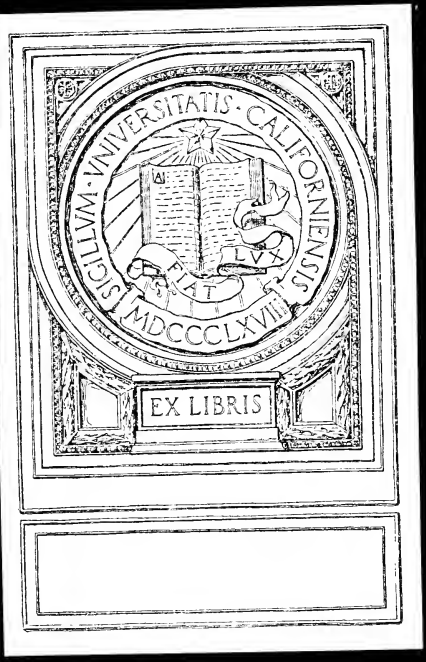
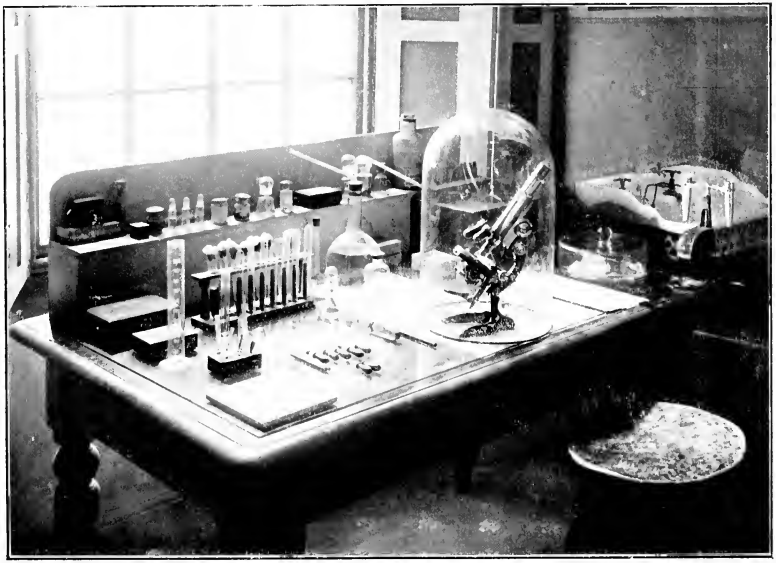
CLINICAL BACTERIOLOGY AND VACCINE THERAPY FOR VETERINARY SURGEONS

W. M. Scott


SIGILLVM · VNIVERSITATIS · CALIFORNIENSIS
LVX
FIAT
MDCCCLXVIII
EX LIBRIS

CLINICAL BACTERIOLOGY AND VACCINE THERAPY FOR VETERINARY SURGEONS

PLATE I.



AUTHOR'S LABORATORY TABLE.

Frontispiece.

CLINICAL BACTERIOLOGY

AND

VACCINE THERAPY

FOR VETERINARY SURGEONS

BY

WILLIAM SCOTT, F.R.C.V.S.

LONDON
BAILLIÈRE, TINDALL AND COX
8, HENRIETTA STREET, COVENT GARDEN
1913

TO

SIR ALMROTH E. WRIGHT, M.D., F.R.S.

DIRECTOR OF THE DEPARTMENT OF THERAPEUTIC IMMUNIZATION,
ST. MARY'S HOSPITAL, LONDON

AS A SLIGHT TRIBUTE TO HIS EMINENCE AS A

VACCINE THERAPEUTIST

PREFACE

If Sir Almroth Wright's prophecy—"the physician of the future will be an immunizator"—is true regarding the future attitude of the practitioner of human medicine towards sero - vaccine therapy, I believe it may with equal truth be applied to the practitioner of veterinary medicine. Certainly there is as much scope for its application in the latter profession as in the former—nay, more: the human practitioner has difficulties and anxious problems to encounter which to the veterinary practitioner are unknown. Every medical man knows with what prejudice his patients look upon any new line of treatment, and particularly so if such treatment is being driven home at the point of the hypodermic needle. For the sake of his own reputation, he is often loath to rouse those latent feelings, with the result that he moves very cautiously—sometimes too cautiously—to the detriment of his patient. Again, a doctor's patients are all possessed of a highly developed nervous system, where the influence of mind over matter is very pronounced, and where emotions and idiosyncrasies are greatly in evidence, requiring a strong personality and the comforting but forcible courage of the trusted physician to quieten; but all these qualifications sometimes avail little, the patient discarding the treatment without fair trial. Or, it may be by a sheer coincidence that after a dose of vaccine has been administered complications in the course of the disease supervene, and although they may have nothing whatever to do with the treatment, vaccine therapy is blamed and condemned, the medical

attendant undergoes a harassing time, his reputation is at stake, and he may perchance lose a lucrative client. The veterinary surgeon is in a much happier position, he does not require to consult his patient, study his whims, or pander to his capricious fancies, or have his reputation held up to ransom if all does not go quite well with the feelings of his patient or the doubtful desires of his client. If he has gained the confidence of his client, he can treat his patient in whatever manner he chooses; all that is required is a restoration of his animal to good health. If, therefore, vaccine therapy is scientifically sound—and who will deny this?—if its results warrant its application in filling a gap no other course of therapeutical treatment can, it behoves the practitioner in his clients' interest and his patients' welfare to take up the treatment, not half-heartedly, which will never lead to success, but giving it wholly and fully his serious consideration, rigidly taking note of every detail, and benefiting from the teachings of each individual case. In the whole field of veterinary science I know of no subject which possesses such opportunities for research and carries with it such active and varied interests than vaccine therapy. Every practitioner can be an immunizer, and to show him that he *can*, with a little diligent effort on his own part, was the primary object I had in view in writing this little book. I am fully alive to the many imperfections it contains, and I know in abler hands there is scope for a much better work. The great portion of this book was written in the small hours of the morning, often after a busy and exacting day, and to those who know the responsibilities and disturbing influences of a busy practice, which are by no means conducive to successful literary effort, not to say mental concentration, I am sure I shall not appeal for their indulgence in vain. I have endeavoured to introduce as little debatable matter as possible in order that the reader may not have to wade through unnecessary detail.

When further information on bacteriology and a fuller

description of the symptoms and diagnoses of diseases is required, the reader must be referred to standard works on these subjects, the primary object of this book being the making and the administration of vaccines.

An exception, however, has been made in the case of two diseases—namely, *tuberculosis* and *swine fever*—where a fuller descriptive detail has been gone into, owing to the prominence given to these two affections by the State and the veterinary surgeon of the present day. My grateful thanks are due to Sir Almroth Wright for kindly admitting me to the Vaccine Department of St. Mary's Hospital, where I obtained much useful and practical information; and to Dr. Poels for so courteously giving me access to the State laboratories and clearly describing the technique of sero-vaccine therapy as practised at Rijksseruminzichting, Rotterdam. I am also indebted to Messrs. Rose and Carless, Dr. Hewlett, Dr. Emery, and Mr. Jowett for the loan of the various photographs appearing throughout the book. For the illustrations of instruments and apparatus I have to thank Messrs. Arnold and Sons and Messrs. Gallenkamp.

Finally, I have to thank Messrs. Baillière, Tindall and Cox, for their courtesy in making alterations and cheerfully carrying out suggestions which appeared to me to be necessary as the work was going through the press.

W. S.

Friarn House,
Bridgewater,
October, 1913.

CONTENTS

APPENDICES

LIST OF ILLUSTRATIONS

PLATES

IN THE TEXT

CLINICAL BACTERIOLOGY AND VACCINE-THERAPY

CHAPTER I

THE LABORATORY

This should be a fair-sized room, and where possible only used for laboratory work. Ample daylight must be admitted, and the whole ought to present a clean appearance. The walls should be washed with a light-coloured hygienic wash or paint, of which there are many.

The woodwork should be painted white, as also the cabinets, cupboards, etc., and the floor covered with a plain linoleum.

As it is essential for media, stains, etc., and even the microscope itself, to be kept at a fairly even temperature, the laboratory should be heated in the winter months by hot-water pipes or fires.

The bench or table must be placed in the brightest place in the room, for preference opposite a window.

An ordinary table will answer one's purpose. This should have a blackened top, along the centre of which runs a piece of white paper, and the whole top should be covered with a sheet of glass.

Plate-glass, of course, is best, but on a large table it is somewhat expensive; and provided the top is perfectly level, ordinary sheet-glass will answer our purpose.

The back of the table ought to carry a rack for holding

stains, etc., and be within easy reach of the operator. These stains should be in glass bottles, with rubber teat droppers fixed in them, and labelled.

The microscope is conveniently placed in the middle of the table, and, of course, accessible to the operator, or in the best position for reflecting the light from the window, a hanging gas-bracket, or an electric pendant.

Other accessories found upon the table should be a test-tube rack, cornet forceps, platinum needles, forceps, watch-glass, various shaped and sized pipettes, spirit lamp, slide trough for staining, distilled water flask and dropper, a slab of plasticine, and one or two glass dishes with covers to them.

Another important addition is a laboratory sink. Any ordinary sink will answer if it is deep enough, and hot and cold water should be laid on, with an additional small tap under which is fixed a bracket shelf, and upon which the glass slide to be washed is placed for the removal of excess of stain, etc.

Two or three rows of shelves should be fixed upon the wall to carry bottles, etc.

A pair of balances will be found most useful, and if put in a cupboard with a glass door and fixed upon the wall at a convenient height they occupy little room, and are within easy access. Another strong shelf should be made to carry an incubator and the two sterilizers (*i.e.*, moist heat and dry heat). Under each of these a gas-tap should be fixed, a hole bored in the shelf, through which a rubber tube passes, and leading to a Bunsen lamp, which is used for the purpose of heating these apparatus.

It is advisable to cover the surface of the shelf with sheet metal to reduce the danger of fire.

In addition one must have glass tubing, one or two glass funnels, graduated measures, and glass flasks. The latter need not be graduated, as they are somewhat expensive. To standardize them, fill with water from a given graduated measure, and with a grease pencil draw a ring round in

PLATE II.

A CORNER IN THE AUTHOR'S LABORATORY.

To face page 2.

a line with the water-mark. Various-sized flasks will be found useful, ranging from 100 c.c. to 1,000 c.c. When the practitioner, for monetary reasons, is compelled to limit his expenditure in fitting up his laboratory, it is astonishing how he can with a little ingenuity economize. For example, a Thermos flask may take the place of an incubator, an opsonizer, and moist-heat sterilizer, the only extra required being a thermometer, which is passed through the cork. Then, again, a cheap dry-air sterilizer can be improvised by using an ordinary baking oven, and suitable for sterilizing glass ware, instruments, etc.

The following is a list of requisites necessary for the laboratory, with an improvised addition :

	Improvised.
Incubator and opsonizer.	Thermostat.
Sterilizer (moist), Koch's.	
Sterilizer (dry).	Baking oven.
Centrifuge (hand, water, electric).	
Pair of scales.	
Thermometers (one registering up to 100° C., and one to 300° C.).	
Oil sterilizer.	Soup ladle fixed on stand.
Vaccine syringe.	Hypodermic syringe
Bunsen burner.	Blow lamp.
Spirit lamp.	
Tubing (glass and rubber).	
Test-tubes.	
Slides.	
Cover-glasses.	
Flasks, funnels.	
Watch-glasses.	
Cedar-oil.	
Canada balsam.	

Platinum needles (straight and looped).
Filter-paper.
Glass-cutting knife.
Pipettes.
Teats (rubber).
Plasticine.
Emery-paper (Hubert's 00).
Cotton-wool.
Bottles, stock vaccines.
Bottle of lysol.
Etc.

The Microscope.

For bacteriological work a sound microscope is essential. It must have a firm, rigid stand, and an accurate fine and coarse adjustment.

It should be fitted, or be capable of being fitted, with substage condenser, mechanical stage, objectives $\frac{2}{3}$, $\frac{1}{6}$, and $\frac{1}{12}$, oil immersion, and two eyepieces giving a magnification up to 1,000 diameters. When higher magnifications are required, it is a question of employing extra eyepieces which possess higher magnifications than those usually supplied with an ordinary bacteriological microscope. These can be obtained from any of the leading microscope-makers.

A comparatively new and important accessory for bacteriological work is the dark-ground condenser constructed for observation of living bacteria in an unstained condition. Under this mode of illumination no staining is required, and the bacteria are so illuminated that they appear as self-luminous bodies upon a dark background.

The microscope we possess is a Leitz, latest model, and is fitted, as above described, with an additional continuous safety micrometer fine adjustment, which prevents any chance of damaging the objectives if accidentally overfocussed and brought into contact with the specimen slide, a most essential point, at least so far as beginners are con-

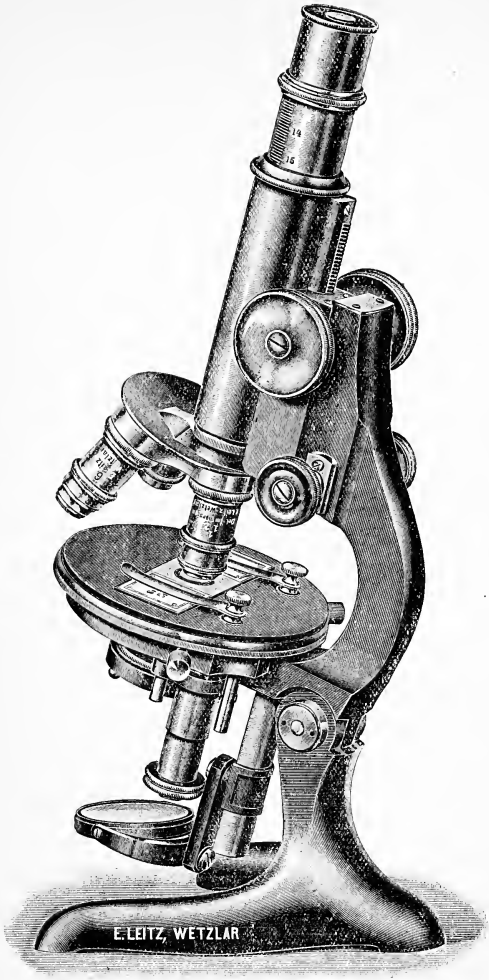


FIG. 1.—THE MICROSCOPE.

This model does not illustrate a mechanical stage, which is a most essential accessory.

cerned. The instrument is also fitted with an arched limb, which gives a large working space above the object stage, and incidentally acts as a convenient handle for lifting the microscope without putting any extra strain upon some of the other vital parts.

The stage is circular, and can be rotated on its own axis; it is also fitted with a centring arrangement, which is an advantage when making an examination of urinary deposits, etc.

The mechanical stage is detachable, and is so fitted to the limb of the microscope that after removal it can be screwed back and occupy exactly the same position each time.

The instrument, of course, inclines to a horizontal position, and possesses all the necessary movements for ordinary bacteriological purposes.

The Use of the Microscope.

Beginners should accustom themselves to use either eye, and the unemployed eye should remain open and passive.

Daylight is the best illuminant, and especially so if reflected from a white cloud. Direct sunlight, of course, is useless. As the majority of practitioners, however, of necessity do their microscopical work when the light of day has departed, artificial illumination is called into requisition. There are some excellent illuminants made by microscope-makers for this purpose, but an ordinary gas or oil light suffices for one's needs.

When examining an object with the dry lens, the concave mirror should be used, which converges the light on to the object. With an oil immersion lens a flat mirror should be used; the rays reflected are converged by the condenser and brought to a focus on the object, so that by the use of the condenser a considerable amount of light is obtained. To reflect the light on to the condenser, the mirror should be moved about until a suitable angle is arrived at.

To a beginner this is often somewhat difficult, in which

case he should remove the eyepiece and look down the tube, moving the mirror until he sees clearly the reflected light.

We will now presume that a slide has to be examined under a $\frac{1}{12}$ oil immersion. If the specimen is only faintly stained, it is well to make a ring with a grease pencil round the field. This serves as a border from which to focus, and is most useful. A drop of cedar-oil should be placed on the specimen, and the tube lowered by the coarse adjustment until the objective almost touches the slide. The beginner should now place his eye on a plane with the stage, at the same time steadily lowering the tube. Immediately the lens touches the oil it will appear to rise to meet the objective. With the fine adjustment then carefully focus until the objects on the field become well defined.

If the specimen contains bacteria in irregular distribution, it is advisable to make one's examination by way of locating the best area with a low power first; in fact, many bacteriologists carry out the process as a routine practice. It may be added an oil immersion lens transmits two or three times more light than a dry lens of the same power, while the Abbé condenser collects the rays of light reflected from the mirror, and concentrates them at a point about 2 millimetres above its upper surface.

If the nozzle of the objective requires cleaning—and this should be done fairly often—the oil can be removed by xylol, turpentine, or benzine on a piece of chamois leather.

The whole instrument should be kept free from dust and other accumulations, and when not in use should be covered with a glass shade.

The Incubator and Incubation.

To grow bacteria outside the animal body some form of incubator is required. This consists of a metal box with double walls, the space between which is filled with water, and the whole encased in some non-conductor, such as felt

or wood. The water is heated at the bottom of the incubator by a gas, oil, or electric lamp. The top of the incubator has three circular holes in it, one for the insertion

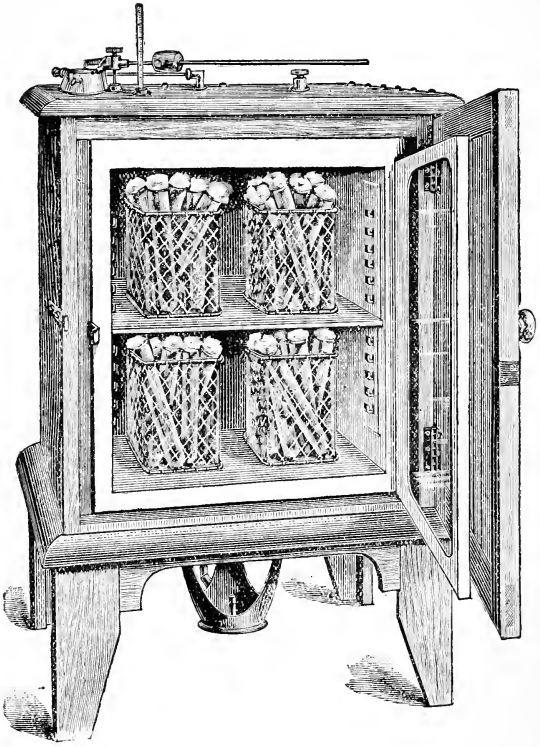


Fig. 2.—Incubator.

of a thermo regulator, one for the thermometer, and a third for the filling of the water chamber. The thermo regulator is composed of a mercurial bulb which passes through a

cork at the top of the incubator, entering the air chamber. The mercury rises and cuts off, or rather limits, the supply of gas entering the burner, and so regulates the heat-supply. The regulator in the writer's possession is in itself regulated by a small screw, so that the temperature may be set at any desired degree. The incubator should be provided with double doors, the inner one of which should be made of glass. The cultivation of bacteria usually takes place in the laboratory at two temperatures—*i.e.*, 20° C. (68° F.), suitable for those germs which grow out-

Fig. 3.—Thermo Regulator.

side the animal body; and 37° C. (98·6° F.), or body temperature. All media are suitable for the higher temperature save gelatin, which melts at about 25° C.

It must also be remembered that bacteria do not grow well in sunlight; they should therefore be incubated in the dark.

Sterilizers and Sterilization.

There are two kinds of sterilizers in use—*i.e.*, dry and moist.

A *dry-heat sterilizer* consists of a double-jacket square box, usually made of strong sheet steel, with a copper bottom and two movable perforated shelves. On the top is a regulating slide and two holes, one for the insertion of a thermometer, and the other for a thermo regulator. The sterilizer is fixed on a raised metal stand about a foot in height, the heat being supplied by a Bunsen burner or by a powerful oil lamp. The articles sterilized by dry heat are glass flasks, plates, test-tubes, pipettes, cotton-wool, etc.

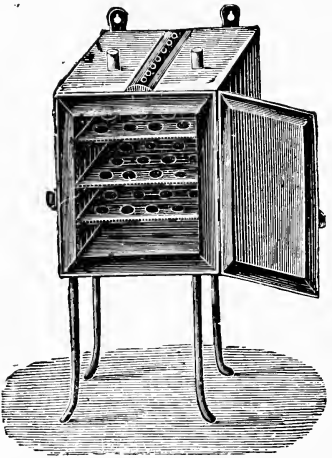


Fig. 4.—Dry Heat Sterilizer.

To completely sterilize any of these, a temperature of 150° C. must be maintained for three-quarters of an hour. When glassware is being sterilized, care must be taken to allow the sterilizer to cool before removing any of its contents; otherwise they are liable to crack when exposed to a lower outside temperature.

Steam or moist-air sterilizers consist of a water-bath over which is a cylinder with a perforated diaphragm fixed

6 inches from the bottom. Several varieties are in use. The one we use is Koch's pattern, and answers all practical purposes. A steam sterilizer is used for sterilizing culture media.

To sterilize media thoroughly, it must be exposed for half an hour daily for three successive days. The first steaming

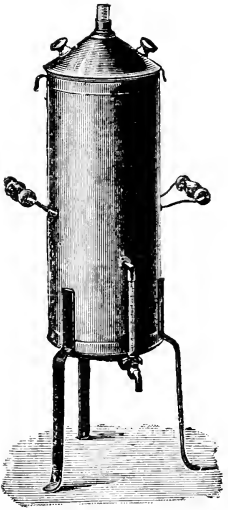


FIG. 5.—MOIST HEAT STERILIZER (KOCH).

destroys all developed bacteria, the second destroys the spore after it has developed into a germ, and the third kills any bacteria which may not have developed from a spore during the first interval.

The Centrifuge.

This is a most essential laboratory accessory, and there are a variety on the market, but whether they are driven by hand, water, or electricity, the principle is the same—

namely, to obtain so many revolutions per minute, varying from 2,500 to 5,000. It is devised for the separation of the solid and liquid elements, blood, pus, milk, urine, and other fluids, for examination purposes.

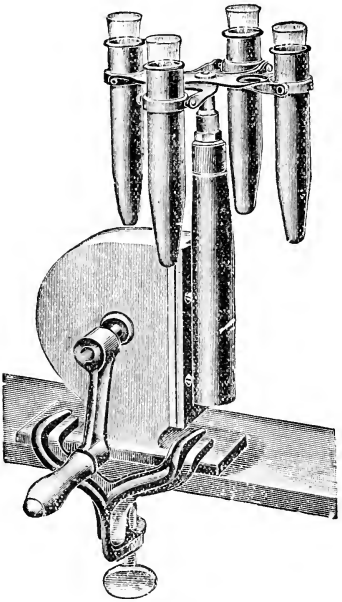


FIG. 6.—HAND-DRIVEN CENTRIFUGE.

The hæmatocrite attachment and tube-holders for purposes of combined lightness and strength are made of aluminium.

A hand centrifuge is conveniently fitted to the table (which must be firm and rigid) by means of a double-tongued clip.

Where one can obtain a sufficient pressure, a water-

driven centrifuge is a thing much to be desired, and which is in most common use.

Centrifugalizing Blood.—The quantity of blood required is placed in a blood-tube, and before coagulation takes place is mixed with citrate solution and centrifugalized. The red cells are thrown down first by reason of their specific gravity, appearing at the bottom as a red layer;

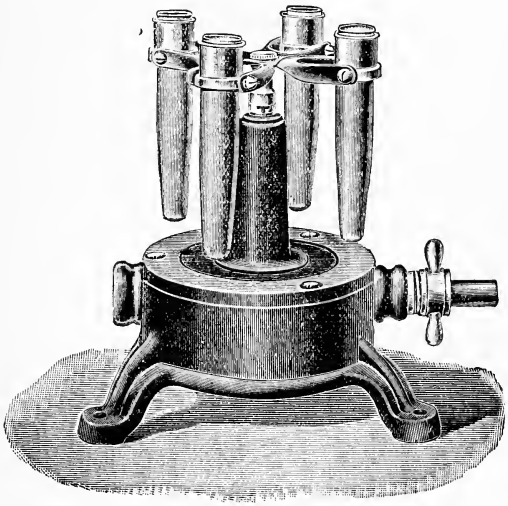


Fig. 7.—Water-driven Centrifuge.

next the white corpuscles as a grey layer; and on the top will be found the liquor sanguinis comparatively clear. To complete this process one has to use the centrifugal force from ten to fifteen minutes.

Centrifugalizing Urine.—To centrifugalize urine, specially graduated tubes, tapering to one end, are used, the speed required being about 2,500 revolutions per minute.

Where one is searching for bacteria in limited numbers,

it will be found good practice to centrifugalize the urine in a large tube first, then pipette the top fluid, leaving about 1 c.c.; this or less may be placed in a small precipitating tube and centrifugalized. By this process a greater concentration is obtained.

Centrifugalizing Milk.—Milk may be centrifugalized for the purpose of isolating a variety of bacteria, and, of course, the most important in cow's milk is the bacillus of tuberculosis so far as veterinary surgeons are concerned.

Mix 20 c.c. of milk with 1 c.c. of a 50 per cent. solution of potash, heat in a water-bath until the solution turns brown, add 20 c.c. of acetic acid. Shake the whole well, heat in water-bath for two minutes, and centrifugalize for ten to fifteen minutes.

The top fluid is now poured off; add 30 c.c. of hot water to the sediment and centrifugalize; pour off the supernatant fluid, and make smears on three slides; fix, stain, and examine.

CHAPTER II

PREPARATION OF CULTURE MEDIA

At the present time a large variety of culture media are in use, but for general routine laboratory work the list may be curtailed to five—*i.e.*, (1) broth; (2) agar-agar; (3) gelatin; (4) blood-serum; (5) potato. These, except potato, can be purchased already prepared in tabloid form, but for economic reasons they should be prepared in one's own laboratory.

Broth.

This is easily prepared, and it forms the basis for the majority of other media.

Method.—Take 500 c.c. of water, boil well for half an hour in a double-contained saucepan, add 5 grammes Lemco, 10 grammes peptone, and 5 grammes of common salt; then boil for thirty minutes. Now test with litmus-paper, and it will in all probability be slightly acid, in which case a solution of soda should be added drop by drop until the reaction is slightly alkaline. If too much alkali is used, a little hydrochloric acid (diluted), to re-acidify, should be added, and then again neutralize. Now add sufficient water (some having been lost in evaporation) to make up 1 litre, and filter through a double thickness of filter-paper into a 500 c.c. flask.

Dry the mouth of the flask thoroughly to keep the cotton-wool from sticking, and plug firmly with absorbent wool. Sterilize at 60° C. for half an hour for three successive days.

Gelatin.

Method.—Take 100 c.c. of broth, add $12\frac{1}{2}$ grammes of finely-cut gelatin, boil thoroughly until all is dissolved, filter into a flask as was done with the broth; but, as gelatin solidifies on exposure to cold, extra heat must be applied during the filtering process. The flame of a spirit lamp placed close to the filter funnel answers the purpose. A double-jacket hot-water funnel can be purchased to serve the same object, or the filter, media, and flask, may be placed in an oven with a temperature registered at 40° C.

Gelatin is also used to grow bacteria for diagnostic purposes, as some microbes produce a digestive ferment and liquefy gelatin, and others do not; again, some liquefy rapidly, and others slowly. Gelatin also may be melted at 25° C., at which temperature bacteria are not destroyed. It is therefore a useful medium for "plating-out" purposes.

Agar-Agar.

Method.—Take 100 c.c. broth, cut up 2 grammes of agar into very fine pieces, soak in acetic acid (diluted).

To make the solution, take 4 c.c. glacial acetic acid, add 500 c.c. water, and soak for fifteen minutes; strain off the acid, wash the agar in water until blue litmus-paper does not become red. Now place the broth in a beaker and boil until all the agar has been dissolved. Neutralize with an alkali, and allow it to cool to about 50° C. To make the media more clear, the white of one egg to each 500 c.c. of media should be added before boiling again. Filter this gently through a double thickness of non-medicated surgical lint which has been previously moistened, and prevent the media from solidifying, as was done in the case of the gelatin.

Blood-Serum.

This medium is somewhat difficult to prepare, but, fortunately, it can often be dispensed with altogether. Moreover, it can be obtained all ready for laboratory work.

Method.—Expose the carotid of a horse with due antiseptic precautions, and insert a sterile cannula into the vessel. The blood should now be collected in a sterile jar by means of a rubber tube fixed to the end of the cannula, and allowed to clot for twenty-four hours.

The serum is then drawn off with sterile pipettes, placed in sterile test-tubes, and solidified by heating to 70° C., the tubes, of course, laid on their sides at a proper angle to give the necessary slope.

Potato.

Method.—The skin should be well scrubbed and peeled sufficiently deep to remove the eyes; cut into long fingers, and round the ends. Now divide into two, cutting obliquely, so that when one is inserted in a test-tube it will have the

FIG. 8.—(*A*) POTATO FINGER. (*B*) CUT DIAGONALLY READY FOR INSERTION IN TUBE.

shape of a sloped gelatin or agar medium (Fig. 8). Soak well in cold water for twelve hours, put each into a test-tube, plug, and sterilize for three-quarters of an hour on three successive days.

Filling and Preparing Test-Tubes for Cultivation.

Having prepared the medium, we require sloped and stabbed test-tubes ready for use. Each tube should be plugged with cotton-wool, and a number thus prepared laid out on the table. A piece of glass tubing should be procured, on one end of which a rubber pipe should be fixed, the glass tubing having been previously marked to allow the taking up of a given quantity of medium each time. The glass end should now be inserted

into the hot medium, the tubing fixed in the mouth by the teeth, the medium sucked up to the volume mark and then expelled into the test-tube, and repeated until all the tubes have been charged. Care must be taken not to allow any of the medium to come in contact with the neck of the tube, in which case the cotton plug is liable to stick. Another method is to procure a glass funnel to which is fixed a piece of rubber tubing. The tubing is

Fig. 9.—Plugged and Sloped Medium Tube.

clipped by a spring clip (clasp forceps answer the purpose well), and the whole fixed upon an iron retort-stand. The funnel is filled with the medium, and, if need be, kept hot by a spirit flame, or a water-jacket funnel used. The tubing should now be passed down the test-tube to near the bottom, and the requisite quantity allowed to run in by loosening the grip of the clip. Here, also, care has to be

taken not to allow the medium to stick on the mouth or neck of the test-tube.

The tubes are usually charged with $1\frac{1}{2}$ to 2 inches of medium. They should now be sterilized in a Koch sterilizer for half an hour on three successive days, and laid on their sides to cool to give a sloped surface, or upright for a stab culture, as the case may be.

CHAPTER III

CULTIVATION OF BACTERIA

Inoculating Medium Tubes.

Having chosen our culture medium upon which we desire to obtain a bacterial growth, either with the idea of assisting or confirming our microscopical diagnosis or of obtaining a bacterial emulsion with the view of making a vaccine, we sow our germ-laden material in the following manner:

Take a platinum loop (this consists of a piece of glass tubing, the end of which has been previously softened by

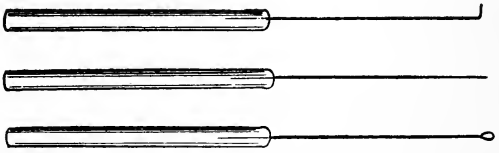


FIG. 10.—PLATINUM NEEDLES.

heat, and while in the flame a piece of platinum wire is forced into the soft glass); pass it through the flame of a spirit lamp to sterilize it. Hold in the left hand between first and second fingers a medium tube, remove the cotton plug and place it between the ring and little fingers of the same hand, taking care at the same time to hold the tube (if solid medium) mouth downwards to prevent ingress of dust, germs, etc. Dip the needle into the morbid material and take up a small loopful; pass it carefully down the tube until it reaches the bottom, letting it rest gently

on the surface of the medium, and draw it slowly along the whole length, care being taken not to graze the surface with the loop end. Withdraw the needle and sterilize it as before. Now take up the cotton-wool with forceps and set it on fire, and whilst aflame thrust it into the mouth of the tube; label the tube with grease pencil—owner's name, animal, and date—and put into the incubator.

Where we desire a stab culture, the process adopted is

Fig. 11.—Plugged Stab Medium Tube.

the same; but in this case we pass the needle, not looped, but pointed, laden with the pus or other material, through the axis of the medium, give it a slight rotation, withdraw, and plug as before.

Plate Cultivation.

Glass plates were originally used for cultivation, but shallow dishes 2 or 3 inches in diameter, with lids, and known as Petri dishes, are now in use.

When we desire to obtain pure cultures, bacteria grown upon test-tube media offer great difficulties. To overcome these, plate cultivation is adopted. This consists of several shallow glass plates, each provided with a lid. The principle adopted is as follows: When suitable, gelatin is usually employed, but agar also may be used. Take three tubes of gelatin medium and melt in a hot-water bath. Gelatin cools to 25° C., and agar to 45° C. Mark the tubes 1, 2, 3. No. 1 is inoculated with a trace of the material from which it is desired to make the pure culture, and completely mixed by rolling the tube between the hands, so that it may permeate thoroughly the melted gelatin. Should this mixture be plated out now, however, it will be found to be too rich in bacteria, necessitating their adhering together when grown, and obscuring their characteristics.

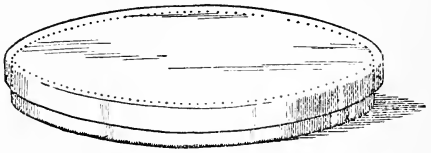


Fig. 12.—Petri Dish, or Plate.

To obviate this, a loopful of No. 1 tube is then taken to inoculate tube No. 2. A further dilution should be made by inoculating in a similar manner No. 3 from No. 2, taking up, say, a half-dozen loopfuls. The Petri dishes having been previously sterilized in a dry-air sterilizer, they are ready for use. The cotton-wool is now removed from the tube, and the mouth sterilized by passing through the flame of the spirit lamp, and the contents of each poured into separate culture plates, the lid being slightly raised for the purpose, and put aside to incubate. They should be examined daily with a hand lens or a low power of the microscope. The latter can be done by tilting the plate and placing it on the stage of the microscope.

The culture in plate No. 1 will usually be too dense to differentiate, but plates Nos. 2 and 3 can as a rule be used for subculture. This is done by taking off a few colonies with the platinum loop and inoculating a test-tube medium and incubating them. Agar plates may be used in a similar manner, but, owing to their rapidly-cooling qualities, the work must be done very quickly. To grow bacteria with the view of procuring a large supply of bacterial emulsion, a convenient method is to pour the medium into flat-sided bottles, allow it to set, and then pour over the surface a small quantity of bacteria-laden broth, taking care it is evenly diffused all over the face of the medium, and that no surplus overflow exists.

Anaerobic Cultivation.

Many bacteria will not grow in the presence of oxygen (free), and various methods are adopted to remove oxygen from the atmosphere they grow in. Some of these methods are very simple, others very complex. The simplest are as follows:

1. Take a narrow test-tube three-quarters filled with sterile agar medium to which has been added a 10 per cent. solution of glucose, place it in hot water, and boil well for five or ten minutes. Cool and make solid by dipping in cold water. The germ-laden needle (a large one) should now be thrust into the axis of the medium, reaching the bottom, rotated and withdrawn, and the sterile plug inserted. The tube is then heated at the upper border of the medium to seal the puncture, and a well-fitting rubber cap applied. The tube is now ready for incubating.

2. Another simple method is to insert a plug of cotton-wool into a test-tube, place some pyrogallic acid on it and moisten with caustic soda or potash, then a little layer of cotton-wool, and fix on the mouth of the tube a tight-fitting rubber cap. As the oxygen becomes absorbed, the cap will be drawn in by suction.

Four grammes of pyrogallic acid and 8 grammes of

caustic soda dissolved in 15 c.c. of water will absorb 1,150 c.c. of oxygen.

3. The following is more complicated, as we require coal or hydrogen gas to replace the oxygen. It is known as "Fränkel's method."

A stout test-tube has inserted into its mouth a rubber cork having two perforations, through which two pieces of glass tubing are passed. One tube reaches into the medium;

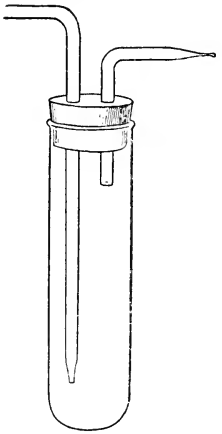


Fig. 13.—Fränkel's Tube for Anaerobic Cultivation.

the other is shorter and does not. Outside the test-tube the tubes are bent at opposite angles to each other, and gradually taper to points. The long tube is connected with the hydrogen supply, and the small tube permits of its exit. The stream of gas should be kept up for several minutes at least, to permeate the whole medium. The short tube should now be sealed off by the blowpipe flame, the long tube in the same way, and the joint at the cork covered with paraffin.

CHAPTER IV

STAINING METHODS AND STAINS

When it is desired to make a microscopical examination of a culture, the following procedure is adopted: Place on a clean slide a drop of distilled water; take the culture-tube between the index and middle finger of the left hand; remove the cotton plug and direct the mouth of the tube downwards if the media is solid; sterilize a platinum needle, and when cool pass it into the tube and pick off a portion of a colony with the loop, taking care not to take too much. Now heat the mouth of the tube with the flame; scorch the cotton plug, and thrust it into the test-tube. The platinum loop, laden with bacteria, should be well mixed with the drop of sterile water previously placed at one end of the slide, and the bacterial emulsion thus made spread out with the platinum needle laid flat on the slide, or a Wright spreader may be used. Such a spreader is made as follows:

Wright's Spreader.

Take the thinnest possible slide, make a nick with a glass-cutting knife* about halfway along its side; then grasp the two ends between the finger and thumb of either hand, and, advancing the thumb of the right hand as far as, or a very little beyond, the intended line of fracture to serve as a fulcrum, break the slide across by putting a transverse strain upon it, at the same time exerting a pull in the longitudinal direction. An arch is now formed; the point which is supported by the thumb will correspond to the

* To make a glass-cutting knife, see p. 47.

crown of the arch.* Cut off the corners by nicking them with a glass-knife, and mount the spreader on the back of another slide, fixing it with sealing-wax.

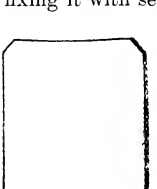


FIG. 14.—WRIGHT SPREADER.

The concavity of the spreader so made will allow for the passage through of the smaller elements, and it follows that as we increase the angle which the spreader makes

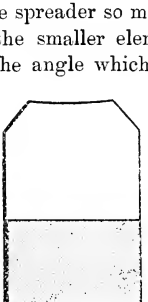


FIG. 15.—WRIGHT SPREADER MOUNTED ON ANOTHER WITH SEALING WAX.

NOTE.—The curve has been exaggerated to make the description more clear.

with the slide we proportionately raise the height of the arch, and so allow the larger elements to pass through.

* Sir A Wright's "technique of the teat and capillary glass tube."

This form of spreader will be found to be most useful in the making of blood-films and in counting bacteria against the blood-corpuscles when we desire to make a standardized emulsion. When dealing with pus only, it is necessary to put a drop on the end of a slide and spread it. If it is too thick, a little distilled water or normal saline solution should be added. Needless to add, to get a perfectly even spread, a clean, grease-free slide should be used. The film so made is now allowed to dry, and fixed by heat sufficient to coagulate the albumin and to retain the bacteria. This heating should be done by passing the slide through the flame of the spirit lamp (specimen face upwards) two or three times, and made just hot enough to bear comfortably on the back of one's hand. The chosen stain is now filtered on to the slide through filter-paper, then washed

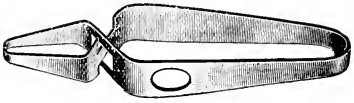


Fig. 16.—Cornet Forceps.

in tap-water, dried with filter-paper, and completely dried over the flame. In the same way cover-glasses are stained, holding them with cornet forceps.

Usual Method of Fixing Films.

1. The simplest method of fixing a film to a slide or cover-glass is to pass it through the flame of a Bunsen burner (specimen side uppermost) three or four times. In this case the albuminous elements are coagulated by heat.

2. Absolute alcohol fixes a specimen in five to ten minutes.

3. Corrosive sublimate (a saturated aqueous solution) fixes in two to three minutes, and even less in some cases.

4. Formalin (a 10 per cent. aqueous solution) fixes in two or three minutes.

As we shall see (page 33), where Jenner's or Leishman's stains are used no fixing is required, as these stains are already supplied with fixing agents.

Stains.

Where one is examining pathological fluids, blood, etc., for bacteria, their presence is more readily detected when they are stained. Unfortunately, however, other elements in the field take up the staining material, thus rendering the presence of bacteria less effective. We possess a method of staining which has the distinct virtue of staining only bacteria, leaving pus cells, débris, etc., intact —*i.e.*, Gram's method. Moreover, this method of staining has another advantage, for by it some bacteria are stained and some are not. It is therefore used as a diagnostic stain, as we have certain bacteria which stain by Gram's method, and called "Gram-positive," and other bacteria which do not stain—"Gram-negative."

Gram's Method of Staining.

