The bacteriology of every-day practice / by J.O. Symes.

Medical Monograph Series

No. II

The Bacteriology of Every-day Practice

SYMES

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MEDICAL MONOGRAPH SERIES.

EDITED BY

DAVID WALSH, M.D.

PREFACE

THE aim of this series is to sketch in brief compass the chief features of given subjects of everyday interest to students and practitioners.

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Medical Monograph Series, No. 2

THE BACTERIOLOGY OF EVERY-DAY PRACTICE.

BY

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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 research 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 depôt. 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 conditions some bacteria have the power of altering their condition

and forming spores. 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.

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 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 essentially 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 true *infection*, such as is seen in septicæmia.

THE

BACTERIOLOGY OF EVERY-DAY PRACTICE.

CHAPTER I.

MATERIALS AND INSTRUMENTS.

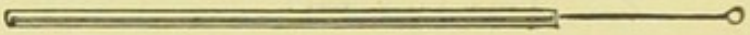
UNDER the heading 'Materials and Instruments' we shall describe only such as are necessary for the preparation of cover-glass preparations or for the making of cultures. The practitioner has not, as a rule, either time or space to devote to more elaborate apparatus for the manufacture of media or the 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 soaking for three hours in a mixture of concentrated sulphuric acid 6 parts, potassium bichromate 6 parts, and water 100 parts; 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.

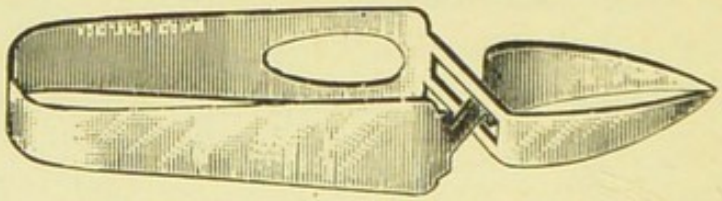
For inoculating tubes, spreading liquids on cover-glasses, or transferring small portions of material, platinum wires fused into glass rods are used. The wire of the most convenient size is No. 12 English



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



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

A hypodermic syringe or exploring needle is often required for drawing off fluids. This must be made of such material as to permit of frequent boiling for the purpose of sterilization. Rubber plungers are soon destroyed if so treated; asbestos or glass plungers are the most serviceable.

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.

Ordinary vaccine lymph tubes may be utilized as pipettes. They are first sterilized by heating in the flame. The open end is then placed in the fluid, a certain amount of which rises in the tube. If this end of the tube be then carefully brought near to the flame, care being taken not to char 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, and the fluid preserved from excessive heating by covering it as far as possible with the fingers.

Culture 'outfits' and culture tubes are sent out by many of the research laboratories, or may be obtained from the larger chemists and dealers. The 'outfits' consist of a tube of some nutrient medium and a throat swab, the latter being a piece of cotton-wool twisted around the end of a copper wire, the whole contained in a plugged test-tube which has been carefully sterilized.

The most useful culture tubes to purchase are those of sterile, solidified 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 made for this purpose.

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. Gentian violet, anilin oil solution—

Saturated alcoholic solution of gentian violet	10 c.c.
Anilin water (3 c.c. anilin to 90 c.c. water)	100 c.c.

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

Grübler's stains are the best. They are most conveniently kept as saturated alcoholic solutions, which may be obtained from Baird and Tatlock, Hatton Garden; Ferris and Co., Bristol, or other dealers.

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 fumigation 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. It is probable that in England it will soon be replaced by the system of spraying all infected surfaces with antiseptic solutions, as is now the practice on the Continent.

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 soda 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 burned. Used coverslips, slides, and glass apparatus should be immediately transferred to a bath of some antiseptic fluid, and similar solutions should be used for disinfecting the hands after bacteriological work.

There is much misunderstanding as to what substances are true disinfectants, that is, 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, and 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.
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 tracks 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 1 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, before 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 and treatment.

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 FILM PREPARATIONS. 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 spring 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, the whole process not occupying more than a couple of seconds, in order to coagulate the albumin, and so 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.

Films may also be prepared by placing a drop of the material between two cover-glasses, which are then squeezed and drawn apart with a sliding movement. The cover-glasses are next dried and fixed. This method is well adapted for the study of specimens of blood, as very thin films are thus secured. The danger in spreading material with a wire is that the film may be too thick, and to prevent this only the smallest quantity should be used.

Blood films, and others in which it is desired to preserve the details of histological structure, should not be fixed by passing through the flame, as they may suffer damage from the heat. They are fixed by being placed for from two to five minutes in a saturated solution of corrosive sublimate, or for about half a minute in a mixture of equal parts of alcohol and ether, or 1 per cent. formalin. They are then washed in distilled water, dried, and are ready for staining.

Staining.—The cover-slip is held in the 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 on 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 is 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.

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 overstained 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.

