of the cork within the bottle being covered with a piece of sterilized oil-silk. The cork should be secured with string and sealed with sealing-wax. For some specimens test-tubes, or small medicine phials previously boiled, are suitable. Special sterilized bottles are provided by bacteriological laboratories.

POST-OFFICE REGULATIONS FOR THE TRANSMISSION OF SPECIMENS.

Deleterious Liquids or Substances.—Deleterious liquids or substances, though otherwise prohibited from transmission by post, may be sent for medical examination or analysis by *registered letter-post* under the following conditions :

‘ Any such liquid or substance must be enclosed in a receptacle hermetically sealed, which receptacle must itself be placed in a strong wooden, leathern, or metal case in such a way that it cannot shift about, and with a sufficient quantity of some absorbent material (such as sawdust or cotton-wool) so packed about the receptacle as absolutely to prevent any possible leakage from the package in the event of damage to the receptacle.

‘ The packet so made up must be marked “Fragile, with care,” and tendered at a post-office for transmission by registered letter-post. It must on no account be dropped into a letter-box or sent by parcel post. These regulations will be rigidly enforced. Any postal packet of the kind found in the parcel post, or any postal packet of the kind, whether registered or not, found in the letter post not packed as directed, will be at once stopped and disposed of as the Postmaster-General shall direct.

‘ Any person who sends by post a liquid or substance for medical examination or analysis otherwise than as provided by these regulations is liable to prosecution,

even if he be a patient sending something to his medical adviser for his opinion, or a medical practitioner sending something to a laboratory or elsewhere.

‘No liquid or substance of the kind may, under any circumstances, be sent by post to or from any place outside the United Kingdom.’

CHAPTER III.

DISCHARGES AND SUPPURATIVE PROCESSES.

ALL true suppurative processes are the result of the growth and activity of micro-organisms, the destruction of the tissue being the result either of chemical bodies contained within the bacteria or of the products of their metabolism. The following is a list of the organisms which have been shown to give rise to pus formation :

<i>Staphylococcus pyogenes</i>	<i>Micrococcus tetragonus.</i>
<i>aureus and albus.</i>	<i>B. pyocyaneus.</i>
<i>Streptococcus.</i>	<i>B. coli.</i>
<i>Staphylococcus cereus, albus,</i>	<i>B. typhosus.</i>
<i>and flavus.</i>	<i>B. pyogenes fætidus.</i>
<i>Staphylococcus citreus.</i>	<i>B. aerogenes capsulatus.</i>
<i>Pneumococcus.</i>	<i>B. prodigiosus.</i>
<i>Diplococcus intracellularis</i>	<i>B. tuberculosis.</i>
<i>meningitidis.</i>	<i>B. anthracis.</i>
<i>Gonococcus.</i>	<i>B. mallei.</i>
<i>Micrococcus pyogenes tenuis.</i>	<i>Actinomycosis.</i>

Of these organisms associated with pus formation, the one that is most frequently found is the *Staphylococcus aureus*, and next in order come the *Staphylococcus albus* and the *Streptococcus*. A short description of these three

organisms will therefore be given, whilst for particulars concerning the others mentioned in the list bacteriological text-books should be consulted.

Staphylococci are spherical in shape, and in films may be found singly, in pairs, or in small grape-like clusters.

They stain easily with anilin dyes. On all forms of nutrient media, gelatine, agar, serum, and broth, they show abundant growth even at room temperatures.

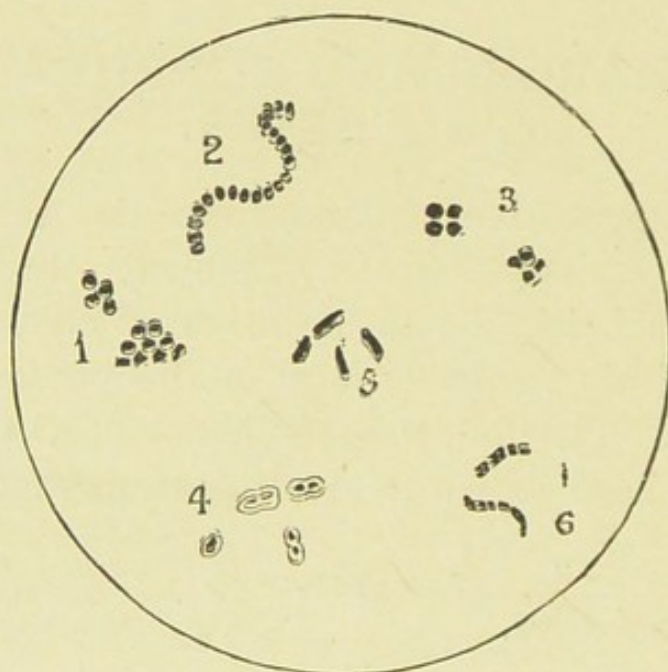


FIG. 4.

- | | |
|--------------------|---------------------|
| 1. Staphylococcus. | 4. Pneumococcus. |
| 2. Streptococcus. | 5. B. Coli. |
| 3. M. Tetragonus. | 6. B. Tuberculosis. |

Staphylococcus aureus, *albus*, and *citreus* all liquefy gelatine at ordinary room temperature, if such tubes be inoculated and kept for from four to ten days.

Streptococcus Pyogenes.—This organism is a spherical body rather larger than the staphylococcus, and distinguished by its tendency to form chains or rosaries. Streptococci stain readily with anilin dyes. On nutrient media growth is slow, and the pale, semi-translucent colonies soon die. They do not liquefy gelatine.

Bacteriological examination of a discharge may demonstrate the presence of one or more varieties of organisms, and if one only is present the infection is spoken of as 'pure,' and if two or more are present as 'mixed.'

Streptococci are most commonly the determining cause of diffuse cellulitic inflammations, spreading traumatic gangrene, lymphangitis, septicæmia, and ulcerative endocarditis.

Staphylococci are more frequently found in superficial and localized suppurations, discharging wounds, ulcers, furuncles, carbuncles, acute osteomyelitis, and pyæmia.

Streptococci have also been described as the causative factor in erysipelas, scarlet fever, and acute rheumatism, and are, it is generally now considered, varieties of one common species, differing only in slight cultural modifications or degrees of virulence.

Both staphylo- and strepto-cocci and many other pyogenic organisms are subject to marked variations in virulence, existing at one time on a normal mucous membrane or exciting a chronic local lesion, whilst at another time they may give rise to a fulminating and rapidly fatal general infection.

A knowledge of the micro-organism concerned in a given case of suppuration may be essential in order to establish a correct diagnosis as to the nature of the original lesion. Thus, films made from the pus of an abscess may show gonococci, pneumococci, *B. coli* or *B. typhosus*, pointing to secondary infections from foci originating primarily in the urethra, lungs, or intestine.

It is not uncommon, when examining films of pus, to fail to detect any microbes. If, after examining a number of slips, this is found to be the case, and if there be no growth on inoculated tubes of culture media, the probability is that the lesion is tuberculous, or that it is a

breaking down gumma. Fresh specimens should then be stained by Ziehl-Neelsen's method in order to demonstrate the tubercle bacillus, and a further quantity of the pus be reserved for testing by inoculation.

The line of treatment may be considerably modified by a knowledge of the bacteriology of the lesion. For instance, an abscess cavity in which the pus shows no organisms, and which therefore may be assumed to be tubercular, may with confidence be scraped and the skin stitched up with the purpose of securing healing by first intention; but if cocci or bacilli are shown to be present, the necessity for drainage is greater. This refers more particularly to the treatment of so-called scrofulous abscesses of the neck.

Similarly, the method of treatment to be followed in a case of empyema may be determined by the organism which is found to be present, and the decision to adopt any method of serum treatment must rest on the same grounds.

Methods of Examining for Pyogenic Organisms.

—The conditions under which it may be found necessary to search for suppurative organisms are numerous and varied. It may be required in surgical practice to test the asepticity of the operator's hands, the instruments, the surrounding surfaces, or the skin of the patient. It may be important, also, to attempt to isolate the pyogenic organism from various surfaces of the body under normal conditions, from discharges of wounds, sinuses, or mucous membranes, from deep-seated abscesses or empyemata.

It is not possible to describe in detail the particulars of each process required. The principle is alike in all, and consists of taking a portion of the material in the loop of the platinum wire, and of inoculating tubes and

making film preparations. In the case of surfaces the wire is well rubbed over the surface so as to get as much material as possible. If the substance in the loop be hard and dry, the film may be made by mixing it with a drop of sterile distilled water on the cover-slip.

In taking cultures from the hands, it is best to obtain scrapings from beneath the nails by means of a straight platinum wire previously sterilized, to cut (with the aid of scissors and forceps sterilized by boiling) portions off the nails, or to snip off fragments of skin. The greatest care must be taken that such fragments are only touched by sterilized instruments, and as speedily as possible transferred to tubes of culture media.

DEEP-SEATED ABSCESSES.

If it be required to examine pus from these and other similarly situated collections of fluid, the exploring syringe is the best instrument to use. The skin over the spot indicated is rendered aseptic by the method already detailed (p. 18), and syringe and needle are boiled for half an hour. A small quantity of pus having been drawn into the syringe, the needle is withdrawn, and by means of a sterilized loop some of its contents transferred to cover-slips for staining, and to tubes for cultural purposes.

If the abscess is to be opened by incision, the pus which first escapes is rejected, as it may be contaminated by the edges of the wound. A loopful is then taken, and inoculations and films prepared.

TUBERCULAR SUPPURATIONS.

If pus be taken from an abscess or other lesion suspected of being tubercular, then the films which are

prepared in the ordinary method are stained by Ziehl-Neelsen's process (p. 22), and examined microscopically for tubercle bacilli. The bacillus appears as a fine beaded rod which has taken the red stain somewhat irregularly, so as sometimes to present almost the appearance of a string of cocci, whilst other organisms and tissue débris are stained blue. It is always necessary to prepare a number of such films, as the bacilli are sometimes present in very small quantities.

As direct microscopical examination of films so often leads to failure to detect the tubercle bacillus even if it be present, it is advisable to adopt other measures with a view to obtaining correct results. A few loopfuls of the discharge should be spread over the surface of blood-serum culture tubes, which if incubated for twelve days may, if the organism be present, develop the small circular white nodules of growth characteristic of the tubercle bacillus.

A further portion of the discharge should be collected in a sterilized glass pipette or test-tube, and despatched to the laboratory for the purpose of being tested by inoculation. The animal generally selected for inoculation purposes is the guinea-pig, and as from two to three weeks' time is necessary for the development of tubercular lesions, no report on the nature of the infection can be obtained until this period has lapsed.

Failure to detect any organisms in a purulent discharge is always suggestive of a tubercular origin. Pus from a freshly-opened abscess may contain large numbers of tubercle bacilli; but if the discharge from the wound be examined twenty-four hours later, it may be impossible to demonstrate their presence. The reason for this disappearance is not known. It is not without interest in connection with the well-known fact that tubercular

peritonitis is occasionally cured by laparatomy and the free admission of air into the peritoneal cavity. In discharges tubercle bacilli are found lying within and outside the cells, singly or in small groups.

The subject of the diagnosis and treatment of tuberculosis by means of tuberculin is referred to in the chapter devoted to Serum Therapeutics.

GONORRHŒA.

In the early stage of gonorrhœa, during the first two or three days of the discharge, the recognition of the gonococcus is a comparatively easy matter. As the discharge becomes more profuse, however, other pyogenic organisms are present in such abundance as to render the detection of the coccus a task of some difficulty.

The gonococcus is a kidney-shaped organism, generally found in the diplococcus form, the adjacent sides of the cocci being flattened (Fig. 5). Although in the early stage they may be found lying free, as the discharge tends to become purulent, increasingly large numbers lie within the pus-cells in groups of eight, sixteen, or thirty-two. They stain with the anilin dyes, but are decolourized by Gram.

Gonococci do not grow on agar or gelatine media, and if it be desired to confirm the diagnosis by means of cultures, the following procedure should be followed: The skin having first been carefully sterilized, a puncture is made in the lobe of the patient's ear. Several loopfuls of the blood so obtained are then spread over the surface of sloped agar tubes, and after allowing a few minutes to elapse for the purpose of drying, the tubes (now known as blood-agar tubes) are inoculated with the suspected

material. On such a medium the gonococcus grows in grayish white dew-like colonies.

To obtain Films.—Wash the meatus urinarius with perchloride of mercury 1 in 1,000. Squeeze the urethra so that a drop of pus exudes, and, rejecting the first portion, take a loopful of the next drop on a sterilized platinum wire. Spread the pus over a series of cover-slips, fix, and stain for ten minutes with methylene blue. If several groups of typical-shaped cocci are seen within the pus-cells, and if, on further staining films by Gram's method, these cocci are decolourized, the diagnosis of gonorrhœa may be regarded as confirmed. In women the specimen for examination is best taken with the aid of a speculum from the urethra or cervix uteri. In chronic cases and in gleet, where there is only a little morning discharge, the patient should be directed to receive this upon one cover-glass, and to spread it out by placing a second upon this, and then to draw them apart and allow to dry. One film is then stained with methylene blue, and the second with gentian violet, so as afterwards to be available for Gram's process.

In cases of chronic gleet, where it is difficult to demonstrate the presence of the gonococcus, a free discharge may be excited by swabbing out or by injecting into the urethra a strong solution of nitrate of silver, and in such a discharge the organism may be detected. 'Prostatic threads' frequently contain gonococci. The threads should be allowed to settle in a urine-glass, or be obtained by means of a centrifuge, and, after being squeezed out between two cover-slips, should be stained with methylene blue and by Gram's method.

Gonococci may be found in the pus in many cases of purulent ophthalmia, both in the newly-born and in adults, in abscesses in connection with the genital organs,

cystitis, pyosalpinx, peritonitis, arthritis, pericarditis, and in malignant endocarditis. Films and cultures should be prepared according to the methods already described. In suspected cases of gonorrhœal arthritis the joints may be aspirated with a sterilized syringe, and a series of films and blood-agar cultures made.