Method.—Spread the fluid on a slide, dry, and fix as already described. Stain for two or three minutes with carbol gentian violet or aniline gentian violet; wash in water to remove excess of stain. Flood with "iodine solution" (iodine, 1 part; pot. iodide, 2 parts; water, 300 parts) for about one minute; wash off in alcohol until no more stain comes away. If the slide is left very pale, one may usually take it for granted there are no Gram-positive bacteria present, or at least very few. The film should now be subjected to a contrast stain, carbol fuchsin (diluted) or neutral red; wash in tap-water, dry with filter-paper, mount, and examine. If the bacteria are Gram-positive they will be dark violet, almost black, in colour; those Gram-negative will follow the contrast stain, as will blood-corpuscles, pus, cells, débris, etc.

The following is a list of the more common Gram positive and negative bacteria:

Gram-positive:

Staphylococcus albus, aureus, citreus.
Streptococcus.
Pneumococcus, diplococcus.
Bacillus of anthrax.
„ of tuberculosis.
„ of diphtheria.
„ of tetanus.
„ of swine erysipelas.
„ of blackquarter.
„ of acne.
Micrococcus tetragenus.
actinomycosis.
discomycosis.
bothriomycosis.

Gram-negative:

Bacillus coli.
„ *mallei.*
„ *pyocyaneus.*
Bacillus of malignant œdema.

The following remarks will apply only to the most useful and common stains required in everyday practice:

1. Gentian violet.
2. Methylene violet.
3. Fuchsin.
4. Carbol-thionin.
5. Neutral red.
6. Eosin.

Any of these stain bacteria, cells, and nuclei.

Aniline Gentian Violet.

Place in a bottle some aniline oil, and add water (always distilled); shake well, seeing that more oil is added than

can be dissolved. Allow this to settle, and then filter through a double thickness of filter-paper. To 9 parts of this solution add 1 part of a saturated alcoholic solution of gentian violet.

This stain keeps much better if carbolic lotion 1 in 40 is added.

Loffler's Methylene Blue.

This stain is made by adding 30 c.c. of a saturated alcoholic solution of methylene blue to 100 c.c. of a 1 in 10,000 solution of caustic potash. The potash solution is made by taking 1 c.c. of a 10 per cent. solution of caustic potash and making up to 100 c.c. with water; shake well, and pour away 90 c.c., making up the remainder to 100 c.c. with water, and shaking thoroughly. Now add 1 minim of the 10 per cent. solution to 2 ounces of water.

This stain keeps well.

Carbol-Fuchsin (Ziehl-Neelsen).

This stain is prepared by adding a saturated alcoholic solution of fuchsin to carbolic lotion (1 in 20) until the fluid has lost its transparency, the preparation usually being about 1 in 9 strength.

The above makes a very powerful stain, and should be diluted, when required, with five times its volume of distilled water.

Carbol-Thionin.

This stain can be used instead of methylene blue. It is made by adding 1 gramme of thionin to 100 c.c. of 1 in 40 solution of carbolic acid in distilled water.

Particular care should be taken to always filter this stain, as it crystallizes very readily; in fact, it is a good axiom to filter all stains before using as a matter of routine. Before use dilute with equal quantities of distilled water.

Eosin and Neutral Red.

These stains are most commonly used as contrast stains. They are particularly clean stains, the process taking place in half a minute to one minute.

Capsule-Staining.

Many bacteria are invested by capsules, and their presence or absence assists in their identification. To stain the capsule, dip the specimen in a mixture of equal parts of carbol-fuchsin and distilled water for a few seconds, rinse in water, stain for fifteen seconds in an aqueous solution of gentian violet, wash in water, dry on filter-paper, and mount.

Flagella-Staining.

Many organisms possess delicate plasmic outgrowths, known as "flagella," and by reason of their delicate construction they are somewhat difficult to stain. To show them to the best advantage, it is advisable to have a well-diluted field of bacteria. Take a very small quantity of young culture and place it on a slide. Dilute with water. From this take a loopful and spread it rapidly on another slide, and allow it to air dry; then fix by passing it through the flame twice, taking care not to over-heat. Various methods are adopted for staining flagella.

Pitfield's Method is as follows:

Take—

(i.) Saturated solution of alum ... ... 10 c.c.
Saturated alcoholic solution of gentian violet ... ... 1 c.c.
Filter and bottle.

(ii.) Tannic acid ... ... 1 gramme
Distilled water ... ... 10 c.c.
Filter and bottle.

Mix an equal given quantity of the two solutions together, and flood the specimen already prepared with it. Hold it over flame of spirit lamp until it nearly boils. Put it aside for one minute, and wash in water; dry, stain with aniline gentian violet for a second or two, wash in water, dry, and mount.

Bonhill's Method.—Take a small quantity of culture material on a platinum loop, and move it about in a little distilled water placed in a test-tube. Leave the tube undisturbed for six minutes, and then place 1 drop on a clean grease-free cover-glass and dry in air. Now fix in the flame, taking care not to overheat, and pour some orcein solution on a watch-glass; float the cover-glass specimen side downwards on the surface of the stain, and heat gently, leaving the specimen in the stain for fifteen minutes. Wash in water and examine, and if satisfactory mount in balsam, but if not re-stain.

Spore-Staining.

The fact that spores are difficult to stain, while the rest of the organism is not, suggests that the former is covered by a specially thick envelope, which prevents the ingress of the stain.

Method I.—Take a film made in the ordinary way, and flood with carbol-fuchsin and warm for fifteen to twenty minutes. Wash in water, and dip for a second or so in 1 per cent. solution of sulphuric acid. Wash lightly, and counter-stain by methylene blue; wash, dry, and mount.

Method II.—Moeller stain:

Prepare slide in the ordinary manner. Keep in absolute alcohol for two minutes, and then in chloroform. Wash in water, allow the slide to stand in a 5 per cent. solution of chromic acid for two minutes, wash, and then stain with warm carbol-fuchsin for ten minutes. Wash, decolorize in a 1 per cent. solution of sulphuric acid. Wash, counter-stain with methylene blue for one minute. Wash, dry, and mount.

Blood Stains.

The two in most common use are Jenner's and Leishman's, and are best bought already prepared.

Jenner's Stain.—A film is allowed to air dry and then flooded with the stain. This stain fixes as well as stains the specimen, taking three minutes to act. It is then poured off, washed in water, dried, and mounted.

Leishman's Stain.—A film is prepared and air dried. The specimen is flooded with the stain and allowed to act for thirty seconds, after which a double volume of distilled water to stain is added on to the film and mixed with a platinum loop. This diluted stain is now allowed to act for five minutes. Wash in water, leaving some water on the film for half a minute to bring out the colour. Dry and mount.

CHAPTER V

IDENTIFICATION OF BACTERIA—GENERAL PRINCIPLES

When examining morbid material or blood for bacteria, it does not follow that because microscopical examination fails to reveal them that they are absent. They may be so few in number as to escape notice, or the material examined may not possess any bacteria in that particular sample. This is commonly seen in tuberculosis of the udder, where the milk may be centrifugalized and yet no bacilli are revealed. Again, in abscess formations the digesting influence of the purulent matter may be so pronounced that the active organisms are only to be found in the cyst wall or its neighbourhood. Moreover, it is well to be careful in forming an opinion of the presence of bacteria taken from animal discharges after dissolution, for extraneous contamination rapidly takes place from the air or from the intestines. In the case of an animal dying from anthrax, for example, we know how rapidly the blood becomes contaminated with putrefactive organisms from the intestines after death, thereby adding greatly to the difficulty of arriving at an accurate diagnosis.

Hanging-Drop Preparations.

Hanging-drop preparations are used principally to identify the motility or otherwise of bacteria, and also to observe their growth and development. Hollow-ground

glass slides are used, the circumference being painted with immersion oil, or a wall may be built up with vaseline. It is most important not to place too large a drop on the cover-glass, for by so doing it will reach the well of the slide, and the fluid will run by capillarity, the hanging drop thereby disappearing. Take a drop of emulsion (bacterial), well diluted with broth or other media, and place it on a cover-glass; invert the hollow-ground slide over it; press it down so that the oil round the well adheres to the cover-glass evenly. Now invert the slide, and the hanging drop is complete and ready for examination. Examine with a low-power lens, and move the slide about when the edge

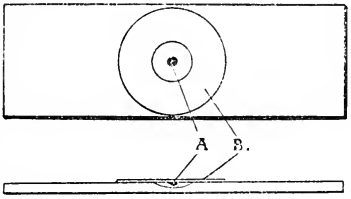


FIG. 17.—HANGING-DROP PREPARATION.

of the hanging drop is seen in the field, opening and shutting the diaphragm until the field becomes faintly illuminated. Now focus a little deeper, and the unstained bacteria will come into view, and note if motile in pairs or clumps, etc. If active they will be darting about in all directions, but if sluggish they can be revived by placing the slide in an incubator for a short time. Needless to add, Brownian movements must not be mistaken for natural bacillary motion. A hanging-drop preparation from cultures should be examined, and the shape, etc., of organisms noted, whether

1. Cocci or bacilli.
2. Motile or non-motile.
3. Grouping—clumps, pairs, chains, etc.

Three films should then be made, dried, and stained:

1. Carbol methylene blue.
2. Carbol-fuchsin (Ziehl-Neelsen).
3. Gram's method.

Differential Diagnosis of the More Common Pathogenic Cocci.

A *hanging-drop specimen* should be made.

A. Non-Motile.—1. Staphylococci, single or in clumps. 2. Streptococci, single, pairs, or in chains. 3. Pneumococci, pairs or short chains. 4. *Micrococcus tetragenus*, usually in fours. 5. *Micrococcus catarrhalis*, single or pairs.

(*a*) *Gram-Positive.*—(1) Staphylococci; (2) streptococci; (3) pneumococci; (4) tetragenus.

Staphylococci.

Biological Characters.—1. *In broth* the medium becomes turbid, with a white, yellow, or brown layer settling at the bottom.

2. *On agar Staphylococcus albus* forms a white, elevated growth, moist and glistening. *Staphylococcus aureus* forms a golden-yellow growth. *Staphylococcus citreus* forms a lemon-coloured growth.

3. *In gelatin* (stab culture) growth takes place along the whole length of the puncture, followed by liquefaction in two or three days, and ending in a deposit.

Staining.—Methylene blue and Gram's.

Microscopical Appearances.—Note characteristic bunches of grape-like formation.

Facultative Anaerobic.—Minimum temperature 6° C.; optimum, 35° C.; maximum, 45° C.

Streptococci.

Biological Characters.—1. *In broth* small granules develop, growing sparingly.

2. *On blood-agar* small distinct colonies develop, with a clear halo round them.

3. *On gelatin* small white granular colonies form. Gelatin is not liquefied. In stab culture distinct colonies form along the track of the needle.

4. *On potato* growth very sparing.

Staining.—Carbol-methylene blue and Gram's.

Microscopical Appearances.—Note characteristic long or short chain formations.

Facultative Anaerobic.—Minimum temperature, 10° C.; optimum, 35° C. Growth also takes place at room temperature.

Pneumococci.

Biological Characters.—1. *In broth* difficult to grow, and present nothing characteristic.

2. *On gelatin* growth seldom takes place.

3. *On blood-agar* small colonies develop, the transparent medium becoming a dirty brown.

Staining.—Carbol-methylene blue for original specimen; Gram's stain for cultivated specimen; and also stain capsule with a capsule stain.

Microscopical Appearances.—The cocci are small, lanceolate in shape, and arranged in pairs. If further proof is required, inoculate a rabbit, make a film of heart's blood, and note capsule by capsular stain.

Aerobic.—Minimum temperature, 20° C.; optimum, 37° C.; maximum, 40° C.

Micrococcus Tetragenus.

Biological Characters.—1. *In gelatin stab* a thick growth takes place, and a thick white disc forms on the surface. On gelatin plates white round colonies form.

2. *On agar* at room temperature moist white colonies appear.

3. *On potato* a thick, irregular slimy patch appears at room temperature.

Staining.—For original pus use Welsh's method. After cultivation use carbol-methylene blue and Gram's method.

Microscopical Appearances.—Cocci are arranged in fours.

Aerobic.—Minimum temperature, 15° C.; optimum, 35° C.; maximum, 40° C.

(*b*) *Gram-Negative.—Micrococcus Catarrhalis.**

Biological Characters.—1. *On gelatin plates* at room temperature, 20° C., the growth is very rapid and the colonies tough.

2. *On agar* colonies very tough and opaque.

Staining.—By Gram's counter-stain neutral red.

Microscopical Characteristics.—The cocci are usually arranged in pairs.

Aerobic.—Growing at room temperature freely. Minimum temperature, 18° C.; optimum, 37° C.; maximum, 40° C.

Differential Diagnosis of the More Common Pathogenic Bacilli.

A *hanging-drop specimen* should be made.

A. Motile.—1. *B. tetani.* 2. *B. œdematus maligni.* 3. *B. pyocyaneus.* 4. *B. coli communis.* 5. *B. anthracis symptomatica.* 6. *B. bronchosepticus* of Ferry.

(*a*) *Gram Positive.—Bacillus Tetani.*

Biological Characters.—1. *In broth anaerobically* the medium becomes clouded.

2. *In gelatin stab* the growth radiates into the medium, liquefies slowly, and forms gas.

3. *On agar* growths form giving the colonies the appearance of very fine wool.

4. *In agar stab* the outgrowths give the appearance of a fir-tree.

* A microbe having close characteristics of the *M. catarrhalis* of man has been isolated by the author from catarrhal discharges of horses suffering from influenza.

Staining on Tissue.—Carbol-methylene blue and cultures by Gram's method.

Microscopically the bacilli when sporulating are like drumsticks, the spores being at the ends.

Anaerobic.—No growth at 20° C. On broth or agar cultivation at 37° C. in anaerobic tubes after subjecting the original material to 80° C. for thirty minutes to kill vegetable forms.

(*b*) *Gram Negative.—Bacillus Œdematis Maligni.*

Biological Characters.—1. *In broth* becomes cloudy.

2. *On gelatin* bright grey colonies surrounded by spreading borders. In stab culture 1 inch below the surface white side-branches form.

3. *On agar* a thick network of threads form.

Staining.—Carbol-methylene blue.

Microscopical Appearances.—The bacilli are long and slender and often in threads, ends rounded, and spores are formed in the middle.

Strongly *anaerobic*, growing well at room or incubator temperature.

Bacillus Pyocyaneus.

Biological Characters.—1. *In broth* the growth makes medium cloudy.

2. *On gelatin plates* flat, irregular colonies form with rapid liquefaction, the whole medium becoming green.

3. *On agar* the colonies form a white upper layer, and a deeper layer is coloured green.

4. *On potato* a greenish-yellow or brown layer forms.

Staining.—Carbol-methylene blue and original culture by Gram's method. Contrast stain neutral red.

Microscopical Appearances.—The bacilli are often arranged in pairs, and form filaments.

Anaerobic, growing either at room or incubator temperature.

Bacillus Coli Communis.

Biological Characters.—1. *In broth* it forms a diffused cloudiness of the medium.

2. *On gelatin plates* it forms iridescent colonies. Gelatin does not liquefy.

3. *In gelatin stab culture* it grows in the form of a nail.

4. *On agar and blood serum* a thick layer forms, moist and slimy.

5. *On potato* the growth at 20° C. is brown and slimy.

6. *In litmus milk* it forms acid, gas, and clots.

Staining. — Original carbol-methylene blue; culture Gram's method, and weak carbol-fuchsin as a contrast stain.

Microscopical Appearances.—Short motile rods, mostly in pairs, sometimes in threads.

Both *aerobic* and *anaerobic*. Grows at room or incubator temperature.

Bacillus Anthracis Symptomatica.

Biological Characters. — 1. *On gelatin plates* in an atmosphere of hydrogen a radiating dull area forms, the gelatin liquefies, and a dark lobulated centre develops.

2. *In deep stab glucose gelatin* gas forms.

3. *In glucose agar* it forms a dense grey-coloured growth, and gives off a pungent-smelling gas.

Staining.—Carbol-methylene blue, but not ordinarily by Gram's method.

Microscopical Appearances.—The bacillus is motile with rounded ends, usually single, sometimes forming threads. Spores form at the ends or in the middle.

Strictly *anaerobic*, growing at room or incubator temperature.

Bacillus Bronchosepticus (Ferry).

Biological Characters.—1. *In broth* cloudiness take place after twenty-four hours.

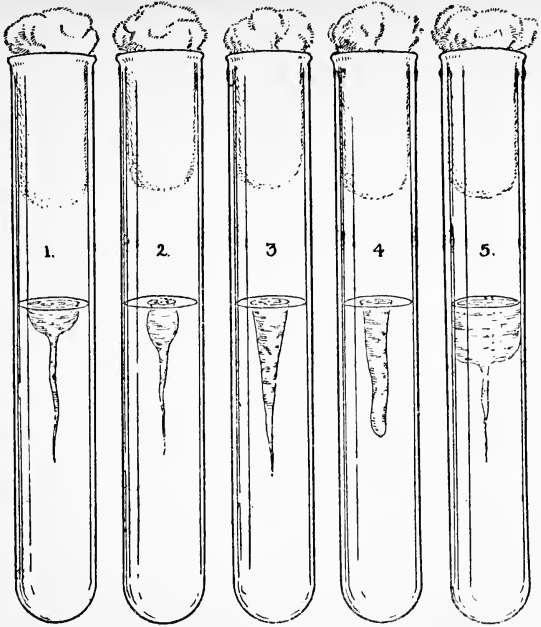


FIG. 18.—TYPES OF GROWTH IN STAB CULTURE.

Liquefying: (1) Crateriform, saucer-shape. (2) Napiform, turnip-shape. (3) Infundibuliform, funnel-shape. (4) Saccate, tubular-shape. (5) Stratiform liquefaction extending to walls of tube.

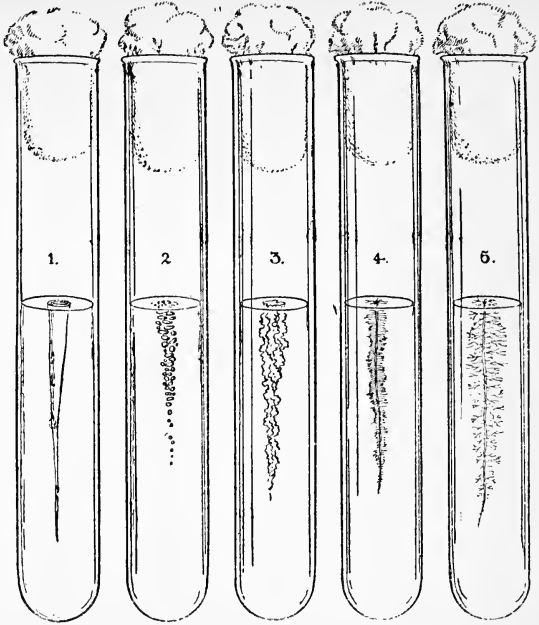


FIG. 19.---TYPES OF GROWTH IN STAB CULTURE.

Non-Liquefying: (1) Filiform, even growth. (2) Beaded, uneven growth. (3) Echinate, beset with acicular extensions. (4) Villous, beset with hair-like extensions. (5) Arborescent, beset with branch extensions like a tree.

2. *In gelatin* (stab culture) after twenty-four hours a filiform growth occurs. No liquefaction of gelatin.

3. *On agar* a moderate growth, slightly raised, surface moist and glistening, of a sticky consistence.

4. *On potato* after twenty-four hours growth rather abundant, surface uneven, moist and glistening, raised and of a light tan colour, which darkens with age.

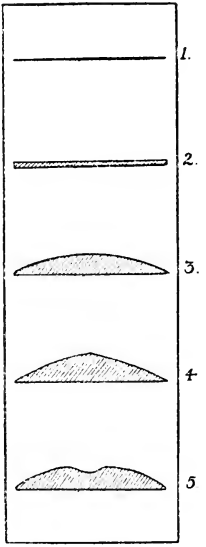


FIG. 20.—SURFACE ELEVATION OF COLONIES.
(1) Flat. (2) Raised. (3) Convex. (4) Conical. (5) Umbilicate.

5. *In litmus milk* after five days colour of medium at top deep blue, with a brown sediment at the bottom.

Staining.—Loeffler's methylene blue.

Microscopical Appearances.—A short, narrow bacillus, single or in pairs, chains, or even filaments.

Aerobic.—Optimum temperature, 37° C.

B. Non-Motile: (*a*) *Gram-Positive.*—Bacilli of acne, anthrax, tuberculosis, diphtheria, and actinomycosis.*

Bacillus Acne Contagiosæ Equi.

Biological Characters.—1. *In gelatin stab* white colonies develop along the course of the needle.

2. *On agar* white colonies growing very slowly.

Staining.—Difficult to stain with ordinary dyes, but by Gram's method.

Microscopical Appearances.—Small ovoid bacilli, single, and also forming chains.

Anaerobic—at least, at first. Optimum temperature, 37° C.

Bacillus Anthracis.

Biological Characters.—1. *On gelatin plates* small white colonies appear, and if examined with an eyepiece, characteristic threads projecting from the edges of the colonies are noted. In gelatin stab after twenty-four hours a thick white central column appears, from which radiate lateral branches, giving the whole growth a triangular appearance, with the apex at the bottom.

Liquefaction begins at the top, extending downwards.

2. *On agar* growth is similar to gelatin plates.

Staining.—Original material ordinary aniline dyes, and by Gram's method.

Note McFadyean's violet colour reaction with methylene blue.

Microscopical Appearances.—The bacilli appear as large rods, and often in threads. In stained specimens the ends are square and the body slightly concave, giving it the appearance, if in thread form, of a bamboo cane.

Facultative Aerobic.—Minimum temperature, 10° C.; optimum, 35° C.; maximum, 45° C.

* Actinomycosis is not a bacillus, but for convenience is described here.

Bacillus Tuberculosis.

Biological Characters.—1. *In broth* a light yellow mass forms, increasing slowly, the medium remaining clear.

2. *On glycerine agar* a dry, wrinkled, brownish-yellow mass forms.

A guinea-pig should be inoculated with the virus for diagnostic purposes as described on p. 183.

Staining.—Ziehl-Neelsen's method, as described on p. 177.

The bacillus belongs to the acid-fast group—*i.e.*, *B. Leprae*, *B. Butyricus*, *B. Smegmatiæ*, bacillus of Johne's disease, bacillus of Timothy-grass, etc.

Microscopical Appearances.—This bacillus is a small thin rod, slightly bent, in culture. A chain of four or of eight may be noted.

This bacillus is *aerobic* and *anaerobic*. Minimum temperature, 27° C.; optimum, 37° C.; maximum, 41° C. Growth slow: about six weeks.

Bacillus Diphtheriæ.

Biological Characters.—1. *In glucose peptone broth* note formation of acid, but no gas.

2. *On gelatin plates* small white columns develop, and growth is very slow, without liquefaction.

3. *In gelatin* (stab culture) at 20° C. surface growth takes place, and the stab growth takes the form of a nail.

4. *On agar* small transparent elevated columns occur. Growth scanty.

4. *On Loeffler's serum* large white colonies grow.

Staining.—Methylene blue or weak carbol-fuchsin, and Gram's method.

Microscopical Appearances.—The bacilli are often arranged in clumps. In shape and size they vary considerably.

This bacillus is *aerobic* and *anaerobic*. Growth takes place between minimum temperature, 10° C.; optimum, 35° C.; and maximum, 48° C.

Streptothrix Actinomycosis Bovis.

Biological Characters.—1. *On broth* the medium is not coloured, but grey masses settle at the bottom.

2. *On gelatin plates* in a week a yellow growth develops.

3. *In gelatin stab* firm outgrowths take place, with slight liquefaction.

4. *On agar* opaque colonies grow.

5. *On glycerine agar*, at 37° C., in three or four days appears a yellow growth, tinged with red, adhering firmly to the medium.

6. *On potato* colonies form of a yellow colour, tinged with red, and look as though covered with fine hair.

Staining.—By Gram's method and methylene violet.

Microscopical Appearances.—If from pus note filaments and spores (Gram-positive). Clubs round periphery of colony, radiating from the centre (Gram-negative).

It is *anaerobic* and *facultatively aerobic.*

(*b*) *Gram-Negative—Bacillus Mallei.*

Biological Characters.—1. *In broth* a diffused cloudiness takes place, ultimately developing into a tenacious sediment.

2. *On glycerine agar* a moist opaque layer is formed.

3. *On potato* a characteristic growth at 37° C. takes place, at first amber in colour, and later developing into a reddish-brown.

Staining.—Young cultures stain readily with the ordinary aniline dyes.

Microscopical Appearances.—The bacillus is a short rod, usually straight, but sometimes curved. They are usually single, rarely in pairs or short chains.

Involution forms are frequently produced; clubs, filaments, and even branches have been noted.

This is a *facultative* bacillus growing with or without oxygen. Minimum temperature, 25° C.; optimum, 37° C.; maximum, 42° C.

Bacillus Friedländer.

Biological Characters.—1. *On gelatin plates* it grows forming small porcelain-like clusters without liquefaction.

2. *In gelatin* (stab culture) at 20° C. a white growth appears along the centre, showing the characteristic nail-head formation. Gas formation on shaking gelatin.

3. *On agar* a white layer is formed.

4. *On potato* a whitish-yellow layer develops, with gas formation.

Staining. — Carbol-methylene blue or weak carbol-fuchsin.

Microscopical Appearances.—Short rods with rounded ends, and surrounded by a distinct capsule under special circumstances of growth.

This bacillus is *aerobic* and *anaerobic*, growing at room or incubator temperature.

CHAPTER VI

GLASS-WORK REQUISITES AND HOW TO MAKE THEM

How to make a Glass-Cutting Knife.

Take an ordinary pocket-knife, heat the blade until it becomes white hot in the flame of a blow lamp, and immediately plunge it into cold water, keeping it there until it has become quite cold; the edge should then be whetted on a rough stone. This process will give it a rough cutting surface, capable of scratching ordinary glass tubing, etc.

When the rough edge wears off, it can always be renewed by rewhetting it on the stone.

Glass Tubing.

For the making of capillary pipettes, etc., tubing of about 5 millimetres external diameter is usually used. The glass should be of the best kind if we desire to make pipettes uniform in shape and calibre, and, as the glass itself is quite inexpensive, there is no need to use inferior quality.

Method of making Capillary Pipettes.

Take a piece of glass tubing, cut it into lengths of about 9 centimetres. This is done by holding a piece of glass tubing in the right hand and against the ball of the thumb, while the same hand is armed with the glass-cutting knife, the edge of which is made to rest firmly upon the glass tube. With the left hand the tubing is now rotated, and

the result will be an even circular scratch, at which point, when pressure is applied, the tubing will break, leaving the two ends clean-cut.

The proper length of tubing thus made should be taken up and rotated by the right and left hand in the flame of the blow lamp, the flame being in the middle of the tubing. When it has become sufficiently plastic to move about freely, it should be removed from the flame and steadily drawn out until it reaches about 6 inches in length. The middle of this tubing should now be allowed to touch the flame, and with gentle traction the ends part, leaving two capillary pipettes.

Many failures will follow the operator's labours at the

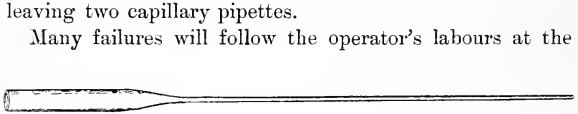


FIG. 21.—A SIMPLE CAPILLARY PIPETTE.

outset, but, as already stated, glass tubing is cheap, and perseverance will bring its own reward.

To reduce the risk of these failures, however, the following points should be noted:

The glass should be uniformly heated from start to finish, and the rotation should be complete, failing which one side would become more plastic than the other, and the resulting capillary would be removed from the central axis of the tube.

Again, the flame throughout does not give off the same amount of heat; therefore it is wise to move the tube up and down in the flame, and also from side to side. Failing this precaution, we shall find a bulb corresponding to the centre of the flame, with capillary taperings on either side, due to the fact that the greatest amount of heat is found at the sides of the flame.

Throttled Capillary Pipettes.

These, by reason of their mechanism, regulate the air transmitted by the rubber teat, and thereby control the movements of the fluid in the narrow stem.

Two such kinds of pipettes are in common use—*i.e.*, (*a*) where the throttle is in the distal end of the capillary stem; (*b*) where a smaller pipette is inserted in the proximal end of the ordinary pipette.

(*a*) Take an ordinary pipette made in the manner already described, and hold the end in the flame of an ordinary wax vesta for a second or so, and then draw it out with a sudden jerk, cut off the end with a glass knife, leaving a very fine point.

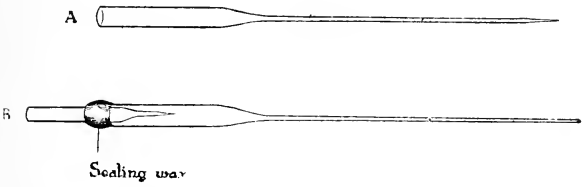


FIG. 22.—THROTTLED CAPILLARY PIPETTES.

(*b*) Take an ordinary pipette, and insert a short pipette of smaller calibre in the proximal end of it.

The smaller pipette is throttled in the manner as above described. A coating of sealing-wax is now placed round its shoulder, the proximal end of the large pipette gently heated, and the small pipette inserted, the wax making a tight-fitting joint.

Method of sealing up a Test-Tube.

When it is desired to seal a test-tube—as, for example, after making a bacterial emulsion, and we wish to break down the bacterial bundles, chains, etc., by shaking—it is imperative that the open end should be closed.—To do this

satisfactorily one requires to exercise great care, and the beginner is well advised to practise on empty test-tubes first. Take a test-tube and rotate in a small flame first, and, when thoroughly hot, rotation should go on in a full flame until the sides of the glass begin to fall in. It should now be drawn out into a narrow stem, and as soon as it has become cool it is reheated in a smaller flame, drawn out into a thin capillary stem, which is broken off, set aside again to cool, and the point sealed in a very small jet, candle flame, or by a wax vesta.

When one is dealing with a fluid-containing test-tube, greater skill and care is required. In the first place, the operator must make sure the glass at the point the flame touches is absolutely dry by careful heating. Then the flame must not come too near the fluid, or it will boil and crack the glass, and probably injure at the same time the

FIG. 23.—DRAWN-OUT AND SEALED TEST-TUBE.

chemical qualities of the fluid. This can be prevented, of course, by holding the test-tube, while being heated in the flame, at a proper angle, and not heating the tube too near the surface of the fluid. Of course, if the flame is applied too near the proximal end of the tube, the glass becomes so hot as to scorch one's fingers.

When there is an excess of the fluid in the tube necessitating this condition, it is wise to heat the mouth of the tube to red heat, holding the base by the left hand and by a piece of glass tubing in the right hand, held also in the flame until it becomes quite hot. Now fix the tubing to the rim of the test-tube, first at one point and then at the diagonally opposed point, and draw the whole into a narrow stem. When cool, put the stem in the flame and draw the two ends apart, sealing up the remaining opening in the stem by applying a small flame to it.

How to seal One End of a Glass Tube.

When we desire to have a tube smaller than a test-tube which will be found useful in working out the opsonic power, etc., we take a piece of pipette tubing, and heat it in the middle in the usual way, drawing it out to make a capillary stem (*A*). This will give us the makings of two tubes by breaking the stem in the middle. We now reduce the flame and apply it to the shoulder, rotating the tube in the meantime with the right and left hands, and when plastic the stem is still further drawn out until it is melted through (*B*). Now apply a full flame to the bottom of the tube, and when

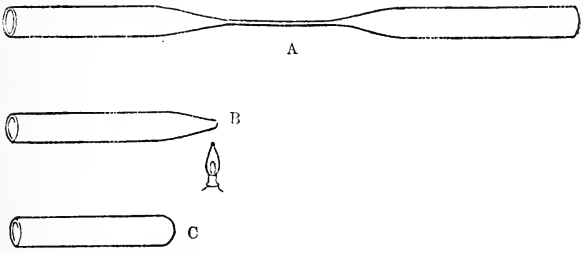


Fig. 24.—Sealing off One End of a Glass Tube.

red hot blow down the tube (*C*). This will make the distal end quite round, and, if too much glass is not left on the bottom, the heat alone will be sufficient to round it off nicely.

How to make Blood-Capsules (Wright's).

These are very useful for the examination and collection of blood to obtain the serum in working out the opsonic power of a case.

Take a piece of glass tubing, draw out into a capillary stem (Fig. 25).

Now heat the barrel in the middle, draw it out into a stem,

and, before it has become too cool to lose its plasticity, bend it towards the body of the tube up to about an angle of 25 to 30 degrees (Fig. 26).

The stem should now be burned through at a short

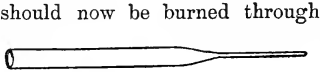


FIG. 25.—WRIGHT'S BLOOD-CAPSULE.

distance from the bend, the complete capsule being the shape of Fig. 27.

To collect blood in this capsule, care should be taken to see both ends are open. If the blood is being taken from a small superficial vein in the horse, after opening the vein

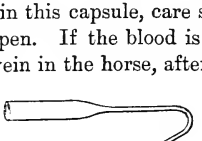


FIG. 26.—WRIGHT'S BLOOD-CAPSULE.

with a lancet, the bent end should be placed in the blood-drop, and by capillary attraction the blood will siphon into the tube, while the air will recede from the straight end. When sufficient blood has been collected, heat the straight end in a small flame, and seal it up. As the imprisoned hot

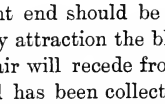


FIG. 27.—WRIGHT'S BLOOD-CAPSULE.

air at this end cools, it will contract, and the blood will be withdrawn from the bent end, which in turn may be sealed. The blood will now clot and separate out the serum. This squeezing out of the serum may be hastened by centri-

fugalizing the capsule, and where need be the serum may be pipetted off into a small tube for further use.

Slides, and how to make Blood-Films.

It is most essential that glass slides should be free from grease; this is done by boiling in lysol, then in soda, next in strong acid, and washing out in spirit.

If a slide is greasy, it is obvious one cannot obtain a perfectly even film; and where one is making a blood-count, for reasons of accuracy it is essential that the objects in

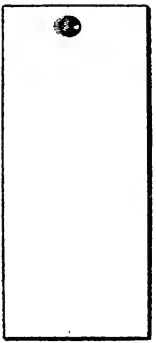


FIG. 28.—SLIDE, SHOWING BLOOD-DROP.

the field should be evenly distributed. By rubbing the surface of the slide with emery-paper, a mechanical roughness is produced, which facilitates an even distribution of the elements in the fluid.

In making a film, it is necessary to ascertain which is the concave and which the convex side of the slide, for it is obvious, to get a good spread, we must place our film on the convex surface. To find out which is which, rotate the slide on a flat smooth surface. On one face it will not spin (concave); on the other it will (convex).

To make a blood-film, place a drop of blood as blood,

or mixed with emulsion (bacterial), which we wish to standardize, on one end of the slide, conveniently at the left-hand border (Fig. 28), which is firmly held between the thumb and finger of the left hand. The end of the spreader* should now be placed in the blood-drop, and moved backwards and forwards and from side to side until the drop is spread out along the whole length of the spreader's edge. Now draw the spreader steadily along the surface of the slide; this will give us a more or less perfectly homogeneous film, composed of red blood-corpuscles, a few small white blood-cells, and blood-plasma. Many of the leucocytes, owing to their size, will be drawn from the field by the spreader.

The film should now be allowed to air dry, fixed with a fixing agent or a fixing stain, and stained.

* How to make a spreader, see p. 25.

CHAPTER VII

THE PROTECTIVE ELEMENTS OF THE BLOOD, WHICH PROTECT THE ANIMAL BODY FROM PATHOGENIC BACTERIA

The science of bacteriology has clearly demonstrated that by far the largest majority of organic diseases from which animals suffer are due to the direct or indirect influences of specific bacteria. To be convinced of this we only require to look through the long roll of bacteria which have been isolated and are capable of producing disease. And, considering the serious ravages that these bacteria have produced in the past, can it be wondered at that the resources of thinking men have been strained to the utmost to find some means of checking their destructive processes?

In evolving a scheme likely to satisfactorily fulfil its intended purpose—namely, the treatment of disease—various methods have been adopted, but the one which concerns us here—*i.e.*, sero-vaccine therapy—has laid open a vast field for research, and offers to those who pursue its subtle courses rich rewards and unthought-of possibilities. To the general practitioner his everyday occupation is a prophylactic and curative one; and whatever line of thought or school of teaching he follows, to be successful he must always be subservient to Nature, and the more he keeps in touch with Nature and her methods, and in his healing art follows her example and emulates her ways, the more certain will be his successes. Many centuries ago the ancients noticed that one attack of a specific disease conferred upon the subject a certain protection against further infection, and it appears the first attempt to utilize this protective principle came from the East,

where we find a mild attack of smallpox was produced by inoculating the discharge from a pustule to ward off dangers of further infection. Following upon this, William Jenner discovered that vaccination with cowpox virus was equally efficacious as a preventive against smallpox in man. This laid the foundation of vaccine-therapy.

Succeeding Jenner's discovery, the actual cause of certain specific diseases — namely, bacteria — was demonstrated. Then came Koch's work upon the method of isolating and identifying bacteria.

At a later date Metchnikoff demonstrated his "power of resistance theory," and showed the action of the leucocytes in the lower animal in their endeavours to devour invading bacteria, and this he called "phagocytosis." It was further proved, however, that the serum exercised some specific influence upon bacteria, and it was shown that if the blood-plasma alone was taken the growth of bacteria was inhibited, and on the supposition that there existed in the plasma some protective elements, Büchner gave them the name of "alexins."

Further, it was demonstrated that an animal suffering from an infectious disease, and recovering, had elaborated within its economy various substances inimical to the growth of the specific bacteria and the creation of their products. These substances appear to be of two distinct kinds: (1) those elaborated consequent upon tissue activities and always present, called *non-specific antibodies;* (2) those manufactured in response to an infection, and named *specific antibodies.*

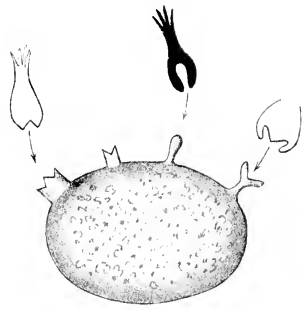
These antibodies vary in character and action, and at the present time little is known about them.

The following are some of the principal antibodies:

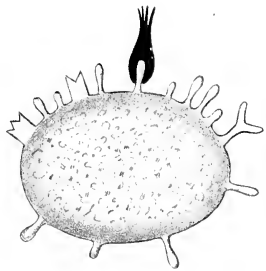
Antitoxins.

These are specific bodies which counteract poisons, or toxins, manufactured within the animal body by disease-producing organisms.

PLATE III.



NORMAL CELL SHOWING VARIOUS SIDE-CHAINS.



FIRST STEP IN THE FORMATION OF ANTITOXIN SHOWING RECEPTOR AND HAPTOPHORE UNITED.

(From Jowett, after Ehrlich.)

To face page 56.

In 1890 Behring showed, if infinitesimal quantities of the toxins of tetanus were injected into an animal, with later succeeding increased doses, the time would arrive when that animal would tolerate such large doses of tetanus poison without ill effect, and which, if injected into an animal whose serum was non-immunized, would cause death from tetanus. Moreover, if the serum of an immunized animal were mixed with an equivalent amount of the poison, and injected into a non-immune animal, no ill-effect would follow. Further, if a dose of immune serum was administered within a short period of time to an animal previously inoculated with the tetanus bacillus, the disease would not develop. This fact is seen daily in the use of antitetanic serum as a prophylactic. Behring shortly afterwards showed that a similar condition existed with regard to the bacillus of diphtheria, when, by treating an animal with the toxins of this organism, a serum could be obtained which is capable of possessing prophylactic and curative effects upon this disease in man.

The toxins manufactured by these two bacteria (tetanus and diphtheria) are known as *exotoxins*, which are soluble, and given off from the bacilli when grown on a suitable culture media—in contradistinction to *endotoxins*, which are insoluble, and are only set free by the death and disintegration of the bacteria themselves, such as staphylococci, streptococci, *Bacillus coli communis*, etc.

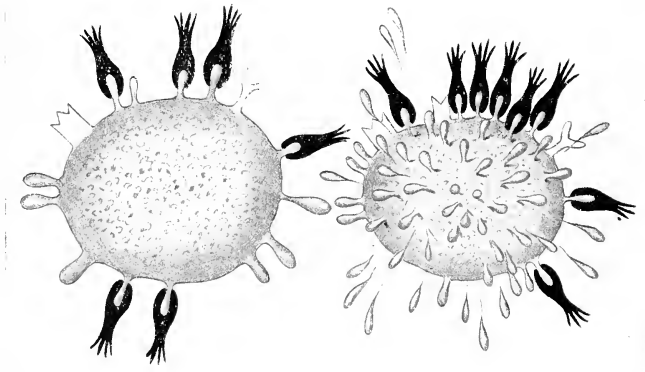
Various theories have been put forward from time to time to explain the production of antitoxins by body cells, but the most universally accepted now is Ehrlich's *cell nutrition and side-chain theory*.