- *Staphylococcus pyogenes aureus and albus.*
- *Streptococcus.*
- *Pneumococcus.*
- *Micrococcus tetragonus.*
- *B. diphtheriæ.*
- *B. tuberculosis.*
- *B. anthracis.*
- *B. tetani.*
- *B. aerogenes capsulatus.*

Decolourized by Gram.	*Gonococcus.*
	Diplococcus meningitidis.
	Pneumo-bacillus.
	B. typhosus.
	B. coli.
	B. pyocyaneus.
	B. influenzæ.
	B. mallei.
	B. proteus vulgaris.

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

1. Stain in aniline oil gentian violet for five minutes, and wash with water.

2. Treat the film with Gram's solution for about a minute until it becomes 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.

Certain micro-organisms, such as the tubercle bacillus, resist the action even of powerful stains, such as carbol-fuchsin, unless applied for a long time, or aided by the application of heat. For such bacteria the *Ziehl-Neelsen* method of staining is commonly applied:

1. Cover the film with carbol-fuchsin stain, and heat until steam arises. Allow it to remain for five minutes.

2. Wash in 20 per cent. solution of nitric 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, react in the same way. Where a number of films are to be stained at one time, 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.

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.

Sloped agar or serum tubes are generally supplied with culture 'outfits.' 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 dispatched for incubation at the very earliest opportunity.

Much care is required in the collection, packing, and dispatch 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.

GENERAL INFECTIONS OF THE BLOOD.

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 *Bacillus 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.

Method of Examining the Blood for Parasites.

The blood is best 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 half a minute 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 dispatched immediately for incubation.

A more satisfactory procedure, but not so easy to adopt in private practice, is to extract from 2 to 5 c.c. of blood from a vein by means of a sterilized hypodermic syringe, and from this larger quantity to inoculate plates and animals.

For the purpose of *diagnosis* the method of film preparations is disappointing. In the majority of cases no organisms will be detected, but this cannot be held to negative a general blood infection, for the number of parasites in the general circulation is so small, and the actual quantity of blood examined so minute, that they might easily escape detection. The best results have been obtained from cases of pronounced sepsis, malignant endocarditis, pyæmia, septicæmia, acute osteomyelitis, and puerperal fever, cultures from the blood yielding numerous colonies of strepto- or staphylo-cocci, or other organisms. The presence of cocci or bacteria in the blood having been once established, the *prognosis* must be regarded as grave. It is apparently only in the more virulent cases, and shortly before a fatal termination, that this dissemination takes place. For instance, in a series of thirty-two cases of pneumonia in which the blood was examined,* the pneumococcus was present in nine only. Of these seven died, and the other two had severe attacks with complications. Of the remaining twenty-three, nine

* Ely, *American Journal of Medical Sciences*, October, 1897.

only died. In diphtheria, too, those cases in which the Klebs Löffler bacillus is found widely distributed after death are, it will be noted, severe cases complicated with coccal infection, and cultures from the various organs invariably show streptococcus growth. The probable explanation of these facts is that ease in finding the parasites in the blood means that they are present in large numbers, thereby increasing the risk to the patient.

Bacteriological examination of the blood, however, is generally undertaken in order to obtain indications for *treatment*, and almost exclusively in connection with the serum treatment of acute sepsis, erysipelas, ulcerative endocarditis, and puerperal fever. The principles of treatment by antistreptococcus serum will be discussed under the heading of Serum Therapeutics, but it is necessary to state here that it is a remedy which can only be administered with success when the disease is the result of an infection with streptococcus. If blood-films when stained show the chains of spherical bodies known as streptococci, then the case may be regarded as one suitable for treatment by antistreptococcic serum. If, on the other hand, the grape-like masses of staphylococci are alone present, then the case is unsuitable. Antistreptococcic serum is, of course, sometimes given when the streptococcus cannot be demonstrated. If under these conditions it fails to effect a cure, the treatment is in no way discredited, as the infection may be of some other kind. Each serum avails only against intoxication by its own specific variety of organism. Although experiments have been made, there is as yet no efficient antistaphylococcic serum. Lest it should be thought that this point has been unduly accentuated, it may be as well to state that the treatment is likely to become discredited, and may fail to get a fair trial, owing to its employment in unsuitable cases.

MALARIA.

In suspected cases of malarial fever the blood should be examined just before or at 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 (1½ inches by ¾ inch), which has been previously provided, is applied to the exuded blood, so that it touches the drop about ⅔ inch from one end of the strip. This is immediately laid, blood-surface downward, on a carefully cleaned cover-glass, 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 fixed by dropping on a little alcohol. The alcohol is allowed to remain for five minutes, and then dried off with filter-paper. The specimen is stained for half a minute with watery solution of methylene blue, washed, dried and mounted in xylol balsam. By this method the *Plasmodium malariæ* is stained a faint blue colour, the nuclei of the white corpuscles a much deeper tint.

There are three well-recognised forms of malarial 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. Thus minute protoplasmic bodies of oval shape (spores) may be observed lying outside the corpuscles, or upon their surface. Rounded bodies with a nucleus and specks of pigment, or rings with the pigment at one side, or rosette-shaped masses lying within the corpuscles, and crescentic and flagellated bodies, are found at different times.

