BACTERIOLOGICAL TECHNIQUE AND SPECIAL BACTERIOLOGY

MANUAL

OF

BACTERIOLOGICAL TECHNIQUE

AND

SPECIAL BACTERIOLOGY

BY

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WITH

ONE HUNDRED ORIGINAL ILLUSTRATIONS

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PREFACE

This Manual of Bacteriological Technique and Special Bacteriology has been written in pursuance of a plan first adopted by the writer while working in the Hygienic Institute, Berlin, and is the result of considerable experience of the wants of students in Bacteriology, both here and abroad. The technique and working methods have been carefully selected, and from the mass of available material on this rapidly growing branch of the subject, only those methods and material have been chosen which possess distinctive benefits. The methods are all straightforward and practical, and when carefully performed give excellent results.

It was thought best to separate the Technique from the Bacteriology of Special Diseases, which latter is included in the Special Bacteriology. This has been made very inclusive, and it is thought that it is quite sufficiently so, for any one not so specially equipped as to go to the original papers and articles. Much that is of importance in the study of the comparative diseases of animals and those transmissible to man has been included, an inclusion very necessary in the light of the growing importance of this to the practical hygienist. Again, to meet the needs of Sanitarians an account has been given of the common bacteria found in water, milk, air, soil, etc. It is generally agreed that a book of this description is not a fitting place for original or controversial work, and that the object to be attained is the presenting of a correct idea of the relative conditions of the contemporary science—consequently little is alluded to of the above nature, and nothing speculative which could be avoided without interfering with inevitable deductions from fundamental experimental principles.

I take the opportunity of expressing my thanks to Dr George Nuttall of the Hygienic Institute, Berlin, for many valuable suggestions,

and for revising the manuscript of the technical portion of this work; to Professor Günther of the same Institute for the many pure cultures he provided me with; to Herr Hänsel, Manager for Carl Zeiss in Berlin, for his assistance in making the negatives reproduced by the colotype process in the four plates at the end of this work, also for valuable assistance in connection with the other illustrations; to Dr M'Lean, Fortrose, N.B., for revising the proof sheets and valuable suggestions regarding same.

I am also indebted to Herr Paul Altman, 52 Luisen Strasse, and Carl Zeiss, 29 Dorotheen Strasse, Berlin, for the loan of the blocks used in illustrating the various Bacteriological Appliances.

The Photomicrographs and Photographs in this work are original, being taken from my own specimens and cultures, with the exception of Figs. 58, 78, and Plate III, 18; Plate IV, 20, 24.

THOS. BOWHILL.

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Edinburgh, *October* 1898.

ERRATA.

Page 6, line 25, *for* "a definite," *read* "an indefinite."
,, 21, ,, 22, *for* "Nielsen," *read* "Neelsen."
,, 27, ,, 30, *for* "symtomatic" *read* "symptomatic."
,, 29, ,, 14, *for* "Part III." *read* "Part VI."
,, 39, ,, 31, *for* "penetrates" *read* "penetrate."
,, 45, ,, 5, *for* "Iodine" *read* "Iodide."
,, 65, ,, 5, *for* "Liborious," *read* "Liborius."
,, 87, ,, 15; page 96, line 13; page 97, line 9; page 208, line 8, *for* "pyocyaneus" *read* "pyocyanus."
,, 94, ,, 10, *for* "Gonorrhœa," *read* "Gonorrhoeæ."
,, 103, ,, 41, *for* "saffronine" *read* "safranine."
,, 110, ,, 33, *for* "no growth in CO_2," *read* "in an atmosphere of CO_2."
,, 113, ,, 18, *for* "Bacillus Œdematis Maligni," *read* "Bacillus des Malignen Œdems."
,, 132, ,, 33, *for* "Schultz" *read* "Schutz."
,, 143, ,, 39, *for* "Morgenrath's" *read* "Morgenroth's."
,, 153, ,, 34, *for* "separated" *read* "situated."
,, 171, ,, 6, *for* "Halz" *read* "Holz."
,, 174, ,, 5; page 175, line 16, *for* "Communis" *read* "Commune."
,, 175 (Table (6), Widal's reaction), *for* "in contact with a genuine case of typhoid fever, *read* "in contact with the serum from a genuine case."
,, 198, line 19, *for* "fleas," *read* "flies."
,, 212, ,, 33, *for* "stongly" *read* "strongly."
,, 222, ,, 29, *for* "germine" *read* "genuine."
,, 244, ,, 32, *for* "hæmatine" *read* "hæmatein."
,, 254, ,, 33, *for* "ductu," *read* "ductus."
,, 274, ,, 8, *for* "Thierartz," *read* "Thierartzliche."

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INTRODUCTION.

The micro-organisms causing putrefaction, fermentation, and infectious diseases belong partly to the lower vegetable, and partly to the lower animal kingdom, and are divided into four groups :—

1. *Hyphomycetes*, or *Mould Fungi.*
2. *Blastomycetes*, or *Yeast Fungi.*
3. *Schizomycetes*, or *Bacteria.*
4. The *Protozoa.*

1. The *Hyphomycetes* consist of cells multiplying only by growth of the distal or point cells, and in this manner forming threads or hyphæ, *i.e.*, chains of cells. The fully-developed mould consists of a mycelium (which might be compared to the roots of higher plants), and of fruit hyphæ which develop out of the former, and bear on their extremities the spores or conidia. The moulds in the absence of oxygen frequently form cloudy mycelial masses within the culture media. Spores are only formed on the surface of media in contact with oxygen. The Hyphomycetes possess no chlorophyll.

2. The *Blastomycetes* consist of ovoid or round cells, multiplying by bud-like processes from the mother cells, also at times by spore formation. There is reason to believe they are allied to the mould fungi. If the newly-formed cells are not detached, a conglomerate cell of buds is formed. On unfavourable, strongly alkaline, and sugar-free media a mycelium is developed.

3. The *Schizomycetes* or *Bacteria* are a large group of unicellular organisms developing from pre-existing cells of the same species; they never appear spontaneously, and multiply by a process of transverse division.

They are spherical, oval, rod-like, and spiral in shape, and are generally devoid of chlorophyll or green colouring matter possessed by the higher plants; owing to this circumstance they are forced to obtain their nutritive materials from organic matters, and therefore exist in either saprophytic or parasitic conditions.

A saprophyte may be defined as an organism obtaining its nutrition from dead organic matter, whilst a parasite exists at the expense of some other living organic creature, known as its host, and strictly speaking cannot develop upon dead matter.

There is, however, a group of so-called '*facultative*' saprophytes and parasites which possess the power of accommodating themselves to existing surroundings, at one time leading a parasitic, and at another time a saprophytic form of existence.

Decomposition, putrefaction, and fermentation result from the activities of saprophytic bacteria, whilst the parasitic bacteria cause changes in the tissues, resulting in disease processes, or causing the death of their host. It has been found convenient in classifying bacteria to describe their chief characteristics by the following terms:—

Chromogenic, for pigment-producing bacteria.
Photogenic, for phosphorescent or light-producing bacteria.
Zymogenic, for bacteria concerned in the various fermentations.
Saprogenic, for bacteria producing putrefaction.
Pyogenic, for bacteria producing suppuration.
Thiogenic, for those converting sulphuretted hydrogen into higher sulphur compounds.

There is another very important saprophytic group which comprises the so-called 'nitrifying' and 'denitrifying' bacteria. The 'nitrifying' group oxidizing ammonia to nitrous and nitric acids; the 'denitrifying' group reducing nitric acid to nitrous acid and ammonia. Through their association (symbiosis) with the nitrifying bacteria and the activity of the latter, as far as we know, leguminous plants are enabled to make up their nitrogen deficit in part from the free nitrogen of the air.

This important discovery gave to free atmospheric nitrogen a biological significance heretofore denied.

Owing to the absence of chlorophyll, bacteria must have the carbon and nitrogen necessary for their growth in the form of decomposable organic substances, and the most favourable media for their development are neutral or very slightly alkaline solutions of proteid materials in one form or another.

Bacteria growing, multiplying, and performing definite functions in the absence of oxygen, and to the existence of which oxygen is positively harmful, are known as 'Anaërobic' bacteria, in contradistinction to the *Aërobic* group, for the proper performance of whose functions free oxygen is essential.

Many organisms can accommodate themselves equally well to both these modes of existence,—exposed to the air they are '*aërobic*,' in its absence they become '*anaërobic*.' Bacteria possessing this faculty are known as '*aëro-anaërobic*' or '*facultative*' organisms.

The most favourable temperature for the development of bacteria pathogenic for warm-blooded animals is that of the human body, viz., 37·5° C., while the so-called normal water bacteria grow best at about 20° C., and the 'thermophilic' bacteria develop, according to some authorities, as low as 34° to 44° C., and as high as 70° to 75° C. From this it will be seen that the range of the activity of bacterial growth depends upon specific differences and may have wide limits: some growing best at low, others at high, and others at medium temperatures. Corresponding to this we find that the limits are defined by minimum, optimum, and maximum temperatures for growth. The minimum, when growth is just possible; optimum, when the growth is most luxurious; and maximum, the highest temperature at which the organism will develop.

Besides a suitable temperature, bacteria require for their development moisture and a medium of suitable composition and reaction.

Bacteria are also influenced in a varying degree by light—most forms develop by far the best in the dark. It is therefore important to have a dark closet in the laboratory for the storing of cultures, and it is, moreover, known that cultures retain their vitality and virulence longer when maintained at low temperatures.

4. The *Protozoa* are one-celled animal organisms, usually considerably larger than the largest bacteria, on which they frequently feed. The cell body is differentiated as a homogeneous ectoplasm and a granular entoplasm containing vacuoles, also one or more nuclei. Some protozoa possess motile organs or flagella, others possess pseudopodia, others cilia. They develop by fission or by spore formation. They may coalesce and form so-called plasmodia, and as in the bacteria the spores may be more resistant than the active organism.

THE CLASSIFICATION AND MORPHOLOGY OF BACTERIA.

The following simple classification of bacteria has been found convenient by medical bacteriologists, though perhaps not quite correct

from a botanical point of view. The three principal divisions are as follows:—

1. *Cocci* or *Micrococci*, or *round organisms.*
2. *Bacilli*, or *rod organisms.*
3. *Spirilla*, or *spiral organisms.*

1. The *Cocci* or *Micrococci* are subdivided as follows:—
   (1.) Staphylococci—growing like bunches of grapes.
   (2.) Streptococci—growing in chains.
   (3.) Diplococci—growing in pairs.
   (4.) Tetrads—developing in fours.
   (5.) Sarcinæ—dividing into fours, eights, etc., as cubes.

All spherical forms belong to this group, the isolated individual members of which are practically of the same diameter in all directions.

2. The *Bacilli* include all straight, rod-like bacteria in which one diameter is always greater than the other. Many of the organisms belonging to this group in the course of development deviate from the simple rod shape, and when very short, *i.e.*, multiplying rapidly, appear like micrococci; the ends of rods also vary in shape according to the particular organism, being either blunt or rounded.

3. The *Spirilla* are subdivided as follows:—
   (1.) Vibrio, or short spirals.
   (2.) Spirillum, or long rigid spirals.
   (3.) Spirochæte, or long flexible spirals.

Different bacteria may, moreover, be characterised by the following peculiarities:—They may form *Zoöglœa*, by which we understand bacterial aglomerations of bacteria in large numbers enclosed in an amorphous matrix, which may give great tenacity to the bacterial mass.

By *Ascococcus* we understand cocci, associated in large numbers in an amorphous matrix, and enclosed in an enveloping membrane.

Some bacteria possess a distinct capsule, and are known as *Capsule cocci* and *Capsule bacilli*, the capsule being more or less evident, depending upon the conditions of growth.

By *Leptothrix* we understand long undulating rods.

By *Cladothrix*, long, straight and branching rods.

By *Streptothrix*, organisms that in their structure resemble at one time the thread fungi, and at other times the bacteria; like the mould fungi, they form cylindrical threads out of round cells, which branch dichotomously, finally becoming visible to the naked eye as irregular radiating thread masses or mycelia.

Involution forms of bacteria usually occur under conditions un-

favourable to the nutrition of the organism causing degeneration, whereby it acquires abnormal aspects, as in old cultures or in media of unsuitable composition.

Pleomorphism.—Recent researches show that the same organism may assume very different aspects, and appear either as bacilli or micrococci. However, pleomorphic organisms are the exception, and it is very unsafe for the beginner to assume, when he finds organisms in his cultures presenting different appearances, that he is dealing with pleomorphic bacteria.

FLAGELLA OR MOTILE ORGANS.

All motile bacteria are provided with flagella, which are distinguished according to their number and position, as follows:—

1. *Monotricha*, one flagellum at one pole.
2. *Amphitricha*, one flagellum at each pole.
3. *Lophotricha*, a bundle of flagella at one pole.
4. *Peritricha*, many flagella given off around the periphery of the organism.

The above terminology being borrowed from that applied to the Protozoa.

(For special method of demonstrating Flagella, see §§ 23, 24.)

THE DIMENSIONS OF BACTERIA.

These vary as much as their form, and are expressed in microns. A micron is the one-thousandth part of a millimetre, and is designated by the Greek letter μ,—*i.e.*, Bacillus Anthracis, 3–10 μ long, and 1–1·2 μ broad. Bacillus Tuberculosis, 1·5–4 μ long, and only 0·4 μ broad, etc.