The fact that the organisms are during the first few days of the discharge found lying free, or upon the surface of the cells, would seem to point to the importance of local treatment at this stage. Later, the action of an antiseptic lotion is diminished by the fact of the cocci lying within the cells, and the presence of pyogenic organisms adds a further complication. So far as gonorrhœal ophthalmia is concerned, it is of practical importance to note that now and then the gonococcal infection may run an extremely mild course, showing only a slight muco-purulent discharge. It is hardly necessary to remark that such cases are centres of active infection.

Urethritis, vaginitis, and vulvitis may be excited by organisms other than the gonococcus, and clinically it may be impossible to distinguish the nature of the infection. An error in diagnosis may lead to very serious consequences, so that in all cases it is desirable that the three special characteristics of the gonococcus should be demonstrated—viz.: (1) occurrence of flattened diplococci in groups of eight or sixteen within the cell; (2) decolourization of this organism by Gram's method; (3) inability to grow on agar or gelatine. The fact that some sixteen or eighteen different species of cocci have been isolated from the normal and diseased urethra render these precautions the more essential.

ACTINOMYCOSIS.

The micro-organism of actinomycosis holds a position midway between the bacteria proper and the higher fungi. The parasite is most frequently found in cattle and pigs, which become infected through eating barley on which the ray fungus is thought to grow. The exact means by which human beings become inoculated is not known. The digestive tract, the lungs, and the skin are common paths of entry. Carious teeth may in some cases prove the point of entrance, and from such a centre there may start those severe inflammatory conditions of the cellular

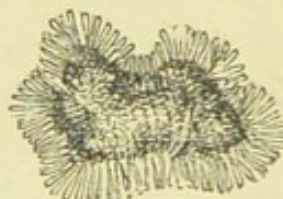


FIG. 5.—PUS CELL SHOWING GONOCOCCI. FIG. 6.—ACTINOMYCOSIS.

tissue of the neck known as Angina Ludovici. The presence of the parasite excites a form of chronic suppuration, with the formation of viscid pus dotted over with minute yellow granules. The inflammatory process has a tendency to spread, forming in the skin raised ulcerating tumours, in the deeper tissues suppurating sinuses, and in the lungs lesions of a tubercular character.

Inflammatory swellings of obscure origin connected with the jaw, or chronic suppurations of doubtful origin with discharges of peculiar character, or the presence of greenish-yellow millet-seed bodies in sputum, may excite suspicion of actinomycosis.

Method of Examination.—The suspected pus or expectoration is spread out over a glass slide, so as to

show up the small granular masses more clearly. If one of these masses be teased out with needles in a 0.75 per cent. solution of sodium chloride, and mounted in 50 per cent. glycerine, the structure of the nodule will be seen to be as follows: In the centre there is a confused mass looking like broken-down débris, but consisting of intermingled mycelium-like threads and cocci. Arranged round the periphery of this mass in lines radiating from the centre is a row of clubs. The clubs are stained by carbol-fuchsin diluted with 5 parts of water or by orange rubin; the central mass by Gram's method. Occasionally in pus mycelium-like threads only are found. Glycerine-agar tubes may be inoculated with portions of the growth.

Treatment.—The ray fungus is of low vitality, and its growth and spread may be readily checked by the local application of antiseptic solutions if these can be brought into sufficiently close contact with the fungus.

Potassium iodide appears to have a specific effect upon the organism, and is given in doses of 10 to 20 grains three times daily.

The bacteriological diagnosis of glanders, anthrax, and tetanus depends largely on the examination of the discharge from the local lesion, and for this reason these diseases, although more properly classified under the head of *general infections*, have been included in the present chapter.

GLANDERS.

Acute glanders is marked by high fever, a pustular rash, purulent nasal discharge, and by multiple abscesses

in the muscles and deep tissues. In the chronic disease an ulcer forms at the site of inoculation, the lymphatics are infected, and a low form of septic fever (unless complicated with other organisms) results. If films be prepared from pus taken from any of these lesions, and stained for five minutes with Löffler's methylene blue, the *B. mallei* may be detected; but most frequently the bacillus fails to take the stain, and the discharge appears sterile. The *B. mallei* is about as long as the tubercle bacillus, straight or curved at the ends, and stains irregularly. Blood-serum tubes may be inoculated with the pus, but the isolation of the bacillus by cultural methods is not easy. The more certain method is to have inoculation experiments made, and for this purpose some of the discharge or scraping from the bulbous pustular patches should be secured in a sterile vessel and despatched to the laboratory.

Treatment.—By treating each pustular spot with strong antiseptics, so as to destroy the bacilli, the severity of the chronic disease may be lessened. Benzoate of soda administered internally has been recommended. Mallein, a substance formed by the growth of the *B. mallei* in peptone bouillon, is chiefly used for the detection of the disease in animals, and if applied universally and followed up by slaughter of infected horses, there is no doubt the disease might be eradicated. It has rarely been employed as a remedy for the disease in man.

Treatment in the acute cases is of little avail. The disease is generally communicated to man by direct inoculation from horses.

ANTHRAX, or WOOLSORTERS' DISEASE.

This disease is communicated to man chiefly by inoculation from herbivorous animals, or from handling infected hides or wool. Infection may be through some abrasion in the skin, or by the intestinal or pulmonary tracts.

In cutaneous anthrax the bacilli are at first entirely confined to the immediate neighbourhood of the malignant pustule. Cover-slip preparations may be made from the serous discharge that oozes from beneath the central necrotic patch, or from the serum contained in the vesicles surrounding the ulcer. The exudate is taken up on the sterilized platinum loop and spread on the cover-glasses, one of which is dried and stained with methylene blue for five minutes, whilst the other is stained by Gram's method, by which the bacillus is not decolourized.

The *B. anthracis* is in shape a thick straight rod with the ends truncated at right angles. It forms long threads. Growth is abundant on most media, with the formation of spores (see Fig. 7), the colonies being characterized by a peculiar wavy outline. Culture tubes should be inoculated from the exudation of the primary pustule or vesicles.

In the pulmonary form of the disease the bacilli may be found in the sputum, or in the serous fluid which generally appears in the pleura. They are, however, difficult to demonstrate unless inoculation experiments are made. This remark also applies to the presence of the bacilli in the intestinal form of the disease, when the organisms are to be looked for in both urine and fæces. It is only towards the termination of the cases that the *B. anthracis* can be demonstrated in the blood. A

drop of blood is taken with antiseptic precautions from the ear, and cover-glass preparations and cultivations are made. Most frequently these give only a negative result.

Treatment.—In the cutaneous disorder, as the bacilli are known to be confined at first to the immediate vicinity of the pustule, early excision and treatment of the surrounding area with strong antiseptics is the obvious indication. In the intestinal and pulmonary forms no line of treatment has met with much success. Preventive treatment is of great importance in this disease. Every hide and all wool from suspected quarters should be



FIG. 7.—B. ANTHRACIS.



FIG. 8.—B. TETANI.

sterilized, and certain precautions should be taken to protect the workmen, such as forced down-draughts to draw off dust from the work-benches, free ventilation, and other recognised methods of prevention.

TETANUS.

Although this disease is generally produced by the inoculation of an open wound with the tetanus bacillus, cases frequently arise in which no such point of inoculation can be detected. The bacillus is present in garden mould and in the excrement of certain animals, particularly the horse. It is possible that the tetanus bacillus is itself capable of exciting suppuration, but more frequently it is found associated with other pyogenic organisms, and this association undoubtedly increases

its virulence. The bacilli remain and multiply at the seat of inoculation, manufacturing at this point a toxin which becomes diffused throughout the whole body, attacking especially the nervous system.

Film preparations should be made from the pus of the wound, and stained with carbol-fuchsin or by Gram's method. The tetanus bacillus is a long slender rod, sometimes presenting a swollen, drumstick end containing the spore (see Fig. 8). If present, it is almost invariably associated with cocci; but frequently it cannot be detected in films, and, as, being anaerobic, it does not grow on ordinary media, recourse must be had to inoculation, pus and scrapings from the wound being obtained in a sterilized pipette, and forwarded to the laboratory for this purpose.

Treatment.—The first indication for treatment is the excision of the original site of inoculation. This having been done, tetanus antitoxin should be injected. Directions for its use will be found under Serum Therapeutics, p. 98.

EMPHYSEMATOUS GANGRENE.

The organisms most commonly associated with this condition are *Bacillus œdema maligni*, *B. aerogenes capsulatus*, and *B. coli*. Films should be prepared from the discharges or juices of the gangrenous parts, and stained with methylene blue and by Gram's method.

The **bacillus of malignant œdema** may be found singly or in chains. It is from 3 μ^* to 10 μ in length, and is decolourized by Gram. Spore formation is rapid, but

* μ = $\frac{1}{1000}$ millimetre.

as the organism is anaerobic, cultures taken on tubes of ordinary media are of no service for diagnostic purposes.

The **Bacillus aerogenes capsulatus** occurs as a diplobacillus in chains. Its capsule may be demonstrated by washing the films with glacial acetic acid, and then staining with carbol gentian violet. The presence of this organ in the tissues after death gives rise to the condition known as 'foam organs.'

Bacillus Coli.—This organism is found not only in emphysematous gangrene, but in a large number of suppurative processes, especially in connection with the gastro-intestinal tract.

It is a motile bacillus, $1\ \mu$ by $0.5\ \mu$ in measurement, grows freely on all the common media, and closely resembles the *B. typhosus* in microscopical appearances.

VACCINATION.

Calf vaccine lymph, as now supplied by the Local Government Board and by private retailers, is mixed with a certain proportion of glycerine, added for the purpose of destroying extraneous organisms and preserving the lymph for storage. Its purity depends upon the fact, discovered by Copeman, that glycerine applied to the vaccine lymph, under certain conditions, has the property of destroying all but the vaccine micro-organisms. Unless the glycerine used is of the very purest quality, and unless the tubes of glycerinated lymph be stored for three or four weeks before being used, the exclusion of pyogenic organisms cannot be relied upon. We have on several occasions examined, by means of plate cultures, specimens of glycerinated lymph obtained from various sources. Pyogenic organisms, moulds, and yeast were present most frequently, and in one at least the *Strepto-*

coccus pyogenes was found. It is desirable, therefore, that practitioners should from time to time test the purity of the lymph supplied to them, both by preparing from it films which may be stained with methylene blue and examined microscopically, and by sending specimens to a bacteriological laboratory for the purpose of having plate cultures made.

CHAPTER IV.

GENERAL INFECTIONS.

BLOOD in the normal condition contains no micro-organisms. Speaking generally, even in diseases due to the multiplication of micro-organisms in the tissues, the blood is not found to be infected. Such, at least, is the generally accepted opinion to-day. Comparatively little attention, however, has been paid to the bacteriological examination of blood, and it seems probable that with increasing knowledge our views may undergo considerable modification. It is certain that in many infections of exceptional severity the pathogenic agent may be and has been detected in the blood. Thus pneumococci have been demonstrated as existing in the general circulation in cases of pneumonia and ulcerative endocarditis; staphylococci, streptococci, gonococci, and *B. coli* in pyæmic states; the tubercle bacillus in acute miliary tuberculosis; and the bacilli of enteric fever, glanders, anthrax, and influenza in severe types of these disorders. From the wide distribution of the diphtheria bacillus in the tissues after death, it is probable that a general infection takes place occasionally in this disease also. The malaria parasite and spirillum of relapsing fever have apparently their natural habitat in the blood.

Methods of Examining the Blood for Micro-Organisms.

In order to determine the presence of micro-organisms in the blood in suspected cases of general infection, the following procedure is adopted: The skin over the front of the patient's elbow is rendered aseptic according to the directions given on p. 18. A bandage is fixed tightly above the elbow, so as to distend the veins below, and the needle of an exploring or serum syringe, previously sterilized by prolonged boiling, is then thrust into a vein (median cephalic or median basilic), and from 5 c.c. to 10 c.c. of blood withdrawn. The blood is at once transferred to tubes of nutrient broth, or spread over the surface of large sloped agar or serum tubes. Occasionally there is a little difficulty in obtaining a sufficient quantity of blood, and in such cases it may be necessary to make a small skin incision and expose the vein, or cultures may be made from the small quantity of blood which exudes from the point of puncture.

In cases where permission to obtain blood by means of the syringe cannot be obtained, the following methods may be adopted:

The blood is obtained by puncturing the finger or lobe of the ear, preferably the latter. At least half a dozen films should be prepared, and, if possible, an equal number of tubes inoculated.

Cover-slips and slides are prepared with the minutest attention to asepticity, passed through the flame of a spirit-lamp, and protected from dust by watch-glasses. The skin having been sterilized in the manner already mentioned, a puncture is made quickly with a triangular surgical needle which has been previously sterilized in the flame. As the drop of blood appears, it is taken up

in the centre of a cover-glass held in sterile forceps, a second cover-glass is placed upon the first, so as to allow the drop to spread out, and then they are slipped apart. Films thus prepared are fixed by placing for five minutes in a solution of equal parts of alcohol and ether, and must be protected from dust whilst they dry. They are stained by exposing to Löffler's solution for five minutes with moderate heat.

As it is possible that the surface may have been contaminated in the process of taking blood for the films, it is preferable when making cultures to re-sterilize the skin and make a second puncture. A series of loopfuls of blood is then taken on a sterilized wire, and as many tubes as possible inoculated. These tubes should be despatched immediately for incubation.

By whatever method the blood may be obtained, there is considerable risk of accidental infection from the skin, and if in cultures a white staphylococcus be found (*Staphylococcus epidermidis albus*), it is well to suspect contamination and repeat the process. Negative results cannot be relied on, as the quantity of blood examined is necessarily small and the germicidal properties of the blood will sometimes be sufficiently strong to inhibit growth.

Micro-organisms are found in blood in cases of pyæmia, septicæmia, malignant endocarditis, puerperal fever, pneumonia, rheumatic fever, and acute osteo-myelitis, and their discovery may have an important bearing on the questions of diagnosis, prognosis, and treatment. In this connection we may quote from an article on the bacteriological examination of the blood* :

Diagnosis.—For purposes of diagnosis the bacteriological examination of the blood is seldom of real practical value. Negative results cannot be relied upon, and

* J. O. Symes, *British Medical Journal*, September 14, 1901.

as a rule the nature of the disease is to be ascertained by ordinary clinical methods long before it is possible to demonstrate organisms in the blood. There are, however, some cases of cryptic infection which may be elucidated by this method; for example, unsuspected pyæmic or septicæmic conditions, following infection from gastric or intestinal ulceration, or gonorrhœal lesions. In cases of suspected infective endocarditis the detection of organisms in the blood may confirm the diagnosis, and indicate the nature of the infection.