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—ten grains at once, followed by 5 grains every four or six hours for the next three or four days.

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.

PLAGUE.

The bacillus of plague was first described by Kitasato in 1894. It is found in the blood and sputum during life, and in the buboes and organs of persons dying of this disease. In form it is a short rod with rounded ends, and stains more readily at the ends than in the centre. Growth is abundant in agar, the bacilli then taking a chain formation. It does not liquefy gelatin. For purposes of diagnosis, films should be prepared from the blood, sputum and from scrapings of the buboes. These are fixed by

heating in a flame, and stained with gentian violet for about a minute. The plague bacilli appear as short, unevenly staining rods, generally in pairs.

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. Treatment by Haffkine's anti-plague vaccine has been the most satisfactory method up to the present. It consists of an emulsion of the dead bodies of plague bacilli, and is administered subcutaneously.

CHAPTER IV.

SUPPURATIVE PROCESSES.

Practically all true suppurative processes are the result of the growth and activity of micro-organisms. Apart from the bacteria of tuberculosis, glanders, and actinomycosis, which excite suppurative processes peculiar to themselves, the following cocci and bacteria may in the course of growth within the human body lead to pus-formation:

Staphylococcus pyogenes aureus and albus.
Streptococcus.

Staphylococcus cereus albus and flavus.
Staphylococcus citreus.
Pneumococcus.
Diplococcus intracellularis meningitidis.
Gonococcus.
Micrococcus pyogenes tenuis.
Micrococcus tetragonus.
Bacillus pyocyaneus.
Bacillus coli.
Bacillus typhosus.
Bacillus pyogenes fœtidus.
Bacillus aerogenes capsulatus.

By far the most common of these are the *Staphylococcus pyogenes aureus* and *albus*, and the *streptococcus*. Of thirty-eight cases of suppuration examined personally during a period extending over twelve months, these organisms, either alone or in conjunction, were found in twenty-three. A short description of them will therefore be given, whilst for particulars concerning the other varieties 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. *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.

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, empyemata, and so on.

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 they are transferred to tubes of culture media.

Deep-seated Abscesses, Empyemata.—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. 15), 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.

The type of lesion produced by streptococcus infection usually differs markedly from that due to staphylococcus. Thus the *streptococcus* is most commonly the determining cause of diffuse cellulitic inflammations, erysipelas, spreading traumatic gangrene, and of abscesses caused by infection of the lymphatic channels. Its dissemination by means of the blood-stream gives rise to the condition known as septicæmia.

The staphylococci, on the other hand, are more frequently found in superficial and localized suppurations, discharging wounds, ulcers, furuncles, carbuncles, and in the pyæmic state. In acute osteomyelitis and ulcerative endocarditis both varieties of cocci have been found on different occasions, the *staphylococcus* being perhaps the more common.

A point worthy of careful attention is that cocci vary in virulence from time to time in the most remarkable manner. This is especially the case with streptococci, which are generally to be found in cultures from normal throats, and are frequently present in scrapings from the puerperal uterus, in both instances their presence apparently giving rise to no symptoms; in other words, they are non-pathogenic. Under certain conditions,

however, which are not fully understood, their virulence develops or becomes greatly intensified, and this is well illustrated by the streptococcal invasion of the fauces seen in diphtheria, scarlet fever, and other forms of angina. It now seems certain that erysipelas is caused by streptococcus of a high degree of virulence.

This variation in the degree of virulence is not so marked in the case of the *staphylococcus*, although there is a striking difference between the acute infections of malignant endocarditis and osteomyelitis and the condition known as latent pyæmia, sometimes met in cases of furunculosis, and in which, although *staphylococci* are present in the blood, they give rise to no symptoms.

A knowledge of the micro-organism concerned in a given case of suppuration may have a distinct bearing upon both *diagnosis* and *treatment*. Films made from the pus may show *gonococci*, *pneumococci*, *bacillus coli*, or *bacillus typhosus*, and thus reveal for the first time the source of the infection. We have ourselves demonstrated the existence of *B. typhosus* in abscesses of bone and in liver substance. The presence of *B. coli* would point to a lesion of or near the intestinal tract. Instances in which *pneumococci*, *gonococci*, and so on, have excited suppurative processes will be found under the paragraphs devoted to special organisms.

It is not uncommon when examining films of pus to be unable to detect any microbes. If, after examining a number of slips, this is found to be the case, the probability is that the lesion is tuberculous, and fresh specimens should be specially stained to demonstrate the tubercle bacillus. In suppuration due to the *Glanders bacillus*, the *Pneumococcus*, and to *amæba coli*, it is sometimes difficult to demonstrate the micro-organism.

With regard to the treatment of general coccal infec-

tion the reader is referred to the paragraphs on general blood infections and on serum therapeutics, in which special reference is made to the use of antistreptococcic serum.

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.

In *empyema* following pneumonia, should the pus drawn off with the exploring needle show, when stained, the presence of pneumococci only, there is a possibility of simple aspiration meeting with success. If cocci are present, it will certainly be necessary to insert a drainage-tube.