As regards *cell nutrition*, Ehrlich believes the protoplasm is made up of many molecules showing an affinity for a large variety of food materials. These molecules are composed of a central portion surrounded by atomic groups, which unite with certain food molecules, binding them to the cell. The affinities of these groups vary: hence they are supposed to be differently constituted, and he calls

them *side-chains* or *receptors*. A side-chain attached to a cell may join to itself a particle of oxygen, fat, carbohydrate, etc., and so take part in the nourishment of the cell. In the same manner it may unite with a molecule of poison, such as a toxin. Now, this toxin, through the medium of the side-chain, becomes part of the cell, and if strong enough will poison it and actually produce death of the cell itself; or it may cause death only of the side-chain, in which case the latter would be thrown off and a new one formed by the cell. Following upon the destruction of the side-chain, several more chains are produced to take its place, with the result that the cell cannot retain them all, and they consequently become detached and are cast off into the lymph around the cell, and eventually reach the blood-stream, constituting free side-chains, whose function now appears to be to unite with the molecules of the toxin before it reaches the cell. In this manner these free side-chains show antitoxin properties which prevent the action of the poison upon the cells, giving thereby to the patient an immunity. Further, if the serum containing these free side-chains is injected into another animal, they will confer upon that animal the same degree of immunity as was possessed by the original immunized animal, which explains the prophylactic and curative action of *antitoxin*.

Toxins are easily destroyed by heat, chemicals, and light, but the loss of toxicity does not result from a complete destruction of the toxin molecule, for it is still able to unite with the antitoxin. It is clear, then, that the toxin molecule is made up of two parts: a *thermostable* portion, which is capable of uniting with cell receptors either in the cell itself or free as antitoxin, and called the *haptophore;* and a *thermolabile* group, which causes the cell injury after union by means of the haptophore has taken place, and called the *toxophore*. When the toxophore group of a toxin has been destroyed, that which remains is called the *toxoid*.

PLATE IV.



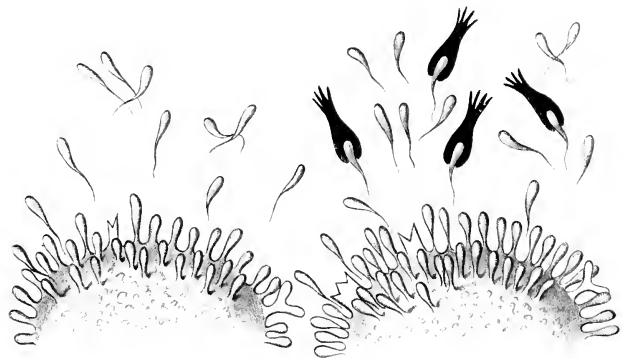
FORMATION OF MANY NEW RECEPTORS, LINKED TO WHICH ARE A NUMBER OF HAPTOPHORES.

OWING TO THE RAPID INCREASE OF RECEPTORS, SEPARATION OF MANY OF THEM FROM THE CELL TAKES PLACE — ANTITOXIN BEGINS TO FORM.

(From Jowett, after Ehrlich.)

To face page 58.

PLATE V.



ANTITOXIN SHOWN FREE IN THE BLOOD.

ANTITOXIN UNITED WITH THE HAPTOPHORE, THEREBY PROTECTING THE CELL FROM TOXIC INJURY.

(From Jowett, after Ehrlich.)

To face page 58.

Agglutinins.

Gruber and Durham have shown, when the serum of an animal suffering from a bacterial disease is added to a culture of the specific bacterium of that disease, they collect together in masses, leaving the rest of the fluid free from their presence. It is supposed they (agglutinins) are formed in lymphoid tissue, marrow, and the spleen, while Metchnikoff noticed the peritoneal exudate was even richer than the blood itself in agglutinins.

Strangely enough, the agglutination of bacteria seems to protect them from the death-producing action of another antibody—*i.e.*, lysin; and if that is so, Nature would appear to be working against her own interests and defeating her

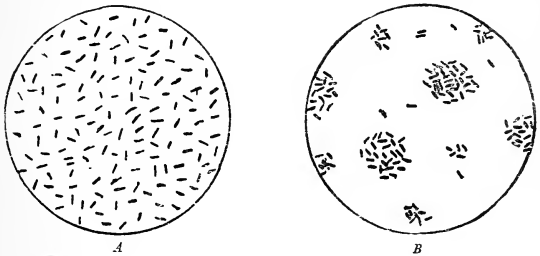


FIG. 29.—AGGLUTINATION OF BACTERIA (JOWETT).

A, Bacteria evenly distributed; *B*, Bacteria agglutinated by serum.

own ends—a very improbable presumption. Allen is of opinion, however, the bacteria are gathered into clumps in the manner just described, so that they may be caught up in the various tissues, such as the liver, the spleen, and lymphatics, where the immunizing bodies are generated, and their destruction thereby brought about.

Widal first suggested using this agglutinating power for diagnostic purposes, and the test now bears his name. It was found that many kinds of micro-organisms were clumped by the serum of animals immunized against them. It was

thought at first the serum taken from a patient suffering from a specific disease had the specific agglutinating power only, but later it was demonstrated that the undiluted serum of normal persons was capable of producing the same effect. It was then decided to dilute the serum. Thus, one loopful of serum was mixed with nine loopfuls of a young fresh bacterial emulsion. Then the strength was made 1 in 50, and when the patient was suffering from the specific disease the agglutinating power would still be in evidence. The length of time allowed for agglutination to take place is usually half an hour.

Widal's Test for Diagnostic Purposes.

Collect the blood from the patient in a Wright's capsule. Allow the clot to shrink, to squeeze out the serum; or, to hasten the process, centrifugalize it, placing the capsule in the centrifuge, having, of course, previously sealed the straight end of the capsule. In this way we obtain our serum. We now take a young culture, preferably grown on broth, make a hanging drop, microscopically examine the specimen, and note the disposition and motility of the bacteria.

We then take a capillary pipette, make a unit mark with the grease pencil on the stem high enough up to contain 1 to 2 cm., aspirate one unit of serum, a bubble of air, one unit volume of 0·86 per cent. saline solution, a bubble of air, one volume of saline solution, and so on, until we have taken up fourteen volumes of the solution, each separated by a column of air. The whole is now expelled from the pipette, and again aspirated to thoroughly mix, and further dilution of this dilution is made—usually three dilutions, 1 in 15, 1 in 25, 1 in 50.

Three hanging-drop slides are next prepared. One loopful of the diluted serum should be placed on three cover-glasses (1 in 15, 1 in 25, 1 in 50), and to each is added and mixed one loopful of broth culture containing the organism. The hanging drops are now examined under the micro-

scope, and note taken of the disposition of the bacteria up to an hour, but usually half an hour is long enough. If the reaction is a positive one, the bacteria, if motile, will lose their motility, and collect themselves into clumps—agglutinate. On the contrary, if the reaction is a negative one no clumping will take place.

Another method of making use of the agglutinative reaction for diagnostic purposes is to add a measured volume of serum to a known quantity of the culture media in a test-tube. In due course, if the reaction is a positive one, a white precipitate will form and settle on the bottom of the tube. This is known as the "precipitation test," and has the advantage of being very simple in its application.

Bacteriolysins.

Under this heading, we find there exists in the blood protective bodies capable of destroying bacteria by solution—bacteriolysins—and the process is known as "bacteriolysis." It appears that the serum of the blood soon loses its bacteriolytic properties when separated from the tissues.

Experiments have proved that the solution of the microbes is brought about by the interaction of at least two substances, one of which is present in all serums in the living body, but disappears on heating and on keeping at room temperature in from five to eight days. The other is more stable, and is produced during the process of inoculation. The former unstable body, found in all animals, is termed "complement"; the latter, produced in the processes of immunity, is called the "amboceptor."

It is presumed the amboceptor links the complement to the bacterium, but the complement remains free if the suitable amboceptor is not present, and bacteriolysis does not take place (Ehrlich) (Fig. 30).

It is upon this process of bacteriolysis that Pfeiffer's reaction depends, which is useful in recognizing the exact species of bacteria.

Method.—Take a mixture of the bacterial emulsion and mix with it a small quantity of serum from an animal immune to the bacterium in question, and inject into the peritoneal cavity of a healthy guinea-pig. After half an hour the peritoneal fluid and exudate is microscopically examined; and if the reaction is a positive one the organisms will be found to have undergone degeneration, in which case the organism will be found to belong to the same species as the one which the already immunized animal was invaded by. On the other hand, if the reaction

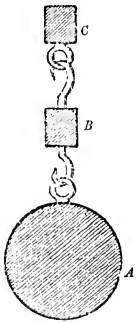


Fig. 30.—*A*, Micro-organism; *B*, Immune Body or Amboceptor; *C*, Complement (Ehrlich).

is a negative one the organism belongs to a different species to that by which the animal was immunized.

Opsonins.

Wright has shown there exists in blood-serum an antibody which possesses the specific virtue of sensitizing the bacteria present, and in this manner prepares them for the devouring activities of the leucocytes. He calls this antibody "opsonin," or "feast-preparer." It appears this opsonic power which the serum exerts upon each kind of bacterium is a specific one, for we find if an animal is

suffering from a bacterial infection and his opsonic index to that infection stand at a certain point a further invasion by another specific bacterium need not alter that index one way or another.

In estimating the opsonic power, we ascertain the following valuable points: (1) what natural resources the animal possesses—in other words, at what point his index stands; (2) during the progress of the disease we can note whether that index rises or falls—in short, what efforts Nature herself is displaying in her endeavours to shake off the invasion; (3) where we are adopting vaccine-therapy, the index will assist us in forming an opinion regarding the suitability of the dose, and to what degree the patient responds to our vaccine stimulus.

Of course, as we shall see later, clinical observations on these three points also are of great assistance to us in forming our conclusions upon the various phenomena and immunizing responses made manifest during the progress of a specific disease. Although in practice the taking of the opsonic power is not essential to success, it gives us a very valuable and very delicate guide to follow, and well repays the practitioner who will take the extra trouble to work it out.

The Usual Method adopted in estimating the Opsonic Power of the Blood.

1. Take a Wright's blood-capsule, and, after sterilizing the skin over one of the facial veins of an animal belonging to the same species as the patient, puncture the vessel with a small lancet, and collect the blood in the capsule. In like manner collect the blood from the patient at or nearly at the same time, since opsonin after a length of time becomes inert.

2. Take the bacterial growth we desire to test, and not more than twenty-four hours old. Add 1 or 2 c.c. of normal saline solution to the culture-tube; shake gently to wash off the colonies. The resultant emulsion should be pipetted off

into a small tube, centrifugalized for a few minutes to assist in breaking down the clumps, chains, etc., and then well shaken, after sealing the tube, as described on p. 51, Fig. 24. This emulsion should be of a faint opalescent colour.

If too many bacteria are in emulsion, it may be almost white, in which case it is too rich, and the leucocytes will take up an excessive number of bacteria, which will render the field difficult to count. Such an emulsion should be further diluted.

3. Prepare an emulsion of living leucocytes in the following manner: Take about 10 c.c. of normal saline solution, and add $\frac{1}{2}$ per cent. sodium citrate solution to prevent coagulation, of the blood;* place this in a centrifugalizing tube and *warm to blood heat.* Then prick the facial vein of the healthy animal and drop into the tube 1 or 2 c.c. of blood. This is now put into the centrifuge and centrifugalized about fifteen minutes, until all the corpuscles have gone to the bottom and the supernatant liquid is left clear.

The deposit will show the red corpuscles at the bottom, and on the top a thin grey layer of leucocytes. Now aspirate the clear fluid with a capillary pipette and teat, taking care not to disturb the layer of leucocytes.

This done, suck up the leucocytic layer and some of the red corpuscles, and put them in a small tube, thoroughly mixing them with the pipette, giving an emulsion of living leucocytes and a few red corpuscles.

4. Take two Wright's pipettes, with rubber teats, and with a grease pencil mark the glass stem at its distal end $\frac{1}{2}$ to 1 inch from the point. Fluid taken up to this mark constitutes one unit volume.

We should now have in front of us on the laboratory table a slab of plasticine, fixed into which are *(a)* a small

* Burroughs Wellcome and Co. make convenient soloids of the following formula:

Sodii chloridi	0·075 gm.
Sodii citratis	0·05 gm.

tubule each containing the patient's serum; (*b*) control serum; (*c*) the washed emulsion of leucocytes; (*d*) the emulsion of bacteria.

We then take up a pipette and immerse the point into the emulsion (bacterial), and draw up by relaxing the rubber teat one volume—*i.e.*, up to the grease pencil mark —withdraw the point from the emulsion, and slightly relaxing the pressure upon the teat, allow the ingress of a column of air. In like manner take up one volume of leucocytic emulsion, and then a column of air. Dip the pipette into the leucocytic emulsion again and take up another volume, then a column of air, and lastly draw up

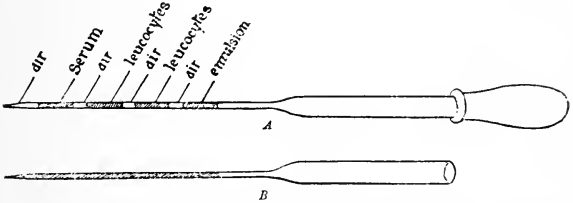


FIG. 31.—*A*, PIPETTE WITH RUBBER TEAT CONTAINING IN ORDER THE INDIVIDUAL ELEMENTS SEPARATED BY COLUMNS OF AIR; *B*, PIPETTE SHOWING THE ELEMENTS THOROUGHLY MIXED AND READY FOR INCUBATING.

one volume of serum. In the pipette we now have, counting from the point to the teat, a volume of serum, a column of air, a volume of leucocytes, a column of air, a volume of leucocytes, a column of air, a volume of emulsion. The operator having laid out a clean slide in front of him, the point of the pipette is made to rest upon it, and the fluid in the stem expelled and sucked up several times to thoroughly mix the contents.

It may be well to note here that the teat should perfectly fit the stem of the glass, failing which we will not obtain the same free aspirating and expelling powers we should have. This point can be proved by pressing the teat, and then sealing the end. If the union is complete, the teat

will remain compressed; if incomplete, it will gradually become inflated.

Again, beginners may find a difficulty in controlling the fluid movements in the stem of the pipette. This with practice will soon be overcome, when the operator acquires that delicacy of touch gained by practical experience, provided, of course, the end of the pipette has been properly drawn out and squarely cut.

When we expel the contents of the pipette containing air-bubbles, of course we find the air-bubble question troublesome when we begin to aspirate. To obviate this condition, raise the glass slide, rest the end of the pipette upon it, squeeze the teat, and allow the fluid to run away from the point.

When bubbles are in existence on the slide, we aspirate from the far side of the drop; in this manner making a kind of strainer.

The contents having been thoroughly mixed, we now aspirate the whole fluid; suck in a column of air at the point, and seal the end in the flame.

The barrel of the pipette should be marked with the grease pencil with the owner's name or number of patient, and placed in the incubator at 37° C. for fifteen minutes, making careful note of the time.

Pipette No. 2 should be treated in exactly the same way, save that the control serum takes the place of the patient's serum, and incubated for fifteen minutes exactly.

When the pipette has been incubated the proper time, it is taken out. A teat is fixed on to the barrel, the pointed or distal end of the stem is scratched and broken off, the whole of the contents expelled on to a slide, and thoroughly well mixed, as previously described.

One or two drops should now be placed upon a clean, grease-free, emery-paper-rubbed slide (on its convex face), and the film made with a spreader. It is well to make three slides in this manner, so that one may choose the best film in the end, and air dried. The application of

heat to fix these films is scarcely practicable, unless in highly skilled hands. As fixing agents we therefore use formalin or corrosive sublimate. The latter is probably the most useful, speaking generally.

The slide is placed in the slide-rack containing a saturated aqueous solution of hyd. perchlor. for half to one minute. It is then washed under the tap, air dried, and stained with carbol-thionin, but in the case of tubercle by carbol-fuchsin (hot).

The best film of each should now be examined by a low power, to ascertain which is the best, and then by a $\frac{1}{12}$ oil-immersion lens, and many of the polynuclear leucocytes will be found to contain bacteria.

A pencil and a piece of paper should then be placed at the side of the microscope, and a count of the leucocytes containing bacteria made up to fifty, first, from the slide with the control serum, secondly, from the slide with the patient's serum, the ratio between the two giving the opsonic index.

Thus, if the control serum film showed that the 50 leucocytes had ingested 150 bacteria, and the patients' serum film showed that the 50 leucocytes had ingested 80 bacteria, the ratio $80 \div 150 = 0{\cdot}53$ gives the opsonic power of the patient; or, assuming the control animal's opsonic power to be normal, the patient's would be nearly 50 per cent. below normal.

In such a condition an animal is a bad subject to resist bacterial invasion. It has been shown that a patient may have a very high opsonic index against a bacterial infection at a certain stage of the disease, and yet die. This state is usually explained by reason of the fact that secondary infection has taken place, and secondary bacteria are the cause of dissolution.

A very low opsonic power suggests either that the animal is suffering from the direct effects of a specific infection, or its resisting powers are so low as to make it susceptible to infection should opportunities occur. This explains largely

why some animals are more predisposed to specific infections than others.

But, of course, it must be pointed out that a high opsonic power does not necessarily mean absolute immunity against bacterial invasion, for, as we have already seen, there are other protective elements in the blood, and in addition the living tissue and organic structures of the body, which play their part in the rôle of conferring and sustaining immunity, and to what extent each can act by itself is at present not known.

Phagocytosis.

The first important theory of disease resistance was put forward by Metchnikoff, who studied the behaviour of the leucocytes in the lower animals, and attributed the destruction of bacteria to their activities. Metchnikoff's theory is that the phagocytes approach and devour invading bacteria, and if they succeed in doing this the disease is checked and prevented. It has been already pointed out that the leucocytes are not in themselves capable of acting in the capacity of invaders, but, as Wright and others believe, the bacteria must be in some manner prepared for them before digestion can take place. On the face of this it appears only reasonable to suppose that phagocytosis is only one link of the complex chain which every living being is daily forging within his economy in his fight against more or less constant bacterial invasions; and it is to the harmonious working of all these protective forces, presenting a strong, vigorous front, that we owe and maintain our existence.

CHAPTER VIII

VACCINES AND THEIR MODE OF PREPARATION

From the previous chapter it will be gathered that within the animal body certain protective forces exist, which, should a bacterial invasion occur, become offensive in their action, and wage war against the invading germs. This warfare is taking place daily in every human and animal body. Sometimes these antibodies are so strong and active that complete protection takes place, and no disturbance is made manifest. At other times the cardinal symptoms of disease present themselves, and after running their course recovery takes place, and the leucocytes and antibodies are thereby fortified. In other cases the bacteria or their products disintegrate, and destroy Nature's protective antibodies, with consequent dissolution of the animal.

Medicinal preparations have long held sway in combating disease, but how many practitioners of medicine are there who are absolutely satisfied with the curative values of drugs in specific bacterial diseases? Nay, more, the longer they are prescribed, the more apparent do their limitations become.

Vaccine-therapy not only carries the therapeutist beyond this limited field, but in many cases he supplants the medical therapeutist altogether, and that, too, often to the great advantage of the patient. Moreover, the logic of vaccine-therapy is based upon a sound reasoning foundation which has the advantage of imitating Nature, and endeavouring to effect a cure in the same way as Nature herself; and in those cases where Nature, usually

through a conglomeration of circumstances, fails to rise to the occasion, the immunizer should step in, and with his vaccines supply the essential stimulus which is needed to produce the various antibodies which she requires to battle against bacterial invasions.

Some animals are born with a lesser degree of resistance to a bacterial infection than others; and also this diminished resistance is particularly noticeable in certain strains of families, and this fact often led the older practitioners astray, when, by reason of the persistent recurrence of the disease in several succeeding generations, they insisted that the condition must be hereditary, when there was nothing more inherited than a weakened resistance. It is in cases such as these that vaccine-therapy can do so much. With this as with all other systems of treatment, an orthodox line laid down and dogmatically followed is almost certain to lead to failure and mistrust, and it is probable that there is no other system of medicine which requires more forethought, more precision, and more careful analytical study, on the part of the immunizer, than vaccine-therapy. For example, an animal may develop strangles, the disease becomes pyæmic, and in due course we clear the system with a streptococcal vaccine. All that now probably remains is a rectal fistula, which we fail to close, and after some further extended trial we give vaccine-therapy up as hopeless. We may even scarify the sinus, plug and dress, and yet fail to get good results. We make a microscopical examination of the pus, and find we are dealing with a mixed infection —for example, a secondary infection by staphylococci— and in addition, being near the anus and rectum, perhaps *B. coli* have become established. The system of treatment has been condemned in failing to complete a cure, but how could a streptococcal vaccine cure a staphylococcal and coli infection? In short, the onus of failure rests with the immunizer in this case. Again, take a case of poll-evil, where, the infection being unusually severe, necrosis of a piece of deep-seated tissue follows, which would in

the ordinary course slough out, but in this case is so imprisoned that separation cannot take place. Needless to add, a vaccine could never effect a cure until the dead slough had been removed, and vaccine-therapy should not be condemned because it failed in what it was never intended to do—*i.e.*, remove a dead slough.

Vaccines are divided into two great classes—stock vaccines and autogenous vaccines. A stock vaccine is prepared from bacteria of the same species, but derived from a different source, and kept in the laboratory for emergency and general purposes. An autogenous vaccine is derived from cultures of the actual bacterium or its strain, already producing the infection in the animal. Again, vaccines are further subdivided into monovalent vaccines and polyvalent vaccines, the former being derived from a single strain of a particular bacterium, and the latter from several strains and races. And, lastly, we have prophylactic and curative vaccines, the former composed of devitalized bacteria, which are injected into an animal whose species are susceptible to the ravages of the bacterium; and by so doing that animal becomes partly or completely immune to the disease-producing influences of that specific bacterium. The latter, as the term implies, are utilized in rousing the bactericidal elements of the animal body to overcome the invading organisms and their death-producing influences. It is only reasonable to suppose that an autogenous vaccine is to be preferred to a stock one, and this is borne out in practice with few exceptions; when one is dealing with an acute disease, such as pneumonia, a stock vaccine should be administered at the outset, inasmuch as it takes time to isolate the causative organism and prepare an autogenous vaccine, for during the time spent in preparation the patient may be getting rapidly worse. To prepare an autogenous vaccine, we take the morbid material from the patient, with careful aseptic precautions to prevent outside contamination; and, as this is an important point, it is perhaps as well to record briefly the method of so doing

in cases as they are seen in practice. If we desire, therefore, to obtain a growth from a nasal discharge, it is wise not to make a culture from the pus taken from the anterior nares, but farther back in the nasal cavity, that region being more certain to contain the specific organisms without so much contamination. Take a piece of stout wire about 15 inches long, and fix on one end a firm piece of cotton-wool (sterile), and pass into the nostril (evading the false nostril). Gently rub the mucosa with the pledget and withdraw it, immediately plunging it into a stout sterile test-tube. Push the free end of the wire through a cork, and bring it down into the mouth of the tube. The pledget will then be firmly fixed in the test-tube. If it is the urine we desire to examine for bacteria, the fluid should be withdrawn with a sterile catheter into a sterile tube and corked. This should be centrifuged, the supernatant fluid withdrawn by a sterile pipette, and one or two loopfuls of sediment placed on slides. One drop of blood should now be taken from the finger-tip and mixed by the pipette with the urine deposit, and spread, dried, and stained. The blood supplies the albumin, which coagulates on heat and fixes the bacteria if present. In cases of thoracic effusion or bursal effusion of joints, these cavities should be aspirated by antiseptic methods, etc., and the fluid placed in sterile vessels. When dealing with milk, it is advisable to asepticize the end of the teat with tincture of iodine, draw a quantity of fluid away before collecting, after which some should be drawn into a sterile vessel, care being taken not to touch the fluid with the hand or fingers. In the case of abscesses, etc., all one requires to do is to smear the pus on the slide, and, if too thick, distilled water should be used to dilute it. It is good practice to make three smears for examination, and to stain them with

1. Carbol-methylene blue.
2. Gram's method (contrast stain, neutral red).
3. Ziehl-Neelsen method (for acid-fast organisms).

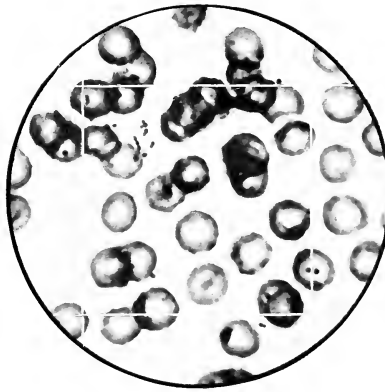
Having arrived at a fair conclusion as to the causative organisms, we proceed to cultivate them. An agar tube, a blood-agar tube, gelatin tubes (two—slope and stab), and one or two anaerobic tubes, should be inoculated and incubated. Growth may be detected in from twelve to twenty-four hours. But if any colonies fail to appear within that period, incubation should be continued for at least three days before the tubes may safely be said to be sterile. When we get a mixed growth, we should start to grow our bacteria on Petri dishes. A Roux bottle is also useful for cultivating bacteria. A first pure subculture is best for the preparation of a vaccine. A careful microscopical examination should now be made of the colonies for means of identification, noting purity, etc. This being satisfactory, 5 c.c. of a 0·1 per cent. saline (NaCl) solution is pipetted into the tube or bottle, and the whole gently shaken. If some of the colonies are difficult to displace, a bent sterile platinum wire or pipette should be used to rake the surface of the medium, taking care, of course, not to graze it. We now have a bacterial emulsion, which should be poured into a sterile test-tube, and which in turn should be heated about the middle in the blow-flame, and, when sufficiently melted, drawn out, and the pointed end sealed by the flame (as described on page 50, Fig. 23), and placed, point upwards, in plasticine or in a tube-rack. The emulsion should now be well shaken for ten minutes to break down bundles or chains of bacteria. To assist in this process, glass beads (sterile) are sometimes inserted also.

Standardization.

Having obtained our emulsion, we now proceed to standardize it, and for this purpose several methods are adopted. The writer has followed Wright's method, which is as follows: A capillary pipette, 6 inches long, with a rubber teat fixed to the open large extremity, is taken, and marked $\frac{1}{2}$ inch from the end of the capillary extremity with a grease pencil. This will indicate one unit volume.

The emulsion-tube should now be scratched at its narrow end with the glass-knife and broken off, but before doing so it is well to sterilize the end in the flame. A ligature should now be placed round one's finger, and the finger pricked; with the pipette one unit volume of blood should be taken up. Then a column of air, one volume of emulsion, another column of air, and one volume of diluent (*i.e.*, a $\frac{1}{2}$ per cent. citrate of soda in a normal saline solution). If the emulsion is very thick, several volumes of diluent may be taken up. This latter prevents the blood coagulating. The teat of the pipette should now be pressed, and the contents expelled on to a clean slide, taken up again and expelled until a thorough mixing has taken place. As soon as the test-tube containing the emulsion is finished with, it should be sealed in the flame and set aside for sterilization. Three slides should now be laid in front of the operator; these slides should have their surfaces roughened by rubbing with emery-paper (Hubert's 00), and a drop of the mixture placed on a corner of each slide, on their convex surface spread out with the spreader, and allowed to air dry. They are then placed in a trough to fix, containing a saturated solution of corrosive sublimate; washed thoroughly under the tap, and stained with filtered carbol-thionin for two minutes; washed again, and dried with filter-paper. The specimen is now ready for examination, and should show, if satisfactory, a fairly even field of bacteria, with blood-cells. Moreover, the bacteria should not be in bundles or chains. If they are, the emulsion must be well shaken again; or if there is a great dearth of bacteria, we must make a stronger emulsion, or, rather, add a smaller volume of diluent, and go over the whole process as before, and make fresh specimens. Should we, however, have a satisfactory field (the beginner may have many unsatisfactory fields before he gets a satisfactory one), we proceed to count the bacteria and the blood-cells under a $\frac{1}{12}$ oil-immersion lens and the highest available eyepieces, so as to limit the field

PLATE VI.



STANDARDIZATION OF BACTERIAL EMULSION, SHOWING BACTERIA AND RED BLOOD-CORPUSCLES.

To face page 74.

view as much as possible. That the field may be divided into squares to facilitate counting, we take a cover-glass and mark cross-lines on it with a pen and ink, or four hairs crossed and stuck on it with seccotine will answer our purpose, and dropped on to the diaphragm of the eyepiece. We now take a pencil, and count in each square the number of bacteria in one column, and in the other the number of red corpuscles. The stage is moved, and another square counted, and so on, until we get a total of 500 red cells.

Sometimes we find a square contains an excess of bundles or chains, or the elements may be badly stained and set out. It is then better to pass on to another square, and estimate from that. The two columns now total 500 red corpuscles as against, for example, 640 bacteria; the ratio is therefore 500 to 640. We know that there are 5,000,000 red cells in a cubic millimetre of blood. Since equal volumes of blood and emulsion have been taken, 1 cubic millimetre of emulsion will contain—

$$\frac{5{,}000{,}000 \times 640}{500} = 6{,}400{,}000.$$

But 1 c.c. contains 1,000 cubic millimetres; therefore the emulsion contains $6{,}400{,}000 \times 1{,}000 = 6{,}400{,}000{,}000$ bacteria per cubic centimetre, and by a process of dilution any suitable strength can be obtained. Now we desire to make our vaccine for future use. The bottles used for storing purposes are rubber-capped, 25 and 50 c.c. capacity, sterilized and filled with ½ per cent. carbolic and NaCl (0·85 per cent.) solution; and we wish every cubic centimetre to contain 1,000,000,000 devitalized bacteria, therefore in our 50 c.c. we must put 50,000,000,000 bacteria, and as we have already seen every cubic centimetre of our emulsion contains 6,400,000,000 bacteria, it is simply a question of dividing the greater amount by the lesser:

$$\frac{50{,}000 \text{ million}}{6{,}400 \text{ million}} = 7{\cdot}81 \text{ (about)}.$$

We therefore must extract from the stock vaccine bottle diluent solution to the quantity of 7·81 c.c., to make room for the like quantity of bacterial emulsion, which has been previously drawn up by the syringe to be injected later into the stock vaccine bottle by passing the needle through the rubber cap, giving us a standardized dose of vaccine of 1,000,000,000 bacteria in every cubic centimetre of solution. Having now obtained this strength, we can give what quantity we desire—*i.e.*, a ½ c.c. containing 500 million; in 1 c.c., 1,000 million; and so on.

As soon as we have made an emulsion and obtained the exact ratio of bacteria, we place the tube in a moist sterilizer, to kill all bacteria, at a temperature of 58° to 60° C. for forty-five minutes. Care must be taken not to overcook, as the therapeutic value of the vaccine is reduced thereby. On the contrary, it is essential to kill all bacteria, and to prove this a small quantity should be pipetted into a culture-tube and incubated. If no growth takes place in from twenty-four to forty-eight hours, one may take it the emulsion is sterile. The stock bottle into which the vaccine is to be placed is filled with (after sterilization) a ½ per cent. carbolic acid and 0·85 per cent. salt solution, and sterile rubber caps applied, and as already explained, an exact quantity of this solution has to be withdrawn from the bottle by a syringe, the needle of which is passed through the rubber caps, to make room for the same quantity of emulsion, which is also injected through the cap with the syringe. To make sure of absolute sterility, the needle should be passed through a drop of lysol put on the cap each time it is punctured.

CHAPTER IX

THE SYRINGE

Syringes of various capacity and make are in use. Probably a 1 c.c. and a 10 c.c. capacity syringe are the two most useful sizes, as they can be used as a vaccine and serum syringe respectively.

They should have metallic or glass pistons, which are much to be preferred to leather-washer ones. It is not necessary to have a large-bore needle, as the serums and vaccines are very fluid. For emergency an ordinary hypodermic syringe will answer one's purpose.

It is advisable to have the syringe made sterile, although the writer must confess in practice to having made several thousand injections, and in many cases failed to sterilize the instrument, and does not recollect having ever had an abscess at the seat of injection or any other sequelæ, but always makes it a point, however, to cleanse the syringe after use by aspirating several capacities of weak lysol solution.

Where one has to be specially particular, and in practice one really cannot be too particular, the syringe can be conveniently sterilized with hot oil in the following manner: Take a tablespoonful of salad-oil and place in a soup-ladle (the latter can be fixed upon a stand), float in the oil a crumb of white bread. Heat over a Bunsen burner or spirit lamp until bubbles of steam begin to rise from the crumb. This will indicate a temperature equivalent to boiling water. Now take up a syringeful of oil, and then expel it into the ladle again; continue to apply the heat until the crumb

becomes brown. A temperature of about 140° C. has now been reached, and at this stage the oil should be aspirated and expelled several times, when the syringe will be found sterile.

Seat of Inoculation.

In the horse and ox the region chosen for inserting one's vaccine or serum is usually one easy to get at, and where

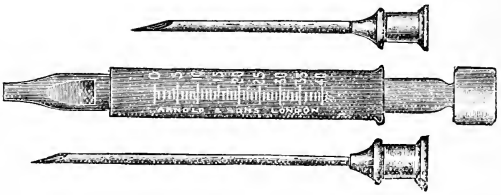


Fig. 32.—Vaccine Syringe.

the subcutaneous tissue is plentiful. Over the scapula or midway up the cervical region fulfils both qualifications. In the dog the most convenient situation is on the inside of the thigh, for here the skin is thin and nearly devoid of hair. Where many injections require to be carried out,

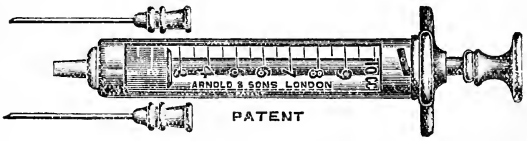


Fig. 33.—Serum Syringe.

it is advisable to operate on one side and then on the other, and upon no account should one injection follow another in exactly the same spot.

The hair should be clipped at the seat of inoculation, and the skin painted with strong tincture of iodine or a little pure lysol lightly applied. This precaution we never fail to

carry through. The syringe is now charged with the necessary dose of vaccine; it is usual to apply a twitch in the horse, although some of them do not resent, especially if the needle is a small one. A fold of skin is then taken up between the left-hand finger and thumb, and the needle firmly plunged through the skin. It is advisable, although not so essential as it is in the human subject, to see all the air is expelled from the fluid-free end of the syringe.

At the seat of injection there may or may not be a little swelling, and in the case of the dog stiffness, but if there is it usually passes off in a day or two.

CHAPTER X

PHENOMENA FOLLOWING ACTIVE IMMUNIZATION BY VACCINES

The immediate result consequent upon a suitable dose of vaccine injected into an animal is a fall in the amount of opsonin present in the serum, apparently due to the gathering up of most of the available opsonin to the bodies of the bacteria introduced. This fall in the index is called the "negative phase," and occupies a period of from twelve to thirty-six hours usually; if the dose is very large it may last for days, if very small it may be eliminated altogether. With this fall of the index there is usually a rise in temperature; the pulse may be slightly quickened, and there may be a slight constitutional disturbance, but in the majority of cases there are no appreciable disturbances. When one is dealing with a local lesion, such as quittor, poll-evil, fistula, etc., an increase in the quantity of the discharge will be noticed, the parts become more swollen, and the animal evinces symptoms of increased pain locally. These phenomena usually last not longer than twenty-four hours, and are replaced by a return to a more normal condition; the pulse-beat becomes less, also the temperature, the discharge lessens, and the animal is brighter. We have now entered upon the positive phase. This is due to the stimulus given by the vaccine; fresh supplies of opsonin are elaborated, during which the opsonic index rises, usually taking a day or two, when the maximum is reached, and a condition is now established which may remain for a day or two, and is called the "phase of increased resistance."

In some patients this phase is a prolonged one; in others it is of shorter duration. What the immunizer should really aim at is to have the positive phase as long as possible, and, of course, the negative phase very short in duration; this can largely be attained by using a suitable vaccine and of proper dosage.

In very acute affections, such as pneumonia, one cannot have the negative phase too short, in which case it is advisable to give a minimum dose at the outset; this will give a quick positive phase, and another larger dose should then be given before the transient positive phase passes off.

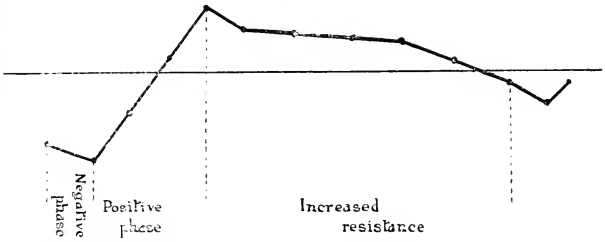


FIG. 34.—CHART SHOWING THE EFFECTS OF A SUITABLE DOSE OF VACCINE.

In some cases the period is not more than twelve to eighteen hours.

It is obvious, in dealing with an acute, depressing disease, the vaccine will produce a very pronounced negative phase, and the patient's resources between the two may be so exhausted that dissolution will follow. This is naturally a condition the immunizer wishes to ward against.

It is therefore a wise axiom to follow: Never give a maximum dose of vaccine in an acute disease with rapid pulse and high temperature, remembering, if the temperature is high, a full dose of vaccine will make it go still higher.

The tracing given in Fig. 34 shows what really should take place after a suitable dose of vaccine has been administered:

When the dose has been too small, we get an immediate positive phase of short duration and of little or no curative value. Thus:

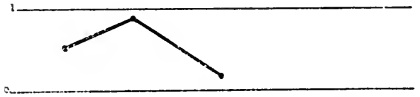


FIG. 35.—DOSE OF VACCINE IS TOO SMALL.

On the contrary, if the dose has been too large, we get an immediate fall of the index. Nature's balance has been so upset she cannot assimilate, so to speak, the dose; great depression sets in, and several days may pass before a restoration to the previous state takes place.

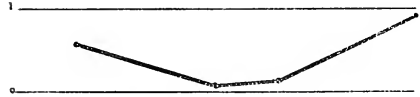


FIG. 36.—DOSE OF VACCINE IS TOO LARGE.

In the horse we have never noticed this phase, and have given doses very many times greater than orthodox present-day teaching suggests. In the case of the dog, we confess to having a few isolated instances of apparent temporary collapse, etc., following upon vaccine-therapy, but never with fatal results.

The immunizer should aim at index No. 1 as his ideal to follow out in practice.

If he get a reaction such as is shown in index No. 2, with no curative results, he should not condemn the treatment, but persevere and increase his dose.

Index No. 3, in the hands of a careful practitioner, should never occur; and if it does, it ought to teach him to be less heroic and more careful in his future dealings with vaccines.

As already stated, and we repeat it again to emphasize the fact, *it is a "sine qua non," when the opsonic index falls,*

the temperature invariably rises; and where one is dealing with an acute disease and a high temperature, it is wise to begin with a minimum dose of vaccine.

When the temperature is very high, and we wish to begin the vaccine treatment, we give a big dose of salicylic acid, wait three to five hours, and then inject. This we find good practice.

Moreover, it is advisable not to give a second dose of vaccine until the improvement begins to fail. In subacute and chronic cases this varies from five to ten days. The second and succeeding doses should, as a rule, be proportionately larger than the first. If one gets no response from the first dose, a second and larger dose can be given almost straight away, and careful notes of the symptoms made.

The Dose of Vaccine.

In deciding upon the most suitable dose of vaccine for a patient, one must be guided by circumstances.

If the disease is an acute one and the temperature high, for reasons already explained, a minimum dose must be administered at the outset; on the contrary, if we are dealing with a chronic condition a full dose is indicated, and is quite safe. Again, certain bacterial vaccines are more potent in their actions than others. For example, a given streptococcal vaccine stimulates the bacteriolytic forces much more effectively than a staphylococcal vaccine of the same strength does.

In judging as to whether a suitable dose has been given or not, one must take note of every little clinical detail; but in reading these off one must not rely upon a single condition alone. It is much safer to total them all together, as it were, and strike averages. For example take a case of acute pneumonia: the temperature after an injection may rise, and at first consideration one might suspect a pronounced negative phase following upon the vaccine; but this rise may largely be a coincidence, the primary cause

being probably an auto-intoxication proceeding from the seat of infection.

Again, the temperature may be undisturbed, while the pulse is increased, the breathing more laboured, and pulmonary effusion increased, and this may be the negative phase of the cycle.

Where we are dealing with a local chronic condition, the clinician is probably in a better position to judge the actual state of affairs, for here we have local manifestations upon which to make our observations and draw our conclusions. These will be given in the succeeding chapters, as their respective diseases are being dealt with.

Although one has to use a certain amount of discretion in deciding upon dosage, failure to effect a recovery may solely depend upon the immunizer being too careful and using too small a dose. On the other hand, we may say the horse and ox in particular are very tolerant to big doses, and make excellent subjects for vaccine-therapy. These conclusions have been formed after watching the effects of several thousand injections.

On the dog one has to exercise perhaps a little more caution.

Many of the vaccines advertised at the present day are of little value, if only for the fact that the doses are too small; we have had repeatedly to give half a dozen in one before getting a suspicion of a negative phase.

To a young colt seven days old, suffering from a streptococcal infection, we have started by giving as a dose 100,000,000 devitalized bacteria, increasing each dose until the maximum of 500,000,000 was reached.

If a young colt can tolerate these doses, how much more so an adult horse!

The Age of Cultures.