Prognosis.—All observers agree that the discovery of micro-organisms in the blood renders the prognosis of the case an exceedingly grave one. This has especially been shown to be the case in pneumonia, in which disease a general blood infection is frequently the precursor of serious complications, or may indicate the speedy approach of a fatal termination. In septicæmia and pyæmia, if organisms can be demonstrated, a fatal termination is seldom long delayed, but in infective endocarditis the patient may survive for many weeks. In other infective fevers, too, such as enteric fever or anthrax, a general blood infection may precede the fatal termination. The frequency with which micro-organisms may be detected in the blood has been a matter of some dispute. In the author's series of examinations they were demonstrated in 29 per cent. of the cases, and the death-rate amongst these was 77 per cent. The death-rate of the twenty-two cases in which no organisms could be demonstrated was 31 per cent., but several cases could not be traced to their termination.

Treatment.—With regard to the question of treatment, the bacteriological examination of the blood is of greater service. The detection of streptococci or pneumococci in the blood would be an indication for the use

of antistreptococcus or antipneumococcus serum, and, indeed, it is only by the collation of a number of cases in which such an examination has been made that a proper estimate of this method of medication can be obtained. Unfortunately, in very few of the cases recorded up to the present time has this procedure been resorted to. Failure to detect the specific organism in the blood should not, however, be an indication for withholding the serum. In several of the cases of septicæmia which I examined and found the blood sterile streptococci were present in the discharges, or in the pus from the peritoneum or from pelvic abscess. One such case terminated fatally. In such cases it would not be rational to withhold the serum on account of failure to find the organism in the general circulation.

Septicæmia and Pyæmia, Infective Endocarditis, Puerperal Fever.

A general infection, with or without the formation of secondary abscesses, may follow a wound or breach of surface the discharges from which have become contaminated by bacteria.

The organisms most commonly found in the blood in cases of **septicæmia** and **pyæmia** are the streptococcus, *Staphylococcus aureus* and *albus*, pneumococcus, and gonococcus.

In **infective endocarditis** the primary seat of infection may not be obvious, and it seems possible that the diplococcus of acute rheumatism can excite this condition. Other organisms which have been found associated with this disease are streptococcus, *Staphylococcus aureus* and *albus*, gonococcus, pneumococcus, *B. coli*, and *B. endocarditis griseus*.

Puerperal Fever.—The denuded surface of the interior of the uterus, or some breach of surface in the genital canal or perineum, affords the point of entry for the infecting organism.

Under normal conditions the interior of the uterus and the upper part of the cervix are sterile, but the puerperal uterus and vagina may contain streptococci, and as these cocci are capable of greatly intensified virulence, there can be no doubt that auto-infection is possible. On the other hand, there is little doubt that the greater danger arises from contamination from without, by means of the attendant's hands or instruments, and that strict attention to antiseptic details in all manipulations is the surest preventive measure.

The organisms commonly found associated with puerperal fever are the streptococcus, *Staphylococcus aureus* and *albus*, *B. coli*, and gonococcus.

Method of Examination.—In all the foregoing general blood infections the blood is drawn off by a syringe and examined by the methods described on p. 44. The most scrupulous attention must be paid to the asepticity of the skin and instruments, and if there be any suspicion of contamination, or if no growth be obtained, the examination should be repeated.

Search should be made for some breach of surface in the skin, mucous membranes, or gums, which might have been the original source of infection, and, when found, cultures and films should be made from the discharges of this primary focus. In the case of puerperal fever, cultures and films should be prepared from the cervix and interior of the uterus by means of a speculum and sterilized wire or probe.

Treatment.—This must be both local and general. The primary focus of infection must be excised, cauterized,

or thoroughly cleansed by the application of strong antiseptics.

The general treatment will consist in the administration of a serum, the choice of which will be determined by the result of the bacteriological examination. Particulars will be found in the chapter devoted to Serum Therapeutics (p. 94).

ERYSIPELAS.

Whilst erysipelas generally may be regarded as due to an invasion of the tissues by a variety of streptococcus, recent researches have shown that other organisms—*e.g.*, *B. coli* and *Staphylococcus aureus*—may set up an acute spreading inflammation, which cannot be distinguished from it.

Cultures of streptococci may sometimes be obtained from a drop of blood from a puncture made in the spreading margin, but it is seldom possible to demonstrate their presence by film preparations. The scales which form as the acute inflammation subsides may, if inoculated upon nutrient media, give rise to the specific microbes. The bearing of this fact upon the necessity for isolating the patient until desquamation has subsided is obvious.

In severe cases of erysipelas treatment by antistreptococcic serum may prove of service. Its value in this disease can only be demonstrated by a more extended trial than it has at present received.

Coley's Fluid.

The toxin elaborated by the streptococcus is sometimes utilized in the treatment of inoperable cases of malignant disease, it having been observed that after an acute attack of erysipelas malignant tumours have sometimes decreased in size and disappeared.

Streptococci obtained from a fatal case of erysipelas

are inoculated through a series of rabbits, and then grown in broth for ten days. At the expiration of that time a culture of *B. prodigiosus* is added, and the two allowed to grow together for ten days. The broth is then heated at 60° C. for an hour, so as to kill the organisms, and the fluid is ready for use. The initial dose is $\frac{1}{2}$ minim, and this is gradually increased daily until the reaction temperature reaches 104° F.

Coley's results with this treatment have been encouraging, especially in the case of sarcomata. A detailed account of his methods will be found in the *Annals of Surgery*, xxv. and xxvi., 1897.

DIPHTHERIA.

In no disease have bacteriological methods of diagnosis been of greater service to the practitioner than in diphtheria. Although appearing at first as a local disease, diphtheria is to be regarded as a general infection due to the development of a bacillus known as the Klebs-Löffler bacillus, and to the introduction into the system of toxins elaborated by this organism.

The Klebs-Löffler bacillus is polymorphic, but there are three well-recognised forms: (1) Irregularly staining, club-shaped varieties (involution forms); (2) long, beaded forms, with darkly staining swollen ends; (3) straight or slightly curved rods, swollen in the centre and tapering at the ends. The diphtheria bacillus grows well on most media, particularly on blood serum or glycerine agar. With Löffler's methylene blue it stains unevenly, showing in places deeply dyed granules. This characteristic method of staining is an important guide in establishing its identity.

Diphtheritic membrane may occur on the throat, nose, pharynx, and upper air-passages, on abraded surfaces of

skin, or on vulva or conjunctiva. So-called fibrinous rhinitis, with discharge, is frequently diphtheritic, and any membranous conjunctivitis, however slight, should be viewed with suspicion.

Methods of Preparing Films and Cultivations.—

If pieces of membrane are coughed up or can be detached with forceps, these should at once be placed in 0.75 per cent. solution of common salt previously sterilized by

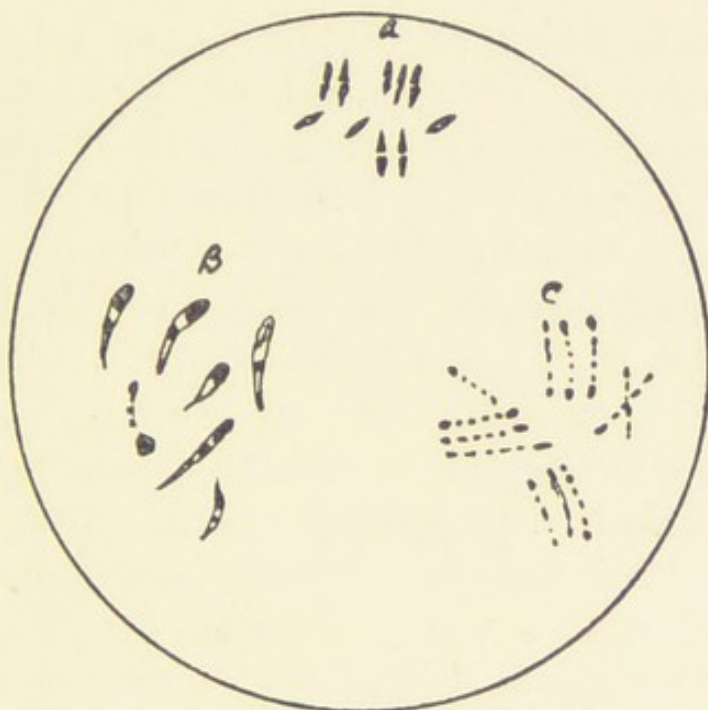


FIG. 9.—*BACILLUS DIPHTHERIÆ*.
(a) Short; (b) involution; (c) long forms.

boiling. The mucus, food débris, and sputum which covered the surface having thus been washed off, the membrane is picked up in a pair of sterile forceps and smeared over the surface of two or three cover-slips. These are dried, fixed by passing through the flame, and stained for three or four minutes in Löffler's methylene blue. Examined microscopically, these films may show the irregular clusters of unevenly stained Klebs-Löffler bacilli, and the diagnosis be at once made. Very fre-

quently, however, the organism cannot be discovered or distinguished from the many other bacterial forms which are present in such films, and tubes should always be inoculated. A platinum loop having been sterilized in the flame, is well rubbed over the surface of the piece of washed membrane, and then passed lightly over the surface of the nutrient material in the culture tube, which should be immediately despatched for incubation and examination.

In cases in which no membrane can be procured, a portion of the exudation is obtained on the swab which is generally sent with culture outfits, or in the loop of a sterilized platinum wire. The fauces and naso-pharynx should be first syringed with distilled water, so as to remove mucus and food débris, and to dilute any remains of antiseptic applications which might interfere with the growth of the bacillus. The swab or wire is then firmly rubbed over affected parts, and, thus charged, is smeared over the surface of the serum or agar tubes, and subsequently, if it be desired, over cover-glasses, which should be at once stained and examined. To insure the successful inoculation of tubes, it is essential that the cultivation shall not be taken immediately after the use of any antiseptic application, that not merely the surface of the exudation be touched, but that the wire or swab be well rubbed in, and that subsequently a large surface of the culture medium be lightly inoculated.

In laryngeal diphtheria, where no membrane can be seen, the bacilli may still be obtained from cultures inoculated with the exudation from fauces (excluding tonsils) and pharynx.

When swabs and culture tubes are not available, a piece of membrane that has been coughed up or detached should be placed in a small wide-mouthed sterilized bottle,

and at once despatched to the laboratory; or a swab should be improvised and forwarded in the same way. *On no account should any preservative, antiseptic or other fluid be added.*

Diagnosis, Prognosis, and Treatment.—The detection of the Klebs-Löffler bacillus enables us positively to distinguish between true diphtheria and all other membranous affections of the fauces and upper respiratory tract. Failure to find the specific microbe may, however, be due to error in inoculating the culture tube, and in suspicious cases repeated cultures should be taken, the wire being introduced not only into the fauces, but into the nasal cavities also. Fatal cases of membranous croup of coccal origin are of not infrequent occurrence. The writer has met several in which repeated examinations both before and after death failed to reveal the presence of diphtheria bacilli.

The great source of error in the bacteriological diagnosis of diphtheria to-day is to be found in the difficulty which is experienced in distinguishing between short forms of the Klebs-Löffler organisms and the so-called '*pseudobacillus*.' Some observers maintain that the pseudobacillus is a modified non-virulent form, and that by various means of cultivation it may be converted into the virulent type. This, however, cannot be held to be established, and in doubtful cases it is most important that every effort should be made to identify the organism, as it is manifestly unfair to the patient that he should be placed in a diphtheria ward, or be detained for weeks in isolation, if there be any doubt as to the nature of the disease.

It is desirable, therefore, when a report of '*short diphtheria bacilli*' is received, in the absence of marked clinical symptoms, to send further swabbings from the

throat to the laboratory, with the request that the exact nature and virulence of the bacilli be determined by means of inoculation experiments. Similarly, if several weeks after an acute attack of diphtheria, bacteriological reports show that 'short forms' are persisting, it is desirable to have a similar test performed, in order that the patient may be released from quarantine if these prove to be non-virulent or pseudo-diphtheria bacilli.

Prognosis.—The discovery of the diphtheria bacillus in any case of angina increases considerably the gravity of the prognosis. Moreover, those cases in which the long variety of bacillus is found are apparently more serious than are infections by the short. Similarly, in mixed infections the prognosis is less favourable if numerous colonies of staphylococcus are detected in the cultivation than if the associated organism be the streptococcus. Complications such as otitis, broncho-pneumonia, and suppurating glands may be attributed to these pyogenic cocci, but their occurrence is with equal frequency due to the diphtheria bacillus itself, which may after death be found in these lesions, and in the heart's blood and the spleen.

Treatment.—From our knowledge of the bacteriology of the disease, treatment should be directed firstly to neutralize the effects of the circulating toxins, and secondarily to the destruction of the microbes manufacturing this toxin. The treatment of diphtheria by serum obtained by immunizing horses against the specific bacillus has now had an extensive trial, and its success is borne out by both clinical experience and by the test of statistical methods.

Particulars of the serum treatment of diphtheria will be found in the chapter devoted to Serum Therapeutics, p. 94.

The dose of the antitoxin will vary with the preparation used. It is essential, however, that the initial dose be a full one, and that it be given as early in the disorder as possible. For this reason it is not desirable in typical or highly suspicious cases to await the result of the bacteriological examination, but to proceed to treatment immediately.

The buttock or skin over the lumbar region is the most convenient spot for injection. The skin should be disinfected, and the syringe sterilized by boiling. The more severe the case, the more the antitoxin should be pushed.

In marked septic cases it has been recommended that treatment by antistreptococcus serum be combined with that by antitoxin. This plan, however, has not met with much favour in this country.