Furunculosis, Boils and Carbuncles are, as it has been previously mentioned, the result of coccal infection, the *Staphylococcus albus* and *aureus* being the varieties most commonly present. A knowledge of this fact will lead to their treatment on strictly antiseptic lines, a method which is far from being as widely adopted as it should be at the present day.

INFECTIVE ENDOCARDITIS.

Although the micro-organisms associated with this disease are most commonly *streptococci* and *staphylococcus aureus* and *albus*, yet many others have been found, including the *gonococcus*, *pneumococcus*, *pneumo-bacillus*, *bacillus*

tuberculosis, *bacillus typhosus*, *bacillus endocarditis griseus*, and so on. It is of great importance before beginning treatment to ascertain the particular variety present in the case under observation.

PUERPERAL FEVER.

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.

Gonococci, *bacillus coli*, and *staphylococci* have all been demonstrated in cases of puerperal fever, either alone or in conjunction with *streptococci*, and the discovery of one or other of these organisms would throw considerable light upon the question of the origin of the infection, and might be sufficient to negative treatment by antistreptococcic serum.

The examination of the blood in puerperal fever has, in the great majority of cases, given disappointing results, in all probability because such small quantities are taken. If two or three cubic centimetres be drawn in a hypodermic syringe from a vein in the manner already described, and allowed before coagulating to flow over the surface of culture tubes or plates, the chances of success are much increased. By means of a speculum and sterilized wire cultures and films should also be prepared from the interior of the uterus, and these may assist in forming

a diagnosis. If the cervical canal be not sufficiently patent to admit a wire, a sterilized probe or sound may be used.

ERYSIPELAS.

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 half a minim, and this is gradually increased daily until the reaction temperature reaches 104° F.

Coley's results with this treatment have been encourag-

ing, especially in the case of sarcomata. A detailed account of his methods will be found in the *Annals of Surgery*, XXV. and XXVI., 1897.

TUBERCULAR SUPPURATIONS.

If pus from an abscess or other lesion be suspected of being tubercular, then the films which are prepared in the ordinary method are stained by Ziehl-Neelsen's process (p. 19), 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. Frequently it is impossible to demonstrate the presence of the bacillus in films, and as it does not grow on ordinary media, another method must be tried, namely, that of inoculation. A small quantity of the pus is collected in a sterilized glass pipette and dispatched to the laboratory for these experiments. Such pus is frequently found to be virulent, and although spores have not been actually demonstrated, it seems probable that these are the true source of infection.

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 laparotomy and the free admission of air into the peritoneal cavity. Tubercle

bacilli are found lying within and outside the cells, singly or in small groups.

If pus when examined microscopically shows no sign of tubercle bacilli or other micro-organisms, and if it shows no growth on ordinary culture media, then a sample should be sent for the purpose of inoculation experiments. A delay of from two to three weeks is entailed, but in this way only is it possible to exclude or verify the existence of a tubercular infection.

Treatment.—The treatment of tuberculosis by tuberculin (Koch) has now practically been abandoned, owing to the risk of exciting a general dissemination of the bacilli from affected centres already in existence. It is still, however, widely used for diagnosing the disease in cattle. Treatment by Koch's new tuberculin, by oxytuberculin, and by the serums introduced by the United States Government, by Marigliano, Paguin, and others, has not met with any marked degree of success.

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. 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. Growth takes place on blood agar, but, as other pyogenic

organisms are apt to crowd out the gonococcus, this is not an altogether trustworthy method of recognition.

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.

Gonococci may be found in the pus in many cases of purulent ophthalmia, whether of the newly-born or otherwise, in abscesses in connection with the genital organs, cystitis, pyosalpinx, peritonitis, arthritis, pericarditis and in malignant endocarditis. Films and cultures should both be prepared according to 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 mucopurulent discharge. It is hardly necessary to remark that such cases are centres of active infection. (See Stephenson's monograph on 'Diseases of the Conjunctiva.')

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 *bacillus mallei* may be detected; but most frequently the bacillus fails to take the stain, and the discharge appears sterile. The *bacillus mallei* is about as long as the tubercle bacillus, straight or curved at the ends, and stains irregularly. 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 dispatched 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 *bacillus 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, however, been employed as a remedy for the disease in man, doses of $\frac{1}{15}$ to $\frac{1}{20}$ c.c. being injected every two or three days for several weeks.

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, dried, and stained with methylene blue for five minutes and with Gram's method, by which the bacillus is not decolourized.

The *bacillus 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, 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 pleuræ. 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 *bacillus anthracis* can be demonstrated in the blood. A drop of blood is taken with all 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 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. If present, it is almost invariably associated with cocci; but frequently it cannot be detected in films, and, as it does not grow on ordinary media, resource 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 treatment of this disease by tetanus antitoxin is, on the whole, encouraging in its results, in cases in which the incubation period has been a lengthened one. In acute cases the antitoxin appears to be of little use, unless injected into the brain substance itself. (See 'Serum Therapeutics.')