Because of involution forms, one must have young cultures, and endotoxins appear to be best suited for vaccine-therapy purposes.

The following is a table of the average culture age of some of the more common bacteria:

Staphylococci (*citreus*, *albus*, *aureus*)	12 to 48 hours.
Streptococci	48 to 72 hours.
Pneumococci	36 to 48 hours.
B. coli communis	4 to 8 hours.
Micrococcus catarrhalis	5 to 10 hours.
Micrococcus tetragenus	5 to 10 hours.
Bacillus pyocyaneus	24 to 36 hours.

Dose of Autogenous Vaccine to Horses and Cattle.

The horse and the ox are very tolerant to big doses of vaccines, and in our experience make excellent subjects for sero-vaccine therapy.

As already stated, when the disease is running a very acute course, smaller doses as a rule should be given; while, on the contrary, in an animal subject to a chronic bacterial invasion the system is more tolerant to much larger doses.

When, however, an animal is suffering from an acute staphylococcal infection, a larger proportionate dose of vaccine can be given here than can be given in the case of a streptococcal invasion with a streptococcal vaccine. After three years' experience of immunization with autogenous vaccines, we find the following range of doses to give the best results, but we would point out the immunizer must ascertain for himself to what extent he may go in dosage with each individual case, remembering that not only does he require to consider the idiosyncrasies of the patient itself, but also the vaccine he is using. In short, there is no absolute rule of thumb to go upon.

	Initial Dose.		
	Minimum.	Optimum.	Maximum.
Staphylococci (three kinds) ...	250,000,000	500,000,000	750,000,000
Streptococci	100,000,000	350,000.000	500,000,000
Pneumococci	75,000,000	250,000,000	500,000,000
Bacillus coli group	200,000,000	350,000,000	750,000,000
Micrococcus catarrhalis ...	250,000,000	350,000,000	500,000,000
Micrococcus tetragenus ...	250,000,000	350,000,000	500,000,000
Bacillus pyocyaneus ...	250,000,000	350,000,000	500,000,000

Dose of Autogenous Vaccine for Adult Dog (Medium-Sized).

	Initial Dose.		
	Minimum.	Optimum.	Maximum.
Staphylococci (three kinds) ...	50,000,000	125,000,000	200,000,000
Streptococci	25,000,000	100,000,000	150,000,000
Pneumococci	20,000,000	75,000,000	150,000,000
Bacillus coli group	50,000,000	100,000,000	200,000,000
Micrococcus tetragenus ...	50,000,000	100,000,000	200,000,000
Bronchosepticus (isolated by Ferry)	50,000,000	100,000,000	200,000,000

CHAPTER XI

SERUMS AND THEIR MODE OF PREPARATION

Sero-therapy is a branch of prophylactic and curative medicine which has made great strides within recent years, its primary object being the neutralizing of liberated toxins circulating in the blood and lymph streams. These toxins are the products of specific bacteria located in certain structures of the animal body, and the serum itself does not prevent the growth of the bacteria.

When these pathogenic bacteria settle in a structure, they incubate, and in the processes of growth pour out a constant stream of toxin, which is continually entering into a kind of combination with the receptors of the cells. It therefore follows that antidotal serum should be used before serious cell changes have taken place. And this is exactly borne out in practice, as, for example, in tetanus, swine fever, and diphtheria in man; and to make certain of a recovery the serum should be injected before the diseases are established.

As the making of serums scarcely comes within the domain of the individual practitioner, a general outline only of the *modus operandi* will be given.

The horse is the animal commonly used both for the making of human and veterinary serums, and it is most essential he should be quite healthy and be free from such diseases as tuberculosis and glanders, for which he is tested by tuberculin and mallein respectively.

The animal is then inoculated with a dose of attenuated toxin prepared by heating the virulent poison or by treating it with a chemical agent, either of which will reduce its strength. Repeated inoculations should be followed up, at stated intervals, with increasingly virulent doses.

The toxins which are injected are prepared by growing the specific bacteria upon suitable fluid media. The cultures are then passed through a Pasteur-Chamberland filter, or a similar filter, to remove the bodies of the bacteria.

When a sufficient degree of immunity has been reached, and three or four days have elapsed after giving the last dose of toxin, the blood is withdrawn under aseptic precautions from the jugular vein by a sterile cannula, and collected in sterile vessels. These are put aside, preferably on ice, to allow the blood to clot, and in due course the serum is decanted into sterile stock bottles, a small quantity of preserving antiseptic added, and they are ready for use.

Antibacterial serums are produced in a similar manner, but the actual bacteria themselves are injected instead of their toxins. Before a degree of tolerance is reached, the dead bodies of the bacteria or an attenuated culture are given at the outset, or a dose of antitoxic serum is administered to mitigate the effects of the first virulent dose.

All serums should be tested before using, to see that they are free from bacterial contamination, by flooding some upon a culture medium and incubating, and to insure non-toxicity the serum should be injected into a small animal first.

Serums are standardized by the physiological test. An animal is taken which reacts in a constant manner to the poison, and dying within a certain time. It is known that a given quantity of poison will destroy an animal of a given weight in a given time. This is taken as a standard. It is then necessary to find what amount of antitoxin is required to neutralize this dose, and we find equal quantities of a given antitoxin will do this, a standard thus being set up.

In therapeutics serums may be used alone or in conjunction with vaccines—in fact, in many cases the combination is a happy one, particularly in the diseases attributed to the streptococcus. Again, in some diseases we are compelled to fall back upon serums alone, as there are certain specific infections where the causative bacterium has never been isolated: hence vaccines—at least, according to the present-day meaning of the word "vaccine"—are not procurable. These specific diseases belong to what are known as the *ultravisible virus group*, of which the following are the principal:

Swine fever.
Canine distemper (probably).
Rabies.
Foot and mouth disease.
Specific pleuro-pneumonia (Bovine).
Rinderpest.
Variolas.
Cape horse sickness.

It may be advisable to point out here there is a distinct phenomena particularly recognized in human medicine following upon the injection of sera under certain circumstances, and it is known as "serum disease." In animals a similar condition has been noted.

Large quantities of serum may be injected into man almost daily, and extending to two or three weeks without ill-effect. If, however, a period of twelve or more days elapse between any two given injections, serious results are liable to follow. The condition of the system is then hypersensitive and is technically know as *anaphylaxis*, and in some cases the result is fatal collapse.

Theobald Smith noticed when guinea-pigs were injected with horse serum and a second injection given after the elapse of ten days, the pig will show signs of hypersensitiveness, and if the dose is sufficient death may follow.

Again, it has been shown that the sera obtained from one

species and injected into another species of animal is more likely to produce anaphylaxis than if the sera is obtained from the same breed of animal we desire to immunize. Thus horse serum should not be used upon the ox, more especially if a considerable lapse of time is allowed to follow any two injections, and *vice versa.*

CHAPTER XII

SPECIAL DISEASES, CAUSED BY SPECIFIC BACTERIA, WHICH ARE SUITABLE FOR TREATMENT BY SERO-VACCINE THERAPY

Diseases affecting the Cutaneous System.

Disease.	Cause.
Abscess	Staphylococci, streptococci, *B. mallei*, *B. pyocyaneus*, *B. coli*, *B. tuberculosis*, actinomycosis, bothriomycosis.
Poll-evil with fistula ...	Streptococci, staphylococci, *B. coli*.
Acne	Acne bacillus, streptococci, staphylococci.
Mange (*Demodex canis*) ...	Staphylococci (secondary infection).
Ulcers	*B. tuberculosis*, streptococci, staphylococci, pneumococci, *B. pyocyaneus*, *B. mallei*.

Abscess.

In practice one sometimes finds cutaneous multiple abscesses which are very intractable to treatment with the ordinary orthodox therapeutical dressings and drugs. It is in cases such as these the immunizer can do much for his patient. As already seen, there are a fair variety of bacteria which are pus-producers and which enter into the formation of abscesses. *Staphylococcus albus* and *aureus* are the two bacteria most commonly found in superficial abscesses; but owing to the situation and the easy means for further infection one often finds a mixed bacterial element.

It is therefore of first importance the immunizer should become conversant with the nature of the infection at the outset. A film preparation should be made and examined after staining; a specimen should also be stained by Gram's

method, and the results compared. Needless to add, the pus should be so taken that there is no danger of outside contamination by other bacteria. If need be, a stroke culture on agar should be made, and a gelatin-tube also inoculated.

Having ascertained the *causa causans*, a suitable autogenous vaccine should be made in the manner already described.

When the abscess or abscesses are very tense and distended with purulent matter, internal pressure should be relieved by lancing, to bring about lymphoid osmosis. Hot fomentations should be applied to them to cause capillary distension, relieve congestion, and increase the flow of bacteriolytic and bacteriotropic blood to the parts; and if the blood is viscid, citrate of soda solution should be applied, with the internal administration of citric acid. Upon no account should strong antiseptics be applied, as they will undo all the good the vaccines are endeavouring to do.

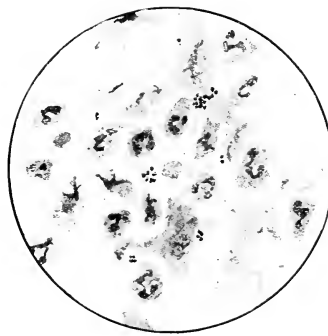
If the abscesses are old-standing, and they usually are, one can begin with full doses; 500,000,000 staphylococci to a full-grown horse may be given at the outset, and the results carefully watched. In five to seven days 1,000,000,000 may be given. A week later 2,000,000,000 may safely be injected, and so on, increasing the dose until one finds the maximum benefit has been gained.

In the case of streptococci, one cannot give such large doses, and we would not advise giving more than 100,000,000 as an initial dose, increasing in the same proportion as for staphylococci. When the streptococcal infection is a severe and acute one, we have obtained considerable benefit by combining the vaccine with the antistreptococcal serum.

Fistula of the Poll (Poll-Evil) and Fistula of the Withers in the Horse.

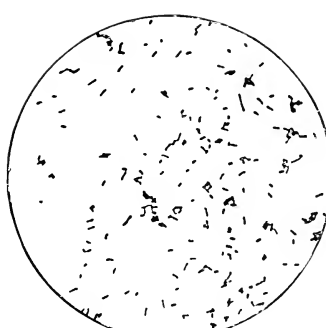
These two conditions may be conveniently taken together. The bacterial cause is the same, the pathological lesions

PLATE VII.



STAPHYLOCOCCI.

(Rose and Carless' "Manual of Surgery.")



BACILLUS COLI COMMUNIS. × 1000.

(Hewlett's "Bacteriology.")

To face page 92.

are identical, and the curative treatment is the same in each case.

The immunizer may be called in to see a case at a stage when there is a considerable accumulation of pus, and by reason of the situation the purulent matter digests the adjacent healthy structures, burrowing deeply—involving vital parts, in the case of poll-evil, the spinal cord and the spinal vertebræ—and often showing no disposition to burst. In this condition one finds a considerable unilateral or bilateral swelling, slightly painful, and hot, and it may be fluctuating or tensely hard. In such a condition vaccine-therapy would be of little or of no use, and the reason is not far to seek. To obtain the full benefit, the antibodies of the blood must gain access to the focus of infection, and where there is a considerable internal pressure through excessive purulent accumulation the infiltration of lymph is dammed back. The blood itself may be very rich in the necessary bactericidal elements, and yet the tissue cells may be dying from nothing short of bacterial intoxication consequent upon auto-bactericidal starvation.

To correct this condition, the pus should be evacuated from the cavity at once, when the curative lymph will flow freely through the diseased area.

If this fails, we must look for other causes. The lymph going to the parts may coagulate too freely, depositing itself upon the cavity wall and adjacent tissues as a coagulum, and thus acting as a barrier to osmosis. To remedy this, flush the cavity with a solution of citrate of soda, and give internally ounce doses every five hours of citric acid until a free flow is established.

Again, the blood itself may be so thick and tenacious as to undergo too rapid and easy coagulation.

Or again, if the case is one of long standing, the abscess walls may be so thickened and indurated by the deposit of lymph, etc., that no fresh healing lymph can pass through. This must be removed by the free use of the curette.

And, lastly, the bactericidal elements of the blood itself

may be so deficient that a complete regeneration of them is required. For the performance of this function, we must look to vaccine-therapy to stimulate the deficiency and supply that which is wanting.

The fact of an animal showing symptoms of poll-evil or fistula to the skilled practitioner is suggestive of one of three conditions: Either the patient's opsonic power to the causative bacterium is too low, indicating a deficiency of opsonins, or the specific infection is a very virulent one, or both.

At first sight it seems rather strange that cutaneous bacterial invasion should select the poll and the withers of horses as the most common seats of entrance. It may be there are three distinct forces at play to account for this:

1. Some localities show a larger percentage of cases of fistula and poll-evil than others. In these districts one invariably finds bad stables and low roofs and unhealthy surroundings, with deficiency of sunlight.

2. Heredity plays an important part. We have known three generations develop poll-evil and fistula, *i.e.*, the dam, the daughter, and the grandson, the latter poll-evil only. In our experience the opsonic power to cutaneous bacterial infections is markedly low in some families compared with others.

3. The bacterium most commonly found in purulent poll-evil and fistulous withers is the *Staphylococcus albus*, in our experience, and as a pathogenic microbe it may be defined as ubiquitous. Certainly the horse's skin, harness, etc., harbours this organism very successfully. Anatomically the poll and the withers are the most prominent points in horses; it therefore follows with low roofs, etc., bruising is more liable to take place in these regions. One sometimes sees in practice an animal receiving a blow or a bruise in these parts; an inflammatory swelling takes place, but in a few days disappears. This the writer does not call poll-evil. Clearly here the skin has not become broken nor bacterial inoculation taken place; but, on the

contrary, should bacteria infect the bruised area, pus soon forms, and, partly by gravitation and partly by digestion, burrows, reaching bursæ and ligamentum nuchæ. The ligament and the bursæ being lowly vascular organs, the bactericidal elements going to these parts are limited. This gives the bacteria a good chance to gain a footing, and, when once established, pus increases, burrows downwards, causing local death to many tissue cells, and even invading the osseous structures and the medullary canal itself.

The *Staphylococcus aureus* may be found in conjunction with the *albus*, and sometimes a streptococcus is present. Very rarely have we found a *Bacillus coli*. The latter's presence is more commonly discovered in fistula of the anus or deep-seated wounds in the region of the rectum. To make an autogenous vaccine, it will be gathered, the proper bacteria should be isolated and examined and cultivated, and an appropriate vaccine made.

A good axiom to go upon in practice is to inject one's vaccines as near the seat of disease, if it be a local one, as possible. And we even go farther than this by injecting the vaccines into various centres round the swelling, if circumscribed ones, such as poll-evil or fistula. Opsonins are largely manufactured—it is believed, in muscular and subcutaneous tissue. Allen has shown that, if limbs of animals or men are perfused with saline solution to remove all the possible blood-elements, and the muscles cooled and minced, and their plasma extracted, this extract has a markedly higher index than that of blood-serum towards the various bacteria—proving at least muscular, subcutaneous, and intramuscular tissue contains more opsonins than the blood-serum itself.

The writer always begins by injecting 500,000,000 staphylococci at the outset, noting the symptoms, and when the improvement appears to be drawing to a close, follow up by giving 1,000,000,000, repeating in five to seven days 2,000,000,000.

We have given up to, as a dose, 10,000,000,000 devitalized bacteria, after a decided toleration has been established. If streptococci are present, a vaccine from this microbe should be made, and the initial dose should be not more than 100,000,000. One has to be more careful with a vaccine from this bacterium than with staphylococci. In five to seven days repeat the dose, giving 200,000,000. As a maximum dose, after several administrations, we have never given more than 1,000,000,000, preferring to combine the vaccine with antistreptococcal serum, if the case requires it.

The writer has had one troublesome case which was a mixed infection in a fistulous withers patient.

The wound was freed of staphylococci, and then the case came to a standstill, the obstinate bacterium being a specially long-chained streptococcus. The vaccine failed to get rid of it by itself, but after combining it with streptococcal serum the patient made good progress, and recovered.

Clinical Phenomena after Injection.

In these cases of poll-evil or fistula, one has the advantage of watching local conditions which, if properly interpreted, are of singular value to the clinician.

There may be a slight constitutional disturbance; this is salutary. Invariably, if the vaccine causes a response, the local swelling increases during the first twenty-four hours; if discharging, the discharge also increases, containing more lymph and looking more healthy. In the case of poll-evil the head is carried more stiffly, and the nose more protruded.

The pus should be carefully noted. The writer has found, in those cases where the opsonic power is low and the bacterial invasion virulent, the purulent elements are of a greyish-yellow colour, thin and watery in consistence, and possessing little or no viscosity. The clinician may safely interpret this condition to mean the lymphoid elements of the blood are either very defective in them-

selves, or else they are unable to reach the purulent cavity. If the former, suitable vaccine will correct the defect; if the latter, surgical aid, as already indicated, should be called into action. Invariably these cases discharging such unhealthy pus are difficult to cure, and are suggestive of a localized necrosis; but when the pus becomes thick and tenacious, or what our forefathers, who made few errors in the field of practical observation, were wont to call "laudable" pus, the indications are most favourable.

The aggravation of these local symptoms after twenty-four hours usually decreases when the positive phase has already set in, and from this period an improvement should be noted. As soon as this appears to be on the wane, another and larger dose should be given. Needless to add, poll-evil and fistula are debilitating diseases, and good food, good surroundings, etc., are essential to the making of a good recovery.

A word of warning may suitably be given here. The practitioner may examine his case carefully, make an accurate diagnosis, prescribe a suitable vaccine, and yet fail to bring about recovery.

There is no system of treatment which has not its limitations, and vaccine-therapy is no exception to this rule. The immunizer, with industry, skill, and adaptability, can drive these limitations farther away, giving himself more scope to work, and in the end obtaining better results. But there are other points to consider. For example, one practitioner gives up a case after a course of vaccine treatment, and condemns the system *in toto;* another practitioner would probe the fistula, and detect, perhaps, a calcifying necrosis on the lower border of the funicular portion of the ligamentum nuchæ, or, maybe, a specific periostitis of the body of the atlas or axis. Now, the most perfect autogenous vaccine and the most skilled immunizer would prove both miserable failures if they followed up vaccine-therapy and failed to curette the necrosed area or, maybe, remove a dead slough.

Again, it is a deep-rooted idea, and even with men of great practical experience, to go on irrigating sinuses and cavities with antiseptic dressings of considerable strength, their logic being that by so doing they are killing out the bacteria present in the cavity, and by getting rid of the *cause*, they reason, the *effect*—*i.e.*, the disease—will disappear. It requires only a moment's consideration to see that such reasoning is entirely wrong; nay, more, the facts are not borne out in practice. To begin with, the most vital and active bacteria are not to be found in the débris of an abscess or in the centre of a sinus. Many of them are degenerates, and therefore possess only slight pathogenic powers. Some are devoured by the phagocytes. Others are rendered inert through the action of the antibodies and the poisonous effects of their own toxins. But in the walls of these compartments they are most active and virulent, for there they find what they want—heat, moisture, and nourishment, and last, but not least, removal from their own exotoxins. Now, how far does an antiseptic fluid, such as an aqueous solution of hyd. perchlor., penetrate into the walls of these cavities, which, be it remembered, are varnished all over with the exuded lymph? Moreover, the mercury salt frustrates its own ends as an antiseptic by coagulating the albuminous lymph and making it a still more perfect varnish. To our mind, there is nothing which detracts more from the healthy healing of a wound, and hampers Nature's efforts so completely, as the repeated use of strong or moderately strong antiseptics. If an antiseptic has to be used, it is much better practice to use one caustic plugging to slough out the germ-laden wall, leaving a healthy granulating surface to complete the repairing process. But even this process has its drawbacks, and must be used with care. The fact of applying dressings strong enough to cause a slough not only causes local death to bring about the slough, but absorption must take place to the surrounding healthy structures, which may be diffused for several inches. All

through this area the protective bodies and tissue cells have had their vital energies depressed by the poison, and their offensive and defensive powers consequently lowered, giving the bacteria a chance to gain another and extended footing.

The only antiseptic solution the writer uses now for these cavities is weak boric lotion, and great results will follow the free use of cold tap-water irrigations. Normal saline solution also makes an excellent dressing as a mild antiseptic, tissue stimulant, and restorative.

Fistula of the Præsternum of the Horse.

This condition, in our experience, is not very common; having only seen six cases, and three of these have occurred within the last four years. The condition is caused by the animal usually running against some hard, fixed object, and bruising the structures from the skin to the cariniform cartilage. A hard or semi-hard swelling results; the animal walks stiffly, with fore-legs held wide apart. In course of time—usually a considerable time—an abscess forms, points, and bursts, the discharge being of a thin, glairy, sometimes brown or dark-grey colour. The swelling tends to subside if the discharge is sufficient, and the animal then goes better; the pus may cease flowing, the wound closes, and the swelling, pain, stiffness return, and when a sufficient purulent accumulation has taken place the abscess again points and bursts, and these processes go on indefinitely. It is probable there is no case of spontaneous cure on record.

As with poll-evil and fistulous withers, we have here also the same forces at work and the same anatomical drawbacks which Nature encounters in her endeavours to bring about recovery—*i.e.*, pus-producing bacteria on the one hand, and the deficient blood-supply going to the injured parts on the other. Sometimes one sees a solitary sinus, at other times there may be two or three, leading either to one or more foci in the sternum.

As with all other bacterial invasions, so here one must

ascertain what causative organisms are present by adopting the usual technique. The *Staphylococcus albus* and *aureus* combined we have found in our three last cases. We are at present treating a case where we detected these two bacteria, and in addition a rather uncommon bacterium—*i.e.*, *B. pyocyaneus* (belonging to the chromogenic class).

In this disease, one has to give full vaccine doses if the best results are to be obtained. The writer usually starts with 750,000,000 *Staphylococcus aureus* and *albus*, and in this latter case combined it with 250,000,000 *B. pyocyaneus*, the doses being doubled each time.

The patient has had up to the present three injections, and progress so far is quite satisfactory: the swelling is becoming smaller and softer, and the stiffness has disappeared. During the negative phase this increased, however, and for twenty-four hours the animal scarcely walked at all, being very stiff on his front-legs. Locally there is one central sinus, which discharges intermittently. The sinus has been curetted and irrigated with soda citrate solution alternated by normal saline solution. Since these notes were made, this case has completely recovered and been in constant work for six months.

Fistula in the Region of the Anus and Rectum.

This condition in equines is seen often as a sequel to strangles running an irregular course, and is most intractable to the ordinary forms of treatment. If the case is one of long standing, the immunizer usually finds the purulent discharge contains more than one bacterium. Streptococci there usually are, staphylococci there may be, and, considering the situation, one has every justification in being on the careful lookout for *B. coli communis;* and if present a suitable vaccine should be made. In human vaccine-therapy, we understand, a stock vaccine of the *B. coli* acts very well.

The writer recalls treating a case with staphylococcal and streptococcal vaccine, giving extremely large doses and fail-

ing to close the sinuses. A further microscopical and biological examination was made, and we found a few shrivelled staphylococci, but an abundant display of very active *B. coli*.

The initial doses in a case of this kind should be—Streptococci, 100,000,000; staphylococci, 500,000,000; *B. coli*, 100,000,000: repeated in five to seven days, according to conditions, in appropriately increasing doses. Of course, if there is any necrosed area in the sinus, this should be removed, as Nature's sloughing efforts in these cases are very indolent. Here also oft-repeated antiseptic dressings should be deprecated.

Acne—Pustular Dermatitis Contagiosæ.

This condition is seldom seen in equines in this country. It occurs in the form of papules or pustules, usually on the parts of the body where the harness rubs. It is due to a bacillus which sets up a purulent inflammation of the skin, destroying the hair follicles, and may spread all over the trunk by means of the harness, grooming utensils, and clothing. The presence of the acne bacillus and the skin lesions it sets up seem to facilitate the growth of the ubiquitous staphylococcus, which invariably is found in the purulent discharges, aggravating the disease and prolonging the condition. Streptococci may also be present in the pustules. The bacillus is anaerobic—at least, at the outset—and is Gram-positive.

It is small and ovoid, occurring singly or in short chains. It is non-motile. In a gelatin stab culture small colonies occur; on glucose agar white colonies grow very slowly.

In this, as in all other mixed infections, the offending bacteria should be isolated, and a vaccine made from each.

To obtain a pure culture of the acne bacillus, take a scraping from the deeper parts of the sebaceous plugs, and mix the material in broth, incubating anaerobically for ten to fourteen days. Should staphylococci be present, by this time they will be inert, while the acne bacilli will have

multiplied. To 10 c.c. of melted 3 per cent. agar rendered acid by hydrochloric acid, add 5 c.c. of fresh sterile ox serum, and pour into a Petri dish.

Now spread a loopful of the acne-bacilli-laden broth over the surface of the plate, and incubate for three days (anaerobically). These may be picked off, replated, and incubated aerobically, and a bacterial emulsion made in the usual way preparatory to the making of the vaccine.

Follicular Mange in the Dog.

This troublesome skin disease of the dog is due primarily to the burrowing of the *Demodex folliculorum*.

In course of time the protective forces of the skin suffer considerably, the animal's health becomes impaired, his opsonic index falls, and the patient is ripe for any bacterial invasion that may come along.

On the surface of the skin is to be found an excellent medium for bacterial growth—blood-serum; and at a body temperature staphylococci soon manifest their presence by excessive pus formation—so much so that, in advanced cases, the writer strongly believes one's greatest concern, so far as a cure goes, is to get rid of the bacteria, and that the acari are but secondary factors at this stage in the causation and continuation of the disease.

An animal in such a condition is clearly one for the immunizer, provided he can use an autogenous vaccine. The obstinate nature of these cases toward orthodox therapeutics lies, not so much in the fact that the acari and the bacteria are so difficult to destroy, but rather they are so ungetatable (if one may use the word), their unassailable seat being principally at the roots of the hair follicles. It is quite obvious, then, to reach them one must use strong penetrating poisons, which, as already indicated (in previous chapters), when dealing with bacterial infections, is to be deprecated, destroying as they do the very elements we wish to preserve and fortify, *i.e.*, the bactericidal antibodies of the blood.

What, then, is the orthodox therapeutist's misfortune should be the vaccine-therapist's opportunity; for the fact that the bacteria are deep-seated gives him a better opportunity, with his vaccine, of driving home in full force Nature's whipped-up, refreshed, and restored antibodies.

Although staphylococci are the usual pus-forming bacteria present, and particularly the *albus*, it does not follow other bacteria may be absent. It therefore behoves the practitioner to make sure by careful microscopical and biological examination what the nature of the infection is.

The dose of staphylococci depends largely upon the size, age, and general condition, of the dog. To a medium-sized dog we usually begin with 75,000,000 devitalized bacteria, watching the symptoms. If one finds a sufficient negative phase has been established, wait about five to seven days, and repeat by giving 100,000,000, the third dose being increased to 150,000,000. After toleration has been established, we have given to a retriever as much as 1,000,000,000 staphylococci. If the improvement is not as much as one would anticipate, do not hesitate to give bolder doses; but it is always well to begin with a moderately small initial dose, and work upwards.

Locally one must endeavour to get the lymph to flow freely, and great benefit is to be derived from boric lint soaked in boiling water, applied to the skin very hot and over this a thick layer of oilskin should be applied and retained. Citrate of soda and boric lotion should also be applied at intervals, and if need be citric acid should be administered internally. Later a course of calcium sulphide tabloids should be carried out. Needless to add, the patient should be fed up, and kept, by being placed in healthy surroundings, from reinfecting himself.

The opsonic index will be very low usually; in one case we found it as low as 0·39. This was a chronic case, and the animal was in a cachexous condition.

Ulcers.

Ulcer formations may be seen in any part of the integument.

They may be single or multiple, benign, and heal with little effort on the part of Nature or the practitioner. They may be malignant, such as cancerous, glanderous, tuberculous, and incurable.

Needless to add, vaccine-therapy in the lower animals excludes these three latter conditions.

When one finds, however, a simple ulcer becoming contaminated by pathogenic bacteria, and assuming serious proportions, with probable symptoms of septic infection or intoxication, the immunizer can do much to relieve suffering and save life with suitable vaccines.

Invariably the infection is a mixed one, and this point must be definitely settled before curative progress can be embarked upon.

Locally the curette should be used, and even a single caustic dressing may stimulate healthy granulation, although it must not be repeated.

When the deep structures of the corium become involved, and if the infection is a virulent one, localized death of tissue may take place. This, of course, must be removed by hot fomentations, the lancet, and free drainage.

One sometimes meets in practice a destructive and troublesome ulcerative keratitis in valuable dogs after distemper, and one would think a suitable autogenous vaccine would be of great service here, saving eyes and eyesight, which are often lost. Of this, however, the writer has no experience, and only throws out the suggestion should others come across a suitable case to try vaccine-therapy upon.

CHAPTER XIII

BACTERIAL DISEASES AFFECTING SYNOVIAL JOINTS

Disease.	Cause.
Traumatic arthritis	Staphylococci, streptococci.
Pyæmic arthritis in foals ...	Staphylococci, streptococci *B. coli*, *B. pyocyaneus*.
Septicæmic arthritis in cattle	Streptococci.

Traumatic Arthritis.

The articular elements of the animal body become the seat of bacterial invasions through two distinct sources—*i.e.*, first, internally, and what might be called "auto-infection," the bacteria being carried usually by the blood or lymph stream; and, secondly, externally, as illustrated by traumatism.

Whatever may be the channel by which bacteria reach these joint cavities, one cannot gainsay the fact that when they do get there they find an admirable breeding-ground, and this fact is soon demonstrated in practice by the very rapid manner in which they develop, disintegrating the vital structures of the joint and adjacent tissues.

In cases of traumatic arthritis the invading bacteria usually belong to the staphylococci group. The practitioner here is not confronted so much with the question of the life and death of his patient as he is with the great desire of limiting the destructive metabolic changes occurring in the joint and caused by these pathogenic bacteria. When destruction of the sensitive articular surface takes place,

anchylosis is certain to follow, and that to an animal of the equine species, of course, means destruction.

We therefore maintain, if the practitioner is called in sufficiently early—*i.e.*, before destructive changes have taken place within the joint—and he adopts a rational course of treatment with suitable vaccines, combined with thermic assistance, he need fear no organic stiffening of the joint. Of course, where pus has been discharging for days, and the whole of the articular surface has been stripped by the digesting action of the bacteria, vaccine-therapy will fail to restore such a joint to a normal condition, although, undoubtedly, it would facilitate the removal of the bacteria.

Retardation of the development of bacteria within the joint is effected by a more or less continuous spray of cold water, and when the circulation is interfered with, or in any way becomes defective, the application of Bier's treatment, by driving an excess of bactericidal elements to the joint, forms an excellent adjuvant to vaccine-therapy. In addition we have used for some years injections of ol. caryoph., with excellent results in open joints, and, unlike most antiseptics, it does not appear to irritate or upset the local tissues it comes in contact with; on the contrary, it seems rather to stimulate than depress Nature's restorative and protective elements.

Pyæmic Arthritis as seen in Young Foals.

The etiology of the condition as seen in practice is far from being complete. Quite a number of bacteria have been isolated as causing the disease, and not a few of these are undoubtedly of secondary origin.

It appears to the writer we have here two distinct conditions—*i.e.*, bacterial invasion *in utero* and umbilical infection after birth.

In the former case the navel cord looks macroscopically healthy and shows little or no retrogressive changes; nevertheless, within a few hours after birth an effusive arthritis has taken place. To those who hold infection

takes place from the navel after birth in these cases, one must reasonably point out the short period which has elapsed between parturition and the first manifestation of organic disease in a distant joint. Moreover, in practice we have ligatured the navel cord at *birth* with sterile catgut, painted the stump with tincture of iodine and then with collodion, and repeated the dressing daily, keeping the animal under the most perfect hygienic surroundings possible, and yet within a varying but usually short period specific arthritis develops. In those cases the animals are nearly always weakly at birth, and if they live they seldom become valuable, being stunted in their growth, etc. The majority, however, die from what appears to be a pronounced septicæmia.

Where joint-evil occurs through bacterial invasion of the navel cord after birth, one usually finds an extended incubation period, or at least an apparently extended period, for one cannot state definitely when the navel stump may become infected. It is reasonable, however, to presume the older the animal becomes, the more the cord shrinks and dries, the less likely one is to get bacterial growth and infection from this source.

Some navel cords appear to be more predisposed to act as bacterial incubators than others; those which are thick and gelatinous at birth, with a more or less serous exudate emanating from them continually, are more likely to lead to joint-evil than small, dried-up, and attenuated cords.

With the class of cases which appear to be affected *in utero* we must confess vaccine-therapy in our hands has been of little or no service, and the reason for this seems to be we have here a bacterial infection primarily affecting the blood-stream—*i.e.*, a typical septic infection.

Where, however, the animal appears to have become contaminated when two or three weeks old, provided the bacteria causing the condition are isolated, and an autogenous vaccine made, good hopes, if the treatment is started fairly early, of a cure may be anticipated.

Before the writer gave vaccine-therapy the serious consideration he now does, he obtained considerable relief in cases where the joint or joints were greatly distended with turbulent-looking synovia, etc., by aspirating them, and giving the fluid to the patient *per oram*, believing in this manner the patient elaborated its own protective vaccine. Certainly in some cases the animal often made a good recovery, and, to say the least, aspirating the joint relieved internal pressure, thereby reducing the painful condition of the joint, which in such young animals so often kills by exhaustion alone. Moreover, we found, if the joint was aspirated moderately early in the course of the disease, one was not so liable to get organic destruction within the joint itself; therefore the consequent anchylosis following upon this condition was considerably reduced, and a better future usefulness of the patient assured. This was, no doubt, due to the fact that the synovia was laden with active bacteria or their products, which in themselves possess an irritating effect upon the synovial membranes and articular surfaces, causing cell proliferation, cell death, rarefying ostitis, anchylosis of the joint, and consequent destruction of its free-moving capacity.

In those cases where the outset of the disease takes place soon after parturition, running a rapid course with great prostration of the patient, giving strong suggestions of a septicæmic condition, little by way of treatment can be done. Lignières inclines to the belief this condition is due to the coccobacillus, in which case his vaccine (polyvalent) may do good; but of this we have no experience.

Considering the ubiquity of the coccobacillus, it is just possible that this bacterium is the primary factor in the causation of joint-evil, and that other bacteria present are more or less accidental and secondary. In like manner, it appears, human tuberculosis claims its many victims, not so much to the specific bacilli of tubercle itself, but to the secondary invasion of streptococci, staphylococci, *Micro-*

coccus tetragenus, etc. The same also may be said of influenza, distemper, and strangles, as we shall see later.

If one aspirates an infected joint, in our experience, in the early stages, centrifugalizes the fluid, and with the sediment inoculates a culture-tube, no growth may follow, and this rather suggests the fact that the bacteria do not enter the capsule of the joint at the outset, but rather remain in the blood or lymph stream, toxins only filtering through, causing turbidity of the otherwise clear synovia; but later on, owing to disintegration of the vessel walls, they are liberated, and find their way into the joint cavity. Be this as it may, we have found the longer standing a pyæmic arthritic is, the greater seems to be the degree of bacterial invasion, and *vice versa*.

A careful examination of the navel cord should be made. The purulent matter should not be taken from the point of the navel. If the cord is fairly long, the tip must be cut off, the hæmorrhage stopped, and a sterile small curette inserted an inch or two into the canal, and a scraping taken from the side of its wall. This should be examined and incubated.

As already pointed out, mixed infection is rather the rule, and it therefore follows, each case, to obtain the maximum of success, should be treated upon its own merits.

The most common bacteria the writer has found are—staphylococci (*albus* and *aureus*); streptococci; in one case the *B. pyocyaneus*, in another a microbe resembling the pneumococcus, and showing its characteristics; and lastly we have isolated the *B. coli communis* from the navel. Considering how readily the navel cord comes in contact with fæcal matter, it is really a wonder one does not see more cases of *B. coli* infection, although such may be so, and have been overlooked in practice.

It is surprising to find how tolerant young foals are to big doses of vaccine. The writer remembers the first two or three cases he injected, feeling rather anxious as to

the results, but in course of time did not hesitate to give a dose of 500,000,000 devitalized staphylococci to a week-old foal. Of course, it should be pointed out, through some idiosyncrasies of the patient itself, vaccines act less seriously in some than in others; and that the vaccines seem to sensitize more acutely some, while others are more refractory to their effects. One must remember also some vaccines possess in a pronounced degree the power of disturbing the metabolism more than others.

As soon as we are called in to a case of joint-evil, we give an initial dose of the following stock vaccines,

Streptococci	50,000,000
Staphylococci	100,000,000
B. coli	50,000,000
B. pyocyaneus	50,000,000

and watch the clinical symptoms.

We then examine a scraping from the navel, aspirate the joint or joints, if more than one is involved, centrifugalize and examine the deposit, cultivate the bacteria present on suitable media, and make the necessary vaccine. Where a joint is distended with fluid, we always insist on removing it; this procedure gives the animal a better chance to recover, and the after-sequelæ, as has already been pointed out, in the joint itself are not likely to be so serious.

If the patient is not making satisfactory progress, and should a streptococcus be present, a combined vaccine and serum gives good results, and should most certainly be tried.

Septicæmic Arthritis in Cattle.

This disease is only seen in cows after parturition, and, in the writer's experience, in complicated parturitions, either where the genital organs have been injured during delivery, or where the placenta or part of the placenta has been retained *in utero*; in short, the condition is due

to a septic metritis developing into a septicæmia, and leading up to a specific arthritis. One or more joints may be involved. The stifle, by reason of its large capacity, and probably its nearness to the centre of infection, is, in the writer's experience, particularly singled out for bacterial invasion.

Of course one sees many cases in practice where the placenta is retained for a long period, and very septic, germ-laden discharges are evacuated, perhaps for weeks, and yet no arthritic lesions develop, nor may we find any indications whatever of septic infection or intoxication; while, on the contrary, the placenta may be retained only a few days, or there may only be slight bruising of the genital passage, and septicæmia and septic arthritis develop. We can only explain these extreme conditions by stating in the one case the bacteriotropic forces were in themselves capable of acting as a resisting barrier against a systemic bacterial invasion, and in the other they were not. So far as our present knowledge goes, it would appear there exists in the blood-serum a specific opsonin for all or nearly all pathogenic bacteria; thus, we may have an antistaphylococcal opsonin, an antistreptococcal opsonin, an anticolic opsonin, and so on. The quantity of opsonin differs in various animals, and even in the same animal at different times; therefore an animal may be, and is, more resistant to bacterial invasion at one time than at another.

This variation is dependent upon many and varied causes, which in themselves are chemically complex and difficult to understand.

But if we do not know why these opsonins and other antibodies diminish in quantity as they do, we do know how to raise them to a standard of proficiency, and so prepare the antibodies to clear the system of offensive bacteria—*i.e.*, by using a suitable vaccine or serum, or both combined.

Where an animal has a high temperature, is off its

appetite, with more than one joint involved, and in addition, perhaps, pneumonic lesions are diagnosed, an acute septicæmia has become established, and little hope of recovery is likely to follow, in such a case as this vaccine-therapy will avail but little; but should the disease be running a slow and insidious course, and the circulation comparatively free from bacteria or their toxins, much can be done by using suitable vaccines.

The writer has isolated from the joints of cattle a streptococcus similar to those obtained from cows infected with endometritis, and in one case a streptococcus has been obtained from the blood of a case suffering from septicæmia and metritis with arthritic complications.

We do not get the same bursal distensions in cows as is seen in foals with pyæmic arthritis, and this is no doubt due to the fact that in the young animal the bursæ are more elastic and distend more freely on pressure than in older animals. We therefore have not the same necessity, nor do we derive the same benefit from aspirating the joint of the cow that we obtain by so doing in the foal.

In treating septicæmic arthritis in cattle, we must endeavour to destroy the bacteria at the source of infection. If it is a retained piece of placenta it must be removed; if it is a septic uterine wound it must be rendered aseptic. Then we must see the blood is kept as free as possible from bacteria or their products, in assisting Nature to get rid of effete material by keeping the eliminative organs free. And, lastly, we must help her to elaborate her protective forces by using appropriate vaccines.

When one has a case showing symptoms of infection or intoxication, with premonitory joint lesions, or when a placenta, even, has been retained, and it appears to be taking a serious turn, one is fully justified in giving as a prophylactic a polyvalent staphylococcal and streptococcal stock vaccine, and, if preferred, combined with an antistreptococcal serum. But to be of any real service the bacterial emulsion should be taken from a culture derived from

the genital organ or joint of a cow suffering from the disease.

Where the immunizer has not a stock vaccine at hand, a culture should be made from the uterine discharge, and also, if possible, from the joint. This latter may be difficult and sometimes impossible to do, by reason of the fact the bacteria may not be present in the synovia at all, as already explained in the case of young foals.

If, however, the animal dies, one can usually obtain active bacteria from the neighbourhood of the joint, and invariably the infection is a mixed one.

Cattle are very tolerant to big doses of vaccine; the writer does not hesitate to give 1,000,000,000 staphylococci and 500,000,000 streptococci as an initial dose, and doubling those doses in five days if necessary.