Locally, the disease is treated with antiseptic lotions with a view to the destruction of the bacilli. Perchloride of mercury 1 in 5,000, and formalin 1 in 250, will be found efficacious, and where possible they may preferably be applied by means of a douche or syringe rather than with a spray. Local treatment alone is insufficient. The infection is a general one, and must be combated as such.

Preventive Measures.—Diphtheria may be spread by fomites, so that it is most important that everything which has been in contact with the infected person, as well as the sick-room, be rigidly disinfected. Milk is another vehicle of the disease, and may become infected, either from the cow or by accidental contamination from persons engaged in milking or in the distribution of milk. Infection by milk may fortunately be guarded against by efficient boiling. Cats also suffer from diphtheria, and may be a source of infection. Insanitary surroundings,

by exciting sore throat, are a predisposing cause of the disease. The chief means of spreading diphtheria is undoubtedly by direct infection from person to person by means of the nasal and buccal secretions. It is therefore of the utmost importance that early cases should be detected and efficiently isolated until the virulent Klebs-Löffler bacillus ceases to be found.

Public elementary schools, where a large number of children at a susceptible age are crowded together within limited floor and air space, and where, in addition to breathing each other's exhalations, they also frequently share pencils, slates, and books in common, are fruitful centres of infection. In an outbreak of diphtheria implicating children attending a common school, not only should these children and members of their household be excluded from school attendance, but the school should be visited and swabbings taken from the noses and throats of all scholars showing signs of faucial inflammation or of rhinitis. In this way unsuspected cases, which might become future foci of infection, may be detected and weeded out.

In the case of patients convalescing from diphtheria the bacilli may remain in the fauces for many weeks or months. As long as typical and virulent forms are to be found, the patient must be regarded as capable of disseminating the disease, and should be isolated. The writer has found the average duration of the infectious period to be about thirty days. Sometimes bacilli remain for months apparently uninfluenced by any local treatment. These bacilli are generally of the short, atypical variety, and when tested prove non-virulent. A plentiful supply of fresh air, the patient spending practically the whole day out of doors, is the most effectual way of causing their disappearance.

ENTERIC FEVER.

In the diagnosis of enteric fever by bacteriological methods it may be desired to isolate the typhoid bacillus from the urine, fæces, or blood, or to test the serum for the specific reaction described by Widal.

The Eberth-Gaffky bacillus is a short thick rod with oval ends, and occasionally forming short chains. It stains well with methylene blue (Löffler), and is decolourized by Gram's stain. Growth is abundant on all ordinary media. It is found in the stools, urine, and blood, and occasionally in the pus of suppurations following an attack.

Methods of Demonstration.—In the fæces the bacilli are present from the earliest stages of the disease, and recent investigations have shown that by using special culture media combined with colour reagents it is possible to differentiate the Eberth-Gaffky bacillus from the *B. coli*, and to establish a diagnosis even earlier than can be done by the agglutination reaction. This procedure is, however, of a delicate and elaborate character, and can only be performed in a properly-equipped laboratory. A small quantity of fæces should be drawn into a sterilized glass syringe, and transferred from this to a wide-mouthed bottle, which is then securely corked and sealed for despatch. No disinfectant should be allowed to come into contact with the stool from which the specimen is taken, and the syringe used should be destroyed in the fire.

In the urine typhoid bacilli are commonly present from the end of the second week of the attack, and may persist for many weeks or months. They are not generally to be found in film preparations, so it is necessary to make plate cultures. The urine should be drawn off by means

of a well-boiled catheter lubricated with glycerine, and should be received into a sterilized glass bottle, which is then securely corked and sealed.

In the blood, again, the organism is not present in numbers sufficient to render it likely that they would be detected in cover-glass preparations. A few drops of blood having been obtained under aseptic precautions by puncturing the lobe of the ear, or by exploratory puncture of the spleen by a hypodermic needle, or by drawing a few cubic centimetres from one of the superficial veins, a series of tubes is inoculated and despatched for incubation.

Of the three methods mentioned for detecting the presence of the *B. typhosus*, we regard the examination of the urine as that calculated to give the best results.

In **suppurations** following enteric fever (boils, periosteal abscesses, etc.) cover-glass films of the pus stained with Löffler's methylene blue for five minutes may, when examined under the microscope, show the specific bacillus. It is advisable in addition to inoculate a series of tubes.

If it be required to confirm the diagnosis at a post-mortem examination, cover-glass preparations and cultures may be made from the spleen or liver, or from local lesions, such as meningitis, pneumonic lung, or suppurative foci.

The **serum diagnosis** of enteric fever is founded upon the observation that if a drop of the blood of a patient who has recently been affected by or is then suffering from enteric fever be added, after proper dilution, to an actively moving culture of the *B. typhosus*, it has the power of checking the mobility of the organism and of aggregating the bacilli into clumps. The value of this reaction from the point of view of diagnosis is great, and its trustworthiness has been tested by a large number of experiments. Of over five hundred

cases tested by the present writer, about one-half of which number were enteric fever and the remainder various pyrexial diseases, the degree of error has not been more than 4 per cent. With increasing familiarity with the technique, and with the higher dilutions now employed, the chances of error should be still further diminished. This reaction may be obtained as early as the seventh or eighth day of the disease, and may persist for several months after convalescence.

Even in advanced cases of typhoid a clinical diagnosis can frequently only be arrived at by a process of exclusion. The method of serum diagnosis has therefore proved of great service, and the fact that it can be quickly made places it far above any process that depends upon isolating and recognising the typhoid bacillus.

It is not, as a rule, possible or indeed advisable for the practitioner to perform this test himself, as cultures of virulent germs should only be kept in places specially reserved for bacteriological purposes. The medical attendant should, however, in cases of suspected typhoid fever, and in all cases of continued fever without obvious cause, secure a sample of the blood for the purpose of submitting it to Widal's test.

If after repeated trials Widal's reaction is still negative, although the clinical aspect of the case is that of typhoid fever, it is possible that the patient is suffering from *paratyphoid fever*, a closely allied condition, and a specimen of blood should be sent to the laboratory with the request that its agglutinating properties be tested on cultures of paratyphoid bacilli.

Method of Obtaining the Blood.—The blood is best obtained in small capillary pipettes with a bulb in the centre, or in the capillary tubes supplied for vaccine lymph. Either the finger or the lobe of the ear may

be punctured, the skin having been first washed and rendered aseptic. If the finger be chosen, the procedure is as follows: A drop of blood is allowed to accumulate over the puncture. One of the open ends of the capillary tube is then inserted into it, and as much blood as can be obtained is allowed to run in. If a sufficient quantity does not enter at the first attempt, the blood already collected may be driven further up the tube by gentle shaking, or by the application of moderate heat; the finger is then squeezed, a second supply taken, and the ends of the tube sealed by holding in the flame. It is advisable to avoid sucking the blood into the tube with the mouth, as such a procedure is not unaccompanied by risk of infection. In sealing the ends of the tubes care must be taken not to heat the blood, or the agglutinating power will be destroyed.

If capillary tubes are not available, the drops of blood may be received upon the surface of glazed note-paper. They are allowed to dry, and are then ready for despatch. The reaction may also be obtained from such secretions as the milk, urine, and tears.

Professor Wright showed that the agglomeration or precipitation took place equally well when the serum of an enteric patient was added to an emulsion of dead bacilli. His emulsions are contained in small glass capsules, and are, of course, non-infective. The mixture of diluted typhoid blood and dead bacilli emulsion is made in tubes especially constructed for the purpose, and is allowed to stand for twenty-four hours. If the fluid remain turbid the blood is not taken from a case of typhoid fever; if it be clear, or nearly clear, the diagnosis of enteric is confirmed. This procedure, which is described at length in the *British Medical Journal* of January 16, 1897, is suitable for use in private practice. The sedimenta-

tion tubes are manufactured by Dean, of 73, Hatton Garden, E.C.

Treatment.—Whilst typhoid bacilli are most numerous in the intestinal tract, mesenteric glands, and spleen, yet the fact of their being found in the urine and blood and in the pus of suppurative complications would point to the infection being a general one. Attempts to prepare an efficient antitoxin and vaccine have, however, up to the present not passed beyond the experimental stage. The administration of antiseptics by the mouth tends to check fermentation in the intestines, and may thus limit the absorption of toxins. They appear to have but little effect upon the growth and activity of the typhoid bacillus.

Preventive Treatment.—The dissemination of typhoid fever is directly or indirectly the result of contamination by dejecta containing the living bacilli. Bed-linen, clothing, feeding utensils, urine-bottles, or bed-pans which have been soiled by the sputum, blood, fæces, or urine of the patient may thus be sources of infection.

If the imperfectly disinfected dejecta be discharged into leaky drains and sewers, or discharged upon the soil, neighbouring water-supplies may become contaminated. Water polluted in this way and used for rinsing milk-pails may infect the milk and give rise to an epidemic outburst. Oysters and water-cresses lying in specifically polluted water-courses have in like manner proved sources of infection. Dust consisting of dried particles of infected matter may propagate the disease, but the part played by sewer gas is difficult to estimate.

The preventive measure of the first importance is the disinfection of the dejecta. The stools should be received in carbolic acid (1 in 20), or in a mixture of four parts of slaked lime in 1,000 of water, and allowed to remain for

one hour. Thorough mixture should be insured by stirring with a piece of stick, which may be afterwards burned. Urine and sputum should be treated in the same way. All utensils should be disinfected by boiling, or by steeping for half an hour in 1 in 20 carbolic lotion. A solution of the same strength is employed for the disinfection of linen before sending it to the wash. The sick attendants must pay scrupulous attention to the disinfection of their hands. In times of epidemic, or when there is likelihood of infection, both milk and water should be boiled.

PLAGUE.

The bacillus of plague was first fully described by Yersen in 1894. It is found during life in the blood, sputum, and buboes, and after death may be recovered from most of the organs. In form it is a short rod with rounded ends, and stains more readily at the ends than in the centre. Growth is abundant on agar, and on artificial media generally there is a tendency to develop coccoid and involution forms. In broth cultures the plague bacillus forms chains. It does not liquefy gelatine, and is decolourized by Gram's method.

Method of Examination.—Films should be prepared from the sputum and blood, fixed by passing through the flame, and then stained with methylene blue. If buboes are present, the needle of an exploring syringe, previously sterilized by boiling, is thrust into the swelling and some of the fluid withdrawn. From this fluid films are prepared and cultures made. The plague bacilli appear as short, unevenly staining rods, generally in pairs and mixed with cocci. In all cases it is desirable to send to the laboratory a portion of the sputum or juice from

the buboes, in order that the diagnosis may be confirmed by inoculation experiments.

The infection of plague is to a large extent spread by personal contact, and the epidemic of 1898-99 has followed the lines of commercial communication by sea and land. Insanitary conditions and overcrowding are favourable to its extension. Perhaps the most important means of dissemination is by rats. These creatures are readily attacked by plague, and die in large numbers. Infection is carried from rat to rat by means of fleas, and it is possible that these insects are also responsible for the spread of the disease to man. For Serum Therapeutics, see p. 99.

CHOLERA.

The cholera spirillum is a short, stout, comma-shaped rod. It grows well on all the ordinary media, and is best stained with carbol-fuchsin diluted with four parts of water, or with Löffler's methylene blue. It is found in the intestine only, forming powerful toxins which, when absorbed, give rise to the typical symptoms.

In suspected cases of cholera a microscopic examination of the stools should be made by means of film preparations. A flake is picked out of the rice-water stools by means of sterilized wires. It is then flattened out between two cover-glasses, dried, fixed by passing through the flame, and stained with carbol-fuchsin. In such a preparation the spirilla may be seen in large numbers lying parallel to each other, and with few extraneous organisms. Diagnosis, however, cannot be based upon microscopical examination alone, for there are other organisms, such as Metchnikoff's spirillum and the Finkler-Prior spirillum, which very closely resemble Koch's comma bacillus. It is therefore necessary to

have cultures made, and for this purpose a small quantity of faecal matter is drawn into a syringe, transferred to a sterile bottle, corked and sealed, and despatched to a laboratory. The syringe used for the purpose is afterwards destroyed in the fire.

Treatment. — Professor Haffkine's antitoxin treatment of cholera has established the fact that an artificial immunity can be established in man, at least for a short period of time. Up to the present his results have been most encouraging, but further observation as to the permanent value must be awaited. The treatment is prophylactic, not remedial.

The infection of cholera is spread in a manner similar to that of enteric fever—by fomites, by water, or by food which has directly or indirectly been contaminated by the dejecta of a patient suffering from the disease. The precautions to be taken to prevent its spread are therefore similar to those described under Enteric Fever.

MENINGITIS AND EPIDEMIC CEREBRO-SPINAL MENINGITIS.

Purulent meningitis may be excited by a variety of organisms, chief amongst which are the tubercle bacillus, pneumococcus, and staphylo- and strepto-coccus.

Epidemic cerebro-spinal meningitis is due to invasion by the *Diplococcus intracellularis meningitidis* of Weichselbaum, and the organism described in association with posterior basic meningitis of infants is probably an attenuated form of the same coccus.

The *Diplococcus intracellularis* is a small coccus arranged in pairs or tetrads, with the opposed surfaces flattened, and is found lying within the pus cells. It stains well

with methylene blue, but is decolourized by Gram's method. Cultures may be made on agar.

Method of Examination.—In order to obtain material for examination it is necessary to withdraw it from the cerebro-spinal meninges, and this is accomplished by means of *lumbar puncture*.