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 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 ·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 with carbol-fuchsin or orange rubine; the central mass by Gram's method. 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 ten to twenty grains three times daily.

EMPHYSEMATOUS GANGRENE.

The two organisms most commonly associated with this condition are *B. œdema maligni* and *B. aerogenes capsulatus*.

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.

The *B. aerogenes capsulatus* occurs as a diplobacillus. Its capsule may be demonstrated by treating with glacial acetic acid, and then with an aqueous solution of gentian violet. It stains with Gram. Its presence in the tissues after death gives rise to the condition known as 'foam organs.' Both organisms are anaerobic.

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 *streptococcus 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 V.

DISEASES OF THE RESPIRATORY SYSTEM.

Phthisis, Tubercular Pleural Effusion, etc.

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. 19.

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 frag-

ments 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 nasopharynx, 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 boiling with 2 per cent. caustic potash, or 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 Exudations may be serofibrinous, hæmorrhagic or purulent in character. Sero-

fibrinous exudations are not, as a rule, of large size, owing to the formation of adhesions; they are not so serious as the suppurative lesions, as there is a possibility of their absorption without operative interference.

To obtain material for bacteriological diagnosis the skin over the affected part is disinfected, and an ounce or two of fluid is drawn off by means of an exploring syringe, which has been previously sterilized by boiling. A portion of the exudation thus obtained is utilized for making a series of films, which, after being stained by Ziehl-Neelsen's process, are examined microscopically for tubercle bacilli. It is, however, but rarely that the bacillus can be demonstrated by so simple a procedure, as they are usually present in comparatively small numbers. The remainder of the fluid drawn off should therefore be poured into a sterilized glass bottle, securely corked and sealed, and dispatched to a laboratory where it can be centrifugalized. Examination of a sediment obtained by centrifugalization will often reveal the presence of tubercle bacilli in an exudation previously regarded as sterile.

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 in 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, as after a case of scarlet fever or diphtheria. 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.

The very high mortality from tubercular diseases of the intestines, tabes mesenterica, etc., amongst children may be attributed to neglect of these precautions. It is

very desirable that dairy-herds should from time to time be tested with tuberculin, and the cows in which there is a reaction weeded out. 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.

PNEUMONIA.

Lobar Pneumonia.—By far the most common of the numerous micro-organisms 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.

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.

Hewlett states that the pneumococcus is found in about half the cases of purulent meningitis, sometimes in cerebro-spinal meningitis, in about one-third of the cases of otitis media and ulcerative endocarditis, and occasionally in peritonitis. It is also sometimes present in follicular tonsillitis, and in some conjunctival inflammations, pericarditis, and localized abscesses.

Method of Examining for Pneumococcus.—The coccus may require to be sought for in sputum or in pus from empyema or other suppurative lesion, such as meningitis, spinal meningitis, pericarditis, or peritonitis. 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 croupous 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 needle, the skin having first been disinfected. In doubtful 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 Fraenkel's diplococcus is very great, and these may occur as complications during the course of an acute attack of pneumonia or as independent affections. Pneumococci have been demonstrated as the determining cause of ulcerative endocarditis, meningitis, epidemic cerebro-spinal meningitis, pleurisy, otitis media, and peritonitis. 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 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 the blood an effective antitoxin. Washbourn uses serum from immunized horses, and the results reported are distinctly encouraging. A much larger number of observations is, however, required to enable definite conclusions to be drawn as to the efficacy of this treatment.

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 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 *influenza bacillus*, 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.

Pleurisy and Empyema.—The organisms most frequently found in serous and purulent pleural effusions are the pneumococcus, streptococcus, and tubercle bacillus.

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 half a minute with carbol-fuchsin, washed, dried, and when mounted examined for strepto- and pneumo-coccus. It is not generally difficult to detect these microbes 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 may be 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. Whilst infection by tubercle bacilli is perhaps the commonest cause of serous pleural effusion, it is far less frequently associated with purulent pleural exudations.

Bronchitis, Putrid Bronchitis, Bronchiectasis.—Cover-glass films from the expectoration in these diseases may sometimes assist in helping to diagnose the cause of the condition. In simple catarrhal bronchitis there are at first few micro-organisms present, but in epidemic influenza the small straight bacilli with rounded ends and polar staining may be detected. The influenza bacillus is best stained by carbol-fuchsin mixed with ten parts of distilled water. The dye should be gently heated and allowed to act for fully ten minutes.

In **Chronic Bronchitis** a great variety of cocci, bacilli, and moulds are present in the expectoration, and in putrid bronchitis these are even more numerous. In the latter disease 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 ray fungus is found in cases of pulmonary actinomycosis, and a short bacillus closely resembling the influenza bacillus has been described as being present in the sputa of cases of whooping-cough in the catarrhal and early convulsive stages.

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 variety; (2) dumb-bells with darkly staining swollen ends; (3) straight or slightly curved rods, swollen in the centre and tapering at the ends. It grows well on most media, particularly on blood serum or glycerine agar. With Löffler's methylene blue they stain unevenly, showing in places deeply-dyed granules. This characteristic method of staining is an important guide in recognising the diphtheria bacillus.