We have found cases of septicæmic arthritis improve more rapidly under vaccine-therapy, lay on flesh more quickly, and not so liable to suffer from subsequent and permanent lameness, thus giving better results than can be obtained by any other mode of treatment.

Moreover, by adopting prophylactic and curative vaccines lives have been saved in our hands which in all probability would have died from auto-infection or intoxication, or both.

It is quite feasible to suspect the *B. coli communis* may attack joints, in a manner similar to streptococci, from the womb, and the immunizer should be on the lookout for such an invasion.

CHAPTER XIV

BACTERIAL DISEASES AFFECTING THE ABDOMINAL ORGANS

Disease.	Cause.
Enteric: Diarrhœa and dysentery	*B. coli* group, *B. pyocyaneus*, *B. proteus*, streptococci, *B. tuberculosis*. Johne's bacillus.
Peritoneal: Peritonitis	*B. tuberculosis*, *B. coli*, staphylococci, streptococci.
Nephritic: Pyelonephritis, cystitis	Pneumococcus, *B. coli* group, streptococci, staphylococci.
Uterine: Endometritis	Pneumococcus, *B. coli* group, staphylococci, streptococci.

Diarrhœa and Dysentery.

As it is only natural to expect, the intestines, by reason of their functions, especially the lower bowels, are infected by a long list of bacteria, some pathogenic, many non-pathogenic. Some of the non-pathogenic bacteria, however, adopt a disease-producing rôle under certain circumstances only, of which several of the *B. coli* group are examples.

Young animals are particularly prone to bacterial intestinal invasion, and the reason probably is that milk is up to a certain period their staple diet, and milk is an excellent medium for bacterial growth and transmission. Moreover, the intestinal mucosa is less resistant in the young animal than in the old. It is just possible, also, as an animal gets older, repeated mild auto-infections from the intestinal mucosæ are continually taking place, conferring upon the subject a degree of resistance, which with age becomes merged into an immunity.

Where one gets isolated mild attacks of diarrhœa in young animals, the ordinary medicinal treatment answers all practical purposes; but when an attack affects a large number of animals, with a high death-rate, prophylactic and curative measures of the most up-to-date kind must be adopted.

It is in cases such as these that vaccine-therapy should be used. In young animals the alimentary canal becomes infected from two distinct sources—*i.e.*, oral and umbilical. To what source one can attribute the greater number of cases it is difficult to hazard an opinion, but the fact that both are capable channels of infection the practitioner should, in investigating any outbreak, always bear in mind.

As already stated, a large variety of bacteria are to be found in the intestinal canal, and it is therefore of the first importance that one should ascertain what bacteria are taking on a pathogenic rôle.

But this is not all. Having isolated from scrapings or excreta, or both, a variety of bacteria, one has to decide which is the most capable of producing the disease in question. Of course, this delicate point confronts the immunizer in all cases of mixed infections, more or less, and it must largely be left to his own individual knowledge upon which data he bases his conclusions, provided they are always in keeping with the general laws of bacteriology. The *B. coli* group appear under certain circumstances to develop very potent pathogenic qualities, and it is probable they are the forerunners of many other grave bacterial intestinal invasions. Some authorities believe the pasteurella group play a most important part.

In addition to these we have the *B. pyocyaneus*, *B. proteus*, and streptococcus; while we find such specific invasions from *B. tuberculosis*, *B. Johne*, and *Streptothrix actinomyces*.

In the condition known as "white scour" in calves,

we have obtained good results from the early injection of a stock *B. coli* vaccine, usually beginning with a dose of 25,000,000 in young calves. It is advisable, where an autogenous vaccine is not used, a polyvalent one should take its place.

A second injection of 50,000,000 should be given three or four days later, and the conditions noted.

In addition to vaccines, in many bacterial intestinal invasions we have had excellent results from the early use and repeated administration of boric acid.

Johne's Disease in Cattle.

It may not be out of place here to mention a rather common bacterial invasion of the intestines of (usually) adult cattle in certain districts by an acid-fast bacterium resembling in many ways the bacillus of tubercle, and named, after the discoverer, Johne's bacillus.

Practitioners in days gone by considered Johne's disease, as seen in cattle, to be a form of enteric tuberculosis.

The specific bacillus can be easily isolated, stained, and demonstrated under the microscope, $\frac{1}{12}$ oil immersion, from scrapings made from the intestines. It is, however, a very shy microbe to cultivate artificially in the laboratory upon the usual media; but, thanks to the well-known research work of Twort and Ingram, a medium has been prepared to suit the growing tastes of this bacterium. These two workers found by cultivating and killing the Timothy-grass bacillus (*B. phlei*), and mixing the result with the nutrient media, the bacillus of Johne grew most successfully, the reason they give being that probably the Timothy-grass bacillus is the "wild ancestor" of the Johne bacillus.

They have made a vaccine, as above indicated, which they assert has a diagnostic value towards Johne's disease equal to that which tuberculin has to tuberculosis, and mallein has to glanders; and they are careful to point out that the maximum rise of temperature is expected

about the tenth hour, which, of course, is earlier than with mallein or tuberculin.

They are hopeful also the vaccine may possess curative virtues as well, but on this point further investigation appears to be necessary.

Peritonitis.

The peritoneum forms in itself an admirable breeding-ground for many bacteria, and probably none grow more luxuriantly than the bacillus of tubercle, especially on the bovine peritoneum.

Bacteria gain entrance to the peritoneum in a variety of ways.

The *B. coli*, existing as it does in the intestines, often finds it way into the peritoneal cavity through abrasions in the intestinal wall, commonly due to the bursting of an abscess, the penetrating effects of intestinal parasites, migration from the womb in endometritis, injuries from without, etc. When they escape from the intestines they multiply with great rapidity, forming toxins which, by reason of the physiological functions of the peritoneal membrane, become readily absorbed into the circulation, producing intoxication, infection, and death. It is obvious in these very acute cases vaccine-therapy will avail little, and, for that matter, any other system of treatment.

When, however, the condition becomes circumscribed and subacute or chronic in contra-distinction to diffused and acute, if the case can be so diagnosed, much good will follow vaccine treatment, and the peritoneum particularly lends itself to such therapy by reason of the fact that it is continually bathed by a process of osmosis with the blood-serum, which contains the protective elements of the blood. Of course, here we find limitations compared with human medicine and surgery, but it sometimes happens, particularly in the smaller animals, abdominal surgery is performed, when we have developed a plastic entero-peritonitis due to the *B. coli* perhaps. The system

can be considerably strengthened and fortified if a suitable vaccine is administered in addition to local treatment and proper drainage.

The *B. coli* may gain a footing in the peritoneum, especially in cows after parturition, through the womb, seen in such cases as metroperitonitis. Here, again, where we suspect such a condition, much can be done by vaccines.

Streptococci may infect the peritoneum through strangles attack running an irregular course, and also from the womb in mares and cows.

The cocci may locate themselves in a mesenteric gland, which in time bursts, the bacteria are liberated, and peritonitis sets in. Here, again, if the condition is diffused and acute, little good will follow any line of treatment, but should it be limited, and not too severe, much benefit will follow the injection of 20 c.c. antistreptococcic serum and a vaccine of 500,000,000 devitalized streptococci to a horse, repeated in three to five days in a double quantity.

It is more than probable that the majority of the peritoneal inflammations we see in practice are of bacterial origin, except those cases due to traumatism; and, of course, they also soon become organismally contaminated, if they are not already so.

Moreover, those acute cases of muco-enteritis which we so often see in equine practice affecting high-conditioned, grossly-fed animals are all probably due, either primarily or secondarily, to bacterial invasion.

Pyelonephritis.

Diseases in general of the urinary system have, on the whole, been seriously neglected by practitioners of comparative medicine. This is partly accounted for by the fact that even grave nephritic conditions may exist, and give no positive indications of their existence to the clinical observer. More important, perhaps, is the undeniable fact that clinical investigations are not as thorough as they might be, nor are they as often applied as they should be.

We are all aware of cases which from time to time come under observation where the kidneys are reduced to a mass of almost useless pulp, and yet during life we never suspected or diagnosed nephritic trouble. Had a careful examination of the urine in such a case been carried out, our diagnosis and treatment might have been different and the results perhaps more satisfactory.

From a fairly extensive bacteriological examination of the urine, especially in equines, we have been struck with the great susceptibility of the urinary system to bacterial invasions. And this is not surprising, considering the anatomical arrangements and excretory functions of the urinary system.

Bacterial infection can take place from two sources: (1) Infection internally from and through the blood-stream; (2 infection externally from the vulva in the female, which is rather common, or in the male through the urethra, which is very uncommon. Considering, therefore, the eliminative functions of the urinary system, and its anatomical arrangements and position, bacterial invasions must needs be nearly always mixed. The most common bacteria found in practice are streptococci, staphylococci, *B. pyocyaneus, B. coli communis, B. tuberculosis.* As an illustration of bacterial infection through the blood-stream, one may cite metastatic strangles, where the streptococci become entangled in the parenchyma of the kidneys following upon metastasis.

External infection is a condition which is not to be wondered at, considering the unhygienic state of the animal rectum, vulva, buttocks, tail, etc., the close relationship the vulva has with the rectum, and, lastly, the nearness of the meatus urinarius to the vulval opening.

In pregnant animals, following upon delivery or abortion, one sometimes finds cystic and nephritic bacterial invasions, as a result of extension from the uterus, vagina, or vulva.

When one suspects disease or derangement of the urinary system, a careful examination of the urine should be made.

A sterile catheter is passed into the bladder, and, after allowing a small proportion of the urine to flow away, the remainder should be collected in a sterile jar and sealed for examination.

The urine thus collected is centrifugalized, and the resultant deposit examined after removal of the supernatant fluid by pipette or pouring.

A hanging-drop preparation should be made of the sediment and carefully examined under a $\frac{1}{6}$ and $\frac{1}{12}$ oil immersion. One will be able thus to detect bacteria if present, and note if they are motile, if single or in pairs, or in chains or bundles, etc. Films should now be made in the ordinary manner and stained. If any difficulty is encountered in fixing the film upon the slide, a drop of one's own blood from a finger-prick and well mixed with the urine will supply sufficient albumin to the field to fix the specimen when heat or other fixing agents are applied.

When a culture is desired, the collection of the urine and the inoculation of culture media requires the most careful application to prevent risk of outside contamination.

Where the kidneys are extensively invaded by bacteria, the process of functional elimination in these organs decreases, and with destructive changes going on in the organs themselves, bacteria and tissue cells are continually passing along the urinary tract. Some of these pathogenic organisms settle in the mucosa, causing cystitis and urethritis; others are expelled with the urine; and superadded in advanced cases one sees typical uræmic symptoms. Drugs in such cases give little relief, although alkalies, urotropin, and the volatile oils, may give temporary benefits.

Having ascertained the causative bacteria, a suitable vaccine should be made in the usual manner, and the phenomena carefully watched.

If uræmia has already set in, much assistance can be given to Nature by lowering the blood-pressure and stimu-

lating the other excretory organs—*i.e.*, the bowels and skin. The subcutaneous or intravenous injection of normal saline solution assists the system also in the removal of an ever-accumulating effete deposit.

Endometritis.

Bacterial invasions of the womb in the lower animals are of rather common occurrence, due largely to contamination at the time of delivery on the part of unskilled attendants, and also by the retention of the placenta.

One also sees many cases following upon difficult parturition, through injuries caused to the mucosa either by the fœtus itself during the process of delivery, the accidental slipping of instruments, and, above all, the want of complete asepsis on the part of the operator.

Some species of animals are more tolerant to the pathogenic influences of bacteria growing in the generative female organ than others. The cow, for example, is probably the most tolerant of all the domestic animals, while the mare is the least.

Why this is so is somewhat difficult to explain, but it is more than probable, in the light of our present knowledge, that those animals which are most resistant are endowed by Nature with the greatest supply of bacteriotropic elements. It is a well-known fact that the uterine fluids at the time of and after parturition possess powerful bacteria-destroying properties, and it may be due to those strong bactericidal qualities also, the possession of which is greater in some species of animals than in others, that we see in practice such variable degrees of resistance versus susceptibility.

As already pointed out, uterine bacterial invasion in practice takes place incidentally to parturition in some form or another, and, indeed, it would seem almost a physical impossibility for the womb to become invaded in animals at any other period, for during a process of quiescence of that organ it is hermetically sealed, and it

would only be the result of some mechanical injury from without that bacteria could reach the organ. Abortion is another prolific source of endometritis and bacterial invasions, owing to the fact of the abortion bacillus appearing to exert a lowering effect upon the protective elements of the womb, and so predisposing it in such a manner to the easy development of secondary infections. The womb also is prevented from closing naturally in many cases owing to a retained placenta, which putrefies, giving an increased impetus to further bacterial invasions. And so we find after abortions, that bacterial infections are nearly always mixed.

Some of these infections, either due to the great activity of the bacteria themselves and the potency of their toxins, or the weakened bactericidal power of the animal itself, run a very rapid and highly fatal course, death taking place in a few hours from septic infection, or even intoxication. In cases such as these the vaccine-therapist can do but little.

When the condition runs a more subacute course, much can be done, however, by using suitable vaccines.

Where the infection is caused by septic hands, slips, instruments, etc., during the course of a difficult parturition, it is invariably a mixed one, the streptococci, staphylococci, *B. coli communis*, etc., being the more common offenders.

To ascertain the causative bacteria, we make it a practice to thoroughly wash the vulva and buttocks with soap and an antiseptic; we then insert the hand and arm, carrying into the womb a sterile test-tube which has been cut down to 2 inches in length. By drawing the mouth along the mucosa close to the neck of the uterus, we obtain sufficient *materia morbis* for examination. This is plugged with a sterile rubber cork, and marked "No. 1." In like manner another tube is carried in, to abstract material in the body of the womb, and marked "No. 2"; while with a third tube material is taken as far forward as one's hand will reach, and marked "No. 3."

By this method we can ascertain—

1. What bacteria we are fighting against.

2. We know nearly what stage of infection the patient is in at the time of examination; that is to say, if the infection is very recent, and the bacteria have not invaded the whole of the womb, material from No. 1 tube will contain more bacteria than are to be found in tubes Nos. 2 and 3. On the contrary, if the invasion is equally diffused over the whole organ, the three tubes should be practically alike.

Of course, if the case is an advanced one and the practitioner is called in late, the womb may be sufficiently closed to prevent the entrance of one's hand unless ill-advised force is used; but, on the contrary, the fact that the womb is in such a diseased condition militates against its natural processes of contraction. In fact, it is this contractile power the practitioner so much desires to be brought about, and to accelerate the condition in practice we make a point of giving full doses of ergot, thereby limiting the risk of extension and the possible development of septic infection or intoxication.

To ascertain the degree of infection in each tube, we place an equal quantity of the material in a sterile tube, and add a given quantity of sterile salt solution, draw off the end of the tube in the blow-flame, seal it up as already described, and mark it with grease pencil "No. 1." The same process is gone through with Nos. 2 and 3.

These are well shaken—each for a given time—to break down the bacterial bundles, chains, etc. A drop of the fluid is now placed on the end of a slide, spread carefully with a Wright spreader, and allowed to air dry and marked "No. 1." The same method is adopted with Nos. 2 and 3. These are respectively examined, and the bacteria, say in half a dozen squares of the microscopic field, are counted and totalled, and each slide total is then compared. If the films are equally spread in each case, the results should be pretty accurate.

Having ascertained what bacteria are present, the immunizer should forthwith make an autogenous vaccine.

In our experience the *B. coli* groups are the most common offenders, and they sometimes take on a very virulent form. After injecting the vaccine, a careful note of the clinical symptoms should be made. A slight rise of temperature may take place, and an increase of the uterine discharge is to be expected. After forty-eight hours this should become gradually less, the patient will look brighter, the temperature falls; and this will go on for a few days, after which another injection should be given.

Locally, of course, much can be done to assist the vaccines.

The womb should be irrigated with a very dilute boric acid solution, to which is added citrate of soda in weak solution.

The temperature of the douche should be about 40° C., and gradually increased to 45° C. The therapeutic value of this is apparent. The womb is a highly vascular organ. By applying heat, and gradually increasing that heat, dilatation of the bloodvessels takes place, more blood reaches the organ, increased osmosis follows, and so long as the blood in such a state can be kept fluid, as it can be with the soda citrate in solution, so long will an increased flow of bacteriolytic and bacteriotropic essentials reach the death-producing bacteria, to the benefit of the patient.

Needless to say, these uterine douches should be repeated at intervals, and the fluid should not remain in the womb longer than a few minutes, it being either pumped or siphoned off if the contractile powers of the womb itself are in abeyance, or the patient is too weak to make an effort.

When streptococci are present, a streptococcal serum should also be used, 20 c.c. being injected daily for several days with increasing doses; but note should be made of the fact that sera only obtained from a bovine animal should be used, such animal having been immunized by the uterine streptococcus for preference.

CHAPTER XV

BACTERIAL DISEASES AFFECTING THE CIRCULATORY SYSTEM

Disease.	Cause.
Septicæmia	*Streptococcus septus*, staphylococci, pneumococci, *B. coli.*
Pyæmia	*Streptococcus septus*, staphylococci, pneumococci, *B. coli.*
Malignant œdema ...	Bacillus of malignant œdema.
Quarter-evil	Bacillus of quarter-evil.
Anthrax	Bacillus of anthrax.

Septicæmia.

The diseases under this heading are more commonly seen in veterinary practice than in human medicine, and the reason is not far to seek. The predisposing causes to all septicæmias, speaking generally, are neglect and unhealthy surroundings.

Septicæmia appears as two distinct conditions—*i.e.*, (1) septic infection, when the blood itself is charged with living bacteria. If such blood is injected into the circulation of a healthy animal a similar disease will be produced in that animal; and (2) septic intoxication, when the blood is charged with poisons emanating from bacteria located in some centre in the body. If such blood is injected into a healthy animal, no reproduction of that disease takes place, thereby distinguishing septic infection from septic intoxication. Although we, therefore, recognize in practice these two conditions as separate ones, the clinician should always remember that an intoxication may become an infection

at any moment, and as practitioners we, therefore, should be on the lookout for such developments.

In septic infection the bacteria gain entrance to the blood-stream or lymphatics usually through a solution of continuity of the skin or mucous membrane, and there must be a tendency towards some defective bactericidal power on the part of the patient, and probably phagocytosic inertia; for, as one knows, so soon as a local centre becomes infected, a ring of active cells is formed round that area, thereby imprisoning the pathogenic elements.

Should this ring remain intact, the most that will follow will be osmosis of toxins through the barrier, which may get into the circulation and set up septic intoxication, with constitutional disturbance, if the toxin is virulent enough and sufficient has been absorbed. If, however, the wall gives way through any defective effort of Nature on the one hand, and extreme activity and virulence of the bacteria on the other, septic infection follows.

It is notorious the number of small punctured but deep-seated wounds which act as a starting-point in the development of a generalized septicæmia compared with superficial and, maybe, more extensive lacerated wounds. This is no doubt due to the fact that small punctured wounds do not look serious in the mind of the layman, and the owner or attendant may even ignore them altogether; whereas a superficial and an extensive wound looks appalling to the lay observer, and he sets about to obtain advice at once. And, again, a punctured wound is infected at its blind end, where sunlight and ordinary dressings can scarcely reach, while a proper temperature for bacterial growth is always maintained, and the result is rapid bacterial development, with grave constitutional phenomena.

The causative bacteria usually found in wound infection are the streptococci and staphylococci; and after castrations, particularly in lambs, we have isolated a bacillus answering the description of the *B. coli communis*—motile and Gram-negative, etc.

Another common illustration of septic infection and intoxication is to be found in cows after parturition in that condition known as "septic metritis." As already explained under the heading of endometritis, bacterial infection takes place through inoculation caused by septic hands on the part of the operator, slips, instruments, injuries after parturition, and retained placenta, particularly after abortion.

Here the *B. coli*, streptococci, and staphylococci play an important rôle. In advanced cases of this kind we have invariably noticed, especially in heifers, in addition to the characteristic septic chocolate-coloured discharge from the womb, a slimy mucoid discharge from the nostrils, accompanied by a persistent bronchial cough. From this discharge the writer has isolated streptococci and diplococci, and has been struck by the smallness of the chains, both from the bronchial and uterine discharges, compared with, for example, the streptococcal chains one sees in strangles or in ordinary wound infections.

We cannot think the pulmonary streptococci had their primary origin in the uterus; much more probable is it they are normal inhabitants of the pulmonary mucosæ, and it is only as the result of debility, caused by the uterine infection, that they become pathogenic and active. Their presence in any case increases the liability of a fatal issue, and the practitioner has to be called in very early if he hopes to bring about a recovery.

Such a case requires most careful attention. A stock vaccine must be given at once, preparatory to the making of a suitable autogenous one; and in a case similar to the one cited above, a mixed vaccine composed of streptococci, *B. coli*, staphylococci, and diplococci should be given. If the case is a bad one, we invariably give a streptococcal serum in addition.

Local treatment, of course, must be attended to, and here, again, we must most strongly deprecate the use of strong antiseptics. In the case of a deep punctured wound,

it must be laid open to permit of free drainage and the admission of dressings. The curette is a most useful instrument for the removal of lodged bacteria on the edges or at the blind end of the wound. If the flow of lymph appears deficient, the wound should be irrigated with citrate of soda solution, or even salt and water; and when the wound appears to be tardy in its healing process, if located in the limbs, we have found considerable benefit from the application of Bier's treatment.

Where one is dealing with septic uterine disease, the womb must be irrigated and a similar treatment carried out as in endometritis.

Medicines in these cases can be of little avail. Should, however, the temperature be high, salicylic acid is indicated, and it is well to keep the eliminative organs active. Thus the bowels should be kept open with salines, the kidneys stimulated, and for this purpose there is no better drug than turpentine; while a useful diaphoretic is liq. ammon. acet. combined with salicylic acid.

Pyæmia.

The bacteria involved in the production of this disease are practically similar to those which cause septicæmia. The characteristics, however, of the two diseases differ in a marked degree. In pyæmia multiple abscesses occurring in various centres of the body, as the result of metastasis, distinguish this disease from septicæmia.

A purulent centre may be the means of producing pyæmia through the protective wall giving way, the specific bacteria entering the blood or lymph stream. Examples of this are to be found in strangles in the horse, purulent thrombophlebitis in foals and calves, and puerperal pyæmia in the mare and the cow. Where abscess formations are found in the internal organs, diagnosis is not always easy, and the disease may have advanced too far for treatment to be of any avail. Moreover, these abscesses are generally tensely filled with pus, which pre-

vents the bactericidal elements from reaching the septic foci. If, however, the abscesses are subcutaneous, evacuation of the pus is easy and essential, and vaccine-therapy can do much to complete a cure.

Where the pyæmia is a sequel to strangles, we always combine the vaccine with an antiserum; and if the temperature is very high, it is advisable to begin with a minimum dose at the outset. If the temperature does not rise too high during the negative phase—in three or five days—we repeat the dose, giving a double quantity.

Malignant Œdema.

This condition is seen in several species of the domesticated animals, and is due to a wound infection by the bacillus of malignant œdema. It has a very wide distribution in Nature, but being an anaerobe, it will not grow on open and exposed wounds or where there is a free circulation, preferring deep punctured wounds, such as those produced by the prong of a pickstaff, the point of a shaft or hook, etc. It is also seen in cases of difficult parturition, with injuries to the vagina or vulva.

Owing to the bacillus evolving gas during its growth, crepitus similar to that seen in quarter-evil is a common symptom.

The disease runs a very acute course, and unless strong measures are adopted very early, little hope of recovery can be entertained.

A protective serum, having a certain curative value if used early, is made by exposing the jugular vein of a horse, and injecting increasingly large doses of virulent material. In this way the animal becomes immune to the pathogenic action of the bacillus.

He is then bled, and the serum collected has prophylactic and curative qualities by reason of its power of stimulating the phagocytes, agglutinins, and antitoxins.

Locally the wound should be attended to. Free use of the knife should be made, to allow all the air possible to

enter the diseased tissues, and, where possible, oxygen should be injected.

The cavities should be irrigated with a solution of potassium permanganate or peroxide of hydrogen, and the circulation should be maintained by repeated doses of strychnine and caffeine hypodermically, and orally ammonia.

Blackquarter (Symptomatic Anthrax).

In some countries and in certain counties this disease affects a large percentage of young cattle; but, owing to the extensive application of vaccine-therapy, it is not so prevalent as it used to be in pre-vaccination days. Curative vaccine-therapy must ever occupy a secondary place so far as blackquarter is concerned. The premonitory symptoms of the disease are so insignificant, and those that are patent are of so short duration, that the immunizer is unable to use his vaccine until the disease has become established; and when diagnostic lesions are in evidence, curative measures, in the writer's experience, are useless. As a prophylactic, blackquarter vaccination has long passed the experimental stage, and as such its position is now assured.

The bacillus of quarter-evil, technically known as the *sarcophysematous bovis* bacillus, gains entrance to the system through some form of wound.

It is motile, anaerobic, easily stained, 3 to 6 μ long, and usually shows at the end a spore, giving the bacillus the appearance of a club, resembling the bacillus of tetanus. Unlike bacilli in general, it is Gram-positive; but, like the bacilli of malignant œdema, during growth it evolves gas.

One attack, if the animal survives, confers immunity for a long period, and it is believed a fœtus *in utero* has complete immunity conferred upon it if the mother contracts the disease and lives.

Several methods have been adopted to produce satisfactory vaccines, but the two most commonly used in

practice are Kitt's single vaccine and Arloing's double vaccine.

Kitt's single vaccine method consists of attenuating the virus by moist heat at a temperature of 100° C. for six or seven hours. The affected muscles should be carefully dissected out and dried in a dry-air sterilizer or oven at a temperature of 35° C., after which it should be powdered and mixed with water to form a paste. This paste is now spread on glass plates, and put in a thermostat and subjected to the temperature already stated above. It should then be dried, and the dose is 1 decigramme, mixed with sterile water and a small percentage of lysol or carbolic acid.

The great advantage of this vaccine to the busy practitioner is that one injection only is required; but the degree of immunity conferred is not so great or so lasting as that obtained in using the double vaccine. We find in practice, for economical reasons, clients do not like the double vaccine, and if the disease is not developing beyond its usual sporadic manner, the single vaccine answers quite well. In some districts where blackquarter obtains serious proportions, however, the double vaccine should undoubtedly be used.

When vaccination as a prophylactic was first introduced into this country, the writer had some unfortunate experiences where the subcutaneous tissue over the shoulder was the site chosen for injection. In several instances the shoulder and leg swelled to alarming proportions, and one animal died, although strict asepsis was observed.

Since then we have always used the tail as the seat of inoculation. Here the cellular tissue is denser, absorption of the vaccine is more gradual, and the danger to life is nil. Against this, however, the degree of immunity may not be so great, and in consequence a more virulent vaccine may be used; and in making our own vaccines now, we make allowance for this fact by subjecting them to a lower temperature.

Arloing's Double Vaccine Method.

Here two vaccines of varying degrees of virulence are used, the first being given to prepare the system for the second and more virulent dose.

They are prepared as follows: The diseased muscle is dried in the sterilizer (dry-air) at a temperature of 35° C., powdered, mixed with water to form a paste, and spread on a glass plate, exactly the same as in Kitt's method.

It is then placed in a thermostat for six hours, and maintained at a temperature of 103° C.

The dried powder is now put in a sterile mortar, ground down finely, placed in a sterile stock vaccine bottle, with rubber cap or glass stopper, and labelled "Vaccine No. 1."

Vaccine No. 2 should be treated in exactly the same way, only instead of being subjected to a temperature of 103° C., a temperature of 95° C. is required.

The dose of each vaccine is 1 centigramme.

No. 2 vaccine should be administered within ten days of No. 1.

The vaccine may be dissolved in normal saline solution to which is added 0·5 per cent. of lysol, and used as a liquid injection; or several layers of thread may be plaited together to form a single cord. Several of these should be steeped in the vaccine emulsion, each cord then constituting one dose.

When an animal is already infected, the vaccine may not be effectual—in fact, during the process of vaccination they are liable to bruise themselves, and often thereby determine the disease. In the writer's experience, complete immunity is not arrived at until the lapse of three weeks, after which period they are usually safe for twelve months. To reduce the risks of bruising during inoculation by the cord method, we find, if there are a number of animals to be inoculated, they can be speedily done if placed in a small house, so that they cannot run about. The operator should catch the tail of an animal so sardined between its

fellows that it cannot move, and, by a firm thrust of the needle, pierce the skin, taking care not to drive the point into the coccygeal bone, in which case the needle invariably breaks. If done in this way, the animals need not be caught or harassed in any way.

When dealing with farms predisposed to quarter-evil and pedigree stock, we always advise calves over six weeks of age being vaccinated, and as a rule animals over three years of age are usually proof against the disease, although we have known a case in a milch-cow of six years old.

Anthrax.

The extreme virulence of the *B. anthracis* and the high percentage of fatalities which follow its invasion offers poor prospects of good results following the application of curative measures. We find in practice some animals, and certain species of animals, which are more resistant to its destructive effects, however, than others; and it appears the greater the tendency for the disease to localize itself, the more certain the prospect of a recovery. Algerian sheep seem to possess an inherited degree of immunity. In cattle the disease usually shows itself as a generalized septicæmia, with consequent rapid death.

In the horse the tendency is for the disease to confine its effects to the glands of the throat, and recovery has been known to follow. When the lesions are in the bowels or lungs, death always follows.

In the pig a favourite seat for infection is the glands of the throat, and not a few recover. One attack seems to confer a pronounced degree of immunity.

Prophylactic vaccines have been made by many workers, but probably Pasteur's vaccine is the most successful now in use. He prepares two vaccines as follows:

Vaccine Treatment.

Vaccine No. 1 consists of cultivating the bacilli on broth at a temperature of 42° C. for twenty-four days.

By so doing the virulence of the bacilli is so attenuated that the vaccine so made kills mice, but not guinea-pigs.

Vaccine No. 2 is prepared in the same manner as No. 1, save that attenuation goes on for twelve days at the same temperature.

This vaccine destroys mice and guinea-pigs, but not rabbits.

For a horse or ox $\frac{1}{4}$ c.c. of vaccine No. 1 is first injected into an animal, and after ten to twelve days the second vaccine is injected—also $\frac{1}{4}$ c.c. About three weeks after the last injection the animal is proof against the disease, which immunity lasts for about twelve months.

These vaccines are not altogether free from danger in practice, and it appears some animals are less resistant to the virulence of the vaccine than others. The writer, seven years ago, vaccinated a dairy of fifty-three cows with Pasteur's vaccine, and one case nearly died after vaccine No. 1. Vaccine No. 2 was not used on her.

An intense painful swelling occurred at the seat of injection; the animal was excessively lame; her breathing was hurried and laboured; her temperature went up to 105° F. Full doses of turpentine, salicylic acid and alcohol, with hypodermic injections of strychnine and caffein, were repeatedly administered, and she eventually recovered. Since then we have used the tail as the seat of inoculation.

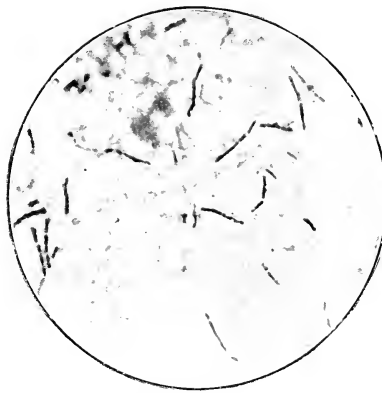
Serum Treatment.

In man several antiserums are in use:

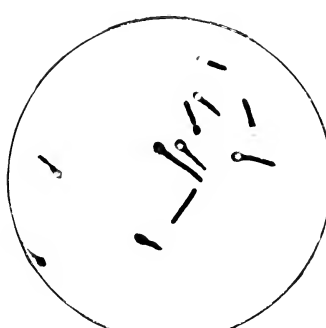
1. *Mendez* immunized horses by injecting them over a long period, six to twelve months, after which he bled them, and obtained the serum in the usual way. He records excellent results by giving doses of 20 c.c. of this serum. In cattle and sheep affected with anthrax he has also obtained recoveries after using his serum.

2. *Sclaro,* after a long period of experimenting, was able to immunize a goat against the bacillus, and produced a protective and curative serum. Later he treated asses in

PLATE VIII.



ANTHRAX BACILLI. × 1000.
(Jowett's "Blood Serum Therapy.")



BACILLUS TETANI. 1500.
(Hewlett's "Bacteriology.")

To face page 134.

a similar manner, and obtained a serum which he found very efficacious. He ascertained, if he injected 2 c.c. of this serum into a rabbit along with 1 c.c. of a fresh virulent broth culture of the bacillus, the animal did not die. In man he injected 30 to 40 c.c. into the flank in several places, and noted a rise in temperature, followed by recovery. He considers the serum is antibacterial, and stimulates the leucocytes.

3. *Legge* treated sixty-seven cases (*British Medical Journal*, 1905) with this serum, and in fifty-six the serum was used alone. He considers the exact mode of action of the serum is unsolved.

4. *Deutsch* has also prepared a serum which appears to be effective in cattle. It agglutinates the bacilli, and contains an immune body capable of destroying the organisms in the presence of a suitable complement.

5. *Sobernhein* has made an antiserum which he combines with a vaccine similar to Pasteur's No. 2, and used the two at the same time with excellent results.

This method appears to be less dangerous than Pasteur's, and, more virulent doses being given, a greater degree of immunity is arrived at.

To be efficacious, it would appear, very large doses of the serum must be given.

CHAPTER XVI

BACTERIAL DISEASES AFFECTING THE NERVOUS SYSTEM

Disease.			Cause.
Rabies	...	...	Ultra-visible virus.
Tetanus	...	...	*Bacillus tetani.*

Rabies.

Fortunately rabies at present is an exotic disease in the United Kingdom, but on the Continent, in India, America, and Africa it is still very rampant.

It is a specific infective disease due to a filterable virus, affecting all the domesticated animals and several of those of the wild species. The virus in its most virulent form is found in the brain, the salivary glands, and the secretions of the affected animal. The virus appears to pass along the nerve fibres, and in this manner reach the brain and the spinal cord, where it attacks the sensory cells causing irritation and producing symptoms of great excitement, etc. This condition of cellular irritability is followed by cellular depression and organic degeneration, and death sooner or later.

There are certain diseases which simulate rabies in many ways so far as the behaviour of an affected animal is concerned, and it is most important that an accurate diagnosis should be made.

An animal having been bitten and showing cardinal symptoms of rabies, however, would suggest little or no difficulty in arriving at an accurate diagnosis. In some cases

the history and symptoms are obscure, and further inquiry is essential in order to arrive at a positive conclusion.

On microscopical examination of sections of the medulla and cerebellum of a rabid animal, Negre found certain bodies. These bodies stain pink with Van Gieson's stain, the nerve cells are blue, and the red corpuscles colourless or faintly yellow.

Further, if medullary extract of an animal suspected of dying from rabies is injected into another animal, and that animal develops symptoms of rabies, the results are conclusive.

Pasteur was the first to demonstrate the fact that a recovered animal could not be artificially affected later with even large doses of the active virus, and on this basis he introduced an antirabic vaccine. He took a rabbit and infected it by giving a subdural injection with fixed virus, after which he carefully dissected out under proper precautions the medulla and spinal cord and suspended it in a long tube at the bottom of which was placed pieces of caustic potash, and subjected the whole to a constant temperature of 23° C. By this process it was found the cord became dry and also lost a large proportion of its virulence. Thus it was ascertained if a cord dried for five days in this manner would, if introduced into an animal, produce rabies on the eighth; if dried for nine days the disease would make itself manifest on the fifteenth day; and if dried for fourteen days the effects of the virus would be nil.

Therefore a medulla and cord subjected to heat for fourteen days would give daily for the same number of days fourteen doses each, varying in virulence.

Pasteur injected into dogs the most attenuated dose first, and by diminishing the attenuation he found the time came when a complete degree of immunity was arrived at. So much so, that a dog so treated, if bitten by a rabid dog, was proof against infection. Moreover, he also proved if up to a given time after an animal had been bitten the

vaccine was injected, development of the disease would be prevented, and that immunity would be maintained for one year at least.

In the year 1885 Pasteur, going forward with the knowledge he already possessed as the result of his experiments upon animals, successfully vaccinated a boy who had been bitten by a rabid dog.

Since then this mode of prophylactic treatment and its modifications has been carried out on an extensive scale, with excellent results. Högyes prepared a vaccine by triturating normal saline solution with virulent spinal cords of rabbits, making emulsions of different strengths—*i.e.*:

1 in 5,000.	1 in 200.
1 in 2,000.	1 in 100.
1 in 500.	1 in 10.

These he injected every two hours, beginning with the most attenuated dose. He found that a dog so treated would resist infection if bitten by a rabid dog or injected artificially with the virus.

These experiments also demonstrated an interesting fact that it is not the attenuation of the virus, as in Pasteur's vaccine, but a reduction of the amount which is important.

Tetanus.

Tetanus is a specific infective disease due to the action of the bacillus of tetanus and its toxins. The bacilli are found most commonly in garden soil, horse manure, and road earth, and there must be an injury to the skin or mucous membrane before an animal can become affected.

The intestines of the horse have been found to harbour the bacilli without producing a pathogenic condition.

The bacilli located in a wound multiply, but seldom leave the focus of infection; they are, therefore, rarely found in the blood-stream or vital organs. During the process of their multiplication they manufacture a specific toxin, which is taken up by the circulation and diffused over the entire

body. Some of the poison is also carried along the nerve fibres. In this manner also the motor and sensory nerve cells become severely charged with the poison, producing the characteristic symptoms. This action of the poison is a very difficult one to explain, but probably Ehrlich's side-chain theory supplies the best material that our knowledge of the subject, as we understand it, can give. Accordingly, each toxin molecule is composed of one non-toxic (haptophore) and one toxic (toxophore) atom group. The protoplasm of the motor nerve cell is composed of a vital nucleus and numerous side-chains, or receptors, of which many possess a special affinity for the haptophore atom groups of the toxin molecule. When such molecules of the tetanus toxin reach the nerve cells, they are anchored by the aid of the haptophore groups to the corresponding receptors of the cell protoplasm, whereupon the toxophore group attack the nucleus of the cell. The opposed affinity between tetanus toxin and nerve substance is demonstrated by the experimental observation, where it is shown that a mixture of toxin and brain substance is non-toxic for a guinea-pig (Wassermann).

When the symptoms of tetanus come on slowly, and the disease itself appears to be running an insidious course, recovery usually follows. On the other hand, if the disease is very acute and the onset rapid, one's prognosis must be unfavourable.

In the latter condition it is probable the toxin has united with the cells, causing vital destruction; while in the former it may only have fixed itself to the free side-chains, or receptors, or those that are approaching freedom, and thereby neutralizing the toxic effects before cell injury can take place. It must obviously follow when one remembers how potent the toxin of tetanus is; to obtain any curative results at all, the poison must be neutralized before it begins to attack the cellular elements. This in practice unfortunately is not always possible, as we have no premonitory symptoms to guide us, the first indications

being a series of established specific phenomena of the presence of the disease itself.

On the other hand, if a liberal liberation of receptors rapidly follows, one would expect that Nature's efforts to neutralize the toxin would take place.

In 1890 Behring immunized rabbits by inoculating them first with 0·3 c.c. of virulent culture (filtrated), and then injecting them with 3 c.c. of a 1 per cent. iodine solution. Such subjects later withstood the effects of a 10 c.c. injection of virulent culture, while 0·5 c.c. of the same culture killed control rabbits. Moreover, they also survived twenty times the fatal dose of toxin, while 0·2 c.c. of their blood-serum protected them against a virulent infection given twenty-four hours later. These experiments laid the foundation upon which serum-therapy as a prophylactic and curative method was built.

Behring adopted the following method in making his vaccine serum: He took a virulent broth culture of the bacillus, and in 80 c.c. he mixed 0·25 per cent. trichloride of iodine; 60 c.c., 0·175 per cent. iodine; 40 c.c, 0·125 per cent.; while he retained another 20 c.c. which he used without mixing at all. He then injected subcutaneously into the horse the most attenuated culture first, and proceeded with the others at intervals of eight days.

Finally he injected the pure culture, 0·5 c.c., doubling the dose every five days.

After the lapse of a given time the animal was bled, and its serum obtained in the usual way.

An animal injected with an immune serum lasts from three to four weeks. The serum may be administered subcutaneously, intravenously, or intracranially, and as such possesses antitoxic properties.

For reasons already explained, the action of antitetanic serum as a curative agent has considerable limitations, and in veterinary practice, at least, its usefulness is mainly prophylactic.

Where we have contused wounds or injuries, caused by

rusty rails, wire fencings, agricultural tools and implements, etc., a dose of antitetanic serum injected will produce complete protection for three or four weeks.

In addition, such wounds should be cauterized and even scarified, and, above all, severely dressed with very strong solutions of tincture of iodine. If the infective wound is low down the leg (below the knee), in such cases we believe we have had benefit by performing neurectomy. The application of Bier's treatment, recommended by some to check absorption, we consider detrimental to the patient. This procedure causes an increase of pain to the patient and aggravates the tetanic symptoms.