MULTIPLICATION.

1. *Micrococci* develop by simple fission (dividing into two transversely).

2. *Staphylococci*, when forming characteristic grape-like clusters by growth and division in different directions.

3. *Streptococci* develop in one direction only, producing chains of varying length.

4. *Sarcinæ* divide regularly in three directions of space, but instead of separating as single cells, remain together in masses like bales of cotton, the individual members, pairs, cubes, etc., being each enveloped in a capsule.

5. The *Bacilli* multiply by a process of division, which is transverse to their direction of growth. The mother cell, having become elongated, develops into two daughter cells—in this way chains of individuals are formed. The length of these chains vary in the different species, and is also influenced by different conditions. Long chains of individuals, when this division is not very distinct, may present a homogenous, thread-like appearance in unstained specimens. The rapidity of the growth is naturally variable, according to the species, nature of the medium, temperature, etc. The most rapid growth observed has been the division of a mother-cell into two daughter-cells within fifteen to twenty minutes.

SPORE FORMATION.

Some bacteria develop what are known as *spores;* by spores we understand a resting stage in the life-history of an organism. Spores are either formed within the mother-cells (endogenous), or certain individual cells prove more resistant. Endogenous spores are formed in the body of the bacilli, usually under conditions unfavourable to their growth,—that is, when they have exhausted their food supply: this is, however, not always the case. Spores are usually resistant, at times highly so, to influences which destroy vegetative forms. They withstand drying, at times for many years, and great elevations of temperature, as well as the effect of chemical agents. Brought under favourable conditions the spores germinate again to the vegetative form or bacillus; they do not as such multiply of themselves, but give rise each to a single bacillus which, dividing, gives rise to a definite number of individuals.

The changes in bacilli about to form spores are as follows:—

1. The protoplasm loses its normal homogenous appearance, and granular refractive points of irregular shape and size appear.
2. The above granular refractive points finally coalesce, the remainder of the cell remaining clear and transparent. Free granules may remain in the vicinity of the spore, as if not required for its development.
3. The complete spore appears as an oval, highly refractive, glistening body, easily differentiated from the remainder of the cell, which now consists only of cell membrane and détritus.
4. The cell membrane eventually disappears, liberating the spore.

A single cell usually produces but one spore, which may be located in the centre or at the extremity of the mother-cell. The cell which produces a spore may assume the shape of a drum-stick or a spindle (known as a Clostridium). Spores cannot be stained by the ordinary methods employed for bacteria (for special methods of staining Spores see §§ 26, 27).

A spore about to develop into a bacillus exhibits the following phenomena:—

1. It gradually loses its highly refractive appearance, enlarges, and appears to assume a consistency approaching to that of the bacillus.
2. The membrane of the spore is ruptured, and the bacillus grows out of the aperture.
3. In some organisms the separation of the spore capsule is more evident than in others, and in this case the remains of the capsule may adhere for some time to the young bacillus. It is on account of the resistance of the spores that the elaborate means of sterilization about to be described are necessary for obtaining media free from germs, by which in turn we can obtain pure cultures—that is, separate cultivations of single species of bacteria. The resistance of the spores is due to a very dense and impenetrable membrane, as well as to the fact that the protoplasm contains less water than that of the vegetative form. More water in conjunction with protoplasm lowers the temperature at which it coagulates.

METHODS OF STERILIZATION.

STERILIZATION BY HEAT.

Sterilization may be accomplished by subjecting the articles to be treated to high temperatures, either in a moist or dry state. Successful sterilization by dry heat cannot usually be accomplished at a temperature lower than 150° C., and the objects should be subjected to this temperature for not less than one hour. The apparatus used for hot-air sterilization is double-jacketed, and made of strong sheet-steel, preferably with a copper bottom, and having two perforated movable shelves. On the top there is a regulating slide, by which a current of air through the apparatus may be secured, and two tubes,

one for a thermometer and the other for a thermo-regulator—which is usually not necessary—and two eyelits for suspending it to the wall, or it can be placed on a stand (see Fig. 1). When suspended against the wall a sheet of asbestos is necessary to protect the wall. It is also important that the copper bottom of the apparatus should be detachable, thus enabling a new one to be fitted without difficulty.

In bacteriological work sterilization by dry heat is limited to such articles as glass flasks, plates, small dishes, test-tubes, pipettes, cotton wadding, and such metal instruments as are not seriously injured by the high temperature. The above articles are sterile when heated to 150° C. for one hour. The usual method is to remove the Bunsen burner when the thermometer registers 170° C., and allow the apparatus to cool before opening, otherwise glass ware may fly in pieces.

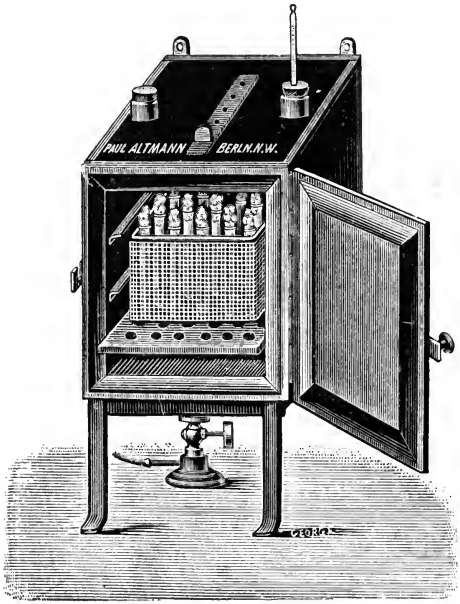


Fig. 1.—Hot-Air Sterilizer.

Fig. 2.—Koch's Steam Sterilizer.

Fluids, culture media, potatoes, etc., in fact, anything that would be destroyed by the great heat of the hot-air chamber, are sterilized in the '*Koch Steam Sterilizer*' (see Fig. 2), or the '*Arnold Steam Sterilizer*.'

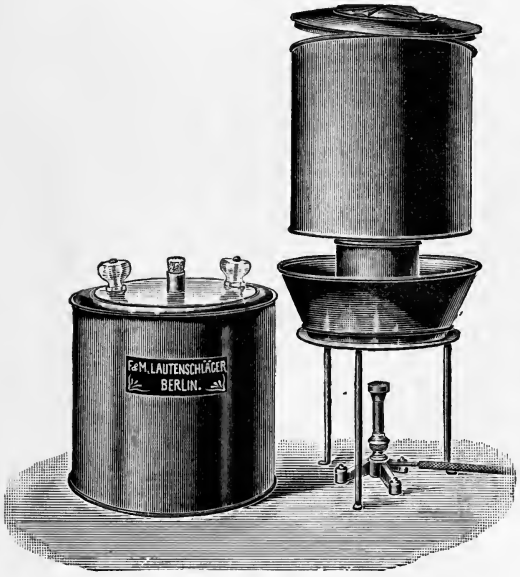
The Arnold apparatus possesses advantages not obtainable with the Koch apparatus. It is smaller but quicker, and is more suitable

for rapid work, the Koch apparatus requiring a long time to obtain the temperature of 100° C., from which moment the period of sterilization can be estimated.

Messrs Lautenschlager, Berlin, have recently, at the writer's suggestion, constructed a useful portable steam sterilizer, costing 27/- (see Fig. 3), showing the apparatus with the outer cover removed, and the inner lid of the steam chamber slightly raised. The outer cover can also be used without the inner lid when a temperature lower than 100° C. is required; a place for a thermometer being provided.

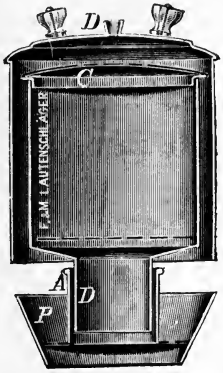
Fig. 4 shows a section of the apparatus, *P* being the water reservoir, and the boiling chamber being constructed similar to the Arnold apparatus. *A* and *D* show the junction of the steam and water chambers by means of a strong detachable telescopic joint, enabling the water chamber to be cleansed, and also facilitating the packing of the apparatus during transportation. The size of the opening admitting the steam into the steam chamber is regulated with a diaphragm fitted on the inside of the steam chamber.

Fig. 3.



Bowhill's Steam Sterilizer.

Fig. 4.



Whereas the above means of sterilization at 100° C. suffice for ordinary purposes, an apparatus for sterilization by steam under pressure, known as an Autoclave (see Fig. 5), is at times very useful,

for the reason that the whole process of sterilization may be shortened from several hours to as many minutes. It can only be used,

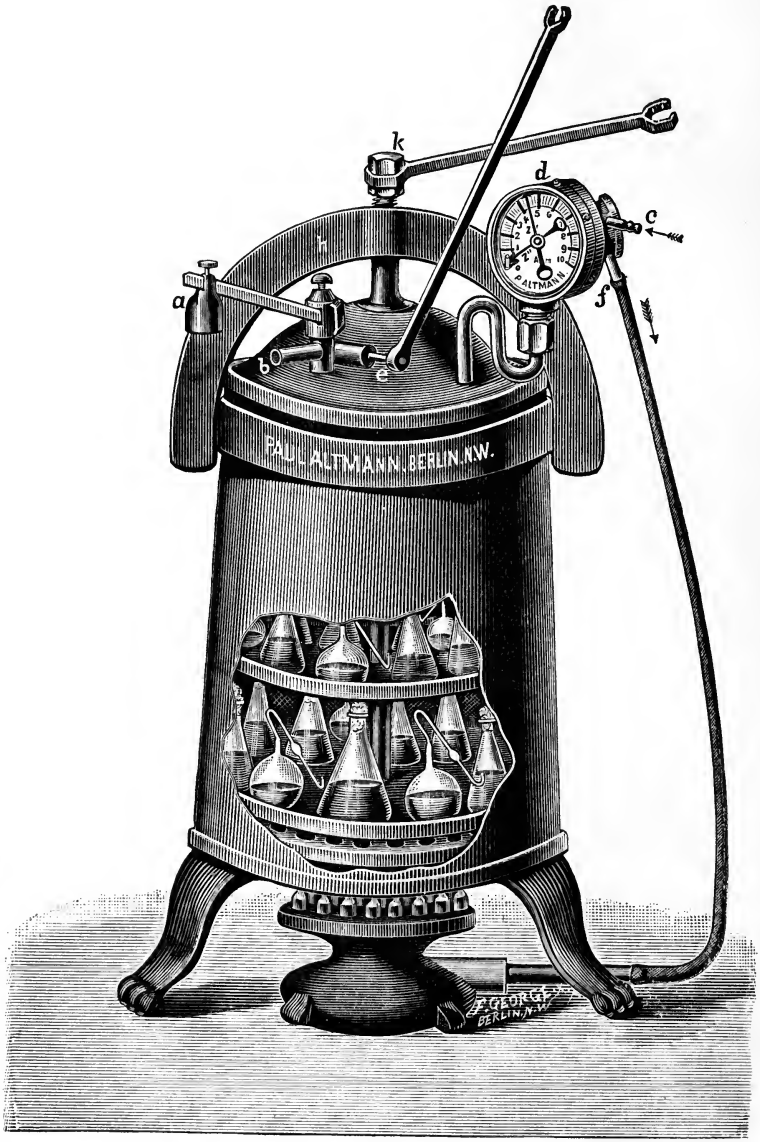


FIG. 5.—Autoclave.

however, for media which remain uninjured by the exposure to the higher temperature obtained in the autoclave.

SIMPLE STEAM STERILIZER 'NOVY.'

The lower part of the apparatus consists of an ordinary water bath, 7 to 8 inches in diameter; the upper part of a copper pail 8 inches high and 8 inches in diameter, with a perforated bottom, and lid with a tube; copper rings are soldered in the interior of the pail to prevent the tube touching the sides, otherwise the cotton plugs would become saturated with the condensed steam. The pail is filled with flasks or tubes, and placed over the water bath, in which the water should be boiling. In five to seven minutes steam will actively issue from the tube in the cover, showing that the interior temperature has reached 100° C. With the apparatus a student can attend to any needed steam sterilization at his own table, and thus save much time. The general usefulness of the apparatus, its compactness, cheapness, and the saving in the gas, will recommend it to those practitioners who desire to equip a small laboratory.

A small steam sterilizer may be readily improvised by standing an inverted funnel of appropriate size upon the water bath. If the funnel is of glass it is well to surround it with a towel, to prevent its cooling too rapidly on removal. Dr Nuttall states that he has used this simple apparatus frequently during the last ten years and found it very useful.

TYNDALL'S DISCONTINUOUS METHOD OF STERILIZATION.

We have stated above that certain bacteria form spores which are highly resistant, whereas the vegetative form of the organism is more sensitive to heat, chemical agents, etc. Tyndall's method of discontinuous sterilization depends upon the principle that bacteria developing from spores in the nutrient media are destroyed by a relatively short exposure to a temperature of 100° C., at which temperature the spores are not affected. To overcome this difficulty presented by the resistance of the spores, the tubes of media or other materials are placed in a steam sterilizer at 100° C. for thirty minutes. The first heating not having killed the spores, the material is placed at ordinary room temperature, or better, at 37° C., when any living spores quickly vegetate. The bacteria to which the spores give birth are killed by a second heating, for twenty or thirty minutes, on the following day. The above operation repeated three, four, or five times, ends in the certain sterilization of the media, all the spores having developed into bacilli, and as such destroyed.