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 ·75 per cent. solution of common salt previously sterilized by 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 frequently, 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 pseudo-bacillus. Some observers* maintain that the pseudo-bacillus 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,

* Hewlett and Knight, 'Transact. Brit. Instit. Prevent. Medic.' (First series).

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.

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 *streptococcus longus* are detected in the cultivation than if the associated organism be the staphylococcus. 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.

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 4,000, 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 secretions. It is therefore of the utmost importance that early cases should be detected, and efficiently isolated until the Klebs-Löffler bacillus ceases to be found.

It is in the detection of such early cases, otherwise

unrecognisable, that the value of bacteriological examination is most marked. In an outbreak of diphtheria in a private household or public institution, systematic bacteriological examination of the throats will often reveal the presence of typical Klebs-Löffler bacilli in persons without symptoms who would otherwise have been unsuspected, and who would have been active centres of infection.

In the case of patients convalescing from diphtheria the bacilli may remain in the fauces for many weeks or months. As long as typical 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 have proved 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.

Membranous Laryngitis. — This condition may closely simulate a true diphtheritic infection. It may be of such severity as to necessitate tracheotomy, and may lead to a fatal issue. Cover-glass films and cultivations on nutrient media show staphylo- and strepto-cocci, leptothrix, sarcinæ, and other micro-organisms. The membrane is much softer and less adherent than in diphtheria.

Acute Tonsillitis, Quinsy, and other Anginas.— 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 anginas 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, and *bacillus coli* are less 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, etc.

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 anginas.

In the initial scarlet fever angina streptococci are present in great numbers, and not infrequently bacilli resembling the short variety of diphtheria bacillus. In the majority of cases further investigation proves these to be pseudo-diphtheritic bacilli; but later, in the convalescent stage, a membranous tonsillitis, the so-called post-scarlatinal diphtheria, is a not uncommon complication, and in this the typical Klebs-Löffler bacillus is found.

CHAPTER VI.

ENTERIC FEVER, CHOLERA, ETC.

Since the introduction of the method of serum diagnosis, the isolation of the *bacillus typhosus* from the dejecta of suspected cases of enteric fever has become a matter of secondary importance.

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.—The work of isolating the *bacillus typhosus* from the stools and urine is of an elaborate character, and can only be done in a properly equipped laboratory. Examination of films is not usually successful. The bacilli are most easily found in the motions during the second and third weeks of the attack, and rapidly disappear with convalescence. 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 dispatch. 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 first week of the attack until the commencement of the second or third week of convalescence. 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 dispatched for incubation.

Of the three methods mentioned for detecting the presence of the Bacillus 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 *bacillus 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 a hundred and twenty 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 persists for months and occasionally years after convalescence.

Even in advanced cases of typhoid, as everyone knows, a clinical diagnosis frequently can 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.

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 with 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 agglomerating 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 of Netley 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 sedimentation 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 for the treatment of the disease have, however, up to the present proved unsuccessful. 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 no 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 scil, 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.

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 bacillus most commonly associated with outbreaks of meat poisoning is the *bacillus enteritidis* of Gärtner.

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-feet earth thermometer reaches 56° F., and the maximum mortality is attained in the week in which the temperature recorded by the 4-feet 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 air-borne, 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*, a variety of streptococcus, and *bacillus enteritidis sporogenes*.

Cover-slip preparations made from the fæces will not unfrequently 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. Not unfrequently, however, such cultures show the presence of cocci only, or of the *bacillus 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 sus-

pected 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.

DISEASES OF THE GENITO-URINARY SYSTEM.

In normal health the urine in the bladder contains no micro-organisms, but in various pathological conditions they may be present. Thus in suppurative nephritis, pyelitis, and cystitis, cocci and *B. coli* are found; in similar tubercular lesions, the tubercle bacillus; in enteric fever, the *bacillus typhosus* or *B. coli;* in gonorrhœal cystitis, the gonococcus; and in general infections, such as glanders, pyæmia, and endocarditis, the various organisms associated with these diseases.

After passing through the urethra the urine invariably contains a number of micro-organisms which have their normal habitat there—the smegma bacillus, diplococci, and streptococci.

Method of Securing Specimens of Urine.—In order to avoid contamination from the micro-organisms in the urethra the following routine should be adopted: The meatus and glans are washed with a solution of perchloride of mercury, and a catheter, which has previously been sterilized by boiling and lubricated with glycerine, is passed. The urine is then received in a

sterilized bottle, which is promptly corked and sealed. Cover-glass films or cultures prepared from the urine as passed are of little service. It requires first to be centrifugalized, and from the sediment thus obtained the necessary preparation may be made. Sometimes, if the urine be mixed with carbolic acid lotion, well shaken up, and allowed to stand in a funnel-shaped beaker, a sufficient sediment remains when the supernatant fluid is poured off.