Where lockjaw is the result of an amputated tail, we reamputate, taking off several inches, if possible, and inject tincture of iodine at the root of the tail.

The intratrachial injections of lugol solution we have found not only to mitigate the severity of the attack in some cases, but in those cases which do recover, the duration of convalescence is considerably shortened.

The subcutaneous injections of mag. sulph. in solution in our hands have proved of no curative value, and the same may be said of the rectal injections of liquor ferri perchlor.

CHAPTER XVII

DISEASES OF THE RESPIRATORY ORGANS

Disease.	Cause.
Bronchitis	Pneumococcus, streptococcus, *Micrococcus catarrhalis.*
Pneumonia	Pneumococcus, streptococcus, staphylococcus, *B. coli*, cocco bacillus.
Strangles	Streptococcus and others.
Purpura hæmorrhagica ...	Staphylococcus, streptococcus, diplococci, and others.
Influenza	Primary: ultra-visible. Secondary streptococcus, staphylococcus.
Glanders	*B. mallei.*
Distemper	Primary: ultra-visible or Bronchosepticus. Secondary streptococci, staphylococcus.
Actinomycosis	*Streptothrix actinomyces.*
Phthisis	*B. tuberculosis.* Secondary infection may include—Streptococci, staphylococci, pneumococcus, *Micrococcus catarrhalis*, *M. tetragenus*, *B. coli.*

Bronchitis.

It may be taken for granted, so commonly is the fact seen in practice, that bacterial invasion of the respiratory tract, from the anterior nares to the pulmonary vesicles, is a mixed one; indeed, the respiratory mucosæ continually harbour pathogenic bacteria whose disease-producing qualities only become manifest through some disturbance of Nature's protective and bacteriotropic forces. No sooner does a specific organism gain a footing, and disease sets in, than other bacteria have placed before them increased opportunities for their development, which opportunities they are not slow to embrace. All atmospheres are charged

with bacteria, but some more so than others. The badly ventilated horse-box, the fusty cow-pen, or the insanitary dog-kennel, pre-eminently carry more than their share, and, needless to add, animals with pulmonary disease inhaling such germ-laden air must be placed at great disadvantage when the crisis of the disease arrives. In fact, we see in practice, some cases where specific invasions of the respiratory organs have taken place under excellent hygienic conditions making good recoveries, while others, less fortunate by reason of their unhygienic surroundings, succumb to pulmonary disease in spite of all efforts to save them. The first point, then, in all respiratory affections the clinician must emphasize is to insist his patient inhaling the purest possible air-supply.

Diseases of the respiratory apparatus seem to be particularly suited to the beneficial influences of vaccine-therapy, the circulatory supply being usually so good, unless, of course, some mechanical or other obstructive lesions are in existence to prevent the bactericidal elements easily reaching the source of infection.

Moreover, the clinical phenomena after vaccine injection can usually be more easily interpreted here than probably in any other system of organs.

Perhaps the most common group of exciting causes productive of derangement of the respiratory system are sudden barometric changes.

A sudden chilling of the respiratory mucosæ drives the blood and its antibodies temporarily away from the parts, the previously latent bacteria often found located upon the respiratory mucosæ become active, and in the process of their growth toxins are formed which still more depress the body-cells. Local cell-proliferation goes on, leucocytosis comes into evidence, and the whole area is bathed in bacteriotropic material. A regular warfare has now become established, and upon the power of the tissue-elements to overthrow the bacteria depends whether the animal lives or dies.

When one is dealing with a mixed infection, it is obvious, if vaccine-therapy is to serve its intended purpose, a vaccine of all the causative bacteria must be obtained. In practice it is difficult to estimate to each bacterium the degree of pathogenicity which that particular bacterium has the power of exercising, and it is probable that in each individual case the degree varies considerably in the same species of bacteria at different times and in the same case.

To ascertain the nature of infection, one must collect the pathological material at one's disposal. In animals we obtain such from the nasal discharges, and the *materis morbi* should be carefully collected, to prevent outside contamination, as already described.

If the patient has had a good previous health record, we begin by giving the following doses, assuming, of course, all these bacteria are present:

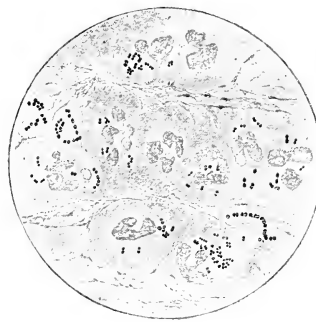
Staphylococci	500,000,000
Streptococci	100,000,000
Diplococci	500,000,000
Micrococcus catarrhalis ...	250,000,000

The clinical phenomena should be carefully noted.

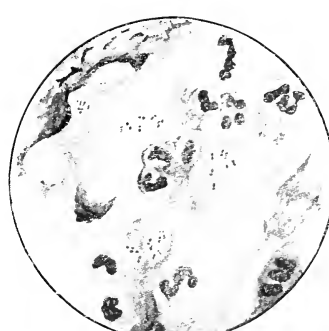
Some twelve hours after injection the temperature will usually rise one or two degrees, the pulse-beat will be increased in number 10 to 20 per minute, and the respiration increases 5 to 15 per minute.

The cough is usually more irritable and the catarrhal discharge increased in quantity, but more tenacious in consistence. On the whole, there is a proportionate general depression. To sum up, these phenomena constitute the negative phase, which are salutary if the vaccine has responded in the manner one desires, and they are to be expected if one anticipates good results to follow. If no such characteristics are noticed, the dose is too small or there is some error in the technique, and a close investigation is called for.

PLATE IX.



MICROCOCCUS CATARRHALIS—HUMAN SPUTUM.
(Emery's "Clinical Bacteriology.")



PNEUMOCOCCI.
(Rose and Carless' "Manual of Surgery.")

To face page 144.

If it is due to the smallness of the dose, an increased dose should be given straight away, amounting to from one-half to double the strength of the original dose.

Following upon the negative phase, a steady improvement should be noted. The temperature falls; the respiration becomes slower and deeper; the pulse-beat lessens, etc. This is the positive phase, which should go on for two, three, or five days.

As soon as the immunizer notes a cessation of the improving symptoms, another dose of vaccine should be given.

It is our experience in pulmonary conditions that vaccine-therapy gives a bigger percentage of recoveries, the disease cycle is cut shorter, and, when recovery is an accomplished fact, the after-sequela are either nil or very slight—in other words, permanent organic disease is not so likely to be left behind.

In severe cases several injections may have to be given, and where progress is not satisfactory, the vaccine should be combined with a polyvalent antistreptococcic serum, beginning with doses of 20 c.c., and increasing daily according to results.

Of course, in the adaptation of vaccine-therapy—and this remark applies not only to pulmonary diseases in particular, but to all other bacterial infection in general—orthodox treatment must not be altogether ignored. In addition, therefore, it is an imperative necessity to place a lung-diseased patient in the best hygienic surroundings, for reasons already explained. One must also, if it is considered advisable and the case warrants such a course, apply counter-irritants to the chest, administer circulatory sedatives or stimulants, according to the condition of the patient—expectorants if the cough is troublesome or the discharge is tardy, antizymotic febrifuges if the temperature requires pulling down, and antiseptic inhalations when advisable.

Nevertheless, when all these so-called curative remedies

are applied—in fact, if they were all collected and totalled into one and placed against the antidotal and protective elements of Nature—one wonders what the ratio would work out at as regards their respective curative values. We would be inclined to place the therapeutic value very low indeed, and this fact must never be overlooked in whatever line of therapeutic action we like to take up.

In short, how far can antizymotics be pushed in the supposed attempt to destroy bacteria circulating in the blood or incubating on the respiratory mucosæ, and leave the leucocytes active and intact? Or to what extent does the inhalation of antiseptic vapours limit the destructive processes of bacteria located in the respiratory mucosæ, or retard their rapid development? One must confess those orthodox means are futile—nay, more, if they are pushed too far, they may become elements of positive harm, hastening on the retrogressive changes which lead to dissolution.

We must, then, fall back upon the protective antibodies of the blood to supply our antidotal remedies, and the more we foster and stimulate them, the more likely we are to effect recoveries.

Pneumonia.

Every practitioner knows the orthodox treatment of a severe case of pneumonia is far from satisfactory.

There are no specific drugs upon whose value we should like to greatly rely, and after attending to the patient's comfort, sustaining him with nutrients to give him strength to face the crisis, and placing him in good hygienic surroundings, we are practically at the end of our resources, and have to fall back upon the *vis medicatrix naturæ*.

In practice we find pneumonia occurring as a complication to such bacterial diseases as strangles and influenza, or it may be seen as an independent and primary condition. Again, we may find a single isolated case in the stud, or several may become affected; but whatever type the disease

may be, it is clearly one for careful bacteriological investigation at the outset.

Lignières in 1897 discovered a bacillus of the pasteurella group which he called the "coccobacillus," and which he considered to be the causative organism of catarrh, bronchitis, pneumonia, strangles, purpura hæmorrhagica, and by its action upon the mucosa, and through it the system, depressing the tissues locally, and the constitution generally, that the more pathogenic bacteria gain a footing, with serious functional and organic consequences, after which the coccobacilli are supposed to drop into insignificance.

Be this as it may, we know for a fact that in typical pneumonias mixed infections are the rule.

As one is generally called in to see the disease in its acute form, it is obvious there is little time for delay, and a stock mixed vaccine, preferably polyvalent, should be injected at the outset. This will give the immunizer time to make an autogenous vaccine, and this rule we rigidly carry out in practice.

The phenomena after injection are practically the same as in bronchitis, and in point of fact the two diseases run a close parallel, so much so that a bronchitis at any moment may merge into a pneumonia, constituting broncho-pneumonia. We strongly believe the verminous pneumonias one sees in cattle and sheep derive their fatal consequences, not so much from pulmonary inflammations due to the irritation of these parasites as to the invasion of pathogenic bacteria at a later stage of the disease, and in such cases as these we have isolated staphylococci, streptococci, and diplococci.

When a pneumonia is running a very acute course, with a high temperature, it is strongly advisable to begin with a very small initial dose; and the reason is obvious: after a vaccine injection the opsonic cycle drops (negative phase), and the temperature rises; conversely, with a rise of the opsonic cycle (positive phase) the temperature drops; but if the temperature is naturally high during the

negative phase, it will go higher at this period, and it is then the danger of a dose too large for Nature to assimilate, so to speak, comes in. This is a principle applicable to all acute diseases, as has already been pointed out, so far as vaccine-therapy goes.

Strangles.

This disease is very prevalent in young horses at certain seasons of the year, and in some years more so than in others. It is a specific infective fever, and when running a simple course it affects the upper air-passages and invades the neighbouring local lymphatic system.

The streptococcus which is responsible for this condition is found in the nasal organs, and possibly exists as a non-pathogenic bacterium in many instances. The disease may run a very mild course, and pass off only as a simple catarrh. This may be due to the low virulence of the bacteria themselves, or the increased resisting powers of the antibodies of the blood itself, or both.

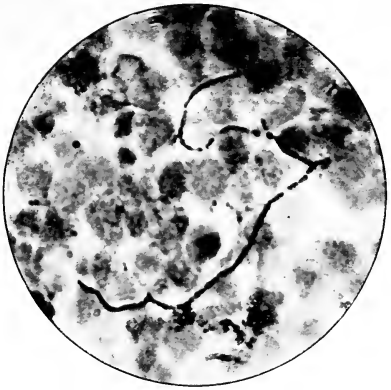
In an ordinary simple case of strangles special treatment is not called for, and all that is necessary is to keep the patient from contracting chills. Complications are, however, liable to supervene, in which case they must be treated upon their merits.

If the disease runs an irregular course, the immunizer can do much to fortify his patient and prevent septicæmic or metastatic lesions developing by the injection of an autogenous vaccine.

Here, too, if the disease is one of considerable standing, a mixed infection has to be looked for; in which case the causative bacteria must be identified, and suitable vaccines made.

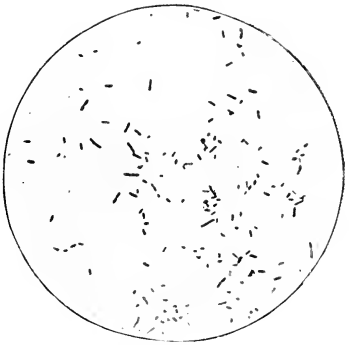
When we have under treatment a bad case, which does not seem to respond to vaccine treatment, we make it a practice of combining the vaccine with antistreptococcic serum.

PLATE X.



STREPTOCOCCUS OF STRANGLES.

PUS FROM A SUBMAXILLARY ABSCESS.



GLANDERS BACILLUS. × 1000.

(Hewlett's "Bacteriology.")

To face page 148

Antistreptococcic Serum.

It has been proved that after injecting an emulsion of devitalized streptococci into the circulation of a horse, and following this up by small and gradually increasing doses of living bacteria, the time arrives when that animal's serum has acquired high antimicrobic powers. Marmorek was the first to demonstrate this fact; and he also showed, if the antistreptococcic serum thus obtained is injected into an animal, and that animal is subjected to the action of virulent streptococci, it will prove itself to be either completely resistant, or, if the disease should develop, it will show itself in an exceedingly mild form.

It is most important to remember that there are many strains of streptococci, and each may be equally virulent; and one can demonstrate this from their appearances easily under the microscope. Take, for example, the plump, well-nourished, globular-looking types one sees in a severe attack of strangles, and compare them with the shrivelled-up, small, almost oval-shaped, short, compressed chains as seen in those cases of bovine endometritis. In making, therefore, a satisfactory serum, a polyvalent serum is to be preferred to a monovalent one. It is the experience of the writer, in the cases where the virulence of the bacteria is very great, that a combination of an autogenous vaccine and a stock serum (polyvalent) gives much better results than if either is used singly.

In fact, so strongly do we hold this view now, that, given a serious case with septicæmic tendencies, we never think of failing to combine the serum with the vaccine.

In some strangles outbreaks the disease takes on a very virulent form, and, in addition to manifesting local lesions, extension takes place, probably through the lymphatic system, to the mesenteric or mediastinal glands.

Or some vital organ, such as the liver, kidneys, etc., may become involved. When the blood-stream itself has become invaded by the bacteria, a typical septicæmia has

taken place, and the consequences are then generally disastrous.

In those cases where pronounced local abscess formations are the order of things, it is imperative the pus cavities should be evacuated as soon as possible. By so doing internal pressure is relieved from the abscess wall, thereby allowing the bactericidal elements, by a process of osmosis, to reach the infective centres. It also tends to prevent the risk of metastatic invasions, a condition always to be feared and warded against in strangles.

We usually begin by giving an autogenous vaccine of streptococci, composed of 250,000,000 as a dose, and if the case is sufficiently bad we add 20 c.c. of antistreptococcic serum.

The serum we repeat the next day (30 c.c.), noting the temperature, pulse, etc. On the third day, if necessary, we give 40 c.c., and if the case is progressing we leave off doing anything further until the fifth day, when we repeat the vaccine, giving 500,000,000. If the negative phase is pronounced, it is better to wait and note progress for three to five days before giving another 500,000,000 to 1,000,000,000 for a dose. If the negative phase is only indifferent, do not wait so long, but within twenty-four hours give 500,000,000 to 750,000,000, and watch the result. In obstinate cases one may have to follow up treatment for a considerable period, increasing the doses as one goes along, and watching the phenomena.

The dose of serum, and the number of times it should be administered, must be items left largely to the individual immunizer upon which to exercise his judgment.

Purpura Hæmorrhagica.

It is probable this disease in equines is never seen as a primary condition, but is met with in practice as secondary to such specific debilitating disorders as influenza, strangles, etc.

That the phenomena produced are due either to a direct

or an indirect effect of one or more species of bacteria or their products no one can deny, but that the bacteriology of the disease leaves a great deal to be desired we must all admit.

In cases of this kind a variety of micro-organisms have been isolated from time to time, including diplococci, staphylococci, streptococci, and bacilli, and, judging from our present bacteriological knowledge, it is difficult to ascribe to which microbe the production of the specific blood-lesions belongs.

When one is dealing with a simple catarrhal fever, an attack of influenza (so called), or an outbreak of strangles, in a stud of horses, the disease generally attacks one animal first, the others slowly or suddenly developing the affection. As the disease goes through the stable, one can invariably isolate, microscopically and biologically, the specific microbe, or, in the case of mixed infections, microbes from all the patients. As a sequel to the outbreak *one* animal may develop purpura hæmorrhagica, for in practice we seldom see more than one case, the disease being very sporadic, and all the others may make a good recovery.

Why should this one animal be singled out for this complication? He has been under the same dietetic, hygienic, and therapeutic management, and his constitutional health and age before the illness began was as good as the others which recovered. Nay, more; for all that we know the clinical phenomena suggested that the specific infection from which he was suffering was identical to the others which recovered without this complication.

We are now face to face with one of two facts: either this animal has had, firstly, a hypervirulent dose, or, secondly, his protective forces are so defective that, not only is he unable to resist the invasion, but the specific invading bacteria find in his economy the very essentials in the most suitable proportion to suit their requirements.

As regards the first hypothesis, it is scarcely tenable; for why should this animal be singled out for a more

virulent dose of infection than the others, considering their surroundings are identical and the infection apparently the same? Clearly, then, there must be in this animal some defective condition which was non-existent in the others.

As we have already seen, purpura makes itself manifest at the tail end of debilitating specific diseases. We know a regular war has been going on between the bactericidal forces of the body on the one hand, and the bacteria on the other.

The serum of the blood, as we have seen, provides these forces, and usually after a bacterial attack the bacteriotropic indices rise and the patient becomes more resistant; but this degree of specificity is only potent to the bacteria present, and while these immune bodies are being elaborated for the purpose of protecting the system against the active pathogenic bacteria, a latent bacterium makes its presence felt by taking on a pathogenic rôle, the antibodies are not sufficiently prepared to resist this fresh invader, the blood-serum undergoes a retrogressive change, the endothelial cells also become affected in some way, and the characteristic petechial lesions and the serous effusions soon follow. This, of course, is only hypothesis, but we venture to think we must look to some alteration of the serum for the production of the purpuric lesions. The point is, what is the nature of the alteration and how is it brought about?

For vaccine-therapy to be of any service in purpura, we must be somewhat empirical in our methods, and we will continue to be so until we know more of the pathology of this disease. In the meantime it becomes us to utilize what knowledge we possess to protect our patient from the destructive influences of this fatal disease.

Autogenous vaccines should therefore be made from the bacteria which have caused the original condition, and they should be combined with serums if considered advisable.

The intratracheal injections of Lugal's solution must also be strongly recommended as an adjuvant.

In fact, in days gone by, when one used to see more cases of purpura than one does now, Lugal's solution was a most useful preparation, and in the writer's hands gave many good results.

Equine Influenza.

The word "influenza," as used in veterinary medicine, has a very wide meaning, and as such, in many cases, is undoubtedly a misnomer—so much so that the clinician recognizes a variety of diseases which may come under the same heading. This is unfortunate, inasmuch as it admits of laxity, and serves no useful purpose, so far as descriptive details go.

Certain it is an endeavour should be made to curtail the meaning of the word, and at the same time there should be drawn up a list of cardinal symptoms and lesions specific in themselves to the disease in question. In this way the meaning of the word itself would be more confined, and the information it conveyed more accurate. One cannot help recognizing, however, there are certain difficulties which will remain until we are in possession of a more accurate knowledge regarding the nature of the causative bacteria. Some authorities hold the disease proper is due to a microbe of the ultra-visible group; others, that it is caused by a coccobacillus which is always present in the primary infective stage, and which is followed later by a secondary infection, the causative bacterium in this secondary infection in most cases being a streptococcus.

Admitting, then, the bacteriology of influenza is in such a nebulous condition, one would suggest that here at least the immunizer has reached his limitations, and at first sight this would appear to be so. On looking more closely into the facts, however, what do we find in practice?

Every practitioner with experience of influenza outbreaks will agree that the disease influenza itself is in nearly every instance a benign one, and that fatal consequences from the primary and direct effects of the disease are practically

nil. We must look, then, for the sequelæ of influenza to account for the percentage of fatalities; and this is just what we see in practice.

If we are, therefore, in a position to fortify the system against the causative bacteria producing the complications, obviously we shall succeed in reducing the death-rate to a large degree. The more important complications, as we all know, are pneumonia, purpura, strangles.

In the majority of influenzal outbreaks in a stud, one animal only at the outset sickens, and most probably this is the carrier of the infection. In the course of one or several days others may show premonitory symptoms. This clearly gives to the practitioner a clue to the degree of infectivity of the virus on the one hand, and the natural resisting forces of his patients on the other. In addition, the first animal to become affected affords material for a bacteriological investigation on the part of the practitioner, which the writer submits he owes as a duty to his client and to his own reputation.

For by so doing an outbreak not only might be robbed of its power of producing monetary losses in the shape of sacrificed lives, but the animals themselves, if they became affected, would be restored to work much sooner by using up-to-date prophylactic and curative measures.

In these days it is no credit to us, as a profession, to lose patients from the complicated sequels following upon influenza, and it is clearly our duty to ward against such risks.

How is this to be done? By immunization.

A careful microscopical examination should be made of the morbid material taken from the nostrils, if any; the purulent discharge from an abscess, if present; and, if need be, a microscopical investigation of the patient's blood should be carried out. In this way a fairly accurate diagnosis of the bacteria present will be arrived at. Biological investigations should now be instituted with a view to confirming and adding to the microscopical diagnosis, and also preparatory to the making of the necessary vaccines.

It may be taken for granted, if one horse in a stud or stable is suffering from influenza, others are sure to follow, and that, in fact, they are invariably already incubating the disease.

Should the outbreak be a severe one, with a tendency to the development of sequelæ, every animal should have, as a prophylactic, an injection of a mixed vaccine, preferably derived from the cultivated bacteria obtained from the first animal to succumb to the disease.

Of course, it does not follow every animal will show the same variety of bacteria, even in the same stable; but it may be taken for granted that a vaccine derived from a patient suffering from the disease similar to the infection to which the others have been subjected, and under the same roof, is more likely to contain the identical or analogous bacteria than if procured from a patient exotic to the diseased area or building.

If streptococci are present, it is an advantage to combine antistreptococcal serum with the vaccine.

When an animal is suffering severely, and sequelæ set in, an autogenous vaccine should be made from the discharges, and the animal treated according to its condition.

In the few cases in which we have taken the opsonic index in influenza sequelæ, it has invariably been very low to streptococci, and in one case it was only 0·39.

Three days after injection another estimate of the opsonic content was made, and it was found to have risen to 1·57.

So far as influenza and strangles are concerned, we have already seen, grave sequelæ may follow either, and in many cases with fatal results. There is a condition, however, where there is no tendency for such fatal terminations, and yet the utility of the animal is greatly handicapped for life, and the monetary losses to the owner are serious. We all know how liable hunter stock are to become roarers and whistlers, particularly if they should suffer from influenza or strangles, and even severe colds.

Young horses at an age when their dentition processes are in full activity are congregated at fairs and sales, come in contact with a focus of infection, and are suitable prey for the many pathogenic bacteria to locate themselves on the respiratory mucosæ. During these eruptive dental periods the natural protective elements of the system are depleted, partly through malnutrition consequent upon indigestion due to improper mastication of the food, the phagocytes and the opsonins, etc., are below the normal healthy standard, and in the case of the Irish hunter sent over to England the nervous and systemic depression following upon a long journey and sudden climatic changes —not to speak of the ill effects of bad boat and rail travelling—make these animals extremely susceptible to all kinds of bacterial invasions in general, but pulmonary in particular.

Here, then, the immunizer has opportunities of rendering good service to the seller and buyer of hunter and blood stock.

One cannot deny that our present knowledge will not permit the use of a perfect prophylactic sero-vaccine as long as the bacteriology of the diseases themselves, which are productive of roaring and whistling, is in an imperfect state. Nevertheless, this fact does not prevent us from using the knowledge we do possess and turning it to good account.

Careful cultivation of the bacteria found in the discharges of animals suffering from influenza or strangles, and also from those obtained low down in the trachea and the smaller bronchi, should be made.

These also should be procured from animals located in various centres in the kingdom. In this manner not only would several strains of bacteria be formed, but varying degrees of virulence would be obtained, and the mixing of them together would give a polyvalent sero-vaccine. This process, of course, would be too elaborate and expensive for individual effort to carry out, but it would be

well worth considering by horse-breeding societies or Government departments.

The individual immunizer, however, can do much for his client, by using prophylactic sero-vaccines when influenza or strangles has broken out in a stud of young blood or hunter stock, to reduce the risk of roaring to a minimum. Every animal should have a dose at the first appearance of the outbreak in a stud, and this should be repeated in three to five days, two to four injections being sufficient, according to the severity of the outbreak. (See Appendix II.)

Glanders.

Mention of this disease is made here, not with the idea of suggesting the use of a curative vaccine, but to note in passing the diagnostic value of mallein.

The toxin is prepared by growing the bacilli on glycerin veal for a month or six weeks at 37° C. in flat-bottomed flasks, by which time a thick yellow surface growth has developed.

The bacteria are then killed by steaming at a temperature of 115° C. for half an hour.

The fluid emulsion is next concentrated to one-fourth its original volume by evaporation, and finally filtered through porcelain to remove the dead bacilli, after which it is mixed with an equal volume of a $\frac{1}{2}$ per cent. solution of carbolic acid and bottled, and kept in a cool, dark room.

If mallein is injected into a glandered horse, a reaction occurs.

The temperature should be taken on the twelfth, fifteenth, eighteenth, and twenty-fourth hour after injection, having been previously taken once at least, or better twice, before injection.

Should the temperature rise 2·7° F., the animal may safely be branded as suffering from glanders, particularly if there is in addition a local reaction in the shape of a swelling at the seat of injection. This swelling will show itself within twenty-four hours of the injection, and persists

for three or four days. If the case is a certain one, the swelling will be at least 4 inches in diameter, and may be double, and is very painful. If the animal is not glandered, a small local swelling may take place at the seat of injection, but this soon subsides.

Widal's agglutination test for diagnostic purposes has been extensively carried out on the Continent, and in those cases where the temperature is persistently high it would appear this means of diagnosis is a useful adjunct.

Canine Distemper.

Wherever a canine population exists, distemper in some form or other is to be found, and as children are supposed to have measles before they reach maturity, so also dogs are expected to develop distemper during their early life.

In approaching the subject of the bacteriology of distemper, one is struck with the number of alleged causative bacteria which have been isolated by various workers.

Rale in 1883 isolated a staphylococcus from pustules, nasal discharges, conjunctival secretions, the internal organs, and the blood.

Vallerio in 1896 discovered an ovoid bacillus in the brain, spinal cord, nasal sinus, and conjunctival discharges, and named it *Bacillus caniculæ*.

Piorkowski in 1905 detected a bacillus similar to Vallerio.

Copeman in 1909 discovered a bacillus of the cocci group, which he grew on gelatin and potato, and produced symptoms characteristic of distemper by injecting 1 c.c. of a week-old broth culture.

Lignières about this period associated the causative organism with the pasteurella group.

Carré, on the other hand, claims that the causative bacterium of distemper belongs to the ultravisible group.

Ferry, in 1910, adopted a system of inquiry contrary to those of the previous investigators, inasmuch as he began investigating the nature of the disease in its initial stage, and isolated from the small bronchi—and sometimes from

the trachea—a short, narrow bacillus, appearing singly, sometimes in pairs, motile, and aerobic.

On agar plates, after twenty-four hours' inoculation at 37° C., small colonies appeared slightly raised and translucent; after forty-eight hours they increased in size, were curved and amorphous, and in a week the edges undulated, and showed a grumose centre.

On potato it grew with a characteristic dark brown colour.

On litmus milk, after seventy-two hours, it grew, giving the upper half a bright blue colour, and in five days the whole tube was coloured.

Ferry also found the serum taken from distemper dogs always agglutinated this bacillus. To this organism he gave the name *Bacillus bronchosepticus*.

M'Gowan, working independently of Ferry, isolated from distemper subjects what appears to be the same bacillus. M'Gowan believes the nasal mucosæ is the primary seat of development of the *B. bronchosepticus*, while Ferry is of opinion it settles on the tracheal mucous membrane at the outset. Torry and Hake also agree with Ferry on this point.

These two latter workers, in addition, give an interesting table of the relative frequency of the *B. bronchosepticus* in the various organs and fluids of dogs suffering from distemper.

Localities Cultivated.	Total of Cases Cultured.	Per Cent. from which *B. broncho-septicus* Isolated.	Per Cent. of Positive Cases from which *B. broncho-septicus* Isolated in Pure Culture.	Per Cent. in which Locality Proved Sterile.
Blood	74·0	5·4	5·4	71·6
Pericardial fluid ...	28·0	0·0	0·0	80·0
Trachea	52·0	77·0	80·0	2·0
Bronchials	77·0	74·0	91·0	6·5
Lungs	89·0	70·0	95 0	6·7
Liver	81·0	30·0	84·0	30·0
Spleen	77·0	17·0	100·0	44·0
Kidneys	74·0	16·0	100·0	43·0
Nose	68·0	56·0	23·7	7·3
Eyes	46·0	2·0	0·0	4·3
Brain	5·0	20·0	0·0	0·0

From an analysis of this table one sees the *B. bronchosepticus* does not confine itself to the respiratory tract, and it is interesting to note the high percentage of cases in which the liver was involved.

These investigators have certainly advanced the bacteriology of distemper further than any other workers, more particularly if the *B. bronchosepticus* proves to be the causative bacterium of distemper proper. In discussing equine influenza we expressed an opinion that the primary causative bacterium was probably of a more or less benign character so far as its pathogenesis goes, secondary infection being responsible for the complications and consequent often fatal sequelæ.

Canine distemper resembles equine influenza in many ways, and so far as mixed secondary infections are concerned, it appears to stand exactly parallel.

The writer believes also that the specific primary infection is seldom responsible, as with influenza, for fatal terminations, and if secondary invasions could be warded against, canine distemper would lose many of its serious aspects.

The most common bacteria found in the secondary infection are streptococci, staphylococci, and *B. coli communis.*

It appears to be evident, from the research work of Ferry, that the *B. bronchosepticus* and its toxins have such an effect upon the system of the patient that the secondary invading bacteria have their way prepared for them, and their development is a very rapid, virulent, and assured one.

If this hypothesis is correct, it will follow, if we desire to combat the ravages of these various bacteria, that a vaccine prepared from the *B. bronchosepticus* must be given in the early stages of the disease—in fact, before the first manifestations of sickening present themselves, and also before the least suspicion of a mixed infection has taken place, if benefit is to follow its administration; for it is obvious that such a vaccine would possess no curative value whatever against the destructive effects of,

for example, a streptococcal infection. It is quite possible the failures recorded against Ferry's vaccine are due to the fact that they have not been used soon enough in the course of the disease.

When secondary infection has taken place, the immunizer must ascertain in the first instance the exact nature of the infection; and, we believe, owing to the variable nature of these infections, to obtain the maximum benefit from sero-vaccine therapy, the immunizer must treat each outbreak or case on its own merits, and for preference making his own autogenous vaccines.

In the past a variety of prophylactic and curative vaccines have been put forward, some being highly spoken of, and some condemned, each having its own exponents as well as its opponents.

In the hands of some the results have been good, while with others they have been unsatisfactory. Why should those diversities of results and opinions be?

Can they be due to any want of skill on the part of the immunizer? This is most improbable, for one capable practitioner praises the vaccine, while another equally capable condemns it. Is it not rather due to the unsuitability of the vaccine to the particular outbreak? and would we not obtain a more even percentage of results if we used autogenous instead of stock vaccines? Certainly, if we did, we would be less empirical in our actions, and probably more successful as immunizers.

When a stock vaccine succeeds, we must reasonably conclude it possesses that specificity of action capable of rousing the attacking forces against the specific bacterium for which it is intended.

The following are the principal sera and vaccines which have been used:

Lignières cultivated many strains from a pasteurella he isolated from distemper cases, and by a process of subculture which he carried on for twelve months, he was able to procure a strain possessing a mild degree of virulence.

These cultures so derived are incubated in broth-tubes for five consecutive days at a temperature of 42° C., and from this Vaccine No. I. is derived. Vaccine No. II. is obtained in the same way, except that incubation goes on for two days only. The dose advised by Lignières is 1 c.c. of Vaccine No. I. to be injected into the thigh of a puppy; ten days later Vaccine No. II. is injected into the opposite thigh.

Lignières at a later date injected his vaccine as above described into horses, beginning with small doses, and increasing them as toleration became established at regular intervals, first under the skin, and later into the vein direct.

The immune serum is then obtained by bleeding the animal, collecting the blood in sterile vessels, allowing it to clot on ice, and the resultant serum placed in stock bottles, and a small percentage of lysol added.

The dose is from 10 to 20 c.c. to each dog.

Phisalix, adopting Lignières' method, made his monovalent vaccine, which he derived also from a pasteurella cultivated on glycerine and broth. The dose he gives is 2 c.c. to each dog, repeating the vaccine in ten days.

Piorkowski prepares a polyvalent serum which is highly spoken of by many clinicians, while others consider it is useless as a prophylactic and curative agent.

As a prophylactic dose he advises 5 to 10 c.c., immunity, it is stated, being maintained thereby for six months, and for a curative dose 10 to 20 c.c. is recommended.

Ferry, after isolating his microbe, the *B. bronchosepticus*, made a protective vaccine mainly to prove whether this bacillus was the real causative organism of distemper, and, in addition, he carried out agglutination tests.

The following is an extract taken from his paper, "The Cause of Distemper in Dogs," and which appeared in the *New York Journal* for July, 1912:

"*Agglutination Tests and Protective Inoculations.*—In

order to strengthen our position it was necessary to carry out tests other than experimental inoculations—namely, agglutination tests and protective inoculations. The agglutination tests were carried out on the following serums: From dogs suffering with the disease spontaneously contracted, from dogs experimentally infected, from dogs immunized to the live organisms, and also those immunized to dead cultures. These were tested repeatedly on the same strain and on different strains. They all gave positive agglutinations, with but one exception; in this case the dog was an old one, and had probably suffered with the disease. The serum from the dogs suffering from the disease spontaneously produced, as those found on the streets, gave an agglutination ranging from 1 in 40 to 1 in 800. The serum from the dogs experimentally infected gave an agglutination from 1 in 100 to 1 in 600; while those immunized to live and dead cultures, gave a reaction from 1 in 800 to 1 in 4,000. The serum which gave the reaction from 1 in 4,000 was tested against several strains of the bacillus, and the results ran from 1 in 600 to 1 in 6,000. Two dogs were given one injection of the live organisms each, one intravenously, and one subcutaneously, and the agglutination reactions ranged from 1 in 100 to 1 in 600. Following the injections of the last dogs, the only marked symptom was diarrhœa. This again emphasizes the symptom upon which I have previously laid so much stress. In all of these agglutination tests we used, as controls, blood of dogs which had never had the disease. They invariably gave negative results.

"The protective inoculations were also very convincing, and proved to our entire satisfaction that we were dealing with the specific bacillus. I will quote from my second article in this connection: 'Forty dogs were used in all; nine were immunized with live cultures, while fourteen were saved as controls. All of these dogs were exposed to at least three dogs suffering with typical symptoms of the disease, including the respiratory, abdominal, and nervous

types. Eight of the controls died, while all of the immunized dogs remained well.' These dogs were all in the same room and exposed to each other, so there was plenty of opportunity to contract the disease from each other had they been susceptible.

"From a practical standpoint we have found these protective inoculations of very great value. From the nature of the work carried on in our laboratory, it is necessary to use from fifteen to twenty dogs a week for one purpose or another, and it has always been our experience to lose nearly all of the young ones, not killed at once, to distemper. Since beginning to give some of these dogs protective injections as soon as they are received from the dog-pound, our experience is just reversed, and we are able to save a large number of them.

"This is not only a great help as far as the experiments are concerned, but a great saving from a monetary point of view, and incidentally corroborates our previous results. All of these dogs are exposed to infection before being brought to the laboratory, and many of them have already contracted the disease, so that the fact that we are able to save so many speaks well for the value of the inoculations. We give three injections, with two or three days' intervals, beginning with 200,000,000 bacteria, and increasing the dose by 200,000,000.

"The dogs have suffered no ill effect whatever from these protective injections."

Ferry advises as a prophylactic the following doses of devitalized *B. bronchocanis,* with intervals of from three to five days between.

First	...	...	...	200,000,000
Second	...	...	...	400,000,000
Third	...	...	...	600,000,000

The vaccine, of course, should be given before symptoms of the disease present themselves. Ferry suggests, for the best results to be obtained, the vaccine should be given a

month previous to exposure to infection. This is not always practicable; but, he adds, "treatment may be instituted at the time of exposure. The disease will not always be prevented at this stage, but the severity of the symptoms, if the dog becomes infected, will be much decreased and the duration of the disease lessened."

For those cases where the disease is established, Ferry prescribes a polyvalent curative vaccine, beginning with the following initial dose:

Bronchosepticus	100,000,000
Staphylococci	50,000,000
Streptococci	25,000,000

The doses are repeated every third or fifth day, each dose increasing by a hundred million bronchosepticus, and the other bacteria *pro rata* according to symptoms and reaction shown. In mild cases four doses may be given, and in severe cases six to seven doses are advised.

Actinomycosis.

This disease is very widely spread in some districts, particularly in low-lying, damp areas, and is most commonly seen in bovines.

It may show itself as a localized neoplasm in the region of the neck, jaw, or throat. The tongue also is a common seat of infection, or it may be diffused, affecting important organs, and simulating in many ways tuberculosis.

Under medicinal treatment many cases made good recoveries. Potassium iodide or hydrargyrum iodium rubrum exerts an almost specific action upon the fungus.

There are, however, cases when these drugs appear only to check or keep the disease in abeyance *pro tem.*, and as soon as they are left off the disease renews its vigour. It is in cases such as these that vaccine-therapy might do good, but on this point we possess no knowledge.

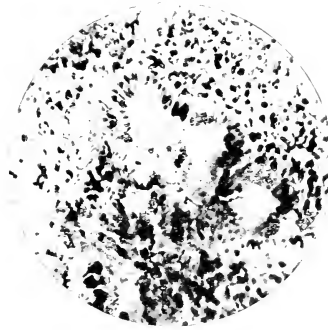
A limited number of cases have, however, been treated in man with fairly good results.

Wynn describes the first case so treated in the *British Medical Journal* of March 7, 1908, thus:

"The infection dates back at least twelve months, and six months prior to admission to hospital extension seems to have occurred from the bronchi to the lung tissue, and much sputum with a fæculent odour was expectorated. Subsequent formation of an empyema required operation, and from the pus a pure culture of streptothrix was isolated, and a vaccine prepared from a forty-eight-hour-old agar culture. The dose employed for each inoculation represented 0·001 milligramme of bacterial substance. Attempts were made to estimate the index, which was approximately 0·3 on January 3, and 0·5 on January 7; on January 8 the first inoculation of 0·001 milligramme was given. Twenty-four hours later the negative phase was apparently over, as the index had risen to 0·7, and by January 16 was 1·2. In a few days the cough became less troublesome, and the sputum and discharge of pus diminished in a remarkable way. The temperature dropped from over 100° F. to normal, and remained normal for three days. Four days after injection the discharge had so diminished that the drainage-tube was removed. A slight rise of temperature resulted, and on the 18th instant a second inoculation of 0·001 milligramme was given. Three days later temperature was again normal, and remained so. Subsequent injections were given on February 11 and 25, and March 11 and 27, each of 0·001 milligramme. Th patient gained 1 stone 6 pounds in weight, and the condition on discharge was a thickened pleura, with a large, dry cavity in the lung. There was no sputum, and only occasionally a dry cough. The patient has continued well."

When local growths are operated upon, there is the possible chance of a recurrence. We venture to think a course of vaccine might do good in cases of this kind, but of this we have no experience.

PLATE XI.



ACTINOMYCOSIS BOVIS. × 1000.
(Hewlett's "Bacteriology.")

To face page 166.

Bovine Tuberculosis.

Clinical and Bacteriological Diagnosis.—Since Koch's discovery of the bacillus of tubercle, our knowledge of this disease as it affects man and the lower animals has ever been accumulative, and although there is still much to learn, probably no disease has been more fully and seriously investigated than tuberculosis.

The disease affects nearly every domesticated animal, some to a greater degree than others, depending partly upon the idiosyncrasies of the breed or strain, and partly upon the environment of the individual, etc. It is not intended here to discuss tuberculosis as it affects all the lower animals, but to confine our remarks strictly to the disease as it is seen in the bovine species.

Tuberculosis may run an acute course and terminate suddenly, or it may, and usually does, develop an insidious character—in fact, the degree of virulence in many cases is so slight that the animal shows no indication of a removal from the usual normal healthy standard.

Recent investigation in human pulmonary tuberculosis has revealed the fact that the bacillus of tubercle is not such a virulent organism as the early bacteriologists led us to believe—in fact, some authorities go so far as to say that if no secondary infection followed the primary invasion of this bacillus, the disease would lose its appalling significance altogether. Certainly this teaching is in accordance with our own views of such diseases as influenza and distemper, where mixed infections are the order of things.