The process of fractional sterilization at low temperatures is conducted in a somewhat similar manner to Tyndall's method, but requires a greater number of exposures to a temperature not exceeding 68° to 70° C., and is employed to sterilize easily decomposable materials which would be rendered unfit for culture purposes by steam—such as blood serum. Sterilization by steam can also be practised by what is known as the 'direct method,' by a single exposure in the Koch Steam Sterilizer, but a prolonged exposure is necessary—at times several hours—to obtain complete sterilization.

When an autoclave is used, a single exposure of fifteen minutes is sufficient to practically destroy all bacilli and their spores, provided the thermometer registers 122° C. The autoclave when used must either remain closed until cool, or until the gauge indicates that pressure no longer exists, for if opened when the steam within is still under pressure, the steam will escape so rapidly that all fluids within the chamber thus suddenly relieved of pressure, will boil violently, and as a rule boil quite out of the tubes, blowing out the plugs.

Open vessels before sterilization are closed with cotton-wool plugs or stoppers. Glass plates and pipettes are sterilized in copper or sheet-iron boxes specially manufactured for the purpose, and removed when required. It is convenient to wrap each pipette in paper.

Petri dishes are most conveniently sterilized in a special cylindrical copper box with a capped lid, there being a round hole in the box and in the lid. These holes are placed opposite each other during sterilization, thus allowing a current of hot air to circulate in the interior of the cylinder. When the process of sterilization is completed, the lid is slightly turned, closing the holes. The interior of the cylinder is fitted with a special apparatus, enabling one or any number of dishes to be withdrawn when required.

STERILIZATION OF POST-MORTEM INSTRUMENTS.

1. Wipe the dirty knife with a piece of cotton wool and dip in a strong solution of caustic soda.

2. Wash in alcohol, heat in a flame, boil in strong soda solution, or place in the steam sterilizer.

Instruments may be quite conveniently sterilized in the laboratory by dipping them in benzine and inflaming the latter. The sterilization is thus completed without exposing the instruments to as high a temperature as when they are drawn through the flame.

PART I.

PRINCIPLES OF BACTERIOLOGICAL TECHNIQUE.

THE WORKING TABLE OR BENCH.

§ I. The bench or work-table must be horizontal and steady, 4 feet by 2 feet 4 inches on top, and 2 feet 4 inches high. The surface of the table is covered with a piece of plain brown linoleum, which can be renewed when necessary. Piano stools make the best seats, being adjustable for height and convenient for any desired position. A sheet of plate or other thick glass is laid on the left hand side of the table, with pieces of black and white paper underneath, forming a background; two pieces of coloured glass can also be used, one piece milk-white and the other black, according as the specimens under examination are stained or unstained. Watch glasses, capsules, slides, etc., under naked-eye examination are placed on either the white or black surface.

1. Unstained specimens or sections are best seen on the black.
2. Stained specimens on the white.
3. The black surface is the most suitable for isolating the caseous portions of suspected sputum.

Opposite and within easy reach of the operator a rack is placed to hold the stains and reagents in daily use (see § 2). A solid block of wood with a number of circular holes bored in it is the most convenient rack. A Bunsen burner is placed on the right hand side of the table, but in the absence of gas a spirit lamp answers the purpose. On a shelf 3 or 4 feet above the right side of the table a large bottle of distilled water is placed, with a rubber tube and water pinch-cock attached to its distal extremity, descending to a circular glass dish within easy reach of the operator, for washing excess stain, etc., from specimens.

§ II. The following articles are also placed on the table:—

1. A large glass dish, 6 inches by 7 inches, with a cover, containing *Disinfecting Solution* (see § 66), for the temporary disposal of old cultures, virulent material, etc.
2. A small glass dish, 3½ inches by 2 inches, containing *Cleansing Solution* (see § 65), for the temporary disposal of dirty slides and cover-glasses.
3. A covered glass dish, containing vaseline and a small camel's-hair brush, for preparing hanging-drop cultures.
4. One large test-tube stand.
5. One small test-tube stand.
6. One small filter stand.
7. One bottle of Canada balsam dissolved in xylol.
8. One small bottle of immersion oil.
9. Several ordinary glass tumblers, some of which are used for holding platinum needles, clean water, scissors, forceps, glass rods, etc. A drawer is necessary in the table to hold clean cover-glasses, slides, test papers, filter paper, watch glasses, staining dishes, etc., when not in actual use.

The following stains and reagents are placed in the aforementioned rack for ordinary use:—

1. Löffler's solution of methylene blue.
2. Watery alcoholic solution of gentian violet.
3. Watery alcoholic solution of fuchsin.
4. Ziehl's carbol fuchsin, or Ehrlich's anilin water fuchsin.
5. Ehrlich's anilin water gentian violet.
6. Roux's double stain.
7. 2 per cent. acetic acid in water.
8. 3 per cent. hydrochloric acid alcohol.

§ III. Methylene blue and gentian violet give excellent staining results, and are useful stains for most bacteria, while fuchsin is one of the simplest and most rapid stains to manipulate. Ziehl's carbol fuchsin is the most stable preparation of fuchsin used in staining Tubercle bacilli. Ehrlich's anilin water fuchsin and gentian violet give excellent staining results, but are very unstable, soon decomposing, requiring to be freshly prepared every three weeks. Roux's double stain is specially adapted for the detection of Diphtheria bacilli, inasmuch as the bacilli stain more readily and with greater intensity than any of the other organisms usually found associated with them. (For the preparation of the above stains and reagents see §§ 49-60.)

§ IV. GENERAL METHODS OF BACTERIOLOGICAL INVESTIGATION.

The Microscope.

For bacteriological investigation, a modern microscope with an Abbé condenser, iris diaphragm, and low and high power lenses, is necessary. The following makers can be selected from :—

Zeiss.	Leitz.
Objective AA.	Objective III.
„ DD.	„ VII.
Homogenous Immersion, one-twelfth.	Homogenous Immersion, one-twelfth.
Eye-pieces II. and IV.	Eye-pieces I. and III.

The low power is used with a narrow diaphragm for the examination of colonies of bacteria developed on plate or Petri dish cultures.

Unstained specimens are always examined with a narrow diaphragm, whilst for stained specimens an open diaphragm is necessary. In examining double-stained sections an open diaphragm is used for the bacteria and a narrow diaphragm for the tissue. The oil immersion lens after use must be cleaned with benzine and wiped with a chamois skin or special lens paper. Excess of immersion oil is removed from the cover-glass with xylol, which can also be used instead of benzine for cleaning the lenses.

§ V. METHODS OF PREPARING COVER-GLASS SPECIMENS.

Fluids.

The platinum loop, previously sterilized in the Bunsen flame, is used to transfer fluids to the cover-glass.

Semi-solid Material.

A drop of sterile water is placed on a cover-glass and the material mixed with the water by means of a sterilized platinum wire or loop.

Tissues, Pieces of Organs, Etc.

Portions of the material are spread on the cover-glass with a sterilized platinum wire, or the cover-glass is drawn quickly across the cut surface of the tissue or organ.

§ VI. THE HANGING DROP.

1. Place directly in the middle of a clean cover-glass with the sterilized platinum loop a minute drop of the fluid to be examined; if a semi-solid, a drop of sterile water, physiological salt solution, or bouillon is first placed on the cover-glass and then inoculated.

2. The edges of the circular cavity in the hanging-drop slide are painted with a narrow strip of vaseline with a camel's-hair brush.

3. Reverse the slide and place it upon the cover-glass so that the inoculated drop is exactly in the centre of the hollow chamber. Apply gentle pressure when the cover-glass adheres to the slide by means of the vaseline, forming an air-tight cavity. The preparation is now turned upwards quickly (*to prevent running of the drop*) and examined as follows:—

The convenient method of beginners, or those examining many drop cultures, is that recommended by Nuttall, consisting of making small rings upon the cover-glass with a mixture of lamp-black and blood serum applied with a camel's-hair brush and a turn-table, and passed in the usual manner through the flame.

1. Cut off the light in the condenser with a narrow diaphragm about the size of a pin-head.
2. Find the edge of the hanging drop with the low power and plain mirror.
3. Regulate the Abbé condenser, and throw the light into the specimen.
4. Place the illumination or light in the centre of the specimen by manipulating the mirror.
5. Screw up the tube, remove the low power, and adjust the immersion lens.
6. Increase the size of the diaphragm to about the size of a pea.
7. Put a drop of immersion oil on the cover-glass.
8. Screw down the tube with the coarse adjustment until the point of the lens touches the oil, and then screw back the tube without breaking the oil connection.
9. Screw down the tube again by means of the coarse adjustment until the object comes in view.
10. Regulate the focus with the fine adjustment.
11. If the field is not evenly illuminated, adjust the mirror without further removal.

The hanging-drop method is used to study the morphobiological characteristics of organisms, and the intravital phenomena connected therewith.

§ VII. NUTTALL'S MICROSCOPIC THERMOSTAT.

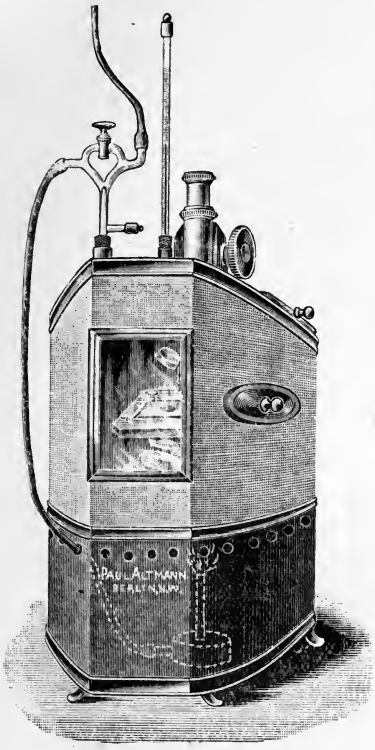


Fig. 6.—Nuttall's Microscopic Thermostat.

This simple apparatus is adapted for use with different microscopes that are placed in the thermostat from behind, the top of the apparatus, which is slanting, consisting of two laterally movable slides, the inner margins of which are made of strips of felt cut according to the shape of the microscope, so that the tube and adjustment remains outside. On the left side is an aperture large enough to admit the hand, by which the slide can be manipulated. In front is a window admitting the light. On the right side screws connected with the mechanical stage can be adjusted; such an arrangement is, however, not usually necessary, and also increases the price. The thermostat is closed by a door at the back. This apparatus is useful for studying bacteria or protozoa in drop cultures, and has also been found useful in studying zoöparasites.

§ VIII. THE COVER-GLASS SPECIMENS.

Ordinary Method.

1. Place a drop of sterile water on a clean cover-glass with a sterilized platinum loop.

2. Inoculate the drop with a small quantity of the material under investigation—mixed and well spread with the platinum loop or wire.

3. Allow the material on the cover-glass to dry in the air.

4. Fix, by passing the cover-glass, preparation side uppermost, through the Bunsen flame three times at intervals of one second, the cover-glass being held with 'Cornet's Forceps.'

5. Stain the specimen by flooding with the desired filtered stain,

allowing it to remain from one to three minutes. (The staining process can be hastened and intensified by 'heating,' not 'boiling,' the cover-glass in the flame until vapour arises.)

6. Remove excess of stain by washing the specimen with the pipette wash bottle, or directly under the tap. (The specimen can be examined at this stage by laying the preparation side downwards on a slide and removing excess of fluid with filter paper, and if of sufficient interest can be permanently mounted.)

7. Remove the excess of water by blowing with or without a glass tube, and dry. (A special blower for this purpose can be made from an ordinary atomizer fitted with glass tubes, and about one-third full of calcium chloride.)

8. Mount in xylol balsam and examine as follows:—

(1.) Place the specimen on the microscope stage so that about the centre of the cover-glass lies in the optical axis.

(2.) Place a drop of immersion oil on the centre of the cover-glass.

(3.) Screw down the tube with the coarse adjustment until the point of the lens touches the oil; the tube is now screwed upwards without breaking the oil connection.

(4.) Remove the diaphragm below the Abbé condenser, and arrange the flat mirror so that the field is somewhat lighted.

(5.) Screw the tube carefully down with the coarse adjustment until the preparation comes in view.

(6.) Regulate the focus with the fine adjustment.

(7.) Obtain the maximal illumination by manipulating the Abbé and mirror regulator.

§ IX. THE CONTACT, OR IMPRESSION SPECIMEN.

1. Lay a clean cover-glass gently on the top of the desired colony on the plate or dish culture, apply gentle pressure, and lift the cover-glass up by one of its edges without lateral movement.

2. Air dry.

3. Fix in the flame, and proceed as with the ordinary cover-glass method, process No. 5, § 8.

The above specimens differ from ordinary cover-glass preparations in that they present an impression of the organisms as they were arranged in the colony from which the preparation was made. It is important to note that 'liquefied-colonies' cannot be used for the preparation of contact or impression specimens.

§ X. THE GRAM METHOD OF STAINING COVER-GLASS SPECIMENS.

1. The 'fixed' cover-glass specimen is stained two to five minutes with Ehrlich's anilin water gentian violet (see § 55).
2. Wash with water.
3. Differentiate with Gram's solution of iodine (see § 60) until the stained surface blackens like a tea-leaf—which usually takes a half to one minute.
4. Decolorize with alcohol until no more stain comes away.
5. Wash in water.
6. Dry, and mount in xylol balsam.