Withdrawal of cerebro-spinal fluid may be practised for purposes either of diagnosis or of treatment. The therapeutic results following the reduction of pressure by this means have not been encouraging; but much light may be thrown upon the case by the chemical, bacteriological, and microscopic examination of the fluid obtained. Lumbar puncture is then chiefly resorted to with a view to assisting diagnosis. The technique of the operation is as follows: The patient, if not anæsthetized, is seated on a chair, and the body is strongly bent forward so as to make as great a space as possible between the spines and laminæ of the lumbar vertebra. The skin over the lumbar vertebræ is then disinfected, being washed with ether, and then with a solution of 1 in 500 perchloride of mercury. A fine cannula and trocar, previously sterilized by boiling, are then thrust into the spinal cavity well below the termination of the cord. The puncture should be made between the second and third, third and fourth, or fourth and fifth lumbar spines, or at the level of a line joining the highest points of the iliac crests. In children the trocar may be placed in the middle line immediately between two spines; but in adults it is preferable to select a spot a quarter to half an inch from the middle line, opposite the junction of the middle with the lower third of a spinous process. The point of the trocar should be directed upwards and inwards, and the dura mater may be pierced 1 to 3 inches from the surface. When the trocar is withdrawn the

amount of fluid that will escape through the cannula will vary according to the nature of the disease; thus in meningitis only a few drops may be secured, whilst in hydrocephalus it may amount to one or two ounces or more. In children, and in persons unlikely to stand the pain of the initial puncture, it is best to employ a general anæsthetic, the patient being kept lying on the side, with the back well arched. With a trocar the operation is usually not more painful than paracentesis thoracis; but the rapid drawing off of fluid with an aspirator sometimes gives rise to much pain, and for this reason the use of that instrument has been abandoned. The wound caused by the trocar is afterwards dressed with gauze soaked in collodion.

Occasionally severe pains extending to the lower limbs result from lumbar puncture, and the rapid removal of the fluid in a case of cerebral tumour has been followed by sudden death. As a therapeutic measure this procedure has been chiefly used for the relief of tension in cases of hydrocephalus, cerebral tumour, and spinal hæmorrhage. It has also been recommended in acute mania, and in some forms of chlorosis. The results in these cases already recorded are not encouraging.

For purposes of diagnosis the fluid should be examined for albumen, blood-cells, and micro-organisms, and a note should also be made of the quantity, reaction, colour, and specific gravity. The quantity of albumen serves to distinguish between a simple hydrocephalus and an inflammatory effusion. In the former not more than $\frac{1}{2}$ per cent. is usually present; in the latter, 1 per cent. is a fair average. Inflammatory effusion such as is found in meningitis is distinguished, too, by its cloudiness, its coagulability, and by the fact that it will usually be found to contain cells. The presence of blood would

point to rupture of a spinal or cerebral vessel, though it is obvious that here error might arise from accidental injury to vessels by the exploring needle. The most useful results have undoubtedly been obtained by bacteriological examination of the fluid, it being possible by this means to distinguish between a meningitis of tubercular origin and the same affection due to other micro-organisms.

The fluid which escapes from the cannula should be collected in a series of sterilized test-tubes. If the fluid be cloudy or if it contains pus the sediment should be allowed to settle, the supernatant fluid poured off, and films prepared and cultures made from the sediment. Films should be stained with methylene blue and by Ziehl-Neelsen's and Gram's method, so as to facilitate the finding of pyogenic organisms, pneumococci, tubercle bacilli, and the *Diplococcus intracellularis*. If there be but a slight opacity in the fluid the sediment must be obtained by means of the centrifuge, and a further portion of the fluid should be despatched to the laboratory for testing by inoculation.

RELAPSING FEVER.

The *Spirillum Obermeieri* is a delicate spiral filament, about five times as long as a red blood corpuscle, and is to be found in large quantities in the blood of patients suffering from relapsing fever during the pyrexial attack. They are easily recognised in preparations of the fresh blood, and in blood-films prepared in the usual manner, and stained with Löffler's methylene blue. Neither the parasite of malaria nor that of relapsing fever can be grown on artificial culture media.

The procedure to be adopted in examining the blood

in the fresh state is as follows: A glass slide and cover-slip are gently warmed. A drop of blood obtained by puncturing the lobe of the ear is received upon the centre of the cover-glass; this is then inverted over the slide, and the blood allowed to spread out between the two. To prevent drying by evaporation a little vaseline is smeared around the edge of the cover-slip. The specimen is then examined, first under $\frac{1}{6}$ and then under $\frac{1}{12}$ inch objectives. At least six cover-glasses should be so examined. This method is also to be followed in suspected cases of malaria, filaria, and trypanosomiasis.

MALARIA.

Malarial fever is a general infection consequent upon invasion of the system by the *Plasmodium malariae*, a body conveyed to man through the bite of a species of mosquito (*Anopheles*).

In suspected cases of malarial fever the blood should be examined just before the time of rigor.

The following are Manson's directions for making stained films. The skin having been cleaned and punctured, a drop of blood about the size of a pin's head is allowed to collect. A slip of tissue-paper ($1\frac{1}{2}$ inches by $\frac{3}{4}$ inch), which has been previously provided, is applied to the exuded blood, so that it touches the drop about $\frac{2}{3}$ inch from one end of the strip. This is immediately laid, blood-surface downward, on a carefully cleaned cover-glass or microscopic slide, and directly the blood is seen to spread out, the paper is drawn along the glass. In this way a very fine film of blood is obtained, which, as soon as dry, is covered by pouring on a little of Jenner's blood stain. The stain is allowed to remain on from three to five minutes, and then rapidly

washed in distilled water and the film dried and mounted. With this method no additional process of fixing is required. Whilst staining, the films should be covered to prevent the dye evaporating. By this method the red cells are stained red, the nuclei of the white corpuscles a deep blue, and the malarial parasites within or without the red cells a pale blue.

Another method of staining is to fix the blood-film by immersing in a mixture of equal parts of alcohol and ether for fifteen minutes, and then staining with a saturated watery solution of methylene blue for five minutes ; wash, dry, and mount. The nuclei are stained a dark and the parasites a pale blue.

A series of films of the fresh blood should be made, in accordance with the directions given on p. 68.

There are three well-recognised forms of malaria parasites—the tertian, quartan, and æstivo-autumnal. The size and shape of these and the presence or absence of pigment will vary with their stage of development.

In stained films the tertian and quartan parasites appear within the red blood cells as faintly stained blue bodies, which, as they increase in size, develop pigment granules, and finally assume a segmented rosette appearance. In both tertian and quartan fever ring-shaped parasites may be found.

In æstivo-autumnal fever the distinctive feature is the presence of a crescent-shaped body, staining faintly blue and containing coarse granules of pigment.

In specimens of the fresh blood the plasmodium is seen as a rounded, pigmented body with amœboid movements lying within the red corpuscle, and if the specimens be kept for twenty minutes or half an hour, flagellated bodies, probably derived from these plasmodia, may be seen freely moving about in the serum. Whichever

method of examination be adopted, the search for the plasmodium should not be abandoned until several slides have been examined, as the number of parasites may be very few. If quinine has been administered in full doses the intra-corporeal forms will not be found, as the drug causes their speedy destruction. Crescent forms and flagellated bodies may, however, be detected well into the convalescent stage two or three weeks after the rigor. The discovery of the plasmodium in the blood is absolutely diagnostic of malaria. With regard to treatment, it is probable that quinine has a direct toxic influence upon the parasite, or that it acts by exciting phagocytosis. According to Manson ('Tropical Diseases'), it should be given as soon as the sweating stage commences—10 grains at once, followed by 5 grains every four or six hours for the next three or four days.

TRYPANOSOMIASIS.

Trypanosomes belong to the order of the protozoa. They have recently been described in the blood and cerebro-spinal fluid of human beings in association with sleeping-sickness in negroes, and long-continued fever with enlargement of the spleen in Europeans. The *T. hominis* is of a worm-like shape, about four times the length of a red blood-corpuscle, one end blunted, the other terminating in a flagellum. The body has an undulating membrane and a nucleus. Trypanosomes are communicated to man by the bite of a tsetse fly (*Glossina palpalis*).

Method of Examination.—The cerebro-spinal fluid obtained by lumbar puncture is centrifugalized and the sediment examined under a $\frac{1}{8}$ -inch objective. Fresh films of the blood are first examined for parasites (see p. 68), and dried films prepared and stained by Jenner's



FIG. 10.

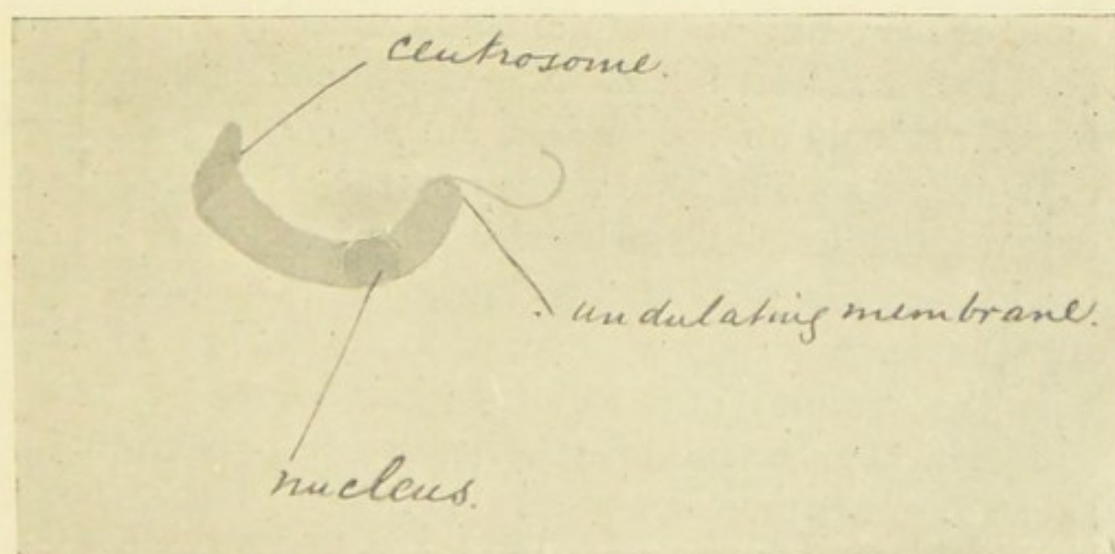


FIG. 11.

TRYPANOSOMES IN CEREBRO SPINAL FLUID.

blood-stain or by Leishmann's modification of Romanowsky's stain. In the fresh specimens the trypanosomes are seen to be actively motile; in films stained by Jenner's method the parasite is stained blue.

CHAPTER V.

DISEASES OF THE RESPIRATORY SYSTEM.

Tonsillitis, Quinsy, and other Anginæ.—By far the commonest organism found associated with acute tonsillar inflammations is the streptococcus. The streptococcus is a normal inhabitant of the mouth, but in angina it becomes altered in character and multiplies greatly. A stained cover-glass film from a case of 'ulcerated throat,' or acute follicular tonsillitis, will show numerous long chains, and an inoculated tube may demonstrate them in almost pure culture. Staphylococci, pneumococci, *B. coli*, *B. pseudo-diphtheriæ*, *Diplococcus rheumaticus*, *B. influenza*, and the fusiform bacillus, are also frequently found associated with faucial inflammations. These organisms are found not only on the surface, but also in the substance of the tonsils. They are frequently the exciting cause of suppuration in neighbouring lymphatic glands and cellular tissues, and may further infect the system, exciting septicæmia, pyæmia, ulcerative endocarditis, and so on.

In the treatment of tonsillitis by gargles, washes, paints and sprays, only such microbes as are on the surface are affected. It is therefore preferable that the fauces should be firmly and frequently swabbed, and that a few minims of carbolic acid (1 in 20) be injected into the substance of

the tonsil. The writer has found this the most efficacious treatment in severe septic angina.

Membranous Rhinitis and Laryngitis.—In all cases of fibrinous rhinitis and of laryngitis swabbings of the nose or throat should be taken and sent for incubation, in order, as far as possible, to exclude the presence of diphtheria bacilli.

True membranous rhinitis and laryngitis may be excited by organisms other than the Klebs-Löffler bacillus. It may be of such severity as to necessitate tracheotomy, and may terminate fatally. The membrane is generally much softer and less adherent than in diphtheria, and the invading organisms are staphylo- and strepto-cocci, leptothrix, and sarcinæ.

Acute Bronchitis.—Many cases of bronchitis are of an infective nature, and although the sputa are exposed to contamination in the mouth, a bacteriological examination of expectoration may make it possible to ascertain the invading organism. The organism most commonly found is the streptococcus, and then in order follow *B. coli*, *Diplococcus pneumoniae*, *B. influenzae*, staphylococci, and *B. diphtheriae*, together with various saprophytic species.

Films should be prepared from the sputum and stained with methylene blue and by Gram's method. Agar tubes should be inoculated.

In **chronic bronchitis, bronchiectasis, and gangrene of the lung**, a great variety of cocci, bacilli, and moulds are present in the expectoration. In putrid bronchitis a specific bacillus, which is said to give rise to the characteristic odour, has been described by Lumniczer. Sarcinæ and leptothrix occur in the sputa from bronchiectatic cavities. The organisms causing gangrene of the lung are highly intensified staphylo-, strepto-, and pneumococci, a bacillus resembling *B. œdema maligni*, and a coli-

form bacillus. To these are superadded saprophytic varieties, such as *leptothrix*, *sarcinæ*, and *spirochetæ*.

Broncho- or Catarrhal Pneumonia.—Although the pneumococcus and pneumo-bacillus are frequently present in cases of broncho-pneumonia, the presence of these or other microbes cannot, therefore, be regarded as the specific cause of the disease. Frequently it is secondary to some other affection. Thus, in diphtheria, the *Klebs-Löffler* bacillus may be found in the consolidated lung, in typhoid fever the *B. typhosus*, in influenza the *B. influenza*, and in septic conditions one or other of the pyogenic cocci. Microscopic film preparations from the sputum cannot be trusted as a means of diagnosing the nature of the infection, but, for the purpose of confirming diagnosis, films and cultures may be made after death from scrapings of the consolidated lung tissue.

LOBAR PNEUMONIA.

By far the most common micro-organism associated with lobar pneumonia is Fraenkel's pneumococcus, whilst next in frequency may be mentioned Friedlander's pneumo-bacillus.

Fraenkel's Pneumococcus, as seen in stained films of pneumonic sputum, is a small diplococcus with lancet-shaped ends surrounded by a clear halo representing the capsule. It is best cultivated on blood agar.