On several occasions we have isolated from the bronchial discharges of cattle destroyed after reacting to the tuberculosis test streptococci rarely and staphylococci commonly, and have used on a limited number of cows, which were certified to be suffering from tuberculosis and which reacted to tuberculin, a vaccine prepared from the bronchial and tracheal discharges, combined with tuberculin and poly-

valent antistreptococcal serum (bovine), in the following combinations :

Streptococci	250,000,000
Staphylococci	500,000,000
Tuberculin	2 c.c.
Antistreptococcal serum (bovine) ...	20 c.c.

To be repeated in three to five days.

The patient's temperature invariably falls, the appetite improves, and the respiration becomes slower and deeper; the coat regains its lustre, the condition generally improves, and the animal puts on flesh. When the disease runs a very rapid course, and the immunizer is called in too late, auto-intoxication may be so far advanced that no recovery can be looked for or expected.

We have been greatly struck with the rapid manner the above vaccine has cleared up the bronchial discharges. The cases which run a latent course are clearly those where secondary invasion has either never taken place, or, if it has, Nature's antibodies have succeeded in resisting the attack to a large extent. We strongly believe that every animal has the power of resisting the bacillus of tubercle, some to a greater degree than others. (See Appendix II.) An illustration of this is seen in the way Nature isolates and throws out an artificial wall round the foci of tubercle so imprisoned, killing them off, the focus often ending in a caseating or calcifying degenerate mass, and all that remains to show what has taken place may only be a cicatrix. Should the bacilli, however, extensively invade the lungs, these foci interfere with the circulation and respiration, and a general toxæmia with extreme depression follows, giving an excellent opportunity for other bacteria to gain a footing. They in turn multiply rapidly, the patient in the end dying from acute toxæmia largely due to a secondary invasion. When an animal is very extensively diseased with advanced tuberculosis, we know tuberculin

may give no reaction. Might this fact not be partly due to the probable condition that all tubercle bacilli have been killed off, not only by their own toxins, but by the toxins and other poisons from the secondary invasion ; and that the animal at this stage is not only *not* suffering from a tuberculosis in the strictest sense, but from an acute specific disease due to the secondary bacterial invasion ?

On looking through the whole symptomatology of tuberculosis, one must confess there does not exist a single diagnostic manifestation which may be taken as proof positive of the presence of this disease. There are, however, several symptoms invariably present, which, if weighed up singly and interpreted collectively (particularly if a process of what might be called symptomatic elimination is adopted also), will give to the trained practitioner strong proof upon which to base his conclusions.

The clinician who relies upon a given set of orthodox symptoms, but fails to interpret minor details even if they be unorthodox, is more likely to make errors of diagnosis than the practitioner who embarks upon his clinical inquiry with a more open mind.

For purposes of diagnosis and descriptive convenience, we propose to divide tuberculosis according to the systems and organs it affects, not forgetting in practice, however, that more than one and even all the systems may be affected at the same time and in the same animal:

1. Tuberculosis of the respiratory system.
2. Tuberculosis of the alimentary system.
3. Tuberculosis of the genital system.
4. Tuberculosis of the nervous system.
5. Tuberculosis of the lymphatic system.
6. Tuberculosis of the mammary system.

Tuberculosis of the Respiratory System.

When a primary invasion of tubercle bacilli takes place, probably through inhalation, an irritation of the bronchial

and tracheal mucosæ follows; and the first indication to the ordinary observer of anything being amiss is a short, dry, strong, and intermittent cough. The animal at this stage may look well, feed well, and give, if a milch cow, the usual quantity of milk; in fact, we have even noticed an increased supply of milk at this stage, probably due to an increase of opsonins thrown into the blood-stream. As time goes on the cough becomes more pronounced, is more persistent, and not so dry; and also there is an expectorate, but, as this is usually swallowed, it is difficult to detect. One can, if standing on the left side of the patient, however, often see it pass along the gullet as a bolus after a fit of coughing.

There may also be signs of pain caused by the act of coughing, as evidenced by rigidity of the costal muscles and the emission perhaps of a grunt. The animal now begins to lose in condition. The appetite may be good, and yet the patient does not seem to thrive as she did formerly, or the appetite may be bad, with resultant falling away of flesh. The respiratory sounds may, and usually do, reveal a variety of conditions. Vesicular murmurs may be either increased or diminished, or even absent. Prominent bronchial blowing sounds may be noticed. There may also be pronounced crepitus over the whole pulmonary area. There are very decided dry or moist râles, which are materially increased on exercise. In pulmonary cases of some standing, digestion becomes impaired, this doubtless being due to the process of auto-intoxication primarily depressing the vagus and leading to general malnutrition, etc. Fermentative changes now go on in the alimentary canal, as evidenced by eructation of gas and foul-smelling fæces. If the mediastinal and prebronchial lymphatic glands are enlarged and diseased—and they usually are—pressure on the œsophagus takes place, rumination is interfered with, and eructation of gas from the rumen is a prominent symptom. When one sees this symptom prominently in a milch cow with an intermittent and troublesome

cough, wasting in flesh, and general symptoms of pulmonary lesions, grave suspicions of pulmonary tuberculosis should be aroused. Where the pharyngeal and laryngeal glands become involved, difficulty in swallowing, stertorous respiration, protrusion of the nose, and fulness in these regions is noticed. These may burst at intervals and lead to intermittent discharges from the nose and the temporary remission of the symptoms.

Percussion may reveal little except patchy areas of dulness. If pleurisy is present, palpation between the ribs makes the animal grunt or groan.

When the pleura is acutely involved, as it invariably is when extensive pulmonary disease exists—the visceral pleura through continuity, and the parietal through contiguity—friction sounds may be detected, particularly in the early stages, although not always.

As time goes on the sounds disappear, and where large growths exist, dulness over these areas is present. The coat in these latter stages is dry and staring, the skin being covered with yellow, scaly dandruff; the patient does not lick herself; the appetite remains in abeyance; rumination and lactation are both suppressed; and persistent fermentative diarrhœa is greatly in evidence. The eye sinks, and a yellow tinged discharge is often seen from the inner canthus. The muzzle is dry and cold, and the discharges on it are not licked off as in a healthy animal. The respiration becomes shallow, the cough weakens, the extremities are cold, and the animal, in a state of persistent and progressive emaciation, collapses, is unable to rise, death taking place from exhaustion, consequent upon a general toxæmia and inanition.

Tuberculosis of the Alimentary System.

Extensive peritoneal lesions may exist, and yet the animal shows no sign of derangement, the condition being often secondary to infection from some other centre. Pressure in an inward and forward direction with the right hand

under the false rib will sometimes assist the clinician in detecting nodules. Examination *per rectum* should be made for visceral or parietal peritoneal proliferations.

Enlargement of the abdominal glands should also be sought for *per rectum*. The subsacral, lumbar, and mesenteric glands can all be felt if diseased and enlarged. To the feel they are hard nodules, which do not fluctuate on pressure.

The liver is often involved, and frequently attains to a great size. It can be felt when greatly enlarged over the hepatic region, with dulness on percussion. The intestines may be extensively diseased, and yet the only pronounced symptoms may be persistent diarrhœa, alternated with constipation. The fæces are usually thin and watery, containing gas bubbles suggestive of fermentation. Sometimes pus and even blood or mucus may be present in the fæces.

Tuberculosis of the Generative System.

This is not uncommon in cows, especially in those cases of generalized tuberculosis, and is characterized by a catarrhal discharge from the vulva, which usually emits a disagreeable odour. The patient either does not conceive, or, if she does, abortion takes place before full term is reached. Examination *per rectum* will reveal a distended womb, which may be hard and fibrous. The adjacent lymph glands are enlarged, sometimes enormously, and are hard and lumpy to the touch, somewhat like bunches of grapes.

Tuberculosis of the Nervous System.

The diagnosis of tuberculosis of the brain or its meninges is by no means easy, but when one is treating or examining a patient whose symptoms are suggestive of tuberculosis—especially generalized tuberculosis—and cerebral disturbances manifest themselves, one may safely suspect tubercular infection of that organ. The symptoms most commonly noticed are as many as they are varied. The

animal, in an advanced case, may have a staggering gait. This must not be confused with the rocking motion due to weakness and inertia; epileptiform movements may also be noted—the animal may shake its head, or hold it to one side. Blindness may be total or partial; one ear may be drooped. The animal may be excited and nervous, or the opposite, much depressed, and semicomatose; muscular tremors may also be present, and there may be partial or complete paralysis.

Tuberculosis of the Lymphatic Glandular System.

When the disease exists in any organ or organs, the neighbouring lymphatic glands are always more or less involved. These glands sometimes swell to a great size. Where the prebronchial and mediastinal lymphatic glands are infected and enlarged, as we have seen, pressure upon the gullet interferes with deglutition, rumination, and consequently digestion. The mesenteric glands also being diseased must interfere with assimilation, and can sometimes be diagnosed through manipulation *per rectum*. When the laryngo-pharyngeal glands are involved, distension of that region is noted; also difficulty in swallowing, and, may be, stertorous breathing, with a more or less virulent nasal discharge, protrusion of the nose, etc. These glands we have often found, by passing into their centres a small, sharp lancet, to undergo rapid calcareous degeneration, which, if the lancet is rotated, demonstrates a distinct gritty feel, giving good diagnostic proof of the nature of the condition.

The prescapular gland may become an enormous size, and show itself as a floating tumour. When this growth has also been cut into prior to or after removal, the same calcareous changes often present themselves.

The precrural and inguinal glands become often involved, and in making a clinical examination should be looked for and manipulated—in fact, enlargement of any of the lymphatic glands affords important assistance

to the practitioner who may be called upon to diagnose a case of tuberculosis.

Tuberculosis of the Udder.

This condition may appear as a single nodule, located in one quarter, giving, when manipulated, a feeling that the surface is irregular, or it may be composed of a collection of nodules, giving the mass considerable dimensions and surface irregularity. These growths are hard, firm, and not painful when pressed upon.

When the growth is confined to one quarter, the adjacent healthy parenchyma of the gland often atrophies, the nodule thereby assuming a more or less pendulous condition.

Where the udder is extensively involved, the lymphatic glands on the supero-posterior aspect are enlarged so much that they can often be seen and invariably felt. The progress of the disease is usually slow, and in some cases a kind of parenchymatous mammitis develops, due perhaps to some form of secondary infection taking place.

The udder may show the nodules for months, and yet to all appearances the milk may be quite normal, and upon careful examination no bacilli may be detected. They may, however, be present, and failure to detect them may be due to the fact that they are eliminated in limited numbers. On the other hand, the organ may appear to be quite healthy, and yet tubercle bacilli will be found in the milk, evidently coming from some infective centre in the system. Also it may be well to note that the udder in these cases perhaps harbours the disease in an invisible form, thereby accounting for the milk contamination. At some period, however, an alteration in the quality of the milk will take place. As time goes on it will become thin and watery, and blue in colour; later it will curdle, and then a mixture of clot and dirty light red water is all that can be milked off, and finally nothing more than a dirty grey or greyish-red watery fluid appears.

Methods of collecting the Materia Morbi with a View to identify the Bacilli of Tuberculosis.

When one is called upon to make a *bacteriological examination* of a case of tuberculosis, one must collect morbid material reasonably suspected of harbouring the bacilli. In the case of pulmonary tuberculosis the sputum is chosen.

In those animals which do not expectorate, considerable difficulty is experienced in collecting the sputum, and various methods have been adopted. Tracheotomy has been recommended low down in the windpipe, and a swab of cotton-wool inserted to remove the bronchial secretion, or a trocar may be inserted between the tracheal rings, and a feather passed through the cannula; the animal coughs, and the sputum is lodged on it and withdrawn. Others recommend covering the nose and mouth with a clean sheet; the animal is then made to cough, and the expectorate will be found lodged on the cloth. The writer finds withdrawal of the tongue, previously covered with a handkerchief to prevent it from slipping from the hand, and pressing sharply the larynx to make the animal cough, expels the discharge, which can be collected into a small plate, easily sterilized by previous boiling. In some cases all we find necessary is to withdraw the tongue as far out of the mouth as possible and make the animal cough. The expectorate will then usually lodge on the roof of the mouth or soft palate. The flow of saliva will in a minute or so dislodge the expectorate, and if the nose is now depressed the saliva will float it on, and in dropping from the mouth it can be caught in a sterile vessel.

When a gland or glands are affected, they may either be dissected *en masse*, or a piece may be removed for microscopical examination of the scrapings therefrom. When suppuration is in evidence, aspirating the fluid may answer the purpose.

In the case of *mammary tuberculosis*, the late Professor Walley years ago recommended the use of a harpoon, which is inserted into the suspicious nodule, and a part of

the tissue removed with due aseptic precautions. When the milk is suspected of containing tubercle bacilli, some of it should be stripped from the quarter and discarded. Brittlebank then advises pressing the udder thoroughly, and collecting the milk in a sterile vessel. This should be centrifugalized for fifteen minutes, the supernatant fluid removed, and the sediment examined microscopically.*

Within recent years antiformin and other substances have assisted investigators in the isolation of the bacilli in pathological fluids. It is a disinfectant mixture of the following composition :

Solution I.	Sodium carbonate	12	grammes.
	Chlorinated lime	8	„
	Distilled water	80	„
Solution II.	Sodium hydroxide	15	grammes.
	Distilled water	85	„

Equal quantities of the two solutions are mixed together.

The morbid material containing the bacteria is placed in a centrifugal tube, and antiformin to about 20 per cent. of its bulk is added. The whole solution is then well shaken, plugged, and put in the dark for twenty-four hours, after which it is centrifugalized and the supernatant fluid pipetted off. Normal saline solution is then added and the whole again centrifugalized, the top fluid pipetted off, and a loopful of the remaining sediment is taken up for the purpose of inoculating a serum tube slope.

The antiformin is credited with the power of destroying all non-acid-fast bacteria, dissolving out mucus, corpuscles, fat cells, etc., while the tubercle bacilli are left with all their vitality unaffected. The examination of the fæces or the urine for the detection of the bacilli is by no means satisfactory, and should not be relied upon.

* The author has devised an instrument the object of which is to aspirate the milk from the gland through the teat. In this way many bacilli lodged on or in the mucosæ are sucked up, thereby increasing their number in a given sample of milk.

PLATE XII.

TUBERCLE BACILLI IN SPUTUM. × 1000.
(Hewlett's "Bacteriology.")

To face page 176.

The Bacillus of Tuberculosis.

This bacillus, in conjunction with *B. lepræ*, Johne's bacillus, and certain non-pathogenic bacteria isolated from fæces, hay, milk, butter, and other sources, have common staining and morphological characters, which associate them under one group, known as the "acid-fast group." As the term implies, they possess the power of retaining stains which resist the decolorizing action of strong acids. They do not stain readily with ordinary aniline dyes, but when stained, they become "acid-fast."

The members of this group are all slender rods, non-motile, having neither capsules nor spores, and are Gram-positive.

Their slight variations, morphologically and culturally, and their considerable differences, so far as their pathogenesis goes, serve to differentiate the various species.

The *B. tuberculosis* is a slender rod, frequently bent, with rounded ends. In length it varies from 0·2 to 3·5 μ. It may stain irregularly, giving the bacillus a beaded appearance.

Save for Johne's bacillus, the other bacteria of the acid-fast group are non-pathogenic to animals, and their presence in animal tissues may be defined as accidental. It is probable they are more commonly present as such than we are inclined to believe. In any case, this probable fact should make us the more careful in drawing our conclusions and giving our opinions. Where one is permitted to do so by law, and time for giving an opinion is unlimited, the presence of tubercle bacilli in morbid material can be most satisfactorily settled by the biological test.

Methods for staining the Bacillus of Tubercle.

Ziehl-Neelsen's Method.—The *materia morbi* having been collected with the usual careful precautions to prevent outside bacterial contamination, and subjected to the methods necessary to facilitate the easy finding of the bacteria, as already described, a loopful of the material is taken up by a sterile platinum loop, and spread by the

needle over a grease-free slide. If the film is too thin, it should be allowed to air-dry, and another layer spread on the top of it, this process being repeated if necessary. In the examination of urine or of the watery secretion of a badly diseased udder, this is sometimes needful.

When the film has become dry, it should be passed two or three times through the flame to fix it.

The slide is then placed upon a metal tray underneath which is a lighted spirit lamp, and carbol-fuchsin filtered on to the specimen.

As soon as steam begins to rise from the slide, it should be removed and decolorized by dipping in a 25 per cent. solution of sulphuric acid for three or four minutes. This has the effect of removing the stain from everything except the acid-fast bacilli. The whole film now shows a yellow tinge. It is then washed in water, when a pale red colour returns to it.

Wash in alcohol until no more stain comes away. By doing so the stain is removed sometimes from acid-fast bacilli other than tubercle, if present, which is of course to be desired.

Wash in water, counter-stain in weak methylene blue for one minute. Wash again in water, and then dry and mount.

By this method the acid-fast bacilli are stained red, while the non-acid-fast bacteria, tissue cells, leucocytes, etc., are stained blue after the contrast stain.

Pappenheim's Method. — This method is credited with the power of differentiating between the bacillus of tuberculosis and other acid-fast bacteria.

The specimen is prepared in the usual way, and stained with filtered carbol-fuchsin without heat for three minutes. The stain is now poured off the specimen, and corallin solution applied; excess of the fluid is then poured off and another application made. Three or four applications in all should be used. It is then washed in water, dried, and mounted.

The solution is made thus:

Corallin	1 gramme.
Methylene blue (saturated alcoholic solution)	120 c.c.
Glycerin	20 c.c.

Tuberculin.

In 1890 Koch introduced his presumed cure for human consumption—namely, tuberculin, which is prepared as follows: A flat-bottomed flask containing 4 per cent. glycerin-broth is inoculated with active *B. tuberculosis* over the surface, and incubated at 37° C. for four weeks. At the end of this period a heavy wrinkled growth appears, and in two months the growth is ready for the preparation of the tuberculin.

The contents of several flasks prepared as above are placed in a porcelain evaporating dish on a water-bath, and concentrated to about a tenth of its original bulk, when the glycerin content becomes about 40 per cent.

Several varieties of tuberculin are prepared by other workers, based upon Koch's original principle, and which are used in bovine practice, not as a curative, but as a diagnostic agent.

Tuberculin as used for a Test for Tuberculosis.

Tuberculin has proved of great value in assisting the clinician to diagnose tuberculosis in the lower animals.

The following are the principal methods in use:

The subcutaneous test.

The ophthalmic test.

The cutaneous test.

The Subcutaneous Test.—The subcutaneous injection of tuberculin is by far the most satisfactory means of diagnosis in veterinary practice.

The cervical or scapular region is the usual site for injection. The hair should be clipped over the part, and

tincture of iodine well rubbed into the skin; and a dose of from 2 to 3 c.c., according to size and age of animal, of tuberculin injected. The temperature should be taken at least once before injection, and on the ninth, twelfth, fifteenth, and eighteenth hours after injection.

The animal should be comfortably housed, moderately fed, and in cold weather allowed chilled water only to drink. Animals in season, in advanced pregnancy, or when suffering from other diseases, or with high temperatures, should not be tested until these conditions disappear. It must be remembered, in estimating reactions, that young animals have a slightly higher normal temperature than adults. When the temperature rises 2·7° F. above the average pre-injection temperature, particularly if the rise should go beyond 103·3° F. on the index, one may safely conclude the animal is tuberculous.

In very advanced cases of generalized tuberculosis, one may obtain no reaction whatever to the test; but the appearance clinically of such cases should arouse grave suspicions in the mind of the clinician, which, with a knowledge of the above fact, will prevent him falling into the "no reaction, no tuberculosis" fallacy. On the other hand, an animal may give a diagnostic reaction, and on post-mortem reveal no macroscopical indication of tubercular lesions. Such cases require very careful and systematic investigation, for the infective centre may be so very small as to escape notice. The various organs may be apparently healthy, and a small gland or chain of glands harbour the disease in its mildest form. It is cases such as these that bring the test into bad repute, when, owing to a superficial examination, an infective centre, due to its smallness, is perhaps overlooked.

When an animal shows a persistent high temperature, one must wait for it to fall before the tuberculin may be used. An animal which has been injected with tuberculin, and reacts, ought not to be injected again for at least a month; for until the full effects of the first injection have

fully passed off no reaction is likely to follow a second injection. This fact is sometimes made use of by unscrupulous sellers, who inject their own animals previous to expert examination and injection. This in itself is a strong plea for the limited sale of tuberculin, and its use restricted to the hands of recognized experts.*

The Ophthalmic Test.—Calmette noticed that a few drops of tuberculin applied to the conjunctiva of a man suffering from tuberculosis was productive of conjunctivitis, and Vallée showed that the same condition took place in animals.

A few drops of tuberculin are placed in the conjunctival sac by means of a sterile glass pipette—that used for opsonic work will answer the purpose—and the eyelids closed and gently moved about. When a reaction takes place, the membrana nictitans becomes very red and its border thickens; the other eye in itself acts as a useful standard by which to estimate the degree of reaction taking place in the tested eye. The reaction sets in earlier than by the subcutaneous method, from six to nine hours, and from twelve to twenty-four hours a purulent secretion forms on the inner canthus, which appears as a long, purulent mass drying into a crust, and finally falling off.

Where one gets a slight conjunctivitis followed by a watery or even a mucous flow, these are of no diagnostic value; the discharge must be purulent. A prior injection of tuberculin has no effect upon the reaction in the ophthalmic test. This should prove useful in those cases of unscrupulous tampering, and also in those animals where the temperature is too high to use the subcutaneous test.

The Cutaneous Test.—Pirquet showed that on inoculating a small quantity of tuberculin into the upper cuticle layers of a tubercular person, a local reddening and swelling took place, and Vallée demonstrated that a similar process took

* If, however, from force of circumstances such a long time cannot be allowed, a reaction may be obtained if 3 to 5 c.c. or more of tuberculin is given.

place in animals. The *modus operandi* advised by Vallée is as follows:

The hair is clipped and the skin shaved over the shoulder-blade about the size of a five-shilling piece, and the cutis scarified with a sterile lancet and a 50 per cent. or concentrated solution of tuberculin painted on with a brush.

If the reaction is a positive one, a visible infiltration of the border of the wound, and an œdematous infiltration of the area surrounding the seat of operation, follows, usually reaching its maximum on the second day. In a negative case there is only a slight indication of irritation, due to the direct effect of the lancet. As it is advisable to have a control area, a similar surface of skin should be scarified on the opposite side, and not treated with tuberculin.

Many theories have been put forward with reference to the tuberculin reaction; the processes which bring it about are probably most satisfactorily based upon what is known as "anaphylaxis."

It has been found that injections of many substances other than bacteria and their products cause reactions on the part of the tissues, with consequent production of antibodies. These substances are called "antigens"; an antigen is therefore a tissue stimulant, and the product of the stimulation is an antibody.

Tuberculin may be injected into a healthy animal, and produce no effect whatever; if injected into a tubercular patient, a reaction follows. There must be some great difference in the state of two such animals, and the consequent reaction in the diseased animal is due to the probable fact that it has become sensitized against the bacterial constituents.

This may also explain why one injection of tuberculin prohibits a reaction to a second injection up to a given lapse of time, the body being in a state of anti-anaphylaxis, the sensitizing elements having become exhausted *pro tem.*, and requiring time for their restoration.

When the tuberculin fails to cause a reaction in these

cases of advanced tuberculosis, it is possible, among other reasons, that the system may be in a state of immunity to the proteins of the bacillus.

Biological Test for Tuberculosis.

Where one possesses the necessary licence, and when the question of an extended period is of no moment before an opinion is required, the experimental inoculation of the morbid material into a susceptible subject offers the best means of forming an accurate diagnosis.

The guinea-pig, by reason of its size and susceptibility to tuberculosis, is the animal usually chosen for such an investigation.

Method.—Three guinea-pigs are tested at the outset with tuberculin to assure their freedom from the disease.

If milk is the medium which is being investigated, a quantity should be milked from the diseased quarter and discarded, after which about a pint should be collected, with great care, into a sterile vessel, and poured into sterile tubes and centrifugalized for half an hour at a speed of 2,500 to 3,000 revolutions per minute.

At the end of this time a semi-solid layer of cream will have collected on the top. Under this is a layer of separated milk, thin and watery, and at the bottom a sediment containing cells, débris, and bacteria. Remove the cream clot, which looks like a plug, and with a sterile pipette withdraw all the liquid from the tube, leaving only 1 or 2 c.c. of the sediment. Triturate this sediment thoroughly and add 10 c.c. of normal saline solution, and centrifugalize again for from ten to fifteen minutes. The supernatant fluid should now be pipetted off, and film specimens made of the sediment in order to detect the bacilli.

The cream should also be examined in a similar manner, as bacilli are sometimes found in this medium.

The experimental animals should now be inoculated with about 1 c.c. of the sediment or cream, usually on the inner

aspect of the bend of the knee, with, of course, due antiseptic precautions.

Each animal is weighed daily. At the end of the second week one is killed, and a careful post-mortem examination made. If no tubercular lesions are shown, kill another at the end of the third week, and if the results are negative the third should be kept alive for six weeks, and then killed.

If lesions looking like tuberculosis exist, a careful microscopical examination for the acid-fast bacilli should be made, and this, combined with the macroscopical appearances, forms conclusive proof.

Macroscopical Post-Mortem Lesions in Bovines.

Having made a careful clinical examination of our subject, and suspecting tuberculosis, we subject her to the test (tuberculin), and if she reacts, and more particularly if we have isolated from the *materia morbi* an acid-fast bacillus, we may with safety certify her to be suffering from tuberculosis, and need not be apprehensive—if our examination technique is accurate—that our post-mortem investigation will not substantiate our ante-mortem opinion.

As the term "tuberculosis" implies, this disease is characterized by the formation of tubercles or nodules, which tend to undergo cheesy degenerative changes.

In the early stages these nodules are small, grey, clear growths, but as they become older they take on a yellow appearance, like a piece of cheddar cheese in colour and consistence—caseation. Later these nodules often become gritty in consistence—calcification.

When the respiratory system is affected, the disease shows itself either as a diffused deposition of a large collection of small foci, on section, in the lung parenchyma—miliary tuberculosis—or large centres of purulent, cheesy, or calcareous masses may be distributed through the lung tissues. The mediastinal lymphatic glands in such cases are usually

extensively involved. On the contrary, no *macroscopical* lesions may be present in the lungs themselves, while the glands may be affected.

Extension often takes place to the pleura, and adhesion of the parietal and visceral layers are common. Characteristic grey nodules are also common on the pleural membrane, appearing as grape-like growths, being the condition known among butchers, etc., as "grapes." When the glands of the throat are affected, they appear as circumscribed growths, and when cut into usually emit a grey creamy pus mixed with calcareous deposits which grate against the knife.

The abdominal organs are also often extensively diseased.

The intestinal mucosa, particularly in young calves reared upon tubercular milk, shows ulceration, and in addition, particularly in adult animals, the mesenteric glands become diseased, are enlarged, caseous, and, later, calcareous.

The liver is commonly the seat of tubercular nodules, and sometimes attains to an enormous size. These nodules are generally full of pus.

The spleen, also, and kidneys may be similarly affected.

The generative organs also become involved. In the cow the uterus and Fallopian tubes may contain a huge mass of tubercular nodules, giving it the appearance of a gravid uterus, and the ovaries also may be extensively diseased.

The mammary gland is a common seat of the disease, where it is characterized by the formation of one or more nodular growths affecting one or more quarters. The formation of small tubercles scattered through the parenchyma, and only detected upon section of the gland, is commonly seen in practice. A kind of interstitial mammitis in some cases is set up, in which case the organ becomes enlarged, firm, and fibrous, and cuts with difficulty.

The lymphatic glands in connection with the udder are always involved and caseous when the mamma is diseased.

CHAPTER XVIII

SWINE FEVER

Swine fever is a specific contagious and infectious disease affecting pigs of all ages, and having a world-wide distribution. It is primarily caused by an ultra-visible virus, and characterized by inflammatory and necrotic lesions due to secondary invasions.

This disease, in common with several other diseases where secondary bacterial invasions play an important part, was, up to the year 1904, thought to be due to a bacillus, whereas in point of fact the bacterium then isolated has now been proved to belong to the secondary group.

In that year Dorset and his co-workers, by injecting the blood-filtrate taken from a diseased pig into a healthy animal produced the disease, and settled once and for all the fact that swine fever is caused by an ultra-microscopic virus.

Symptoms.—These are by no means constant, nor are their interpretations always accurate, the former largely depending upon which causative factor predominates.

Where intestinal lesions are the most pronounced, abdominal symptoms will predominate. Where the pulmonary organs are most severely affected, thoracic symptoms will be evident, and where both systems are involved, a complexity of symptoms will be seen.

In the *septicæmic form* the disease usually runs a very rapid and equally fatal course. In this condition the animal isolates itself, refuses food, and lies down constantly. The bowels at first are constipated, but this soon

gives way to diarrhœa. The fæces often contain blood and mucus.

Vomition may be present, the vomit often containing mucus, bile, or even blood.

Acute purulent inflammation of the conjunctiva is also commonly seen, whilst the temperature is usually high.

In the *abdominal form* the animal isolates itself, refuses its food, lies about, usually hidden in a dark corner.

The bowels, constipated at first, become replaced with diarrhœa, the fæces being very fœtid. Emaciation rapidly takes place; vomition as a symptom may be present, and the vomit is often mixed with bile.

The mucosæ of the mouth and pharynx are inflamed, and are often covered with a tenacious exudate of a greyish-yellow colour. Invariably there is difficulty in deglutition, and sometimes respiration. The temperature is always elevated and the pulse quickened.

The disease may run an acute and rapid course, terminating fatally in a few days. In other cases it may take on an insidious form, and recovery follow in two or three weeks. Such subjects, however, seldom become valuable for commercial purposes.

In the *pulmonary form* the disease is characterized in most cases by symptoms indicative of acute pneumonia. The respirations are hurried and laboured, and sometimes oral, and in hot weather, if the animal is exerted, it will roll over from sheer exhaustion and a sense of suffocation.

To increase the chest capacity and facilitate respiration, the animal will often sit on its haunches. The temperature is usually very high. There is always present a short, husky cough, and a slimy discharge may be noticed coming from the nostrils.

Discoloration of patches of the skin may be present. Emaciation may be very rapid, death soon taking place from exhaustion. On the other hand, the disease may take on a chronic phase. The cough usually persists, with somewhat laboured respiration.

Fœtid diarrhœa may be present, and a chronic arthritis may set in as an additional symptom.

The animal may eventually sufficiently recover to put on flesh, but seldom thrives well.

The skin may be the seat of pronounced lesions located under the abdomen and chest, or on the thighs, and in the region of the anus. These may show various degrees of severity, from red spots to vesicles and crusts.

In somes the animal may be a subject of paralysis.

Bacteriology.—The consensus of opinion seems to favour the views of Garbert and Uhlenhuth that the bacteria in the secondary infections in cases of swine fever are to a greater or lesser degree normal and non-pathogenic inhabitants of the pig. Thus we find the *Bacillus suipestifer* in the intestines, and the *B. suisepticus* in the blood and lungs, and it is only due to the debilitating effects of the ultra-visible virus that the bacteria of secondary infection take upon themselves an aggressive rôle. Be this as it may, we know that most pronounced lesions are set up by the secondary bacteria, which not only adds to the gravity of the disease, but prolongs its course.

It is presumed the filterable virus produces a catarrh of the intestinal follicles, and while in this condition the *B. suipestifer* penetrates and multiplies with great rapidity, producing cellular proliferation and necrosis. At first a nodule forms, followed by an ulcer, and showing a necrosed centre. Absorption of the bacteria takes place into the lymph-stream, and in passing through the mesenteric glands sets up a cellular inflammation, and, later, a dry caseous necrosis.

In like manner the *B. suisepticus* locates itself in the circulation, producing a typical hæmorrhagic septicæmia; or it may invade the lung tissue, causing pneumonia, and in some cases the pleura becomes involved, constituting a typical pleuro-pneumonia.

The *B. suipestifer* belongs to the paratyphus B. group: it is mobile; in culture it appears as long

chains or even threads. In the tissues it is single or in pairs.

Stains aniline dyes, Gram-negative; it is anaerobic and aerobic.

In broth it becomes turbid with a resultant sediment.

On gelatine bluish transparent colonies form and do not liquefy.

On agar bluish colonies also appear.

On potato a brownish-yellow growth takes place.

Minimum temperature 10° C., optimum 37° C., maximum 40° C.

The *B. suisepticus* belongs to the pasteurella group, and appears in the form of short rods, is non-mobile, and aerobic. It stains with the ordinary dyes, and is Gram-negative.

In peptone broth the medium becomes cloudy.

On gelatine the colonies appear like dewdrops, at first transparent, but later opaque.

On agar they grow with a whitish colour, very tough in consistency, and firmly adherent to the medium.

Minimum temperature 15° C., optimum 27° C., maximum 35° C.

Macroscopical Post-Mortem Lesions.

When swine fever is investigated in its septicæmic form, hæmorrhages occur on the serous and mucous surfaces, and, in addition, serous effusions take place. The lymphatic glands are swollen and infiltrated, and also the parenchyma of such organs as the spleen, liver, and kidneys.

In the alimentary form the intestines are most commonly and acutely involved, and particularly the cæcum and large intestines. The mucosæ of the colon shows round ulcers, yellow or dark grey in colour, and particularly round the ileo-cæcal valve, and extension of similar ulcers may take place to the adjacent small intestines. In the less acute cases small nodules may take the place of ulcers. These stand out from the surface, and constitute the so-

called "buttins" of swine fever. In acute conditions a typical gastro-enteritis with petechial hæmorrhages may be noted.

In some cases necrosis of the mucosæ over a large area of the large bowels is pronounced, giving the surface a dirty greyish-yellow appearance.

The lymphatic glands are extensively involved, infiltrated, and often hæmorrhagic, and, later, patchy necrosis is seen.

On the pharyngeal mucosæ hæmorrhagic patches with ulceration and necrotic areas are noted, and the same conditions may be seen in the larynx and the root of the tongue.

Where the pulmonary organs are involved, we find a typical croupous pneumonia, and in advanced cases multiple necrotic centres are distributed through the parenchyma of the lungs. In addition, a typical pleurisy may be present, which tends to become plastic, and sometimes pericarditis is noticed.

Sero-Therapy.

It is obvious that so long as the bacterium of swine fever remains undetected so long will it be impossible to make a prophylactic or curative vaccine. To prevent or cure the disease we must therefore fall back upon serum-therapy.

As far back as the year 1897 Preisz obtained the blood from a pig which had recovered from a natural attack of swine fever. From this he made a serum, injecting 10 c.c. each into thirty pigs, and produced a degree of immunity. These thirty pigs were placed together with thirty healthy pigs and with a few affected ones. Eighteen of the inoculated pigs sickened and nine died, while all of the thirty control pigs died.

Since that period great improvement in the technique has taken place, and to-day on the Continent very great value is placed upon the serum treatment for swine fever both as a prophylactic and as a cure.

The writer has recently had the privilege of visiting the State Serum Institute at Rotterdam, and by the courtesy of the Director—Dr. Poels—a very full and detailed description of the *modus operandi* of swine-fever serum was gone into, which is briefly as follows:

Four young pigs about eight weeks old are each injected with 2·6 c.c of defibrinated virulent blood. When the disease is established, they are killed and their blood collected, the serum of which is used to hyper-immune an adult pig. Into this pig 1 c.c. of virulent serum with 10 c.c. of immune serum is injected.

14 days later	1 c.c.	virus,	100 c.c.	immune serum.
14 „	„		200 c.c.	„
14 „	„		„	„
14 „	„		„	„
14 „	„		„	„

The pig in all has now had 900 c.c. of immune serum, and is hyper-immune. Dr. Poels emphasized the interesting fact that pig serum can only produce antibodies against swine fever, and that horse serum is quite useless. The drawback to this is obvious. The pig being a small animal a limited quantity of serum can only be obtained at a reasonable price.

The hyper-immune pig (such pigs, by the way, must be possessed of long tails) is now bled to procure the immune serum. After thoroughly disinfecting the tail and buttocks, the animal is placed in a kind of stocks to keep it in a fixed position. The tail is then cut at the end, and the stump placed in a glass tube with a capacity of 200 c.c., and 700 to 1,000 c.c. of blood collected; seven days later the operation is repeated. As a rule seven to fifteen bleedings—the latter, if the tail is a long one—can be obtained from one pig. The pig is then killed by bleeding, and from 5 to 7 litres of serum is collected. The serum thus

collected from the tail and throat is mixed together, a preservative added of the following:

Ac. carbolic.	...	...	5·5 c.c.
Glycerine	...	...	20·0 c.c.
Aquæ dest.	...	...	74·5 c.c.

100 c.c. of the above is added to 1 litre of serum, and is placed in 10, 20, 30 c.c. bottles and sealed ready for use.

The dose for an adult pig is from 10 to 30 c.c.

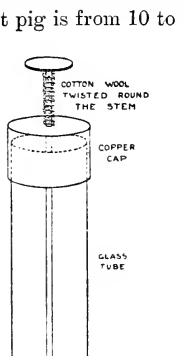


Fig. 37.—Vessel used at the Serum Institute for collecting Blood from the Tail of an Immune Pig.

Pigs from five to ten days old receive 5 c.c. The serum is injected subcutaneously behind the ear. In those cases where a pig is being hyper-immunized and large quantities of serum is used, one-half is injected behind one ear and the other half behind the other.

When pigs are injected with the immune serum, immunity is only conferred for a period of three weeks. This period, of course, is of too short a duration to be of any practical use. Should, however, the pig after injection

become infected with the virus in a *natural* manner, that pig is immune for life.

It is therefore imperative for the successful immunization of a herd of pigs that the virus of infection must be always present, and without it no permanent immunity can be conferred.

The writer inquired of Dr. Poels what his experience was of *artificial* infection in event of his failing to have the means of natural infection—*i.e.*, a diseased pig—at hand, and his answer was, it was not serviceable, but that he was experimenting by feeding pigs with the natural virus, and was hopeful in time to overcome the present difficulty of no natural infection, no immunity.

The procedure adopted in Holland is this: When a pig shows symptoms of swine fever, the whole herd is injected with serum. The badly affected pigs are separated from the slightly affected ones, and the experience of those in authority is very favourable to serum therapy. In Dr. Poels' words, "The slightly affected always recover; the very badly not often."

Dr. Von Velseim's experience is that after the yearly injection of 2,000 pigs, 6 to 8 per cent. die.

In Holland there are no restrictions placed upon swine fever areas, no compulsory notification, and no slaughter.

APPENDIX I

SERO-VACCINE THERAPY IN HOLLAND AND OTHER COUNTRIES

Sero-vaccine therapy has made greater advances in Continental countries than it has in Great Britain, for the obvious reason that the respective Governments of these countries recognize what a valuable asset the principle of the treatment is, and freely place funds at the disposal of experts, who give their time and energy to the advancement of the science. Those in authority see the great gains agriculture obtains from scientifically applied preventive and curative medicine.

Thus we find institutions erected, laboratories fitted up and efficiently staffed by excellent men well paid, whose duty it is to study animal diseases, carry out research work, and adopt scientific measures in the treatment of animal diseases.

Such an institution is the State Serum Institute of the Dutch Government, which is doing a very great work for the agriculturist in particular, and the whole State in general.

The sceptic might say this is all very true, but sero-vaccine therapy is a comparatively new method of treatment with few disciples, and it has not withstood the test of time.

In an exact sense this is not so, for all animals and human beings themselves are by Nature vaccine therapeutists —nay, more, this fact is shrouded in antiquity, for it dates back to the period of the development of the first pathogenic microbe. We know we are daily admitting into our bodies disease-producing germs through ingestion and inhalation, through abrasions of the skin and mucosæ, and we are continually manufacturing in our economies protective vaccines to fight against these deadly bacteria. If it were not so, it is appalling to think what the conse-

quences would be—most probably complete annihilation of the whole human and animal race.

The vaccine therapeutist is following out Nature's own mode of treatment, which she has been adopting for generations, and it is only when, for a complexity of reasons, she fails or appears to fail that the science and art of vaccine therapy need to be called in; and so by stimulating the depressed antibodies and strengthening them to overcome the bacterial invasion, she restores an infected and diseased animal to a healthy and normal condition.

Again, the sceptic may look and obtain food for thought at the rapid progress made by those Continental institutions.

Take, for example, the Serum Institution at Rotterdam, and what do we find? It was established in the year 1904, and could only claim all told 15 horses supplying serum. To-day there are 150 horses, 50 cattle, and 100 pigs used in the production of serum.

During this period 23,000,624 animals have been treated by serums and vaccines supplied from the institution. Such figures as these speak for themselves and prove conclusively that the institution and the principle of treatment it adopts has become a valuable asset and a national necessity.

At the present time the following sera and vaccines are in daily use :

1. Serum and vaccine against abortion in mares and cows.
2. „ „ anthrax.
3. „ „ quarter-evil.
4. „ „ swine erysipelas.
5. Serum against contagious pneumonia in horses.
6. „ strangles.
7. „ tetanus.
8. „ foot-and-mouth disease.
9. „ white scour in calves.
10. „ septic pleuro-pneumonia in calves.
11. „ metritis.
12. „ mammitis.
13. „ swine fever.
14. „ fowl cholera.