The Gram method is employed to differentiate Bacteria into two divisions:—

(*a*) Those staining according to the method.

(*b*) Those decolorized according to the method.

According to Lehmann, recent researches show that the result depends very much upon the age of the culture and condition of the organism at the time the specimen is stained, as well as the method employed.

The Bacillus of Black Quarter, or 'Symptomatic Anthrax,' can also be stained under certain circumstances, although most authors write to the contrary.

In order to check the Gram test and place results beyond doubt, proceed as follows:—

> Take a clean cover-glass and put a small quantity of the material to be stained on one half, and on the other half a small quantity of a young culture of Bacillus anthracis. Air dry, fix, and stain by the ordinary Gram method above mentioned, so that both materials on the cover-glass are thus subject to the same reagents and conditions.

The Bacillus anthracis staining readily according to the Gram method, one can thus judge whether the bacterial species under investigation stains or decolorizes by this method.

§ XI. THE FOLLOWING BACTERIA STAIN ACCORDING TO THE GRAM METHOD.

1. Bacillus anthracis.
2. Bacillus of Tuberculosis.

3. Bacillus of Leprosy.
4. Bacillus of Mouse Septicæmia. } Different varieties of
5. Bacillus of Rouget, or Swine Erysipelas. } the same species.
6. Bacillus of Tetanus.
7. Streptococci of Pyæmia and Erysipelas.
8. Staphylococcus pyogenes aureus.
9. Staphylococcus pyogenes citreus.
10. Staphylococcus pyogenes flavus.
11. Staphylococcus pyogenes albus.
12. Micrococcus tetragenus.
13. Diplococcus pneumoniæ, 'Fraenkel.'
14. Actinomyces hominum.*
15. Actinomyces bovis.*
16. Actinomyces musculorum suis.
17. Botryomyces 'Bollinger.'†
18. Discomyces 'Rivolta.'†
19. Botryococcus ascoformans 'Kitt.'†
20. Bacillus of Diphtheria.
Bacillus of Black Leg, or } Only under certain conditions and circumstances.
'Symptomatic Anthrax.'
21. The Lactic Acid Bacillus of Hueppe.

§ XII. THE 'CLADIUS' CONTRAST METHOD FOR COVER-GLASS SPECIMENS.

By adding $\frac{1}{4}$ per cent. solution of picric acid in water to 1 per cent. solution of methyl-violet in water a blue indigo colour is the result, insoluble in water, very soluble in alcohol, chloroform, anilin, and clove oils.

Method of Procedure.

1. Place the material on a cover-glass and air dry.
2. Pass three times through the Bunsen flame.
3. Stain one minute in a 1 per cent. watery solution of methyl-violet.
4. Wash in water and dry with filter paper.
5. Pass through $\frac{1}{4}$ per cent. picric acid solution (see § 61) one minute.
6. Wash in water; dry with filter paper.

* At stage 4, during decolorization with alcohol the cover-glass must be kept moving, otherwise, if left still, the Actinomyces are almost entirely decolorized.

† Named according to investigators.

7. Decolorize in chloroform or clove oil, and repeat the application until the decolorization is complete. 'Clove oil is the better of the two for permanent specimens.'

8. Dry and mount in Canada balsam, dissolved in xylol.

Result.—The bacteria are stained a deep indigo blue.

This stain has very little affinity for fungi cells and other histological elements, whilst its affinity for certain bacteria is somewhat extraordinary. The bacteria stained by the Gram method also stain by this method, as also the bacillus of malignant œdema.

§ XIII. ZIEHL-GABBET METHOD OF STAINING TUBERCLE BACILLI IN COVER-GLASS PREPARATIONS.

1. Spread a small portion of the material, 'sputum, caseous mass, mucus, etc., that is most likely to contain the bacilli,' upon a clean cover-glass in as thin a layer as possible. '*Günther considers it is dangerous in making cover-glass specimens from sputum to rub two cover-glasses together and then separate them, as the cover-glasses might break, cutting the finger, and thus inoculating the operator.*'

(In examining the sputum of suspected tubercular and asthmatic patients much time is saved by using the sputum attachment of the hand centrifuge, see Fig. 7, § 20.)

2. Air dry, and fix by passing three times through the Bunsen flame.

3. Place some Ziehl-Nielsen's carbol fuchsin (see § 51) in a watch glass or small porcelain evaporating dish, and float the cover-glass on the surface, material side downwards; heat the stain until vapour arises, and set aside one minute to allow the stain to work. 'Another method is to flood the cover-glass with the stain, and heat three or four times until vapour arises.'

4. Wash the cover-glass in water to remove excess stain.

5. Place the cover-glass in Gabbet's solution (see § 52) one to three minutes, according to the thickness of the preparation and the intensity of the fuchsin stain.

6. Wash in water, dry.

7. Mount in xylol balsam.

§ XIV. METHOD OF STAINING TUBERCLE BACILLI IN COVER-GLASS SPECIMENS WITH EHRLICH'S ANILIN WATER STAIN.

This method is recommended by Günther.

1. Prepare the material on the cover-glass in the same manner as described under 1 and 2 processes, Ziehl-Gabbet method, § 13.

2. Place some Ehrlich's anilin water fuchsin or gentian violet (Ziehl's carbol fuchsin can also be used) in a watch glass or porcelain evaporating dish, float the cover-glass on the surface, material side downwards (if it sinks it does not matter), heat the stain until vapour arises, and set aside one minute to work.

3. Withdraw the cover-glass with the small forceps, and wash off excess stain with water.

4. Lay the cover-glass, preparation side upwards, in a watch glass containing 3 per cent. HCl. alcohol (see §. 70) for one minute, and move to and fro, when it is decolorized.

5. Wash with water, and dry.

6. Stain the cover-glass with a few drops of weak alcohol or watery solution of methylene blue or malachite green for fuchsin stains, and Bismarck brown for the violet stain, being careful not to stain long enough to obliterate any stained Tubercle bacilli.

7. Wash in water, and dry.

8. Pass through the Bunsen flame. 'If passed many times through the flame the stained bacteria do not fade so readily. Unna employs this method for Leprosy bacilli.'

9. Mount in xylol balsam.

§ XV. LOFFLER'S METHOD OF STAINING GLANDERS BACILLI IN COVER-GLASS SPECIMENS.

1. Make a smear preparation from a young nodule, air dry, and fix.

2. Stain in Löffler's methylene blue solution (see § 54) five minutes.

3. Place the preparation one second in a 1 per cent. solution of acetic acid in water, which is rendered a Rhine wine colour by the addition of a few drops of a watery solution of trapäolin OO.

4. Wash quickly with distilled water.

5. Dry, and mount in xylol balsam.

'*The addition of the trapäolin OO acts as follows: The cell plasma is entirely, and the nuclei partially decolorized while the bacilli retain their colour.*'

§ XVI. METHOD OF STAINING COCCI AND GONOCOCCI IN COVER-GLASS SPECIMENS.

1. Prepare the cover-glass specimen in the ordinary manner, air dry, and fix.

2. Flood the specimen with concentrated watery solution of methylene blue.

3. Heat in the flame until vapour arises.
4. Wash in water.
5. Dry with filter paper.
6. Mount in xylol balsam.

§ XVII. KNACK'S METHOD OF STAINING GONOCOCCI IN COVER-GLASS SPECIMENS.

1. Make the cover-glass from the pus in the ordinary manner, air dry, and fix.
2. Stain with methylene blue.
3. Place the cover-glass in a 1 per cent. solution of argonin four minutes.
4. Wash and dry the specimen.
5. Place the cover-glass in a watery solution of fuchsin 1-20 for ten seconds.
6. Wash, dry, and mount in xylol balsam.

§ XVIII. NEISSER'S METHOD FOR THE DIFFERENTIAL DIAGNOSIS OF DIPHTHERIA BACILLI IN COVER-GLASS SPECIMENS.

1. Cover-glass specimens are prepared from cultures grown on Löffler's blood serum, at 34° and 35°, not exceeding 36° C.; the cultures must also not be younger than nine and not over twenty to twenty-four hours' old; air dry, and fix the specimen in the flame.
2. Stain three seconds with Neisser's No. 1 solution (see Stains, § 58).
3. Wash the specimen with water.
4. Stain three to five seconds with Neisser's No. 2 solution previously filtered (see Stains, § 58).
5. Wash in water, dry, and mount in xylol balsam.

Result.—The body of the Diphtheria bacillus is stained brown, containing blue granules—as a rule two—one at each end, or only one at one end, seldom any in the middle. The granules are oval, and never found free. In other organisms resembling the Diphtheria bacillus, these are round, and always situated at the end, close to one another.

§ XIX. UNNA'S METHOD OF STAINING FUNGI.

Solution A. Concentrated watery solution of methylene blue.
„ B. Unna's glycerine-ether mixture.

1. Place the suspected material (crusts, etc.) on a slide, and saturate with acetic acid.

2. Crush the material with a second slide laid crosswise.
3. Heat over the flame until the acetic acid cooks.
4. Separate the two slides and dry over the flame.
5. Remove fatty substances with ether.
6. Cover the prepared surfaces with solution A, and heat over the flame until vapour arises.
7. Wash with water.
8. Decolorize in solution B, three or four seconds.
9. Wash with water.
10. Dry with filter paper and over the flame.
11. Mount in xylol balsam.

The fungi are stained a deep blue colour, while epithelial structures, owing to the glycerine-ether mixture, appear green. If this differentiation is not desired, the decolorization with glycerine-ether can be dispensed with.

§ XX. ORDINARY METHOD OF EXAMINING MILK FOR BACTERIA IN COVER-GLASS SPECIMENS.

1. Dip a sterilized platinum wire into the milk, and draw the wire sharply across the surface of a clean cover-glass.
2. Air dry, and fix in the flame.
3. Hold the cover-glass between the fingers, and flood the preparation side with some sulphuric ether to remove the fat.

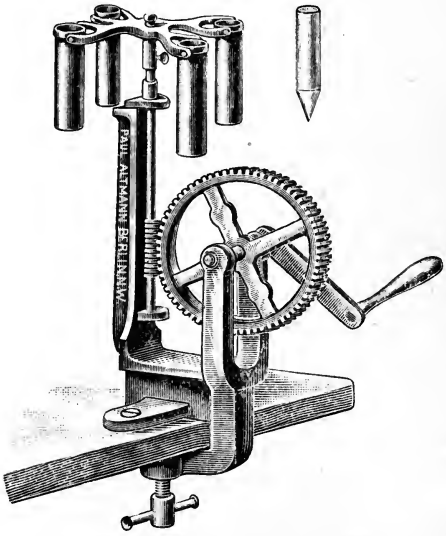


FIG. 7.—Altmann's Hand Centrifuge.

4. To demonstrate ordinary bacteria stain with methylene blue, as it does not colour the background so intensely as fuchsin or gentian violet. To demonstrate Tubercle bacilli, stain the specimen according to the special method given on page 21. The centrifugal machine can also be used (see Fig. 7), and the resulting sediment examined, the same way as ordinary milk.

The advantages of the centrifugal machine are that sedimentation can be carried out very quickly, and the discovery of Tubercle bacilli rendered easier.

§ XXI. ROTH'S METHOD OF EXAMINING BUTTER FOR TUBERCLE BACILLI AND OTHER BACTERIA IN COVER-GLASS SPECIMENS.

1. About two to four grammes of the suspected butter is placed in a test-tube with a small spatula or a glass rod, the test-tube filled about three-quarters full with water, placed in a water bath at 50° C., and heated until the fat is thoroughly melted.

2. Place a cork in the test-tube and shake well a few times in order to separate any Tubercle bacilli, etc., from the fatty substance.

3. Place the test-tube cork downwards in the warm bath for fifteen minutes, until the fat is again thoroughly dissolved.

4. Place the test-tube in a cool place, so that the butter fat again solidifies.

5. The fluid contents are either placed in the centrifugal apparatus or set aside until the sediment forms.

6. Some of the sediment is placed on a cover-glass, air dried, slightly heated, laid in a mixture of one of ether and three of alcohol, removed, dried, and fixed in the flame.

7. The specimen is stained according to the ordinary process for Tubercle bacilli (see § 14).

'To isolate bacteria by this process from the butter a sterilized platinum loop is dipped in the fluid, and gelatine cultures instituted.'

§ XXII. METHOD OF STAINING CAPSULE BACTERIA IN COVER-GLASS SPECIMENS.

Treat the fixed preparation as follows:—

1. Stain with 2 per cent. gentian violet watery solution, and intensify the staining action by heating until vapour arises.
2. Wash with water, and dry.
3. Decolorize the capsules with 2 per cent. acetic acid in water, six to ten seconds.
4. Wash with water.
5. Mount preferably in water, as the high refractive index of balsam renders the capsule less visible.

Fuchsin can also be used in place of gentian violet.

Many species of bacteria which were supposed not to possess a capsule show them very distinctly by this method.

It is of especial value in differentiating the capsule of Bacillus anthracis according to 'Johne.'

§ XXIII. METHODS FOR STAINING CILIA OR FLAGELLA.

Löffler's Method, Modified by Günther.

1. Take a young culture grown on the surface of agar-agar media, and make a hanging-drop specimen, examine it under the microscope, and note if the bacteria are motile.