Friedlander's Pneumo-bacillus closely resembles the above, but is a short, stout rod with rounded ends. It possesses a capsule, but differs from the pneumococcus in that it is decolourized by Gram's staining process. Lobar pneumonia should be regarded as an acute general infection, of which the pulmonary hepatization is a leading feature, but in which the pneumococcus is free in the

blood stream, and may excite inflammatory and suppurative lesions elsewhere, such as pericarditis, otitis, meningitis, empyema, peritonitis, phlebitis, ulcerative endocarditis, and tonsillitis.

Methods of Examination.—The pneumococcus may be sought for in the sputum or in the pus from one or other of the suppurative lesions named above. Its presence in the sputum cannot, however, be held to be diagnostic of lobar pneumonia, as the organism is frequently found in healthy sputa, in tonsillitis, in bronchitis, and in broncho-pneumonia. There is, however, strong presumptive evidence of lobar pneumonia if in rusty or white gelatinous sputum we find the lanceolate coccus in pure or almost pure culture. The expectoration should be examined in as fresh a condition as possible, for if it be allowed to remain for some hours the capsules appear to undergo a process of digestion which renders their demonstration by staining methods a matter of some difficulty. A small quantity of expectoration having been obtained in a sterilized vessel, cover-glass films are prepared and fixed. These are then stained for about half a minute in carbol-fuchsin, washed, and mounted. The diplococcus appears deeply stained, surrounded by a clear halo. A second cover-glass should then be stained by Gram's method, the fact of the organism not being decolourized serving to distinguish it from the pneumobacillus.

From localized abscesses and empyemata the pus is drawn off by means of a sterilized syringe, the skin having first been disinfected. In all cases a little of the exudation should be collected in a sterile pipette for examination by cultural or inoculation methods.

Prognosis, Diagnosis, and Treatment.—As we have mentioned elsewhere, the pneumococcus is not

generally to be found in the blood, but if its presence be demonstrated in that situation the prognosis is most grave. The variety of lesions which may be caused by the pneumococcus is very great, and these may occur as complications during the course of an acute attack of pneumonia or as independent affections. Sometimes two or three acute suppurative processes will complicate a case of pneumonia. Thus, we have recently investigated cases of lobar pneumonia in which there was accompanying middle-ear infection, infection of middle ear and peritoneum, and infection of middle ear and cerebral and spinal meninges. In the two first of these pneumococci were found in the spleen after death, showing that the infection was widespread and general. A knowledge of its bacteriology will lead to a considerable modification of our conception of pneumonia, so that we shall regard it more as the local manifestation of a specific general infection than as simply an inflammatory condition of the lung.

Cerebral symptoms and pain in the ears should lead to an inspection of the tympanic membranes, and if pus be present in the middle ear this should be evacuated by means of an incision. The extension of the suppurative process to the cerebral meninges may thus be checked, and a fatal termination averted.

The grave symptoms of the disease are caused by the absorption of toxins from the seat of the local lesion, and in view of this fact attempts have been made to immunize animals against the pneumococcus, and to obtain from their blood an effective antitoxin. See Serum Therapeutics, p. 97.

PLEURAL EFFUSIONS.

The organisms most frequently found in serous and purulent pleural effusions are the pneumococcus, streptococcus, staphylococcus, and tubercle bacillus. These may occur in pure or in mixed cultures, or may be associated with putrefactive bacteria giving rise to offensive smell, or with *B. coli* or *B. termo*.

Clear serous effusions are generally due to invasion by the tubercle bacillus, and later, when they become purulent, the pus is thin and flaky. Staphylococci are often associated with tubercle bacilli. When the pneumococcus is the infective agent the pus is greenish, thick, and creamy, with the *B. coli*, *B. termo*, and putrefactive bacteria offensive, and perhaps accompanied by a formation of gas.

Method of Examination.—A portion of skin over the chest-wall having been disinfected, a sterilized exploring needle is introduced, and a small quantity of the effusion drawn off. By allowing a drop to fall from the syringe on to the platinum loop, tubes may be inoculated for incubation and a series of films prepared. One film should be stained for a few seconds with carbol-fuchsin, washed, dried, and when mounted examined for strepto- and pneumo-coccus. It is not generally difficult to detect these organisms when present, but the case is very different with the tubercle bacillus. Although a large number of films stained by Ziehl-Neelsen's method may be examined, the result is frequently negative, and similar failure may attend the examination of the sediment obtained by centrifugalization. In such a case the only trustworthy method of establishing the presence or absence of tubercle is by means of inoculation experiments.

Prognosis. — Pneumococcal empyemata generally

warrant a more favourable prognosis than those due to any other organism. It is claimed that such may be cured by simple aspiration, but this is not our experience.

The prognosis in cases of streptococcal invasion is very grave, whilst tubercular empyemata, although affording less imminent risk to life, are very slow to recover. This last remark also applies to staphylococcal empyemata, probably because they are in many cases complicated by the presence of tubercle bacilli. If the pus be found to contain *B. coli* or a variety of putrefactive organisms, the pleura has possibly been invaded by an abscess in connection with the abdominal viscera, and the case may be one of subphrenic abscess. Foul-smelling empyemata, and those in which free gas is found, heal with great difficulty.

INFLUENZA.

Influenza reappeared in this country in 1889, and since then there has been a succession of epidemics. The bacteriological diagnosis of the disease is a comparatively easy matter, and it is to be regretted that it has not been more frequently applied in doubtful cases. The *B. influenzae* was first described by Pfeiffer in 1892. It is the smallest of the known pathogenic bacteria, 0.5μ in length, a fine, short rod with rounded ends. It often occurs in pairs or chains, and sometimes in stained specimens shows an enveloping halo, in these respects resembling the pneumococcus, from which it is to be distinguished by its smaller size and decolourization by Gram's stain. The *B. influenzae* does not stain readily, but prolonged staining in methylene blue or exposure for five minutes in carbol-fuchsin, diluted with 5 parts of water, will yield good

results, and demonstrate the fact that the ends stain more deeply than the body of the cell.

Method of Examination.—A sterile swab, such as is used in making diphtheria cultures, is passed into the nose or throat, and from this films are prepared and cultures made.

The films, after being dried, fixed, and stained, may in cases of influenza show large numbers of the bacilli almost in pure culture lying within and without the cells. In cases of bronchitis with expectoration the sputa should be received in a vessel containing a little normal saline solution, washed by gently agitating for a minute, and then picked out with a platinum loop and transferred to tubes of culture media, or sent to the laboratory in glass test-tubes previously sterilized by boiling. The best medium for the growth of the *B. influenzae* is blood agar (see p. 33).

PULMONARY TUBERCULOSIS.

Sputum.—The demonstration of the tubercle bacillus in the sputum of cases of suspected tubercular infection of the lungs or respiratory passages is one of the commonest bacteriological tasks which falls to the lot of the practitioner. This method of diagnosis is of peculiar value, as it permits of the recognition of the disease at its earliest stage, before physical signs are pronounced, and when treatment is most likely to be effectual.

Method of examining Sputum.—The sputum first coughed up in the morning should be taken, as it is more solid, less contaminated with food particles, and represents the mixture of the various secretions more fully than that coughed up at other times. A little is spread

out on a slide against a dark background, and from this the small yellowish specks, streaks or curdy masses, are picked out by means of the platinum loop. The fragment thus selected is squeezed between two cover-glasses, and when the sputum is spread out as far as possible the two are separated by sliding them apart, when each presents a delicate film. When dry the films are passed through the flame and fixed. They are then stained by Ziehl-Neelsen's method, as described on p. 22.

Prepared in this way the tubercle bacilli appear as delicate red rods, more or less beaded, straight or slightly curved. Other organisms, such as pus cocci and fragments of cells and tissues, are stained blue. The colour and beading of the bacilli varies in different cases, and they are described as being of a much brighter red colour and more markedly moniliform in acute than in chronic cases.

The number of bacilli in a sample of sputum cannot be regarded as any measure of the acuteness of the process of disease. In tubercular ulceration of the larynx or of the naso-pharynx bacilli appear in the sputum. These, however, are generally secondary infections, and it is impossible in such cases to decide from what source the bacilli have come. If hæmoptysis be present, the bacteriological examination had better be deferred until the sputum is nearly free from blood. The presence of tubercle bacilli in the sputum may be regarded as pathognomonic of phthisis pulmonalis, but failure to detect the organisms in ordinary cover-glass preparations cannot be held to exclude the disease. In some cases the bacilli are very few in number, and of a score of films only one will be found to show the tubercle bacillus. It is advisable, therefore, in doubtful cases to have a further

examination of the sputa. For this purpose some of the morning sputum is collected in a wide-mouthed glass bottle, previously sterilized by boiling, and this, after being hermetically sealed, is sent to the laboratory, where it is centrifugalized after shaking with 1 in 20 carbolic acid. A still more decisive test is the subcutaneous injection of the sputum into animals.

It is not usually practicable to obtain cultures of the tubercle bacillus by inoculating tubes with the sputum or other discharges.

Tubercular Pleural Effusions (see p. 77).

Prevention and Treatment.—Whilst there are certain causes which predispose to tuberculosis, such as dampness of soil, imperfect ventilation, overcrowding, deficient light, nature of employment, and hereditary tendency, yet the actual determining cause must be the entrance of the tubercle bacillus into the tissues. This may be effected either by inoculation or by swallowing or by inhalation. The introduction of the organism by means of inoculation is chiefly confined to cases of accidental contamination of wounds in surgical practice, or of the hands of persons conducting post-mortem examinations, and is becoming increasingly rare since the introduction of antiseptic methods. By far the greater danger arises from the dissemination of tubercle bacilli by means of the sputa of phthisical patients. In this way all vessels, clothing, surrounding objects, and the walls of the room in which the patient lives, may become contaminated and prove a source of infection to others. The expectoration, when dried and powdered, is capable of widespread dissemination, in the form of dust particles containing active bacilli or their spores. This being so, it becomes imperative that the strictest precautions be taken. Sputa should be received in 5 per cent. carbolic

lotion, or in small pieces of linen or paper, and burned. As far as possible a room and all utensils should be devoted exclusively to the use of the patient, and in a fatal case all clothing, bedding, etc., should, together with the apartment, be disinfected. In advanced cases the dejecta should be mixed with some disinfectant, and all the dusting of the apartment should be done with a cloth moistened with some antiseptic solution. It is difficult to apply these regulations to incipient cases of phthisis which are not confined to bed, but they should be instructed as to the nature of the danger, and urged to exercise every precaution. The removal of phthisical patients from the wards of general hospitals, and their treatment in separate sanatoria, is much to be desired. Food is a constant vehicle for the introduction of the tubercle bacillus. The milk from cows with tuberculous disease of the udder has infective qualities, and the flesh of animals with only localized tuberculous lesions may become infected in the process of cutting up. Boiling the milk and the thorough cooking of meat appear to be efficient safeguards.

It should be mentioned here that Koch has recently denied that there is any relationship between human and bovine tuberculosis, but his statements have not been generally accepted in this country.

The compulsory notification of phthisis would permit of the issue of useful information to the relatives of the sufferer, and insure the disinfection of rooms at the termination of the case.

The serum treatment of phthisis has not up to the present met with such a measure of success as to warrant its general adoption. The open-air treatment in suitable cases, and under favourable conditions of climate, is most beneficial, and depends no doubt to some extent

upon the known inhibitory effect of sunlight and fresh air upon bacterial growth and activity.

Cod-liver oil, creosote, and other drugs extensively employed in the treatment of phthisis can in no way be regarded as having specific action. In this disease, as in most others due to specific microbes, the hope for the future lies in prevention.

CHAPTER VI.

DISEASES OF THE ALIMENTARY SYSTEM.

THRUSH.

IN certain disorders of the digestive tract, especially when associated with feeble energy and impaired nutrition, the buccal mucous membrane becomes the seat of growth of a mould, the *Saccharomyces* or *Oidium albicans*. Occasionally the growth may spread to the œsophagus, stomach, and intestines. This condition, known as thrush, is especially liable to attack infants in the first few months of life, giving rise to the formation of thin, opaque, whitish specks of membrane on the tongue, cheeks, and pharynx, occasionally accompanied by slight superficial ulceration.

Method of Examination.—A small portion of the white membrane should be detached and teased out in glycerine. Microscopically it will be seen to consist of epithelium and débris, in which lie the long mycelial threads and the spores of the thrush fungus. The cells of the mycelium are about $5\ \mu$ thick, and ten to twenty

times as long. The filaments end in roundish cells which produce one or more spores.

The vitality of the thrush fungus is very low, and it is readily killed by the action of some weak antiseptic such as glycerinum boracis. The mouth should be first cleansed by swabbing and the drug then applied. Careful cleansing of nipples, feeding-bottles, spoons, etc., may prevent the appearance of the parasite or its conveyance to other children. In all cases the constitutional treatment is the most urgent necessity.

DILATATION OF STOMACH.

In the vomit of persons suffering from chronic dilatation of the stomach there may occur certain micro-organisms the recognition of which may afford considerable help in diagnosis. Chief amongst these are *sarcinæ*, *torulæ*, and the *B. filamentosus* of Boas and Oppler.

Method of Examination.—The vomit, or stomach contents drawn off by siphonage, is allowed to stand for one hour, and then several loopfuls of the scum which rises to the surface are examined diluted with water on a slide with a $\frac{1}{8}$ -inch objective.

Sarcinæ ventriculi are large ovoid cells grouped together in cubes of eight, the sides which are in contact being slightly flattened, and the whole resembling a wool-pack. *Sarcinæ* may occur in great profusion in stomach contents, and can be picked out and cultivated on gelatine. They are best stained with weak carbol-fuchsin (1 in 10).

Yeast cells, *Saccharomyces cerevisiæ*, are seen as large

ovoid cells with vacuoles and nucleus. They are readily distinguished by the fact that many of the cells are undergoing a process of budding.

The **B. filamentosus** described by Boas and Oppler is said to be pathognomonic of malignant disease of the stomach. It is generally to be found in those cases of malignant disease which are accompanied by delay of stomach contents, absence of hydrochloric acid and presence of lactic acids. The *B. filamentosus* is a long, thin, thread-like organism, sometimes thicker at one end than the other. It is non-motile, and grows slowly on glycerine-agar. In the vomited material it may form large tangled masses, which are readily seen in film preparations stained with methylene blue. In our own experience the detection of this organism has been of great diagnostic service.