Tubercle bacilli may be detected in films stained by Ziehl-Neelsen's method, and a careful examination of the accompanying cellular débris may help to indicate whether the lesion is located in the bladder or kidney. The smegma bacillus has a similar staining reaction to the tubercle bacillus, but if the urine be drawn off with the precautions indicated above, there is little chance of contamination by this organism.

The tubercle bacillus is generally found in the urine in small groups, accompanied frequently with pyogenic cocci and pus-cells. Sediment deposited from the alkaline urine of cystitis, examined microscopically, shows *Proteus vulgaris*, *bacillus coli*, the *bacillus ureæ* (not unlike the tubercle bacillus), and various micrococci. Sarcinæ and the yeast fungus also occur in urine.

In a condition known as bacteriuria the urine may be passed so loaded with parasitic life as to present a cloudy appearance. The organisms most commonly present in this condition are micrococci and *bacillus coli*. The urine, which is acid, may have an offensive smell, but the bladder-wall on cystoscopic examination is seen to be healthy, and the condition apparently gives rise to no symptoms.

Whilst it seems likely that the *bacillus coli* escaping from the intestinal tract may excite spontaneous cystitis

and pyelitis, and although it is certain that septic emboli containing staphylo- or strepto-cocci may excite suppuration in the kidney, yet there can be no doubt that the greater danger arises from the introduction from without of germs upon instruments such as catheters, bougies, the lithotrite, and the cystoscope. It is therefore of the utmost importance that these should be carefully sterilized before use, and that as lubricants pure glycerine or sterilized olive-oil only should be employed. Roosing* maintains that with the greatest precautions organisms may still be introduced into the bladder. He recommends the use, in cases of daily catheterization, of an application to the bladder of a 1 per cent. solution of phenosalyl, and in all cases of a single introduction of an instrument the injection of 40 to 50 c.c. of a 2 per cent. solution of silver nitrate.

The centrifugal machine is of especial value in the examination of urine for bacteria. One of the best forms is the Hæmatocrit made by R. and J. Beck. In this apparatus tubes containing about one ounce of urine are placed in the revolving arm and rotated until a sediment forms. This sediment is then pipetted into small precipitating tubes with tapering ends, which are then fixed to the holder and rotated at the rate of about 4,000 revolutions per minute, and the resulting sediment examined in microscopic film preparations. Sputum and blood may also be examined in the same manner.

CHOLERA.

The cholera spirillum is a short, stout, comma-shaped organism. It grows well on all the ordinary media, and is best stained with carbol-fuchsin diluted with four parts

* *British Medical Journal*, October 29, 1898, p. 1309.

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, 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 fæcal 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.

POST-MORTEM EXAMINATIONS.

Many interesting points in connection with infective processes may be elucidated by the study of films and cultures taken from the tissues after death. The specific organisms of septic and pyæmic conditions, or of localized suppurations, may be detected, the nature of pneumonic processes or serous exudations determined, or the germs of a zymotic fever isolated.

It is necessary, however, that the post-mortem examination should be made early, 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 per-

chloride of mercury, drying, cutting into their 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 needle, 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 with methylene blue or carbol-fuchsin.

CHAPTER VII.

SERUM THERAPEUTICS. LUMBAR PUNCTURE. PARASITES.

THE treatment of an infective disease by the injection of serum obtained from the blood of an animal previously rendered immune to this same disease is now undergoing an extensive trial. Experimental research has shown that the serum of animals rendered immune to the toxins or micro-organisms of a certain disease has the power, when injected into the healthy individual, of protecting against infection by that particular disease, and may in some cases exercise a curative effect if early symptoms of the infection have appeared. *The action of these serums or antitoxins is in all cases specific; for instance, antistreptococcus serum will protect against streptococcus and against streptococcus only.* Curative serums are prepared alike for intoxicative processes, such as tetanus, or general bacterial infections like that from strepto- or pneumococcus. As to the method of their action, there is still

some doubt. On the whole, however, it would appear that when introduced into the human body the curative antitoxins do not act by directly killing the infecting organisms, or by chemically neutralizing the toxins elaborated by them, but that their effect is produced by stimulating the body-cells to increased activity in resisting the toxic influences or microbial invasion.

Directions for Using Antitoxins.—In all cases it is desirable 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 (p. 23); 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 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, and the syringe is boiled for half an hour before use. It is seldom necessary or advisable to inject more than 10 c.c. into the same place. 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.

It cannot be claimed that in the majority of these the treatment has met with any great measure of success, and in none has the result been so decided as to warrant the abandoning of all the old-established remedies. Many of the better known antitoxins can be obtained from the British Institute of Preventive Medicine, Grosvenor Road, Chelsea, S.W.

Diphtheria.—Antitoxin treatment has given better results in this than in any other disease. The committee appointed by the Metropolitan Asylums Board reported that with its use there was 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 that generally the clinical course of the disease was milder. The bulk of evidence goes to show that diphtheria antitoxin does not excite such complications and sequelæ as nephritis and post-diphtheritic paralysis.