2. Put a drop of distilled water on a clean cover-glass with a sterile platinum loop, inoculate the drop with a little of the culture material, and spread gently over the surface of the cover-glass.

3. Allow the specimen to air dry, and then pass three times through the flame, but be careful not to heat too much.

4 Filter a few drops of Löffler's mordant (see § 62) on the cover-glass, and allow it to remain one-half to one minute. (Heat is unnecessary, as it only yields dirty, unsatisfactory specimens.)

5. Remove the mordant with a fine stream of water from the wash-bottle, and dry the cover-glass in the ordinary manner by blowing, etc.

6. Filter a drop of anilin water fuchsin solution on the surface of the cover-glass, or without filtering place a drop of fresh watery alcoholic solution of fuchsin on the cover-glass with a pipette, and heat the cover-glass over the flame until steam arises, remove, and allow the warm stain to remain one minute on the cover-glass, then wash off with water.

7. Dry quickly, and mount in xylol balsam.

The addition of acids or alkali to the mordant are now no longer considered necessary, and Günther considers heating the mordant unnecessary.

§ XXIV. BOWHILL'S METHOD OF STAINING FLAGELLA AND BACTERIA SIMULTANEOUSLY WITH ORCEIN.

1. A small quantity of material is taken from the surface of a young agar-agar culture (previously tested by 'the Hanging Drop'), and a suspension made in boiled distilled water in a test-tube.

2. Leave the tube undisturbed for five minutes, then place one drop of the bacteria-suspension on a clean cover-glass, and air dry.

3. Fix in the flame, holding the specimen between the fingers to prevent excessive heating.

4. Pour some orcein solution (see Stains, § 59) in a watch glass, float the cover-glass, preparation side downwards, on the surface of

the solution, and heat gently—*do not boil*—leaving the specimen in the solution ten to fifteen minutes.

5. Wash the preparation in the ordinary manner with water. Examine in water, and if satisfactory mount in *xylol balsam.* The advantage of examining the specimen in water is that the flagella appear more distinct than in balsam, and if too faintly stained, the specimen can again be placed in the orcein solution, and the process *repeated.*

§ XXV. THE FLAGELLA OF THE FOLLOWING BACTERIA STAIN BY THE ABOVE METHOD.

1. Spirillum choleræ Asiaticæ.
2. Bacillus typhi abdominalis.
3. Bacillus coli communis.
4. Klein's Bacillus of Swine Fever.
5. Bacillus subtilis.
6. Bacillus violaceus.
7. Bacillus fluorescens liquefaciens.
8. Bacillus prodigiosus.
9. Proteus vulgaris.
10. Vibrio Finkler-Prior.
11. Vibrio Metschnikoff.
12. Vibrio aquatalis.
13. Vibrio Berolinensis.
14. Vibrio Rugula.
15. Bacteria in hay infusion.
16. Bacteria in oat-straw infusion.
17. Infusoria and various bacteria in canal water.
18. Bacillus tetani.
19. Bacillus œdematis maligni.
20. Bacillus of Symtomatic Anthrax.

§ XXVI. METHODS OF STAINING SPORES.

Endospores possess a firm membrane or capsule of great resistance towards staining reagents, and can only be stained with certainty on cover-glass specimens.

Ordinary Method.

1. Prepare the specimen in the ordinary way (see § 8), air dry, and fix in the flame by the ordinary method.

2. Float the cover-glass, preparation side downwards, on a watch

glass or small porcelain evaporating dish of Ehrlich's anilin water fuchsin or gentian violet, or Ziehl's carbol fuchsin, heated until vapour arises, when it is set aside to cool, and allow the stain to work in for one minute. '*This process is repeated five times.*'

3. Decolorize with 3 per cent. HCl. alcohol, allowing one minute to work in; the spores remain stained.

4. Wash the cover-glass with water.

5. Contrast stain, with methylene blue, if the spores are stained with the fuchsin stain; if with the violet stain, then use a Bismarck brown as a contrast.

6. Wash in water, dry, and mount in xylol balsam.

Result.—With *fuchsin*, the spores are stained red and the bacilli blue; with *gentian violet*, the spores are stained blue and the bacilli brown.

§ XXVII.—SPECIAL METHOD OF STAINING THE ENDOSPORES OF BACILLUS ANTHRACIS.

1. Make a hanging-drop culture (see method, § 6) from the heart's blood of an inoculated mouse.

2. Place the hanging drop in an incubator from twenty-four to forty-eight hours, at 35° C., until spores are developed.

3. Remove the cover-glass from the slide.

4. Air dry.

5. Remove the vaseline with xylol.

6. Fix in the flame in ordinary way.

7. Proceed as at process No. 2. (Ordinary method of staining Spores, see § 26).

§ XXVIII. THE EXAMINATION OF BLOOD BY COVER-GLASS METHODS.

1. Handle the cover-glass with forceps only, as the heat of the hand injures the specimen.

2. Place an extremely small drop of blood on the cover-glass, either directly from the living subject or with the platinum loop.

3. Spread the drop of blood by placing a second cover-glass on the top of the first. Avoid pressure, and draw the two cover-glasses apart in a horizontal direction.

4. Air dry under a glass cover.

5. The cover-glass can be fixed by either of the following methods:—

(*a*) Exposure to a temperature of 110–115° C. in hot-air chamber for a few minutes.

(*b*) Immersion in absolute alcohol.

(*c*) Immersion in equal parts of absolute alcohol and sulphuric ether for thirty minutes.

(*d*) Immersion in a solution of formalin for five minutes.

(*e*) According to Ehrlich's method, on a metal plate, which is heated.

The above methods of fixing are specially adapted for detecting corpuscular changes, etc. For ordinary bacteria proceed as at No. 4, general method, § 8.

6. To stain for bacteria, use the ordinary stains for that purpose. The special stains for Malaria are described under 'Malaria' in Part III.

Precautions.—In examining specimens of blood for bacteria an excess of hæmoglobin is liable to exist, which interferes with the isolation of the organism. Remove the excess before fixing, by placing the cover-glass specimen in a three per cent. solution of acetic acid for a few seconds, wash with distilled water, dry, and proceed as above at No. 4, general method, page 17.

§ XXIX. METHOD OF REMOUNTING AND RESTAINING COVER-GLASS SPECIMENS.

1. Heat the slide in the flame of the Bunsen to warm the balsam; when it is melted remove the cover-glass, and transfer to another slide.

2. To restain the cover-glass specimen, place it, after removal from the slide, in xylol for twenty-four hours, which is changed several times to ensure thorough removal of the balsam.

3. The cover-glass is now placed in absolute alcohol to remove the xylol, and stained according to the method desired.

§ XXX. NECESSARY PRECAUTIONS IN MANIPULATING COVER-GLASS SPECIMENS.

1. Never handle a heated cover-glass with cold forceps, as it generally breaks.

2. All specimens must be thoroughly air-dried, otherwise the albuminoids coagulate under the influence of heat. Some bacteria, *i.e.*, Bacillus anthracis, change their form, break up, are surrounded by a halo, and lose their affinity for the stain.

3. During the process of fixing, the specimen must on no account be scorched in the flame, otherwise the form and staining properties of the bacteria, etc., are entirely lost.

4. To remove immersion oil from the cover-glass of a freshly mounted specimen absorb the most of the oil with a piece of filter paper, then the remainder can be removed with xylol when the balsam sets.

§ XXXI. METHODS OF EXAMINING SECTIONS OF ORGANS AND TISSUES FOR THE DETECTION OF BACTERIA.

There is no method of demonstrating bacteria in unprepared specimens. All tissues, organs, etc., must be cut into sections to study the relative position of the bacteria present in the tissue; and before cutting into sections, the tissue, organs, etc., must be thoroughly fixed and placed beyond the reach of cadaveric changes as follows:—

1. Cut the portion of organ or tissue into pieces the size of a nut before putrefactive changes can take place.
2. Place the squares of tissue on small pieces of filter paper, write the name of the organ or tissue on the side of the paper, and place in alcohol, when the block of tissue becomes firmly fixed to the filter paper, which also assists in keeping the portion to be hardened above in the anhydrous stratum.
3. The blocks of tissue remain two or three days to harden in the alcohol, which is changed several times.
4. Pieces of kidney, liver, and muscle, after remaining in alcohol two or three days, can be fastened to a block with the following preparation:—

Gelatine . .	1 gramme.
Water . .	2 c. c.
Glycerine . .	4 c. c.

Heat and dissolve to a thick consistency, when the tissue is fastened to the block with the above adhesive mixture, place in alcohol, preparation side downwards, and in twenty-four hours the gelatine fixing will be sufficiently hard to admit of the tissue or organ being cut into sections with the microtome.

The above method is suitable for firm tissues and organs, but delicate structures must be imbedded in celloidin, or frozen, before they can be cut into sections.

Formalin can also be used for hardening tissues, etc., but the pieces must be very small, as formalin lacks the power of great penetration; however, small pieces of tissue, etc., can be laid in one part of 40 per cent. formalin and ten parts of water for six to eight hours, and then placed in absolute alcohol.

§ XXXII. METHOD OF EMBEDDING IN CELLOIDIN.

1. Transfer the hardened tissue or organ from the alcohol into equal parts of alcohol and sulphuric ether for twelve hours.

2. Transfer the tissue, etc., into a solution of celloidin made with equal parts of alcohol and sulphuric ether and enough celloidin to form a thin syrup; small pieces of tissue, etc., remain in this solution two days, while larger pieces remain four or five days.

3. Remove the piece of tissue and place in a small porcelain evaporating dish, pour in some of the lower or thicker solution of celloidin until the tissue is covered, when the dish is set aside until the celloidin is quite hard; at least twenty-four hours, sometimes longer, are required.

4. Cut the celloidin away from the sides of the porcelain dish, place the imbedded tissue on a block, and fasten with a little celloidin solution. Expose the block to the air until the celloidin hardens—about two hours—and then place in 80 per cent. alcohol, and keep until required. Absolute alcohol must on no account be used, as it dissolves the celloidin.

§ XXXIII. METHOD OF PREPARING TISSUES FOR FREEZING.

The method of cutting sections with the freezing microtome is conducted with a special apparatus sold with most of the modern microtomes, consisting of an ether spray and special platform for holding the block of tissue. A small microtome, manufactured by Jung of Heidelberg, known as the Students' Microtome, is specially adapted for frozen objects and those imbedded in paraffin, being fitted with a mechanical knife-guide, enabling the most inexperienced to make serial sections without difficulty (see Fig. 8).

§ XXXIV. BEFORE FREEZING.

All traces of alcohol must be removed from the hardened tissue as follows:—

1. Place in a 1 per cent. solution of 40 per cent. formalin in water for two hours. Pieces of fresh tissue are prepared in a similar manner.

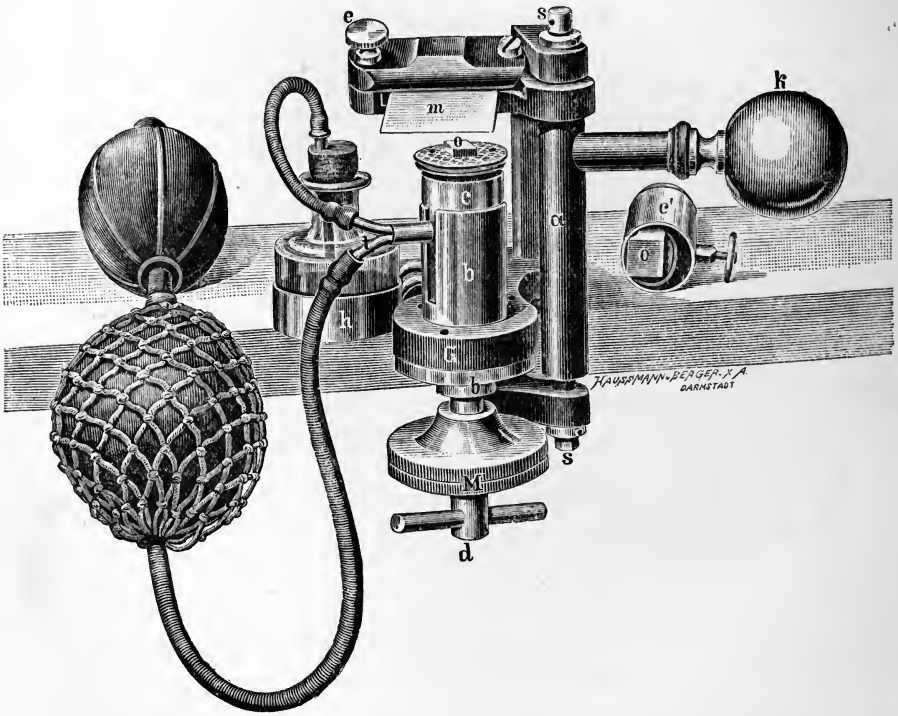


Fig. 8.—Jung's Students' Microtome.

2. A piece of Joseph's paper or ordinary lens paper is laid on the freezing plate of the microtome, the tissue laid on top of it, and frozen with the ether spray. When hard it is cut into sections, and the cut sections placed in water.

§ XXXV. For cutting sections of tissue embedded in celloidin a microtome, with the knife working at an angle, is necessary, and a most serviceable microtome is manufactured by Schanze, Leipzig (see Fig. 9).