The institute is divided into twenty-four sections, the most important of which are—

1. The preparation of serum.
2. The preparation of vaccines.

3. The control of serums and vaccines.
4. The examination of the immunity.
5. Inquiry into diseases affecting horses, cattle, sheep, swine.
6. Inquiry into unknown causes of diseases.
7. The examination of milk.
8. The examination of tuberculosis of cattle.
9. The examination of secret remedies.
10. The chemical section.

The institution is presided over by that distinguished veterinary surgeon, Dr. Poels, and an able staff numbering about fifty.

APPENDIX II

ADDITIONAL NOTES

The Bacteriology of "Whistling and Roaring."

On p. 157 we discussed incidentally the advisability of using prophylactic sero-vaccines in cases of influenza and strangles, with the view not only of saving life and shortening the attack, but also preventing the occurrence of roaring as a sequel. It may not, therefore, be out of place here to briefly discuss a phase of the etiology which, so far as we know, has not obtained the prominence it demands or the investigation it deserves.

The anatomical arrangement of the horse's larynx with its peculiar nerve-supply, the general conformation of the neck and chest, the exerting character of the work hunters and blood horses are called upon to perform, must ever remain *exciting causes* in the production of the conditions known as "whistling" and "roaring" proper. The *actual cause*, we venture to think, is attributed to the specific action of bacteria and their products in the majority of cases.

The inflammatory sore throat one sees in practice is due to bacteria. The nasal, laryngeal, and pharyngeal catarrhs are bacterial in origin; influenza, bronchitis, pneumonia, and pleurisy are all caused by specific micro-organisms, and we know roaring is a common sequel to any or all of these conditions. Horses also are liable to become grunters following upon these conditions, and grunting invariably is the precursor of roaring.

The fact that a horse grunts when "ribbed" suggests a general derangement of the nervous system, the nature of this derangement being hyper-sensitiveness, owing to malnutrition and a consequent probable toxæmic neuritis.

In addition to these constitutional disturbances we must also look to local conditions. There we find the laryngeal

mucosæ acting as an excellent medium upon which a rich bacterial growth is taking place. Consequent upon this growth the whole surface is bathed with endotoxins and exotoxins derived from the pathogenic bacteria.

Absorption of these takes place into the deeper structures, including the muscular elements of the larynx extending to the nerve endings, the nerve fibres, and nerve trunks themselves, and, according to the structures involved, we now find a specific mucositis, cellulitis, myositis, and neuritis set up. This is followed by degenerative changes; the trophic functions of the recurrent nerve are soon interfered with, and muscular atrophy is the result. These conditions are aggravated by the strain placed upon the already depressed set of muscles, and the degenerative changes which have already taken place in the nerve are increased by the jerking of the aorta round which the nerve passes.

The muscles on the right side of the larynx are subject to the same degenerative causes, and no doubt temporary atrophic changes take place, but owing to the different disposition of the nerve-supply, the trophic interference is not called into play.

Undoubtedly there are cases where the mildness of the attack has only been capable of producing slight degenerative and organic changes both in the muscles and the nerve, and, given time, a complete recovery follows. This may explain a very common fact we see in practice—namely, an animal recently recovered from a catarrh, etc., may be a pronounced whistler, and even a roarer, and, in time, with exercise, good food, and conditioning, may become quite sound in his wind, the obvious conclusion being the retrogressive changes were so slight that Nature was able to bring about a complete restoration before hopeless organic chaos took place.

If these foregoing conclusions are correct, it is obvious, if we succeeded in preventing bacterial invasions to the upper air passages, we should hear very little of roaring and whistling in horses, but such a task, of course, would scarcely be practicable. We can, however, reduce the severity of a bacterial attack, and in many cases even prevent its development altogether by sero-vaccine therapy.

It may be pointed out heredity plays an important part in the production of roarers and whistlers, and with this, in a limited sense, we agree. Conformation is undoubtedly

hereditary, and we know horses with long necks, narrow intermaxillary spaces, long backs, narrow chests, etc., are all liable to become roarers. But this point does not interest us here so much as the fact that some animals inherit in a pronounced degree a defective resisting-power to bacterial invasion.

We know some horses are more predisposed to the disease-producing effects of the virus of strangles and influenza than others, one animal suffering slightly or not at all, and another severely. The protective antibodies of the one are more resistant than in the other, and we are of opinion that it is an inherited deficiency of the antibodies which largely accounts for the practical conclusions of roaring appearing most often in certain strains, and from one generation to another.

The Drain of Antibodies through the Milk-Supply as a Probable Predisposing Cause of Tuberculosis.

On p. 168 we stated our belief that every animal has the power of resisting the bacilli of tubercle, some to a greater degree than others. In such a complex mechanism as the animal body, provided as it is with a variety of protective bodies, many of which are little understood, it may be that more than one antibody is deficient, and it is possible a variety of forces are at play to account for this deficiency.

We know milch cows are more prone to tuberculosis than any other class of our domesticated animals. Moreover, some strains appear to be more predisposed to the disease than others, and we invariably find the deeper the milker the greater the predisposition to tubercle.

The pertinent question may here be asked, Why should this be ?

The depletion of the system through the milk-supply undoubtedly accounts for a lowering of the vital forces, and thereby making the animal a fit subject for bacterial invasion. But why should the physiological act of lactation, and even excessive lactation, predispose the animal to tuberculosis ? Such a normal act should not disturb the metabolism, and make the subject a suitable medium for bacterial growth.

It is, therefore, not enough to say the vital forces are lowered by milking. There must also be a drain upon the

bacteriotropic and bacteriolytic elements of the blood, which find their outlet by the milk stream. We know opsonins are to be found in the urine and in the perspiration, and why not in the milk, which, in addition to being a secretion, is also an excretion up to a point. And if this is true of opsonins, why not the other antibodies of the blood also? A system so depleted of its protective antibodies must therefore be liable to bacterial attacks, and more so if these antibodies are specific to the causative bacteria.

We have over and over again noticed cows suffering from tuberculosis in a moderately mild form give a larger volume of milk *per diem* than cows deemed free from the disease, but it would appear to be the quantity is increased at the expense of the quality. These cows are generally in very poor condition, and even in advanced stages of emaciation. Now, if such cows are allowed to become "dry," it is astonishing how the symptoms common to tuberculosis abate, and the animal begins to thrive and often do well, and in time an apparent complete cure follows.

Some practitioners will explain that this is due to a check upon the nutritive material in the form of milk leaving the system and disturbing the metabolism. This may be, and undoubtedly is, true up to a point, but that fact would not in itself sufficiently explain the improvement taking place in the diseased organs. This improvement, we believe, is brought about by a rise in the opsonic index owing to a check upon the outflow of opsonin into the milk stream; and, of course, an increase of opsonin in the system means a more powerful offensive and defensive force attacking the invading bacteria, and bringing about their destruction, and the restoration of the patient to a more healthy standard.

APPENDIX III

WEIGHTS AND MEASURES

(*a*) The initial unit of the Metric System is the metre (m.).

(*b*) The unit of mass is the gramme (g.), which equals the weight of 1 cubic centimetre of water at its maximum density.

(*c*) The unit of the measure of capacity is the litre (l.), which equals the volume of 1 kilogramme of water at its maximum density.

Length.

(μ)	1 micron	=	$\frac{1}{25000}$ inch.
(mm.)	1 millimetre	=	$\frac{1}{25}$ inch.
(cm.)	1 centimetre	=	$\frac{2}{5}$ inch.
(1″)	1 inch	=	25 millimetres.

Mass.

(mg.)	1 milligramme	=	0·01543 grain.
(g.)	1 gramme	=	15·4323 grains.
(kg.)	1 kilogramme	=	2 pounds $3\frac{1}{4}$ ounces.
(lb.)	1 pound (Avoirdupois)	=	453·592 grammes.
(oz.)	1 ounce „	=	28·35 grammes.
(gr.)	1 grain „	=	0·0648 gramme.

Volume or Capacity.

(c.c.)	1 cubic centimetre	=	16·9 minims.
(l.)	1 litre	=	35·196 fluid ounces.
($\bar{3}$)	1 ounce	=	28·42 cubic centimetres.
(O.)	1 pint	=	568·34 cub. centimetres.

To convert	grammes into grains - -	× 15·432.
„	grammes into ounces - -	× 0·03527.
„	kilogrammes into pounds -	× 2·2046.
„	cubic centimetres into ounces	× 0·0352.
„	litres into fluid ounces -	× 35·2.
To convert	grains into grammes - -	× 0·0648.
„	ounces into grammes - -	× 28·35.
„	ounces into cubic centimetres	× 28·42.
„	pints into litres - - -	× 0·568.

THERMOMETRIC SCALES

On the Fahrenheit scale the freezing-point of water is 32°, and boiling-point 212°; on the Centigrade scale the freezing-point is at zero, and the boiling-point at 100°.

The following formula is used for converting Fahrenheit into Centigrade and *vice versa*:

F. into C. = (degree — 32) ÷ 9 × 5.
C. into F. = degree ÷ 5 × 9 + 32.

Appended are two complete tables:

TABLE FOR THE CONVERSION OF FAHRENHEIT INTO CENTIGRADE DEGREES

Fahr.	Cent.	Fahr.	Cent.	Fahr.	Cent.	Fahr.	Cent.
32	0·0	78	25·6	123	50·6	168	75·6
33	0·6	79	26·1	124	51·1	169	76·1
34	1·1	80	26·7	125	51·7	170	76·7
35	1·7	81	27·2	126	52·2	171	77·2
36	2·2	82	27·8	127	52·8	172	77·8
37	2·8	83	28·3	128	53·3	173	78·3
38	3·3	84	28·9	129	53·9	174	78·9
39	3·9	85	29·4	130	54·4	175	79·4
40	4·4	86	30·0	131	55·0	176	80·0
41	5·0	87	30·6	132	55·6	177	80·6
42	5·6	88	31·1	133	56·1	178	81·1
43	6·1	89	31·7	134	56·7	179	81·7
44	6·7	90	32·2	135	57·2	180	82·2
45	7·2	91	32·8	136	57·8	181	82·8
46	7·8	92	33·3	137	58·3	182	83·3
47	8·3	93	33·9	138	58·9	183	83·9
48	8·9	94	34·4	139	59·4	184	84·4
49	9·4	95	35·0	140	60·0	185	85·0
50	10·0	96	35·6	141	60·6	186	85·6
51	10·6	97	36·1	142	61·1	187	86·1
52	11·1	98	36·7	143	61·7	188	86·7
53	11·7	99	37·2	144	62·2	189	87·2
54	12·2	100	37·8	145	62·8	190	87·8
55	12·8	101	38·3	146	63·3	191	88·3
56	13·3	102	38·9	147	63·9	192	88·9
57	13·9	103	39·4	148	64·4	193	89·4
58	14·4	104	40·0	149	65·0	194	90·0
59	15·0	105	40·6	150	65·6	195	90·6
60	15·6	106	41·1	151	66·1	196	91·1
61	16·1	107	41·7	152	66·7	197	91·7
62	16·7	108	42·2	153	67·2	198	92·2
63	17·2	109	42·8	154	67·8	199	92·8
64	17·8	110	43·3	155	68·3	200	93·3
65	18·3	111	43·9	156	68·9	201	93·9
66	18·9	112	44·4	157	69·4	202	94·4
67	19·4	113	45·0	158	70·0	203	95·0
68	20·0	114	45·6	159	70 6	204	95·6
69	20·6	115	46·1	160	71·1	205	96·1
70	21·1	116	46·7	161	71·7	206	96·7
71	21·7	117	47·2	162	72·2	207	97·2
72	22·2	118	47·8	163	72·8	208	97·8
73	22·8	119	48·3	164	73·3	209	98·3
74	23·3	120	48·9	165	73·9	210	98·9
75	23·9	121	49·4	166	74·4	211	99·4
76	24·4	122	50·0	167	75·0	212	100·0
77	25·0						

TABLE FOR THE CONVERSION OF CENTIGRADE INTO FAHRENHEIT DEGREES

Cent.	Fahr.	Cent.	Fahr.	Cent.	Fahr.
0	32·0	34	93·2	68	154·4
1	33·8	35	95·0	69	156·2
2	35·6	36	96·8	70	158·0
3	37·4	37	98·6	71	159·8
4	39·2	38	100·4	72	161·6
5	41·0	39	102·2	73	163·4
6	42·8	40	104·0	74	165·2
7	44·6	41	105·8	75	167·0
8	46·4	42	107·6	76	168·8
9	48·2	43	109·4	77	170·6
10	50·0	44	111·2	78	172·4
11	51·8	45	113·0	79	174·2
12	53·6	46	114·8	80	176·0
13	55·4	47	116·6	81	177·8
14	57·2	48	118·4	82	179·6
15	59·0	49	120·2	83	181·4
16	60·8	50	122·0	84	183·2
17	62·6	51	123·8	85	185·0
18	64·4	52	125·6	86	186·8
19	66·2	53	127·4	87	188·6
20	68·0	54	129·2	88	190·4
21	69·8	55	131·0	89	192·2
22	71·6	56	132·8	90	194·0
23	73·4	57	134·6	91	195·8
24	75·2	58	136·4	92	197·6
25	77·0	59	138·2	93	199·4
26	78·8	60	140·0	94	201·2
27	80·6	61	141·8	95	203·0
28	82·4	62	143·6	96	204·8
29	84·2	63	145·4	97	206·6
30	86·0	64	147·2	98	208·4
31	87·8	65	149·0	99	210·2
32	89·6	66	150·8	100	212·0
33	91·4	67	152·6		

APPENDIX IV

(8798.)

ORDER OF THE BOARD OF AGRICULTURE AND FISHERIES.

(*Dated 13th February*, 1913.)

TABLE OF CONTENTS.

TUBERCULOSIS ORDER OF 1913.

The Board of Agriculture and Fisheries, by virtue and in exercise of the powers vested in them under the Diseases of Animals Acts, 1894 to 1911, and of every other power enabling them in this behalf, do order, and it is hereby ordered, as follows:

Interpretation.

1. In this Order—

"The Board" means the Board of Agriculture and Fisheries:
"Local Authority" means a Local Authority for the purposes of the Act of 1894:
"The Act of 1894" means the Diseases of Animals Act, 1894:
"Inspector" includes Veterinary Inspector:
"Bovine animal" means a bull, cow, ox, heifer, or calf:
"Cow" includes a heifer that has calved:
"Milk" includes cream and separated or skimmed milk.

Other terms have, where the context so permits, the same meaning and scope as in the Act of 1894.

Notice of Disease.

2.—(1) Every person having in his possession or under his charge

(i.) any cow which is, or appears to be, suffering from tuberculosis of the udder, indurated udder, or other chronic disease of the udder; or
(ii.) any bovine animal which is, or appears to be, suffering from tuberculosis with emaciation

shall without avoidable delay give information of the fact to a constable of the police force for the area wherein the animal is, or to an Inspector of the Local Authority, and the constable or Inspector shall transmit the information to the Local Authority, who, if not themselves the Sanitary Authority, shall inform that Authority.

(2) The person in possession or having charge of the animal shall forthwith take such steps as are necessary to

secure compliance with Article 9 (*Precautions to be adopted with Respect to Milk, etc.*) and Article 10 (*Detention and Isolation of Suspected Animals*).

Notification of Disease by Veterinary Surgeons.

3.—(1) A veterinary surgeon or veterinary practitioner who in his private practice is employed to examine any animal, and is of opinion that the animal, if a cow, is suffering from tuberculosis of the udder, indurated udder, or other chronic disease of the udder, or, if a bovine animal, is suffering from tuberculosis with emaciation, shall with all practicable speed give notice of the existence or suspected existence of such disease to an Inspector of the Local Authority, who shall transmit the information to the Local Authority, who, if not themselves the Sanitary Authority, shall inform that Authority.

(2) A veterinary surgeon or veterinary practitioner who under and in accordance with this Article gives notice of the existence or suspected existence of disease to an Inspector of the Local Authority shall be entitled to receive from the Local Authority a fee of two shillings and sixpence for each notification.

(3) Where two or more animals are examined by a veterinary surgeon or veterinary practitioner on the same premises and at the same time, and are found to be diseased, one fee only shall be payable to him under this Article in respect of the notification of the existence or suspected existence of disease in such animals.

Inspection and Examination of Animals.

4.—(1) Where a Local Authority, by reason of information received under the preceding Articles or otherwise, have reasonable ground for supposing that on any premises in their District there is a cow which is suffering from chronic disease of the udder or giving tuberculous milk, or a bovine animal which is suffering from tuberculosis with emaciation, the Local Authority shall with all practicable speed cause such veterinary examination of the bovine animals on such premises to be made by a Veterinary Inspector as in the opinion of the Local Authority is necessary to ascertain whether any cow thereon is suffering from tuberculosis of the udder or giving tuberculous milk,

or whether any bovine animal thereon is suffering from tuberculosis with emaciation, and for that purpose the Inspector may, with the previous consent in writing of the owner of the animal or of his agent, but not otherwise, apply the tuberculin test to any cow which the Inspector suspects of suffering from tuberculosis of the udder, or of giving tuberculous milk, or to any bovine animal which he suspects of suffering from tuberculosis with emaciation.

(2) For the purpose of such examination, a Veterinary Inspector may at all reasonable hours enter on any part of the premises and examine any bovine animal thereon, and require any cow to be milked in his presence, and may take samples of the milk, and the milk from any particular teat shall if he so require be kept separate, and separate samples thereof shall be furnished.

(3) The Inspector may also take samples of the fæces or urine of any bovine animal on the premises, or of any abnormal discharge from any bovine animal thereon.

(4) The occupier of the premises and the persons in his employment shall render such reasonable assistance to the Inspector as may be required for all or any of the purposes of this Article, and any person refusing such assistance shall be deemed guilty of an offence against the Act of 1894.

(5) The Inspector shall as soon as possible send to the Local Authority a report showing the result of his inspection and examination and of the examination of any sample taken by him. The Local Authority, if not themselves the Sanitary Authority, shall send a copy of the report to that Authority.

(6) If the report of the Inspector as to any animal does not show that it is suffering from tuberculosis of the udder, or giving tuberculous milk, or suffering from tuberculosis with emaciation, the Local Authority shall forthwith give notice in writing to the owner or person in charge thereof that the provisions of this Order relating to precautions to be adopted with respect to milk and detention and isolation of suspected animals have ceased to apply to the animal.

Slaughter of Diseased Animals.

5.—(1) Where a Local Authority are satisfied by the report of the Inspector that in their District there is a cow which is suffering from tuberculosis of the udder, or giving tuberculous milk, or a bovine animal which is suffering

from tuberculosis with emaciation, the Local Authority shall with all practicable speed give notice in writing (in the Form set forth in the Schedule hereto or to the like effect) to the owner or person in charge of the animal and also to the Board, and cause the animal to be slaughtered; provided that if, before the slaughter is carried out, the owner of the animal, or any person on his behalf, gives notice in writing to the Local Authority, or to their Inspector or other officer directed to carry out such slaughter, that the owner objects to the animal being slaughtered under the provisions of this Order, it shall not be lawful for the Local Authority to cause the animal to be slaughtered without the special authority of the Board first obtained; provided also that this special authority shall not be given in the case of any animal valued under this Order at more than thirty pounds, if and so long as the animal is detained and isolated, and the milk (if any) is dealt with in accordance with the provisions of this Order.

(2) If the value of an animal proposed to be slaughtered, as agreed or certified under this Order, exceeds thirty pounds, the Local Authority shall not proceed with its slaughter unless so directed by the Board.

Valuation for Compensation.

6.—(1) Before the slaughter of an animal the Local Authority shall either agree in writing with the owner of the animal the value thereof in its condition at the time of valuation, or if they shall fail so to agree shall cause such value to be ascertained by a valuer appointed by them or appointed on the application of the Local Authority by the Board, but paid by the Local Authority, and such valuer shall give to the Local Authority and to the owner a certificate in writing of the said value.

(2) In ascertaining the value of an animal, regard shall be had to any Act, Order, or Regulation dealing with the sale or use of milk, milk products, or carcases for human food.

(3) The value shall be ascertained both on the basis of the certificate of examination hereinafter required showing that the animal was suffering from tuberculosis, and also on the basis of its not showing that the animal was suffering from tuberculosis, and the amount to be paid for compensation shall depend on such certificate accordingly.

Post-Mortem Examination of Slaughtered Animals.

7.—(1) In the case of every animal slaughtered under this Order, the Local Authority shall cause the carcase, at the time of slaughter or as soon as practicable thereafter, to be examined by a Veterinary Inspector of the Local Authority, or (if so required by the owner or person in charge of the animal before it is slaughtered) by some other veterinary surgeon, who, failing agreement between the Local Authority and such owner or person, shall be nominated by the Board, but paid by the Local Authority.

(2) The Veterinary Inspector or other veterinary surgeon shall at the conclusion of his examination give to the Local Authority and to the owner of the animal a certificate of the result of the examination in the Form set forth in the Schedule hereto or to the like effect.

Compensation.

8.—(1) If the Local Authority fail to carry out the examination required by the preceding Article, or if the certificate of such examination does not show that the animal was suffering from tuberculosis, the Local Authority shall, by way of compensation, pay to the owner thereof a sum equal to the value of the animal as agreed or certified in manner aforesaid, and a further sum of twenty shillings.

(2) If the certificate of the examination shows that the animal was suffering from tuberculosis (not being advanced tuberculosis), the Local Authority shall, by way of compensation, pay to the owner a sum equal to three-fourths of the value of the animal as agreed or certified in manner aforesaid, after deducting from such sum one-half of their reasonable costs of any valuation of the animal by a valuer appointed by the Board, and of any examination of its carcase by a veterinary surgeon other than the Veterinary Inspector.

(3) If the certificate of the examination shows that the animal was suffering from advanced tuberculosis, the Local Authority shall, by way of compensation, pay to the owner a sum equal to one-fourth of the value of the animal, as agreed or certified in manner aforesaid, or the sum of thirty shillings, whichever sum is the greater, after deducting from such sum one-half of their costs of valuation and examination as in the preceding case.

(4) For the purposes of this Order an animal slaughtered under this Order shall be deemed to have been suffering from advanced tuberculosis

(*a*) when there is miliary tuberculosis of both lungs;
(*b*) when tuberculous lesions are present on the pleura and peritoneum;
(*c*) when tuberculous lesions are present in the muscular system, or in the lymphatic glands embedded in or between the muscles; or
(*d*) when the carcase is emaciated and tuberculous lesions are present.

Precautions to be adopted with Respect to Milk, etc.

9.—(1) The milk produced by any cow which is, or appears to be, suffering from chronic disease of the udder or tuberculosis with emaciation, shall not be mixed with other milk until the cow has been examined by a Veterinary Inspector in accordance with the provisions of this Order, and until the owner or person in charge thereof has been notified that this Article has ceased to apply to the cow; and all milk affected by this Article shall forthwith be boiled or otherwise sterilized, and any utensil in which such milk is placed before being so treated shall be thoroughly cleansed with boiling water before any other milk is placed therein.

(2) A Local Authority, or a Veterinary Inspector on their behalf, may by written notice apply the restrictions imposed by this Article to the milk produced by any cow specified in the notice which is suspected of giving tuberculous milk and is being examined under this Order, and such restrictions shall apply accordingly.

Detention and Isolation of Suspected Animals.

10.—(1) Every person having in his possession or under his charge any cow which is, or appears to be, suffering from chronic disease of the udder, or any bovine animal which is, or appears to be, suffering from tuberculosis with emaciation, shall keep the animal isolated as far as practicable from other bovine animals, and also keep the animal in his possession or under his charge, until the animal has been examined by a Veterinary Inspector in accordance with the provisions of this Order and the owner or person

in charge thereof has been notified that this Article has ceased to apply to the animal; provided that the animal may at any time be slaughtered by the owner or person in charge.

(2) A Local Authority, or a Veterinary Inspector on their behalf, may by written notice apply this Article to any bovine animal specified in the notice which is being examined under this Order, and such Article shall apply accordingly.

Suspected Animals in Markets, Fairs, and Sales.

11.—(1) A Veterinary Inspector of a Local Authority may by notice served on the owner or person in charge of a bovine animal exposed in a market, fairground, or saleyard, which appears to him to be

(i.) suffering from tuberculosis of the udder, indurated udder, or other chronic disease of the udder; or

(ii.) suffering from tuberculosis with emaciation,

require the animal to be removed from the market, fairground, or saleyard, to the premises from which it was brought thereto, or, if the owner or person in charge so desires, to any other suitable premises, to be specified in the notice, and thereupon the animal shall forthwith be moved by the owner or person in charge to those premises for the purpose of examination under the foregoing provisions of this Order.

(2) Where the premises to which the animal is required under this Article to be moved are not in the same District as the market, fairground, or saleyard, the Inspector serving the notice shall forthwith send a copy of the notice to the Local Authority of the District in which the first-mentioned premises are situate.

Cleansing and Disinfection.

12. The occupier of any premises on which there has been a cow suffering from tuberculosis of the udder or giving tuberculous milk, or a bovine animal suffering from tuberculosis with emaciation, shall, if so required in writing by an Inspector of the Local Authority, cleanse and disinfect at his own expense, and to the satisfaction of the Inspector, that part of any shed or other erection in which the animal has recently been placed or kept.

Reports to the Board.

13. Every Local Authority and their Inspectors and officers shall send and give to the Board such reports, returns, and information, as to their proceedings under this Order, as the Board require.

Extension of Certain Sections of Diseases of Animals Act, 1894.

14. Tuberculosis shall be a disease for the purposes of the following sections of the Act of 1894 (namely):

Sections nineteen and twenty (*Slaughter in Disease and Compensation generally*);

Section forty-three (*Police*);

Section forty-four (*General Administrative Provisions*); and also for the purposes of all other sections of the said Act containing provisions relative to or consequent on the provisions of those sections and this Order, including such sections as relate to offences and legal proceedings.

Information to be given as to Certain Animals or Animals in Contact therewith.

15. Article 36 of the Animals (Transit and General) Order of 1912 (*Information to be given as to Diseased or Suspected Animals or Animals in Contact therewith*) shall apply to

(i.) any cow which is, or is suspected of, suffering from tuberculosis of the udder or giving tuberculous milk; and

(ii.) any bovine animal which is, or is suspected of, suffering from tuberculosis with emaciation.

Offences.

16. Every person who—

(i.) fails to give the notice required by Article 2 or Article 3 of this Order; or

(ii.) fails to comply with any provision of this Order relating to precautions to be adopted with re-

spect to milk or relating to detention and isolation of animals; or

(iii.) fails to comply with any notice directing removal of an animal from a market, fairground, or sale-yard; or

(iv.) fails to cleanse or disinfect any erection which under this Order he is required to cleanse or disinfect,

shall, according to and in respect of his own acts and defaults, be deemed guilty of an offence against the Act of 1894.

Extent.

17. This order extends to England and Wales and Scotland.

Local Authority to enforce Order.

18. The provisions of this Order, except where it is otherwise provided, shall be executed and enforced by the Local Authority.

Commencement.

19. This Order shall come into operation on the first day of May, nineteen hundred and thirteen.

Short Title.

20. This Order may be cited as the TUBERCULOSIS ORDER OF 1913.

(L.S.) In witness whereof the Board of Agriculture and Fisheries have hereunto set their Official Seal this thirteenth day of February, nineteen hundred and thirteen.

SYDNEY OLIVIER,
Secretary.

SCHEDULE.

FORMS.

TUBERCULOSIS ORDER OF 1913.

Form of Notice of Intended Slaughter.

(Article 5.)

To of .

The Local Authority for the county [borough or burgh] of hereby give notice that they are satisfied that [*insert description of animal*] which is now kept at [*insert description of premises where it is kept, stating parish*] is

*(*a*) suffering from tuberculosis of the udder;
*(*b*) giving tuberculous milk;
*(*c*) is suffering from tuberculosis with emaciation,

and that they propose with all convenient speed to slaughter the animal.

* *Strike out the part which is inapplicable.*

(Signed)

By direction of the Local Authority.

Dated 191 .

NOTE.—If the owner of the animal, or any person on his behalf before the slaughter is carried out, gives notice in writing to the Local Authority, or to their Inspector or other officer directed to carry out the slaughter, that the owner objects to the animal being slaughtered, it may not be slaughtered without the special authority of the Board first obtained.

The compensation payable by the Local Authority is regulated by the Order.

TUBERCULOSIS ORDER OF 1913.

Form of Certificate of Result of Post-Mortem Examination.

(Article 7.)

I, *A.B.*, a Veterinary Inspector of the Local Authority for the county [borough or burgh] of [*or* a veterinary surgeon acting under the Tuberculosis Order of 1913], do hereby certify that my examination of the carcase of [*here describe the animal slaughtered*], which was caused to be slaughtered by the Local Authority for the

county [borough or burgh] of on the
day of 191 , and which animal belonged to
of , does not show that the animal was affected with tuberculosis [*or* shows that the animal was affected with tuberculosis (not being advanced tuberculosis within the meaning of the Tuberculosis Order of 1913)] [*or* shows that the animal was suffering from advanced tuberculosis within the meaning of the Tuberculosis Order of 1913].

(Signed) *A.B.*

Dated 191 .

BIBLIOGRAPHY

Allen : Vaccine Therapy.

Bowhill : Manual of Bacteriological Technique and Special Bacteriology.

Buchanan : Veterinary Bacteriology.

Emery : Clinical Bacteriology and Hæmatology.

Friedberger and Fröhner : Veterinary Pathology.

Hewlett : Manual of Bacteriology.

Hutyra and Marek : Special Pathology and Therapeutics of the Diseases of Domesticated Animals.

Jowett : Notes on Blood-Serum Therapy.

Law : Veterinary Medicine.

Wright : Technique of the Teat and Capillary Glass Tube.

INDEX

Baillière, Tindall and Cox, 8, Henrietta Street, Covent Garden, London

Abridged List of Veterinary Works

PUBLISHED BY

BAILLIÈRE, TINDALL & COX.

BANHAM'S Table of Veterinary Posology and Therapeutics, with Weights, Measures, etc. Third Edition. Pp. 236. Price **2s. 6d.** net, postage 3d.

BANHAM'S Anatomical and Physiological Models of the Horse and Cow. Half life-size; composed of Superposed Plates mounted on linen and coloured to nature. With explanatory text. Mounted on strong boards. Price each, **25s.** net, packing boards and carriage 2s.

CADIOT & ALMY'S Treatise on Surgical Therapeutics of Domestic Animals. Translated by A. LIAUTARD, F.R.C.V.S. Vol. I. Pp. xviii + 580, with 118 Illustrations. Price **20s.** net, postage 5d.

CALKINS' Protozoölogy.
Pp. 349, with 125 Illustrations and 4 full-page coloured Plates. Price **15s.** net, postage 6d.

COURTENAY & HOBDAY'S Manual of Veterinary Medicine.
Third Edition. Revised by FREDERICK T. G. HOBDAY. F.R.C.V.S. Pp. xii + 460, with 77 Illustrations. Price **10s. 6d.** net, postage 6d.

FLEMING'S Text-Book of Veterinary Obstetrics.
Third Edition. Revised and Edited by J. CRAIG, F.R.C.V.S.

> "Professor Craig has omitted the superfluous material of the original work, and has added a very considerable amount of recently-acquired up-to-date knowledge not found in any similar treatise. . . . The style in which it is written is free and pleasant to read. The illustrations are good, and include many not published in the original work."—VETERINARY JOURNAL.

Pp. viii + 528, with 160 Illustrations. Price **15s.** net, postage 5d.

FLEMING'S Text-Book of Operative Veterinary Surgery.
Second Edition. Edited by J. MACQUEEN, F.R.C.V.S., and W. OWEN WILLIAMS, F.R.C.V.S. Pp. xliv + 716, with 687 Illustrations. Published at **22s. 6d.** net; reduced to **10s. 6d.** net, postage 6d.

GALVAYNE'S XXth Century Book on the Horse.
Third Edition. Pp. xxvi + 346, with 200 original Illustrations. Price **21s.** net, postage 9d.

HERZOG'S Text-Book on Disease Producing Micro-Organisms. Pp. 644, with 214 Illustrations in black and 14 coloured Plates. Price **21s.** net, postage 6d.

HOARE'S Manual of Veterinary Therapeutics and Pharmacology. Second Edition. Pp. xxiv + 780. Price **15s.** net, postage 6d.

HOARE'S System of Veterinary Medicine.
By various Writers (edited by E. WALLACE HOARE, F.R.C.V.S.).

"The list of collaborators is in itself a guarantee for the excellence and reliability of the matter contained in this work. The price is ridiculously small compared with the enormous amount of really up-to date information it contains."—VETERINARY JOURNAL.

"The reproach can no longer be laid at the door of the British veterinarian that he must look to foreign literature if he would be up-to-date in the science of his profession."—LIVE STOCK JOURNAL.

In Two Volumes. Vol. I. : Microbial Diseases. Pp. xvi + 1328 ; Vol. II. : Constitutional Diseases, etc. (*in the Press*). Royal 8vo. Price, complete, **£2 2s.** net. Weight for postage, 12 lbs.

HOBDAY'S Surgical Diseases of the Dog and Cat.
Second Edition. Pp. xxiv + 366, with 243 Illustrations. Price **10s. 6d.** net, postage 4d.

HUTYRA & MAREK'S Special Pathology and Therapeutics of the Diseases of Domestic Animals. Translated and Edited by J. R. MOHLER, V.M.D., and A. EICHHORN, D.V.S. Large royal 8vo. In Two Volumes. Vol. I. : Infectious Diseases ; Diseases of Metabolism ; Diseases of the Blood, Spleen, Urinary, and Circulatory Organs. Pp. xvi + 1134. With 10 Coloured Plates and 198 Illustrations in the Text. Vol. II. : Diseases of the Respiratory Organs, Digestive Organs, Nervous System, Organs of Locomotion, and Skin. Pp. xviii + 1018, with 5 Coloured Plates and 163 Illustrations in the Text. Price for the Two Volumes, **£3 3s.** net.

JOWETT'S Notes on Blood-Serum Therapy, Preventive Inoculation, and Toxin and Serum Diagnosis. Pp. viii + 204, with 47 Illustrations. Price **5s.** net, postage 4d.

KITT'S Text-Book of Comparative General Pathology.
Pp. xiv + 472, with 4 coloured Plates and 132 other Illustrations. Price **25s.** net, postage 5d.

LANDER'S Veterinary Toxicology.

"This work is very complete, and practically the only one in English on the subject. The author is peculiarly fitted by training and actual experience to produce a work of this kind."—AMERICAN JOURNAL OF VETERINARY MEDICINE.

Pp. xii + 314, with 39 Illustrations. Price **7s. 6d.** net, postage 5d.

LAVERAN & MESNIL'S Trypanosomes and Trypanosomiases. Translated and Edited by DAVID NABARRO, M.D. Pp. 538, with coloured Plate and 81 Illustrations. Price **21s.** net, postage 6d.

LAW'S Text-Book of Veterinary Medicine.
In Five Volumes. Vol. I. : General Pathology ; Diseases of the Respiratory and Circulatory Organs, of the Bloodvessels and Lymphatic System. Pp. 560. Price **20s.** net. Vol. II. : Diseases of the Digestive Organs, Liver, Pancreas, and Spleen. Pp. 598. Price **20s.** net. Vol. III. : Diseases of the Genito-Urinary Organs, Skin, Eye, and Nervous System ; Constitutional Diseases. Pp. 612. Price **20s.** net. Vol. IV. : Infectious Diseases, Sanitary Science and Police, Microbes, Immunization Methods, Serum Therapy, and Specific Infections. Pp. 788. Price **25s.** net. Vol. V. : Parasites and Parasitism. Pp. 622. Price **20s.** net.

LITTLEJOHN'S Meat and its Inspection.
Pp. xii + 400, with 5 Plates and 31 Illustrations. Price **10s. 6d.** net, postage 5d.

MALKMUS' Outlines of Clinical Diagnosis of the Internal Diseases of Domestic Animals. Fourth Edition. Pp. 259, with 57 Illustrations. Price **12s. 6d.** net, postage 5d.

MERILLAT'S Animal Dentistry and Diseases of the Mouth. Pp. 262, with 160 Illustrations. Price **12s. 6d.** net, postage 4d.

MERILLAT'S Principles of Veterinary Surgery. Pp. x+669, with 114 Illustrations. Price **18s.** net, postage 6d.

MULLER'S Diseases of the Dog and their Treatment. Third Edition. Translated by A. GLASS, M.A. Pp. xx+700, with 100 plain and coloured Illustrations. Price **25s.** net, postage 7d.

NEUMANN'S Treatise on the Parasites and Parasitic Diseases of the Domesticated Animals. Second Edition. Revised and edited by J. MACQUEEN, F.R.C.V.S. Pp. xvi+698, with 365 Illustrations. Price **21s.** net, postage 6d.

OSTERTAG'S Handbook of Meat Inspection. Fourth Edition. Translated by E V. WILCOX, M.A. Pp. xxxvi+886, with coloured Plate and 260 Illustrations. Price **31s. 6d.** net, postage 7d.

REEKS' Common Colics of the Horse. Second Edition. Pp. xvi+260, with 15 Illustrations. Price **5s.** net, postage 4d.

REEKS' Diseases of the Horse's Foot. Pp. xxi+458, with 165 Illustrations. Price **10s. 6d.** net, postage 5d.

ROBERTSON'S Meat and Food Inspection. Pp. x+372, with 40 Illustrations. Price **10s. 6d.** net, postage 5d.

SCOTT'S Clinical Bacteriology and Vaccine Therapy for Veterinary Surgeons. Pp. xiv+222, with 12 Plates and 37 Illustrations in the Text. Price **7s. 6d.** net, postage 5d.

SMITH'S Manual of Veterinary Physiology.

"The subjects are presented in such an interesting manner that the book will afford pleasure for leisure hours, and can be read with marked profit by the practitioner. After its perusal he will recognize that it is the work of one who is not o ly an accomplished scientist, but also a skilled clinician—a combination which, we may remark, is very seldom met with."—VETERINARY NEWS.

Third Edition. Pp. xvi+715, with coloured Plate and 160 Illustrations. Price **15s.** net, postage 5d.

SMITH'S Manual of Veterinary Hygiene. Third Edition. Pp. xx+1036, with 225 Illustrations. Price **15s.** net, postage 7d.

SMYTHE'S Veterinary Parasitology.

Owing to the increased attention paid to the study of parasitology, there has arisen a need in our literature of a text-book dealing with the more common parasites of the domesticated animals. It has been the aim of the author to fill the gap with a volume which, while covering the course laid down for the student, shall, at the same time, be of practical value to the veterinarian as a book of reference.

Pp. xiv+130, with 29 Illustrations. Price **4s.** net, postage 4d.

THOMPSON'S Elementary Lectures on Veterinary Science.

A particular feature of this new edition is, that the whole of the illustrations have been redrawn. For its particular purpose this book is the best of its kind in existence. It should form part of the equipment of every veterinary student.

Fourth Edition. Pp. xvi + 544, with 54 Plates and 17 Illustrations in the Text. Price **10s. 6d.** net, postage 5d.

TWORT & INGRAM'S Johne's Disease.

In this book an attempt has been made to summarize existing knowledge on the subject, and the authors have brought before English readers the investigations of Continental workers, which have not been generally available to veterinary surgeons in this country. The volume also includes their own investigations, which have recently been carried out at the Brown Institution.

Pp. xii + 180, with 9 Plates. Price **6s.** net, postage 4d.

WILLIAMS' Principles and Practice of Veterinary Medicine.

Ninth Edition. Revised by W. OWEN WILLIAMS, F.R.C.V.S., and F. S. H. BALDREY, Major I.C.V.D., F.R.C.V.S., D.V.H. Liv. Pp. xviii + 986, with 25 Plates, mostly coloured, and 90 Illustrations in the text. Price **25s.** net, postage 7d.

WINSLOW'S Veterinary Materia Medica and Therapeutics.

(According to the British and United States Pharmacopœias.) Fifth Edition. Pp. vii + 857. Price **25s.** net, postage 6d.

WOODRUFF'S Economics of Feeding Horses.

"The object of the work is to explain the principles of feeding, and to show how those principles can be put into practice with economic advantage. It is written for the use of veterinary surgeons, for horse owners and managers of studs, to all of whom the subject is of the greatest importance."—VETERINARY JOURNAL.

Pp. x + 118. Price **3s. 6d.** net, postage 3d.

TROPICAL VETERINARY BULLETIN.

Published Quarterly for the Tropical Diseases Bureau, Imperial Institute, and Edited by A. L. SHEATHER, B.Sc., M.R.C.V.S. Single numbers, **3s.** net. Annual Subscription, **10s.**

VETERINARY JOURNAL.

A Monthly Review of Veterinary Science. Established 1875. Edited by FREDERICK HOBDAY, F.R.C.V.S., F.R.S.E., formerly Professor in the Royal Veterinary College, London. Price **1s.** Annual Subscription, **12s.** post free.

VETERINARY NEWS.

Established 1904. Published every Thursday morning. Price **2d.** Annual Subscription, **10s. 6d.** post free.

LONDON:

BAILLIÈRE, TINDALL & COX, 8 HENRIETTA STREET, COVENT GARDEN.