DIARRHŒA.

Diarrhœa may be caused by the ingestion of food which has undergone putrefactive changes as the result of bacterial activity. Some cases of meat and fish poisoning are of this character, and the diarrhœa of infants fed from imperfectly cleansed bottles may have a similar origin. Latterly it has been recognised that diarrhœa may be excited by the growth and multiplication of bacilli and cocci within the alimentary tract, and that the process may be a true infection. In these cases the meat or other article of food may not have undergone putrefactive changes, and nothing wrong can be detected by taste or smell. The exciting organism thus introduced multiplies with great rapidity, and the train of symptoms excited by the toxins formed in the bowel

may be fatal in a few hours or days. In virulent cases with gastro-enteritis the infection becomes a general one, and after death the specific organisms may be cultivated from the heart's blood, spleen, and liver. The bacilli most commonly associated with outbreaks of meat-poisoning are the *B. enteritidis* of Gärtner, *B. botulinus*, and *B. coli*.

The summer diarrhœa of infants is of microbial origin, being probably in part due to a direct infection, and in part to the ingestion of milk and other food containing toxins elaborated by putrefactive organisms. It is essentially a filth disease, aggravated by overcrowding and want of light and fresh air. Ballard has shown that the temperature of the soil plays an important part in its epidemicity. The rise of diarrhœal mortality does not begin until the mean temperature recorded by the 4-foot earth thermometer reaches 56° F., and the maximum mortality is attained in the week in which the temperature recorded by the 4-foot earth thermometer attains its mean weekly maximum. The micro-organism or micro-organisms have their habitat in organically polluted soils. They multiply at a certain temperature, become airborne, and find a suitable nidus in food either inside or outside the body; from the food they elaborate a chemical poison which is the direct exciting cause of diarrhœa. No one specific organism can be designated as the cause of infantile diarrhœa, but amongst those most commonly associated with the disease may be mentioned *Proteus vulgaris*, *Streptococcus enteritidis*, and *B. enteritidis sporogenes*.

Cover-slip preparations made from the fæces will not infrequently show one or other of these organisms to be present in enormous numbers. They may also be demonstrated by inoculating agar or gelatine tubes from the excreta, or by taking cultures from the organs after

death. Frequently, however, such cultures show the presence of cocci only, or of the *B. coli* or other organisms.

Diarrhœa being a filth disease, all efforts for its prevention should be framed with a view to improving the cleanliness of soil, of surroundings, of food, or of person. 'Made ground,' or any ground open to organic soil pollution from cesspools or drains, should be avoided as dwelling sites. Back-to-back, dark and ill-ventilated houses, should be condemned, and strict attention paid to cleanliness in the preparation of food and drink. Especially important is the boiling of milk and of suspected water, and the preservation in a state of scrupulous asepticity of infants' feeding-bottles. With regard to meat-poisoning, it seems probable that many outbreaks would be avoided were the food raised to a higher temperature and cooked more thoroughly.

Whilst it is possible that putrefactive changes may go on after the cooking, and that bacteria may gain access subsequent to this operation, yet it is more probable that bacteria are introduced at an earlier stage, and, owing to imperfect cooking, are not destroyed.

DYSENTERY.

A variety of conditions have been included under the term 'dysentery,' many of them being acute or chronic inflammatory lesions of the bowels excited by various bacteria, such as those described by Shiga and Flexner. Tropical dysentery and abscess of the liver are associated with the presence of an amœba, which, although closely resembling the amœbæ normally found in the colon, differ from these in its larger size, coarser granules, and

greater abundance. The lesions are situated most commonly in the upper part of the great bowel.

Method of Examination.—A small portion of the stool, as soon as possible after it is passed, is allowed to spread out beneath a thin cover-slip on a warmed slide. The amœba appears as a cell five times as long as a red blood-cell, with nucleus, vacuoles, and dark granules, and possessing pseudopodial movement. It may be stained by running a drop of hæmatoxylin under the cover-glass. Fæces for examination should be despatched promptly to the laboratory, and kept warm during transit. The pus from a liver abscess is best examined from twenty-four to thirty-six hours after the abscess has been opened, as amœba are frequently absent from the first discharge.

In cases of bacillary dysentery a small portion of the fæces should be collected in a sterile bottle and despatched to the laboratory, together with a specimen of the blood, the latter to be tested for the agglutination reaction.

CHAPTER VII.

DISEASES OF THE GENITO-URINARY SYSTEM.

FOR the efficient bacteriological examination of the urine it is essential that it shall be obtained free from accidental contamination, and that when so obtained it shall be possible to secure sufficient sediment.

To obtain a specimen free from such organisms as are found in the anterior part of the urethra the following routine should be adopted: The meatus is washed with a solution of perchloride of mercury (1 in 2,000), and a rubber catheter which has previously been sterilized by

boiling, and lubricated with glycerine, is passed, the urine being received in a sterilized vessel, which is then promptly secured.

To obtain a sediment it may be sufficient in a cloudy and heavily loaded urine to allow the specimen to stand in a conical glass for several hours, and then to pick up the solid matter with a pipette. Far better results, however, are obtained by the use of the centrifuge, by means of which any and all bodies in suspension are collected and made available for examination.

The high-speed hand-driven centrifuge made by Beck and Co. answers this purpose excellently, and we have found Purdy's model, supplied by the same firm, but driven by electricity, an even more useful appliance.

BACILLURIA.

The simplest form of urinary infection is the condition known as bacilluria, in which the urine, without necessarily being altered in composition or giving rise to subjective symptoms, is loaded with bacteria, which may give it a cloudy appearance. The organisms most frequently found in bacilluria are *B. coli* and *B. typhosus*. Typhoid bacilluria most frequently occurs during and after the fourth week of the disease, and may persist for weeks or months. Invasion of the urine by *B. coli* is commonest in women, and the infection may take place either from the kidney, from neighbouring viscera, or *per urethram*. In cases of diphtheria, advanced tuberculosis, and general septicæmia, the specific organisms may be present in the urine. If bacilluria be suspected, a specimen of urine is withdrawn into a sterile bottle, and a drop immediately mounted and examined under a $\frac{1}{12}$ -inch oil immersion lens, when the field may be seen to be crowded

with organisms. Films are then stained, and cultures made from the urine. When the organisms are not so abundant, the urine, securely sealed, is sent to the laboratory, where it is examined after several days' incubation.

Bacteria, when free in the urine, are readily destroyed by weak antiseptics, of which one of the best, when administered by the mouth, is urotropin. This drug should be given in doses of $7\frac{1}{2}$ grains four times daily, and in cases of enteric fever its administration should be a routine treatment during the weeks of convalescence, as in this way a fruitful source of infection may be obviated.

URETHRITIS.

A condition of simple urethritis may be excited by many of the pyogenic organisms, whose presence may be demonstrated by means of stained films and cultures. Careful examination of these preparations will serve to differentiate the condition from gonorrhœa, especial attention being given to the shape and arrangement of the organisms, their situation within or without the cell, and their reaction to Gram's method of staining.

Gonorrhœa, see p. 33.

CYSTITIS.

A large variety of organisms are associated with cystitis, some being undoubtedly direct exciting causes of the disease, and others superadded saprophytic forms. The organisms most commonly causing this condition are staphylococci, streptococci, gonococci, *B. coli*, *B. tuberculosis*, *B. lactis aerogenes*, and *B. proteus*.

In cystitis of coccal origin and in mixed infections the

urine is generally alkaline; with *B. coli*, *B. typhosus*, and *B. lactis aerogenes* the reaction is acid.

Method of Examination.—The urine is drawn off and sedimented with the strictest aseptic precautions. Films are made from the sediment, and stained with methylene blue and by Gram's and Ziehl-Neelsen's methods.

If the sediment has been obtained by means of a high-speed centrifuge, there is seldom much difficulty in demonstrating the tubercle bacillus if it be present; and if the urine has been drawn off with the proper precautions and the films well washed with spirit before counter-staining, there can be little danger of confusing this with the smegma bacillus. Tubercle bacilli in urine are generally found in clumps, and are shorter than when obtained from sputum. In doubtful cases the presence or absence of the tubercle bacillus should be established by means of inoculation experiments.

Urinary antiseptics do not act uniformly in cases of cystitis. Thus urotropin, whilst it controls readily infection by *B. typhosus*, does not succeed in infection by *B. coli*, and cases of cystitis secondary to enlargement of the prostate improve much more readily than do those secondary to gonorrhœa. Generally it is advisable not only to render the urine antiseptic, but also to change its reaction. See article by author on 'Some Urinary Infections,' *Bristol Medico-Chirurgical Journal*, March, 1902.

PYELITIS, PYELONEPHRITIS, AND SUPPURATIVE NEPHRITIS.

The principles governing the examination of the urine in these diseases are identical with those given under the head of Cystitis, but wherever it is possible an attempt

should be made to collect separately the urine from the two kidneys by means of catheterization of the ureters. The kidney and ureter may be infected through the general circulation, or by extension from the bladder. The latter is the more common method. The organisms most commonly present are *B. tuberculosis*, *B. coli*, staphylococci, and streptococci. The reaction of the urine in pyelitis is generally acid, but in acute non-tubercular cases it may be alkaline, possibly from admixture of blood. It is generally possible to demonstrate tubercle bacilli, if present, in films prepared from the centrifugalized deposit. In doubtful cases inoculation should be resorted to. Only by consideration of the clinical symptoms, combined with a careful examination of the epithelial debris, is it possible to say from what part of the genito-urinary tract the deposit is obtained. Urinary antiseptics do not exert a marked beneficial effect on cases of infection of the body or pelvis of the kidney.

CHAPTER VIII.

DISEASES OF THE SKIN AND HAIR.

FOR the examination of the following parasites a lens ($\frac{1}{8}$ inch), capable of 300 to 500 magnifications, is sufficient:

Ringworm.—There are two varieties of this parasite—the *Microsporon Audouini* and the *Trichophyton megalosporon*. The former attacks the scalps of children, and its masses of spore, 3 to 4 μ in diameter, may be readily detected on the surface and in the substance of diseased hairs. The *Trichophyton megalosporon* is divided into two classes—the endothrix, which occurs chiefly in the hairs of adults, its spores lying within the hair; and the

ectothrix, which attacks the nails, skin, and beard, the spores being arranged in chains and resting on the outside. The spores of *Trichophyton megalosporon* measure 4 to 12 μ . To prepare hairs for microscopical examination: Pull out a short hair from the margin of the patch, wash in ether, and then soak in liquor potassæ (B.P. 6.8 per cent.) for six hours. Mount in glycerine jelly. (For rapid work the ether may be omitted, and the action of the liquor potassæ hastened by warming the slide.)

To obtain stained specimens, wash first in ether and then stain for two minutes in the following solution: 5 per cent. alcoholic solution of gentian violet 1 part, anilin water 3 parts. Dry in blotting-paper, and then stain in Gram's solution for two minutes; again dry, and treat with iodine in anilin oil; clear with anilin oil; wash in xylol; mount in balsam.

Tinea Versicolor.—The scales from an untreated patch are scraped on to a slide, and a drop of liquor potassæ and one of glycerine are added and mixed with them; a cover-glass is then pressed down over the whole. The parasite—the *Microsporon furfur*—is then seen as a fine branching network, consisting of short threads with rounded ends, and interspersed with masses of rounded spores resembling bunches of grapes.

Favus.—The favus organism (*Achorion Schönleini*) is the largest of any of the vegetable parasites. It may attack the skin, hair, or nails. A portion of a crust should be ground up on a slide with glycerine and liquor potassæ, and covered with a cover-glass. The mass will on microscopical examination be seen to consist of short mycelial threads branching at right angles, and of large oval spores resembling those of trichophyton, but much larger.

Erythrasma.—Scrapings should be made of the

reddish or brown patches in the skin, and the scales mounted in a mixture of glycerine and caustic potash. The parasite—the *Microsporon minutissimum*—is seen as a network of fine, pale, unjointed, interlacing threads with scattered spores. The threads are of very small diameter, and do not branch.

The above-mentioned vegetable parasites do not grow well on ordinary media.

Specimens should be sent to the laboratory wrapped in oil-silk which has been previously sterilized by boiling.

CHAPTER IX.

SERUM THERAPEUTICS.

SERUMS may be divided into two classes: 'antitoxic' and 'antibacterial.'

Antitoxic serums are prepared by injecting toxins of a given organism into the horse, with the result that there is formed a new body known as **antitoxin**. If now the serum of the horse containing antitoxin be collected and injected into a patient suffering from a disease caused by the given organism, the antitoxin introduced combines with the toxin being formed and neutralizes it. Diphtheria and tetanus antitoxins belong to this class.

Antibacterial serums are prepared by injecting into an animal living or dead cultures of a bacillus. The serum of such animals is then found to contain certain bodies which, when they unite with other bodies (complements) in human serum, confer protection against certain diseases by securing the destruction of the invading organisms. Antiplague, anticholera, and anti-streptococcic serums belong to this class.

Antitoxic serums have slight protective properties, and antibacterial serums feeble antitoxic powers.

The action of these serums is in all cases specific ; for instance, antistreptococcus serum will protect against streptococcus and streptococcus only.

Directions for using Antitoxins.—In all cases it is necessary that the true nature of the infection shall be determined by bacteriological examination. Thus, in supposed cases of pneumococcus or streptococcus infection, the cocci associated with these diseases should be sought for in the blood ; and in more localized diseases, such as diphtheria, tetanus, and glanders, the demonstration of the specific organism should be a matter of the first importance. Want of attention to this point has done much to bring serum treatment into disrepute.

The dose of an antitoxin will vary according to the strength of the preparation put upon the market by the manufacturer. There is a great need of a universal standard of strength and concentration for antitoxins, for some definite officinal instructions as to their constitution, mode of preparation, and storage. At present the profession has to depend entirely on the makers.

The dose should be proportionate to the age and size of the patient and the intensity and duration of the infection. It is advisable that treatment commence with a large dose, smaller doses being given at frequent intervals. The earlier in the disease the treatment is commenced the better are the chances of success ; this has been especially demonstrated to be true in the case of diphtheria. Liquid forms of antitoxin are to be preferred to the solid, as in dissolving the latter there is risk of sepsis, and undissolved material is apt to block the needle.