It is important to remember that whatever microtome is used in cutting tissues embedded in celloidin, botht he preparation and knife must be kept continuously wet with alcohol, and the cut sections

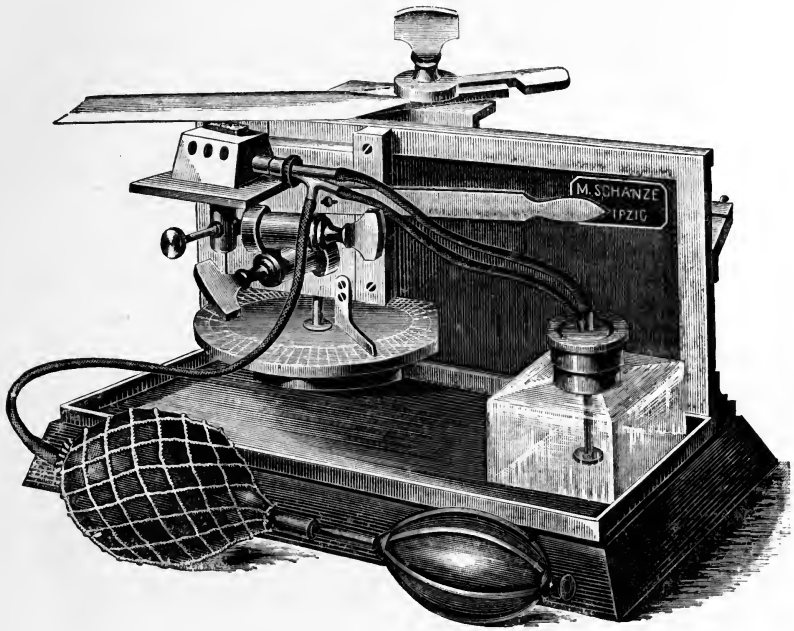


Fig. 9.—Schanze's Microtome.

removed from the knife with a camel's-hair brush, into 60 or 70 per cent. alcohol. The following conditions are absolutely necessary :—

1. A sharp microtome knife.
2. Hard tissue.
3. The tissue firmly attached to the block.

In cutting sections of tissues for the demonstration of bacteria they need not be any thinner than 0·02 mm.

§ XXXVI. To stain bacteria in sections, the section is, with few exceptions, always brought directly out of the alcohol into the colouring solution.

1. Sections remain longer in the dye than cover-glass specimens.
2. It is often necessary to warm the staining solution either in

the incubator or with a spirit lamp or Bunsen burner, '*but only till vapour arises.*'

3. Decolorizing processes have the effect of decolorizing the nuclei and rendering them obscure; to prevent this, stain the sections first with lithio- or picro-carmine. (See Günther's modification of the Gram method, § 38).
4. To reduce or prevent the bleaching effects of alcohol during decolorization, add to the alcohol a small quantity of the stain with which the section was treated in the first place.
5. In clarifying sections do not use clove oil for anilin dyes (except in the Cladius method), as it has the power of removing the stain. Use oil of cedar, and best of all for bacteria is xylol.

§ XXXVII. METHODS OF STAINING BACTERIA IN SECTIONS OF ORGANS AND TISSUES.

Weigert's Original Method.

The section is carried from one watch-glass to the other with a needle or spatula.

1. Remove the section out of alcohol into water, or directly into the stain one-half to one minute.
2. Into stain 'methylene blue' one to two minutes.
3. Wash the section in water.
4. Into one-half per cent. solution of acetic acid in water for one minute.
5. Into absolute alcohol thirty seconds, spreading the section well out.
6. Again in absolute alcohol thirty seconds.
7. Into xylol thirty seconds.
8. Place on a slide, dry with filter-paper, and mount in *xylol balsam*.

Anthrax bacilli can be detected in sections by this method, as also many of the bacteria belonging to the Septicæmiæ Hæmorrhagicæ group of organisms, but care must be used not to carry the acid and alcohol treatment too far. The Glanders bacilli can also be demonstrated in sections of tissue with this stain.

§ XXXVIII. THE GRAM-GÜNTHER METHOD FOR SECTIONS.

1. Remove the sections into water for two or three minutes.
2. Into a solution of picro-carmine two to five minutes.

3. Wash the section in four or five changes of water.

4. Into alcohol.

5. Into Ehrlich's anilin water, gentian violet, or methyl violet (see § 55) one to two minutes.

6. Remove section into Gram's iodine solution (see § 60) for two minutes, and spread the section well out.

7. Into alcohol thirty seconds.

8. Into 3 per cent. HCl. alcohol (see § 70) for ten seconds.

9. Now into alcohol many times until a maximal decolorization is obtained, *i.e.*, until no more colouring matter comes away.

10. Into xylol one-half to one minute.

11. Remove the excess of xylol with filter-paper, and mount the section in xylol balsam.

When the Gram method is used for sections without Professor Günther's modification, only the bacteria are stained, but by first staining with picro-carmine a beautiful double-stained specimen is the result, enabling the relation between the bacteria and the tissue to be distinctly observed.

The Gram method does not stain the cells except plasma or granule cells, which are sometimes mistaken for clusters of micrococci. Some of the epidermis cells also stain, and liver cells decolorize with difficulty.

The same bacteria are stained in sections by the Gram method as are stained in cover-glass specimens (see § 11).

§ XXXIX. THE CLADIUS 'CONTRAST STAIN' FOR SECTIONS.

1. Stain the section with 1 per cent. watery solution of methyl violet for two minutes.

2. Wash in water, and dry with filter-paper.

3. Into picric acid solution (see § 61) for two minutes.

4. Decolorize in clove oil, and dry with filter-paper.

5. Into xylol.

6. Mount in xylol balsam.

This stain acts in a similar manner to Gram's method, and for many bacteria it is preferable. For organisms stained by the method, see Gram's method, § 11.

The original paper does not mention the method of applying the clove oil; but in conducting and testing the special action of this stain it was found necessary to place the section on the slide during the process of decolorizing with the clove oil, to prevent its curling up.

§ XL. EHRLICH'S METHOD OF STAINING TUBERCLE BACILLI IN SECTIONS OF TISSUE, ORGANS, &c.

(*a*) Alcohol Sections.

1. Place sections in Ehrlich's anilin water fuchsin* (see § 55), and set the glass dish in the incubator at 37° C. for one hour and a half, and at room temperature twenty-four hours.
2. Wash in water five minutes.
3. Into 3 per cent. HCl. alcohol one minute, and move round, not allowed to simply lie in the alcohol.
4. Wash in water.
5. Contrast stain with methylene blue or Bismarck brown.
6. Wash in water.
7. Into alcohol to remove water.
8. Into xylol.
9. Mount in xylol balsam.

* Ehrlich's anilin water gentian violet can also be used, or Ziehl's carbo fuchsin.

(*b*) Frozen Sections.

1. Transfer the sections from the salt solution into 80 per cent. alcohol for five minutes to harden; or,
2. Harden in corrosive sublimate, 1 to 1000-1500 for one-half to one hour, and wash in water, and proceed as above at process No. 1.

§ XLI. LÖFFLER'S UNIVERSAL METHOD OF STAINING SECTIONS.

1. Bring the sections out of alcohol into Löffler's methylene blue (see § 54) for five to thirty minutes.
2. Into 1 per cent. acetic acid.
3. Into absolute alcohol.
4. Into xylol.
5. Mount in xylol balsam.

The amount of time the section remains in the acetic acid solution will depend on the variety of organism the operator is working with By this method

Bacilli are stained = dark black-blue.
Cellular constituents = blue.
The Protoplasm = light blue.

This method can be employed for almost all bacteria.

§ XLII. WEIGERT'S METHOD FOR FIBRIN OR BACTERIA IN SECTIONS.

1. Harden section in alcohol.
2. Stain in Ehrlich's anilin water gentian violet (see § 55) five to fifteen minutes.
3. Wash the section in 6 per cent. solution of chloride of sodium.
4. Dry the section with filter-paper.
5. Place in a 1 or 2 per cent. watery solution of iodide of potash for two or three minutes.
6. Dry with filter-paper.
7. Decolorize in the following solution :—

Anilin oil, . . .	2 c. c.
Xylol, . . .	1 c. c.

8. Place the section in xylol to remove the anilin oil.
9. Mount in xylol balsam.

§ XLIII. LÖFFLER'S METHOD OF STAINING THE GLANDERS BACILLUS IN SECTIONS.

1. Stain the section in Löffler's methylene blue (see § 54) for a few minutes.
2. Place the section in the following solution :—

Aqua distilli, . . .	10 c. c.
Concd. Sulphuric acid, . .	2 drops.
5 per cent. sol. of Oxalic acid, .	1 drop.

for five seconds.
3. Decolorize and dehydrate in absolute alcohol.
4. Place the section in xylol.
5. Mount in xylol balsam.

§ XLIV. ANOTHER METHOD OF STAINING THE GLANDERS BACILLUS IN SECTIONS OF TISSUES.

1. Transfer the sections from alcohol to distilled water.
2. Transfer from the water to a slide, and dry thoroughly with filter-paper.
3. Stain with a few drops of the following stain for thirty minutes :—

Carbol. fuchsin, . .	10 c. c.
Distilled water, . .	100 c. c.

4. Remove superfluous stain with filter-paper, wash the section three times with 3 per cent. acetic acid, and do not allow the acid to act for more than ten seconds each time.

5. Remove all traces of the acid with distilled water, absorb the water with filter-paper.

6. Dry the section with gentle heat according to the dry method of Unna (see § 46). Clear in xylol, and mount in xylol balsam.

§ XLV. KÜHNE'S METHYLENE BLUE METHOD FOR STAINING SECTIONS.

1. Bring the sections out of alcohol into Kühne's methylene blue (see § 56) from a half to one hour (leprosy sections remain longer).

2. Wash quickly in water.

3. Wash in water containing 1·5 to 2 per cent. hydrochloric acid until the section becomes light blue in colour.

4. Transfer the section to a solution of lithium carbonate, prepared as follows:—

Concentrated watery solution of carbonate of lithia, 68 drops.
Water 10 c. c.

5. Place the section in clean water three to five minutes.

6. Immerse the section for a short time in absolute alcohol, to which a little methylene blue in substance is added.

7. Rinse the section completely in pure anilin oil.

8. Place the section in thymol or oil of turpentine for two minutes.

9. Place in xylol.

10. Mount in xylol balsam.

The advantages of this method are that the bacteria are not decolorized, whereas the tissues are sufficiently so to render the bacteria visible, and admit of the use of contrast stains.

§ XLVI. UNNA'S 'DRY METHOD' FOR SECTIONS.

Many bacteria during the washing process become entirely decolorized, and to prevent this result the dry method of Unna is employed as follows:—

1. The section is removed from the staining solution directly into water.

2. Spread the section out on a slide, and remove excess of water with filter-paper.

3. The slide must be carefully heated over the flame, 'not cooked,' until the section is dry, when it is allowed to cool and mounted in xylol balsam.

Contrast stains by this method are of little use, as the tissue cells are too much altered by the heating. Professor Günther recommends the 'Dry Method of Unna' as the only way to obtain permanent specimens of tubercle bacilli in sections.

§ XLVII. NECESSARY PRECAUTIONS IN THE MANIPULATION AND STAINING OF SECTIONS.

1. When staining sections, never do a number at once until you have tested the correctness of your method in a few first.

2. In the enumeration of the various staining methods precise directions as to time, etc., have been given; it is well, however, to remember that these directions are for uniform preparations of the stains, solutions, etc.

3. By staining, the difference in length of bacteria, however slight, is demonstrated, also the extremely characteristic shape of some species.

4. By staining, an insight is gained into micro-organismal life existing in the tissues, while double staining enables the bacteria to be distinguished from the tissues with remarkable precision. It is an art requiring great care and study, invaluable in the hands of those who know how to employ it.

§ XLVIII. THE FOLLOWING ARE THE MOST COMMON STAINING FAULTS.

1. Portions of organs left too long before being put in alcohol commence to putrefy, *i.e.*, putrefactive bacteria and fungi gain access, and in staining sections from such a specimen, for a definite organism, the putrefactive bacteria are also stained, and such being the case, particular attention must be given to the distribution of the bacteria throughout the section, as putrefactive bacteria penetrates the organ or piece of tissue from without, their numbers are therefore found to diminish in proportion to the distance from the outside surface, while the inner portions are usually quite free from putrefactive bacteria.

2. Many staining solutions, *i.e.*, carmine, hæmotoxilin — even distilled water, and some of the anilin colours often contain bacteria.

3. Faulty staining is liable to occur by the Gram method, when

part of the colouring matter may be deposited on the surface of the sections. The use of too strong acids sometimes cause rod organisms to break up into beads, when they are liable to be mistaken for a chain of cocci. In tissues, extreme caution is necessary in the examination of plasma or granule cells. These cells under the action of anilin dyes behave directly the opposite of all other cells. They generally occur as large flat formations of the outer wall of vessels, consisting of a nucleus and very fine grained protoplasm. It is only the protoplasm of those cells that stains, the nucleus does not, therefore it requires close observation to find it, while the cells contain a deeply stained mass of granules, strongly resembling a colony of micrococci. They are, however, distinguished by the granules being of unequal size. The nucleus present, when sought for, and the occurrence together of, one or more cells of the same size. When the section is stained with methylene blue, the plasma cells are stained a deep violet colour. Plasma cells also sometimes stain according to Gram's method, which further leads to their being mistaken for micrococci. Some parts of the epidermis also stain by Gram's method, and liver cells decolorize with difficulty.