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. — Aronson's antitoxin is sold in 5 c.c. or 10 c.c. phials, the strength being 100 units per c.c. Two thousand units are given as an initial dose, the size and frequency of subsequent doses being governed by the progress of the case. Behring's serum is given in doses of about 600 units, and that of the British Institute of Preventive Medicine in doses of 1,500 units.

Tetanus.—In acute cases with short incubation period the antitoxin treatment has proved a failure. In the more chronic cases the results are encouraging. The dose of Behring's serum is 5 c.c. (500 units), of the British 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.

Much more 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. Of the antitoxin of double strength sent out by the Pasteur Institute 2½ c.c. are injected on each side. The hypodermic medication is continued in addition to this.

Antistreptococcus Serum.—This serum, prepared from the blood of horses and asses rendered immune to the *Streptococcus pyogenes*, has been employed in septicæmia, puerperal fever, ulcerative endocarditis, erysipelas, scarlet fever, and acute anginas. 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 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 British 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.

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. Washbourn advises 10 to 20 c.c. every twelve or twenty-four hours.

Cholera.—Injections of the dead bodies of attenuated cholera vibrios, or of their toxins, have been extensively utilized in India, more especially for prophylaxis, and the reports speak highly of their value for this purpose. The work has been conducted chiefly by Professor Haffkine.

Plague.—Yersin's serum is obtained from a horse previously immunized by intravenous injections of living virulent cultures of the plague bacillus. Though extremely valuable as a prophylactic agent, its curative value is not yet so well established. Repeated doses of 10 to 20 c.c. are advised. Haffkine's vaccine is well spoken of.

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 ·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.—None of the many serums brought

forward for the treatment of tubercular phthisis appear to have any marked influence upon the disease. Koch's new tuberculin, Hirschfelder's oxytuberculin, Maragliano's and Paquin's sera, have all had more or less extensive trials, but have proved disappointing.

Many other antitoxins, amongst which may be mentioned those for the treatment of *enteric fever*, *snake-bite*, etc., have given most successful results in laboratory experiments. They have, however, as yet hardly emerged beyond this stage, and the result of their employment in cases of disease in human beings has yet to be awaited.

On the whole, it may be confidently anticipated that a brilliant future awaits the method of serum therapeutics. At the same time it is clear that results of present value are thereby placed within the grasp of the practitioner of medicine, notably in the treatment of diphtheria. In the foregoing pages an endeavour has been made to supply him with information that will guide him in daily practice, not only as regards his curative efforts, but also in the oftentimes not less important directions of diagnosis, prognosis, and prevention.

LUMBAR PUNCTURE.

Withdrawal of cerebro-spinal fluid may be practised either for purposes of diagnosis or 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 ½ 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. Thus Furbinger (*Berlin Klin. Woch.*, April, 1898) found tubercle bacilli in twenty-seven out of eighty-six cases investigated, and verified the diagnosis by post-mortem examination.

In three other cases which terminated fatally, and in which tubercle bacilli were found, no post-mortem was held. One case of spinal suppuration showed pneumococcus, and a second the micrococcus of cerebro-spinal meningitis. Seven cases in which the bacteriological

examination was negative proved on post-mortem examination to be tuberculous. Jacoby, who has had a wide experience of lumbar puncture, reports an interesting case in which by this means it was possible, in a case of middle-ear suppuration, to diagnose a secondary streptococcal cerebro-meningitis (*New York Medical Journal*, December 28, 1895). When the fluid drawn off is purulent, it is frequently possible to demonstrate the organism exciting the disease by growth on culture media or by means of film preparations. In suspected tubercular cases, and always when the fluid is cloudy, it is best to receive it in a sterile vessel, and reserve it for centrifugalization and inoculation.

The technique is by no means difficult, especially in children, and may readily be obtained by practice in the post-mortem room.

THE MICROSCOPICAL DETECTION OF CERTAIN PARASITES.

(*For the examination of these parasites a lens 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. The method of preparing hairs for micro-

scopical examination is as follows: Pull out a short broken hair from the margin of the affected patch, wash in ether for a few minutes, and then soak in 7 per cent. caustic potash solution for six hours. Dry and mount in Canada balsam.

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 one part, anilin water three 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 Schönleinii*) 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.

Thrush.—This is due to an organism named the *Saccharomyces* or *Oidium albicans*. 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 μ thick, and ten to twenty times as long.

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

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.

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 (× 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 *substage 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 1½ 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 lamplight and saves the eye. These blue glasses are generally supplied with the chimneys. Use the flame edgewise. A bullseye 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 (× 4) and No. 4 (× 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 6½ 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 nosepieces, 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 $\frac{1}{6}$ 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.		160 mm.	160 mm.	170 mm.
Objective		Zeiss A.	Zeiss D.	Leitz.
Power		$\frac{1}{4}$ inch.	$\frac{1}{6}$ inch.	$\frac{1}{12}''$ oil.
Eyepiece	2.	50	240	680
	4.	90	420	1000

$\mu = \frac{1}{1000}$ of a millimetre,

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THE END.

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