Bottles containing serum should be kept in a cool place, and not exposed to light.

The subcutaneous tissue of the flank or buttock is generally chosen as the spot into which the injection is made, and specially constructed syringes of 10 c.c. capacity are employed for the purpose. It is not necessary to penetrate the muscles. The skin over the point selected is rendered aseptic in the manner already described (p. 17), and the syringe is boiled for half an hour before use. A piece of antiseptic gauze, soaked in collodion, should be applied to the puncture. The syringe should again be boiled immediately after use. Any inflammation or suppuration resulting at the point of puncture should suggest a want of proper cleanliness in the instrument used.

The following is a list of the chief diseases in which serum medication has been tried :

Diphtheria.	Scarlet fever.
Tetanus.	Tuberculosis.
Cholera.	Rabies.
Plague.	Pneumonia.
Septicæmia.	Enteric fever.
Puerperal fever.	Syphilis.
Erysipelas.	Cancer.
Ulcerative endocarditis.	Snake-bite.

The most successful results of serum therapy have been obtained in the treatment of diphtheria. In many of the other diseases the treatment has not passed beyond the experimental stage, and in all cases the older methods of treatment should not be omitted.

Diphtheria.—It is difficult to estimate the reduction of mortality in this disease brought about by the use of diphtheria antitoxin, as the issue is confused by the fact

that bacteriological methods of diagnosis have altered our ideas as to what is and what is not true diphtheria. There is, however, undoubtedly a very marked decrease in the mortality of cases treated in the first and second days of the disease, a decrease in the general mortality, marked relief in laryngeal cases and in cases requiring tracheotomy, and generally the clinical course of the disease is milder.

As a prophylactic agent this antitoxin has not been given as extensive a trial as it deserves. In school outbreaks or in localized epidemics of diphtheria much might be done to check the spread of the disease if all those who have been, or are likely to be, exposed to infection were inoculated with a minimum dose of this remedy.

Dosage.—To obtain the best results it is necessary that diphtheria antitoxin be given in sufficiently large doses and at the earliest possible date in the disease, if necessary before the diagnosis is confirmed by bacteriological report. In a severe case, as judged by the extent of the membrane and the gravity of the constitutional disturbance, the dosage should be as follows: An initial dose of 4,000 units repeated every six hours during the first day, and doses of 2,000 units on each succeeding day until the membrane has cleared. In mild cases the initial dose of 4,000 should be followed in twelve hours' time by a second of 2,000 units, and on subsequent days, provided progress is satisfactory, doses of 1,000 units should be injected. With this drug, as with any other, the effect has to be watched and the dose graduated according to the effect produced. An objection to large doses of antitoxin was formerly that they entailed the injection of large amounts of fluid, which caused considerable pain and discomfort. Such an objection should not hold good to-day. A good antitoxin should contain at least 2,000

units in 5 c.c. of fluid, and some are made of double the strength, so that in no case is it necessary to inject more than 20 c.c. (about half an ounce). For prophylactic purposes the dose is 500 units.

Tetanus.—In acute cases with short incubation period the antitoxin treatment has proved a failure. In the more chronic cases the results are more encouraging. The dose of Behring's serum is 5 grammes (500 units), of the Lister Institute of Preventive Medicine serum 10 to 20 c.c., and this may be repeated every six or twelve hours. The remedy has been used in traumatic and so-called idiopathic tetanus, and in trismus neonatorum. As the prophylactic power of the antitoxin is great, although short-lived, it might be advisable to use it in cases where the onset of the disease is to be suspected.

Favourable results have followed the injection of antitoxin into the substance of the brain. A small trephine opening is made just in front of the motor area of each hemisphere. Through this opening a round-pointed needle attached to a syringe with a screw piston is passed, and the antitoxin very slowly allowed to soak into the substance of the brain. The hypodermic medication is continued in addition to this.

Septic Infections.—Antistreptococcus serum prepared from the blood of horses and asses rendered immune to the *Streptococcus pyogenes* has been employed in septicæmia, pyæmia, puerperal fever, ulcerative endocarditis, erysipelas, scarlet fever, and acute angina. So few cases have been reported in which serum treatment has followed the demonstration of the streptococcus that we are not yet in a position to fully judge of its merits. Its indiscriminate use in cases of infection other than that by the streptococcus is likely to confuse the issue.

Antistreptococcus serum, as supplied by the Lister

Institute of Preventive medicine, is given in doses of 10 to 20 c.c., and may be administered twice daily. It may be obtained in both the liquid and dry forms, and should be injected as near the site of the lesion as possible.

In septicæmia due to invasion by staphylococci, the antistaphylococcic serum of Messrs. Burroughs and Wellcome may be tried in 10 c.c. doses. If the *B. coli* be the infective agent, the anticollic serum of the same firm in 10 c.c. doses is worthy of trial.

Pneumonia.—Antipneumococcic serum has been difficult to obtain, and only a few cases treated by this method have been reported. Klemperer's results with serum obtained from immunized rabbits were good, but a much wider experience of its use is required before a definite opinion as to the value of the treatment can be formed. The dose is 10 to 20 c.c. every twelve or twenty-four hours until the crisis occurs.

Cholera.—Haffkine has prepared two vaccines for the prophylactic treatment of cholera. These vaccines are emulsions of cholera vibrios in sterile broth, and are injected into the subcutaneous tissue of the abdomen, the weaker vaccine being followed by the stronger after an interval of five days.

The vaccination diminishes the risk of contracting the disease, but does not lessen the risk of a fatal termination.

Plague.—For prophylactic purposes Haffkine's vaccine has met with considerable success. A subcutaneous injection of from 2 to 2.5 c.c. is employed, and it is claimed that immunity is secured for a period varying from four to six months.

The treatment of cases of plague by means of anti-plague serum has not, so far, been very successful.

Yersin's serum is the best known, and should be injected in doses of 20-40 c.c. twice on the first day and once on subsequent days of the disease.

Rabies.—True serum treatment of rabies, as devised by Baber and Lepp, is seldom adopted, Pasteur's remedy, which is in reality immunization by intensive vaccination, having met with greater favour. In Pasteur's method emulsions made from the spinal cords of rabbits that have died from rabies are injected into the patient. The treatment extends over two to three weeks, each day a stronger virus—that is, an emulsion taken from a fresher cord—being used. In cases bitten by wolves, or bitten on the face or head, an 'intensive' course of treatment—ten injections distributed over the first three days—is adopted.

The results of Pasteur's treatment have been most satisfactory. In the case of persons bitten by animals subsequently proved experimentally to be suffering from rabies, the mortality after treatment has never exceeded 1 per cent., and in 1897 was 0·7 per cent. It is important that the treatment be begun not later than six days after the bite has been inflicted. If commenced later in the incubation period, or after symptoms of hydrophobia have manifested themselves, the chances of recovery are very small.

Tuberculosis.—The treatment of tuberculosis by anti-tuberculous serum, by tuberculin, or by tuberculin R., has at present fallen into abeyance. The injection of old tuberculin for diagnostic purposes is occasionally of service. A dose of $\frac{1}{1000}$ c.c. for an adult, or $\frac{1}{2000}$ c.c. for a child, is injected subcutaneously. In tuberculous cases a rise of temperature results, and signs of inflammation appear in the part affected. In healthy persons no reaction is obtained, even though tested with increasingly large

doses. Fisch and Maragliano have produced serum said to have curative value.

Snake-bite.—The poisons of all snake-bites are apparently similar in nature. Antivenomous serum is obtained by inoculating horses and donkeys with gradually increasing doses of venom. The serum is administered by intravenous injection in doses of from 10 to 30 c.c. as soon as possible after the bite. It is least potent against the bite of the rattle-snake and viper.

Glanders.—Mallein, a growth of the glanders bacillus in glycerinated broth, is chiefly used as a diagnostic agent in suspected cases of glanders in man or the horse. Its value as a curative agent in chronic glanders in human beings is as yet undetermined. Mallein (Lister Inst. Prev. Medicine) is injected in doses of 1 c.c. for diagnostic purposes; complete reaction comprises a rise of 2.7° F., and an extensive local inflammation.

Enteric Fever.—Attempts to produce an antitoxic curative serum for enteric fever have not met with success. As a prophylactic measure, Wright's antityphoid vaccine has given encouraging results, but these need further confirmation.

APPENDIX.

By D. S. DAVIES, M.D.

THE MICROSCOPE.

BACTERIA can, it is true, be recognised, especially when the grouping is characteristic, with a $\frac{1}{4}$ -inch, $\frac{1}{6}$ -inch, or $\frac{1}{8}$ -inch objective and a No. 2 ($\times 4$) eyepiece; but a higher power is necessary for satisfactory work, and a $\frac{1}{12}$ -inch oil immersion objective is most generally useful. Useful objectives of this power can now be obtained of most English makers at £5. Baker, Swift, Watson, or Leitz supply objectives at this price, while Beck supplies a satisfactory glass at £4. These high powers require more light for the proper illumination of the field than can be obtained with the concave mirror and usual stop or diaphragm. This is secured by the use of the *sub-stage condenser*, which is a compound lens so arranged as to bring the light to a focus from below, in the plane of the object.

Most modern microscopes (even the smaller histological models from £4 4s.) have an understage fitting, into which these condensers slide—the universal size fitting is $1\frac{1}{2}$ inches diameter; but it is convenient to have a screw focussing substage, which can be obtained at a cost of about an extra £1 from Messrs. Baker, Watson, or

Beck. The condenser itself, with iris diaphragm, can be obtained for from 15s. to 30s., not achromatic, but satisfactory for bacteriological work. In the instruments specially constructed for bacteriological work, the substage is fitted with centring screws, but the makers usually centre the fittings with sufficient accuracy, except for very critical work.

The *substage condenser* is constructed to utilize parallel rays of light or divergent rays; it will not work well with convergent rays. Consequently the *plane* mirror should always be used in working with a substage condenser. In use the substage condenser is racked up close under the glass slide, and focussed until the fullest light is obtained. The best light is daylight from a white cloud. If artificial light is used, a paraffin light is best, fitted with an iron chimney, which is grooved to receive an ordinary (3×1) glass slide; a pale-blue glass modifies the lamp-light and saves the eye. These blue glasses are generally supplied with the chimneys. Use the flame edgewise. A bull's-eye on the lamp, used plane side towards and close up to the lamp, parallelizes the light for the condenser, but is seldom necessary. The most useful eyepieces are No. 2 ($\times 4$) and No. 4 ($\times 7$) or equivalent ones.

In buying objectives, it should be ascertained at what tube length the objective works best; the tube length is reckoned from the bottom of the collar, into which the objective screws, up to the *top* of the eyepiece in position. Foreign objectives generally work best with a short tube, 160 mm. to 170 mm. (about $6\frac{1}{2}$ inches), and the English with the draw-tube extended to 10 inches. All objectives must have the universal screw. The fine adjustment must be delicate and free from backlash or sideshift.

The microscope outfit for bacteriological work, if a

new microscope has to be purchased, should include eyepieces 2 and 4, objectives $\frac{2}{3}$ -inch, $\frac{1}{6}$ -inch, and $\frac{1}{12}$ -inch oil, or Zeiss A, D, and $\frac{1}{12}$ -inch oil, substage condenser and iris diaphragm, and large stage, and may be bought from Baker, Watson, or Swift at an inclusive price of about £15. If centring substage is supplied, as with Baker's 'Advanced Student's' or 'D.P.H.' Microscope, or Watson's 'Model G.,' the price will be £18 to £18 18s. The English or Jackson foot is steadier than the continental horseshoe. The higher-priced microscopes have draw-tubes to 10 inches. Always order a microscope or objective on approval, and let someone test the working of the lenses and the fine adjustment, etc. Every microscope should have an easy running rackwork coarse adjustment. Baker's objective changer is more handy than the somewhat heavy and cumbrous triple nose-pieces, and is about the same price.

To find Stained Bacteria on Cover-slips.—Put on No. 2 eyepiece and $\frac{1}{12}$ -inch oil immersion objective. Rack up substage condenser close under glass slide; open iris diaphragm to full aperture. Put a drop of cedar oil upon centre of cover-glass. Rack down carefully with coarse adjustment until the objective dips well into the oil, keeping the eye on a level with the stage whilst doing so, to avoid coming down on cover-glass. Now look through microscope, adjust light with plane mirror, focus substage condenser gently until best light is obtained.

Now rack up objective slowly with coarse adjustment until the stained bacteria appear. Use fine adjustment to define. Do not attempt to *find* the field with the fine adjustment; the power of the micrometer screw is proportional to its slowness of action, and cover-glasses (No. 1) are easily broken.

To find Living Bacteria in a Hanging Drop.—To

examine living bacteria in a hanging drop, as, *e.g.*, the bacillus of enteric fever for Widal reaction, use Zeiss D, or $\frac{1}{4}$ -inch or $\frac{1}{8}$ -inch dry objective, and cut down the light a little with the iris diaphragm until the bacilli are defined. If the light is unsatisfactory, the substage condenser may be racked down slightly, or its top lens removed for low-power work; or the condenser may be removed altogether, and the concave mirror (which is adjusted on the microscope so as to bring parallel rays to a focus in the plane of the object) may be used, with the ordinary stop diaphragm, beneath the stage.

For stained bacteria in tissues, use microscope as for stained bacteria on cover-slips, with iris diaphragm fully open, and condenser racked up and focussed on object; the bacteria will be well defined against the ill-defined structure of the tissue in which they lie. By closing the iris diaphragm the structure of the tissues will become more clearly defined.

APPROXIMATE MAGNIFICATIONS IN DIAMETERS.

Tube length.	150 mm.	160 mm.	170 mm.
Objective ..	Zeiss A.	Zeiss D.	Leitz.
Power	$\frac{1}{4}$ inch.	$\frac{1}{6}$ inch.	$\frac{1}{12}$ -inch oil.
Eyepiece {	2.	50	240
	4.	90	420
			680
			1000

$\mu = \frac{1}{1000}$ millimetre.



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THE END.