STAINS.

§ XLIX. STOCK SOLUTIONS.

1. Concentrated alcoholic solution of fuchsin.
2. Do. do. do. gentian violet.
3. Do. do. do. methyl violet.
4. Do. do. do. methylene blue.

In preparing the above stock solutions, use about half an ounce of each dye, and place in 8-ounce bottles with glass stopper, filled with alcohol. These solutions are not directly employed for staining purposes.

§ L. ORDINARY STAINS FOR DAILY USE.

(1) Take of the undissolved dye 2 grammes, and distilled water 85 c. cm.

Boil five to ten minutes, and after cooling, add 15 c. cm. of 90 per cent. alcohol ; mix thoroughly and filter.

Or (2) mix 5 c. cm. of the concentrated alcoholic solution of the dye desired with 50 c. cm. of distilled water.

Or (3) fill an ordinary test-tube three-quarters full of distilled water, and add enough of the concentrated alcoholic or watery solution of the dye, until you can just see through the solution, which is ready for use.

§ LI. SPECIAL STAINS FOR COVER-GLASS SPECIMENS AND SECTIONS OF TISSUES.

Ziehl's carbol. fuchsin.

Fuchsin	1·0 gramme.
Acid, carbolic	5·0 grammes.
Alcohol	10·0 c. cm.
Aqua distilli	100 „

§ LII. GABBET'S SOLUTION.

Methylene blue	1 to 2 grammes.
Aqua distilli	75 c. cm.
Concentrated sulphuric acid	25 „

This solution is used in the Ziehl Gabbet method for demonstrating the presence of the bacillus of tuberculosis; the acid decolorizes, whilst the methylene blue acts as a contrast stain, and is of especial value owing to the simplicity and rapidity of its action.

§ LIII. ROUX'S DOUBLE STAIN.

Dahlia or gentian violet	·5 grammes.
Methyl green	1·5 „
Aqua distilli	200 c. cm.

Method for Cover-Glasses.

1. Air dry and fix cover-glass.
2. Stain from five to ten seconds.
3. Wash in water.
4. Dry and mount in Canada balsam dissolved in xylol. Sections remain in this stain twelve hours, then washed, dehydrated, and mounted. This stain is, as already mentioned, of especial value in the examination of diphtheritic specimens.

§ LIV. LÖFFLER'S METHYLENE BLUE.

Concentrated alcoholic Solution of methylene blue, 30 c. cm.
Watery solution of caustic potash (1 : 10,000) 100 „
This stain is specially adapted for staining the glanders bacillus.

§ LV. EHRLICH'S ANILIN WATER, GENTIAN VIOLET, FUCHSIN, OR METHYL VIOLET.

Anilin oil	4 c. cm.	This is known as anilin water.
Distilled water	100 „	

Shake the above well together, filter, and add 11 c. cm. of a concentrated alcoholic solution, gentian violet, methyl violet, or fuchsin, whichever stain is required; shake the mixture, and set aside from twelve to twenty-four hours before using.

These stains yield good results in staining many forms of organisms, especially tubercle bacilli, and in conjunction with Gram's method; but they have one drawback, they are very unstable, and soon decompose, requiring to be renewed every three weeks.

§ LVI. KÜHNE'S METHYLENE BLUE.

Methylene blue	1·5 grammes.
Absolute alcohol	10·0 „
5 per cent. acid carbolic in water	100·0 „

Mix the methylene blue and the alcohol, rub in a mortar until the methylene blue is thoroughly dissolved, and then add the 5 per cent. solution of carbolic acid.

§ LVII. CHLOROFORM FUCHSIN SOLUTION.

(1) One or two crystals of fuchsin are dissolved in chloroform 2 to 3 c. cm.

Or (2) concentrated alcoholic solution of fuchsin, three or four drops chloroform 2 to 3 c. cm.

Aren's method of staining tubercle bacilli in fatty substances, milk, etc., etc.

§ LVIII. NEISSER'S STAINS FOR DIFFERENTIAL DIAGNOSIS OF DIPHTHERIA.

I.

Methylene blue	1 gramme.
96 per cent. alcohol	20 c. cm.

When dissolved, add—

Aqua distilli	950 c. cm.
Glacial acetic acid	50 „

II.

Vesuvin	2 grammes.
Aqua distilli boiled	1000·0 c. cm.

§ LIX. BOWHILL'S ORCËIN STAIN FOR FLAGELLA AND BACTERIA.

A saturated alcoholic solution of orcëin (this solution possesses greater staining powers if allowed to stand ten days before use).

A 20 per cent. solution of tannic acid in water (dissolved by heating before use. The above solutions are mixed as follows with distilled water :—

Saturated alcoholic solution of orcëin .	15 c. cm.
20 per cent. watery solution of tannic acid	10 „
Distilled water	30 „

Mix and filter.

MORDANTS.

For Demonstrating certain Bacteria.

§ LX. GRAM'S SOLUTION OF IODINE.

Iodine crystals, ...	1 gramme.
Iodide of potash, . . .	2 „
Distilled water, ...	300 c. cm.

§ LXI. M. CLADIUS'S METHOD.

One-half per cent. solution of picric acid in distilled water, . . .	50 c. cm.
Distilled water, ...	50 „

Used with a 1 per cent. watery solution of methyl violet.

For the Demonstration of Cilia or Flagella.

§ LXII. LÖFFLER'S MORDANT.

Dissolve by heating together

Tannin, ...	2 grammes.
Distilled water, ...	8 c. cm.

And to this solution add

Concentrated cold water solution of ferrous sulphate, . . .	5 c. cm.

And

Concentrated alcoholic solution of fuchsin,	1 c. cm.

Shake well together, and after filtering, it is ready for use.

§ LXIII. BUNGE'S MORDANT.

Ferric chloride B.P. solution diluted 1 to 20 of water, ...	25 c. cm.
Saturated aqueous solution of tannin, .	75 „

N.B.—Recent researches by Günther, Fischer, and Lehman, show that the addition of alkalis or of acids, according to Löffler, are superfluous, as also the hydrogen per oxide solution added by Bunge to his mordant.

§ LXIV. SOLUTION FOR CLEANSING AND DISINFECTING —FOR COVER-GLASSES.

Newly purchased cover-glasses are placed in a solution of nitric acid and water for twenty-four hours; removed, washed in water, and kept until required in alcohol. The advantages of really clean cover-glasses and slides are appreciated by experienced microscopists.

§ LXV. SOLUTION FOR DIRTY COVER-GLASSES, SLIDES, AND OTHER GLASSWARE.

Water	50 c. cm.
Methylated alcohol . . .	45 „
Strong ammonia . . .	5 „

§ LXVI. DISINFECTING SOLUTION FOR POTATOES, HANDS, &c.

Bichloride of mercury . . .	1 gramme.
Water	1000 c. cm.
Hydrochloric acid, 'strong' . .	5 „

§ LXVII. LÖFFLER'S METHOD FOR CLEANSING COVER-GLASSES.

1. Warm the cover-glasses for some time in concentrated sulphuric acid.
2. Rinse in water.
3. Place until wanted in a mixture of equal parts of alcohol and ammonia.
4. Before use, dry with a cloth, from which all fat has been extracted.

SPECIAL REAGENTS.

§ LXVIII. 'SOLIDS.'

1. Boracic acid.
2. Celloidin.
3. Acid, carbolic.
4. Ferrous sulphate.
5. Iodine crystals.
6. Mercuric bichloride.
7. Acid, oxalic.
8. Potass. causticum.
9. Potassium, Iodine.
10. Picric acid.
11. Pyrogallic acid.
12. Sodium,bicarbonate.
13. Sodium causticum.
14. Tannin.
15. Zinc (metallic).
16. Paraffin.
17. Grape sugar.
18. Milk sugar.

§ LXIX. 'LIQUIDS.'

1. Acetic acid (glacial).
2. Alcohol, absolute.
3. Ammon. fort. liqr.
4. Anilin oil.
5. Chloroform.
6. Ether, sulphuric anhydrous.
7. Ferric chloride.
8. Formaline, 40 per cent. solution.
9. Glycerine (Price's).
10. Acid, hydrochloric.
11. Acid, nitric.
12. Acid, sulphuric.
13. Water, distilled.
14. Celloidin.
15. Lysol.

The purposes of the above reagents are described in the chapters dealing with specific methods, etc., etc.

§ LXX. DIFFERENTIATING AGENTS.

1. Distilled water; 2. Absolute alcohol; 3. Acid alcohol, made as follows :—

90 per cent. alcohol . . .	100 c. cm.
Aqua distilli	200 „

and the desired percentage of either hydrochloric, nitric, sulphuric, or oxalic acids.

4. Anilin oil, 2 parts . .	Weigert's.
Xylol, 1 part . . .	

5. Oil of cloves; 6. Gram and Cladius's solutions (see §§ 60, 61).

§ LXXI. SPECIAL ACTIONS OF THE ABOVE REAGENTS

1. Distilled water, remove surplus stain.
2. Absolute alcohol, dehydrate and remove stain.
3. The acid alcohols are used to decolorize cover-glass specimens

and sections, in order to differentiate the micro-organism which do not, in some instances, decolorize as readily as the tissues.

4. The anilin oil and xylol are recommended by Weigert in his 'Fibrin, or Bacteria Stain in Sections.'

5. Oil of cloves is used in the Cladius's method for sections.

§ LXXII. CLEARING AND MOUNTING MEDIA.

1. Xylol.
2. Oil of cedar.
3. Turpentine.
4. Oil of origanum.
5. Canada balsam dissolved in xylol is the best mounting medium.
6. Vaseline for preparing hanging drop preparations.

PART II.

THE PREPARATION OF NUTRIENT MEDIA—BOUILLON MEDIA.

§ LXXIII. ORDINARY BOUILLON.

1. Cut 500 grammes of lean beef or veal, free from fat, into small pieces, add 1 litre of distilled water, and set in a cool place for twelve to twenty-four hours. In hot weather place in an ice chest, or boil over an open flame for one hour, stirring continually.

2. Place the mixture in a clean piece of cloth or fine muslin, strain into a flask, and squeeze until you obtain 1 litre of meat infusion. It has an acid reaction.

3. Add, Peptone siccum, 10 grammes.
Common salt, 5 „

4. Heat the flask in the water-bath for one to one and a half hours, or warm directly over the flame, using an ordinary enamel sauce-pan with several pieces of wire gauze or a sheet of asbestos intervening between the flame and the pan.

5. Make *slightly* alkaline with a saturated solution of sodium bicarbonate, '*if too much alkali is added the bouillon will never clarify.*' The white of a hen's egg can be added, but it is not absolutely necessary.

6. Sterilize in the steam sterilizer one to two hours, remove and filter through two thicknesses of filter-paper when '*it is cold.*' If filtered when hot, fatty substances pass through, '*it must be filtered very slowly.*'

7. Place again in the steam sterilizer for one hour, and if on removal the bouillon is of a clear golden colour '*it is good.*' If it is '*turbid*' it is allowed to cool, is filtered, sterilized for one hour, and the process repeated until it is thoroughly clarified.

8. Pour the bouillon into clean tubes (about 10 c. c. in each), or else into small Erlenmeyer flasks, and close with cotton wadding plugs. (*In making a cotton wadding plug, twist the cotton together, and when placed in the tube the relaxation of the twist ensures a tight fit.*)

9. The tubes or flasks are finally sterilized in the steam sterilizer for fifteen to thirty minutes on each of three successive days. The bouillon is ready for use if on cooling it still remains perfectly clear.

§ LXXIV. GRAPE SUGAR BOUILLON.

Put half a gramme of grape sugar into a small Erlenmeyer flask, add 100 c. cm. of bouillon after it is filtered, and proceed as in process No. 7, ordinary bouillon, § 73.

Milk Sugar Bouillon.

Prepare the same as grape sugar bouillon, but add 2 per cent. of milk sugar instead of ½ per cent. of grape sugar.

§ LXXV. GLYCERINE BOUILLON.

Add 4 to 6 grammes of glycerine to 100 c. c. of bouillon either at process No. 3 or at process No. 7, ordinary bouillon, § 73, and continue the following processes:—

Instead of using lean beef or veal to make a meat infusion, Liebig's Meat Extract can be substituted, using 10 grammes of the extract to 1 litre of distilled water, but the resulting medium is brown coloured, whereas with the meat infusion it is colourless.

POTATO MEDIA.

§ LXXVI. ORDINARY METHOD.

1. Select some good potatoes and wash thoroughly with the potato-brush and water, cutting out any eyes with the potato-knife.

2. Lay the cleansed potatoes in sublimate solution, (see § 66), for thirty minutes.

3. Sterilize in the steam sterilizer one-half to three quarters of an hour.

4. Wash the hands in sublimate solution, remove the potatoes, and cut in two with a sterilized potato-knife.

5. Lay the cut pieces of potato in plate culture dishes with a piece of filter paper previously soaked in the sublimate solution in the bottom of the dish. '*The potatoes are not used until cold.*'

6. Use the platinum loops to inoculate the cut surface of the potatoes, and spread the material over the desired amount of surface with a sterilized potato-knife.

§ LXXVII. ESMARCH'S METHOD.

1. Thoroughly wash some potatoes.
2. Peel and cut off the ends.
3. Cut into sections 1 cm. thick and place in clean water.
4. Place the slices in Esmarch dishes.
5. Sterilize in the steam sterilizer three-quarters of an hour.
6. If the Esmarch dishes are not sterilized before the slices of potato are put in, then sterilize on each of three successive days as follows:—

First day, for 30 minutes
Second day, for 15 to 20 minutes
Third day, for 15 to 20 minutes
} In the steam sterilizer.

§ LXXVIII. ROUX AND GLOBIG'S METHOD.

1. Some potatoes are thoroughly washed, the ends cut off, bored through with a cork-borer, and the cylindrical pieces of potato laid in water.

2. The potato cylinders are cut diagonally in two and placed again in water.

3. Take some ordinary test-tubes, place some cotton wadding or a piece of glass rod in the bottom, add a little water, put a piece of potato in, and shake it down until it touches the material in the bottom of the tube, plug the tube with cotton wadding.

4. Potatoes as a rule have a slight acid reaction, and to render them faintly alkaline, lay the potatoes in a 1 per cent. solution of sodium carbonate before placing in the tubes or before sterilizing.

5. Sterilize on each of three successive days as follows:—

First day, for 30 minutes
Second day, for 15 to 20 minutes
Third day, for 15 to 20 minutes
} In the steam sterilizer.

§ LXXIX. HOLZ'S METHOD.

Potato Water for Bacillus Typhi Abdominalis.

1. Wash and peel 500 grammes of potatoes, grate very fine, and squeeze the juice through a linen cloth.

2. The juice is left twenty-four hours in an ice-chest before filtering, or is filtered immediately through animal charcoal.

3. Sterilize one hour in the steam sterilizer, add 10 per cent. of gelatine (Elsner adds 1 per cent. of iodide of potash to the gelatine before use), sterilize again one hour in the steam sterilizer, and fill into test-tubes.

4. Sterilize the tubes on each of three successive days as follows:—

First day, for 30 minutes
Second day, for 15 to 20 minutes
Third day, for 15 to 20 minutes
} In the steam sterilizer.

§ LXXX. POTATO WATER.

For Cultivating Bacillus Tuberculosis.

1. Grate 500 grammes of clean peeled potatoes, add 500 c.c. of water, and place in an ice-chest for twelve to twenty-four hours.

2. Decant the liquid portion and add enough distilled water to make 1000 c.c.

3. Cook one hour in the water-bath, filter, add 4 per cent. of glycerine, and fill into tubes.

4. Sterilize the tubes on each of three successive days as follows:—

First day, for 30 minutes
Second day, for 15 to 20 minutes
Third day, for 15 to 20 minutes
} In the steam sterilizer.

PEPTONE MEDIA.

§ LXXXI. PEPTONE WATER SOLUTION, 'KOCH.'

Witte's peptone	2 parts.
Sodium chloride	1 part.
Distilled water	100 parts.

§ LXXXII. DUNHAM'S PEPTONE SOLUTION.

Dried peptone . . .	1 gramme.
Sodium chloride . . .	0·5 „
Distilled water . . .	100 c.c.

Filter, place in tubes, and sterilize by the ordinary discontinuous method for three successive days.

This solution is used as a medium to determine if an organism under consideration possesses the property of producing indol as one of its products of nutrition.

§ LXXXIII. PEPTONE ROSOLIC ACID SOLUTION.

Dunham's peptone solution . . 100 c.c.
and 2 c.c. of the following solution :—

Rosolic acid (Coralline) . .	0.5 gramme.
Alcohol (80 per cent.) . .	100 c.c.

are boiled, filtered, placed in test-tubes, and sterilized by the ordinary discontinuous method for three successive days. This medium is used to study the reactions produced by different bacteria.

MILK MEDIA.

§ LXXXIV. The milk must be quite fresh and the reaction must not be acid. Place about 10 c.c. in each tube, plug, and sterilize in steam sterilizer for one hour on each of three successive days. When the tubes are not in the sterilizer they must be kept at about 20° C.

The sterilization of milk media is very important, as the spores of some of the milk bacteria are most resistant.

§ LXXXV. Milk media for testing the reactions of certain bacteria are prepared by adding a few drops of tincture of litmus to the medium before sterilization until it is slightly blue in colour. Ordinary milk media acquires a light brown colour after sterilization.

EGG MEDIA.

§ LXXXVI. HUEPPE'S METHOD.

1. Wash a fresh egg with a brush and soap and water.

2. Sterilize a glass dish with sublimate solution, wash with sterilized water, and dry with sterilized wadding.

3. Make an opening at one end of the egg with a hot needle and inoculate the egg.

4. Close the opening in the egg with a small piece of silk paper, and then apply a coating of collodion.

§ LXXXVII. GÜNTHER'S METHOD.

1. Wash one end of an egg with soap and water and a brush.

2. Sterilize one end of the egg with the flat surface of a clean hot potato-knife.

3. Heat a steel needle, and when cool, make an opening in the sterilized spot, large enough to admit the platinum needle with which the egg is inoculated.

4. Close with sterile paper and collodion, or with hot sealing-wax.

§ LXXXVIII. EGG ALBUMEN METHOD.

Take the white of an egg and distribute it in tubes, slant, and coagulate as with blood serum (see § 99.)

GELATINE MEDIA.

§ LXXXIX. NUTRIENT GELATINE.

1. Make 1 litre of meat infusion (see Bouillon, processes Nos. 1 and 2, § 73).

2. Gelatine . . . . 100 grammes.
   Peptone siccum . . . . 10 „
   Common salt . . . . 5 „

are placed in a glass boiling flask, and the meat infusion added last.

3. The gelatine and peptone are thoroughly dissolved in a water-bath, between 40° and 50° C., or directly over the flame, using an ordinary enamel saucepan, with several pieces of wire gauze or a sheet of asbestos intervening between the flame and the pan.

4. Test the reaction, make alkaline with a concentrated solution of sodium carbonate (the same caution is requisite as described at Bouillon, process No. 5, § 73), and add a fresh egg.

5. Place in the steam sterilizer for one and a quarter hours (too long boiling lowers the solidifying point of the gelatine), remove and filter through *two thicknesses* of filter paper. (*Fold the filter paper with a sharp point pushed well down into the neck of the glass funnel,*

which is placed obliquely in the flask, and a small Bunsen flame fixed at an angle a short distance from the neck of the glass funnel. The gelatine filters easily by this method, and a hot water funnel is unnecessary.)

6. The reaction is again tested, and if still faintly alkaline, fill into tubes, about 10 c.c. in each, and close with cotton wadding plugs. ('The Koch method of sterilizing wadding and tubes before use is now dispensed with, except for blood serum media.')

7. Sterilize the tubes of gelatine in the steam sterilizer for fifteen minutes on each of three successive days. The gelatine must finally be as clear as glass.

§ XC. GRAPE SUGAR GELATINE.

To 100 c.c. of nutrient gelatine, before it is sterilized (see § 89, No. 6), add 2 grammes of grape sugar.

§ XCI. MILK SUGAR GELATINE.

Add 2 grammes of milk sugar to 100 c.c. of gelatine in the manner described for grape sugar gelatine.

§ XCII. THREE PER CENT. COMMON SALT GELATINE.

Add 3 per cent. instead of the $\frac{1}{2}$ per cent. of common salt used in the ordinary process of making nutrient gelatine.

AGAR-AGAR MEDIA.

§ XCIII. ORDINARY AGAR-AGAR.

1. Make 1 litre of meat infusion (see Bouillon, processes Nos. 1 and 2, § 73), add

1 per cent. of peptone siccum	. .	10 grammes.
$\frac{1}{2}$ „ common salt	. .	5 „

2. Place the above in a clean flask, heat in a water-bath one to one and a half hours, and add 1 to 2 per cent. ($1\frac{1}{2}$ is the amount generally used) of agar-agar, finely powdered or cut into very small pieces, and boil five to eight hours until the agar-agar is thoroughly dissolved.

3. Make alkaline with sodium bicarbonate solution.

4. Place in the steam sterilizer one to two hours.

5. Filter through ordinary filter paper or flannel in the steam sterilizer.*

6. Fill the desired quantity into test-tubes, and close with cotton wadding plugs.

7. Sterilize in the steam sterilizer for fifteen minutes on each of three successive days.

§ XCIV. GRAPE SUGAR AGAR.

Add half a gramme of grape sugar to 100 c.c. of agar-agar medium after it is filtered, and proceed as at § 93, No. 6. In filtering grape sugar agar into tubes it is customary to fill the tubes two-thirds full, as this medium is used in the investigation of anaërobic and gas-forming bacteria.

§ XCV. GLYCERINE AGAR.

Add 4 to 6 grammes of glycerine to 100 c.c. of agar-agar medium, either before or after filtering, and proceed as at § 93, No. 6.

§ XCVI. WURTZ'S LACTOSE LITMUS AGAR.

1. To ordinary slightly alkaline agar-agar add 2 to 3 per cent. of lactose.

2. Fill into tubes and sterilize by the ordinary method for three consecutive days.

3. When sterilization is complete, add enough *sterilized* litmus tincture to give the medium a decided pale blue colour.

Bacteria causing fermentation of lactose, when grown on this medium, develop into colonies of a pale pink colour with a corresponding reddening of the surrounding medium. Bacteria incapable of producing fermentation grow in pale blue colours and cause no reddening of the surrounding media. (Instead of agar-agar, ordinary nutrient gelatine can be substituted in the preparation of this medium.)

§ XCVII. BLOOD AGAR.

1. A few drops of human or pigeon's blood, obtained under sterile precautions, are spread over the oblique surface of some ordinary sterile agar-agar tubes.

* The author filters agar through two pieces of moleskin cloth under pressure.

2. The prepared tubes are placed in the incubator at 37° C., and the non-sterile tubes placed on one side. (This medium is specially adapted for the gonococcus and Influenza bacillus.)

§ XCVIII. AGAR-AGAR GELATINE.

This medium is prepared in the same way as ordinary agar-agar, (§ 93), using 1·5 grammes of agar-agar, and when it is dissolved adding 50 grammes of gelatine.

BLOOD SERUM MEDIA.

§ XCIX. FLUID BLOOD SERUM.

1. Collect the blood from a living animal into a large sterile glass jar, under sterile precautions, and close it tightly.
2. Place the jar in an ice-chest for twenty-four hours.
3. Remove the serum into tubes with a sterile pipette.
4. Place the tubes of serum in the incubator at 37° C.
5. Remove any tubes that become turbid.

§ C. SOLID BLOOD SERUM.

1. Collect the blood the same as in No. 1 process for fluid blood serum.
2. About fifteen minutes after it has begun to clot, pass a sterilized glass rod between the clot and the wall of the jar, breaking up the adhesions that prevent the clot from sinking.
3. Close the jar tightly, and place in an ice-chest twenty-four to forty-eight hours.
4. Draw off the serum with sterilized pipettes into sterilized tubes, and close with sterilized cotton wool plugs. (*This is the only medium where the tubes and plugs are sterilized before filling.*)
5. Place the tubes in a blood serum sterilizer, and heat to 65° or 68° C. for one hour on each of five successive days, and place in the incubator at 37° C. for several days to test whether they are sterile.
6. Place the sterile tubes in Koch's apparatus for solidifying blood serum (see Fig. 10, p. 56), and heat to 75° or 80° C. until the serum is solidified.

§ CI. BLOOD SERUM 'QUICK METHOD.'

Processes 1 to 4, the same as § 100, solid serum.

5. Place the tubes in the serum sterilizer and heat at once to 90° or 95° C. for one hour or more.

6. Place the tubes in an inclined position in the steam sterilizer for one hour or more. The temperature must not run too high, otherwise bubbles form in the serum.

7. Place the tubes in the ordinary wire baskets, and sterilize as usual for fifteen minutes on each of three successive days.

Serum prepared according to this method is more opaque than when prepared according to method § 100, but answers all practical purposes, saves time, and avoids imperfect sterilization, somewhat common under the former method.

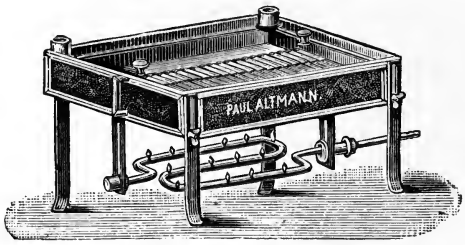


Fig. 10.—Koch's Apparatus for Congealing Blood Serum.

§ CII. LÖFFLER'S METHOD.

Special Media for Cultivating the Bacillus Diphtheriæ.

1. Take blood serum, 3 parts; 1 per cent. grape sugar; Bouillon 1 part. Mix well together, and fill into test-tubes.

2. Solidify between 60° and 70° C.

3. Sterilize for ten minutes in the steam sterilizer on each of three consecutive days.

Dr Nuttall has devised a bulb for the collection of blood serum. It is a sterilizable vessel made of glass, by which 10 to 100 c.c. of blood can be collected, and under proper precautions no contamination takes place. The method of procedure is as follows:—

Expose a femoral or carotid artery, and adjust two ligatures; the one distant from the heart is tightened and the proximal one left loose between the latter and the heart. The artery is clamped; a small slit is now made in the wall of the artery, the point of the bulb—the sealed end of which has been broken off and rounded in the flame—is introduced, and the artery bound tightly around it with the loose

ligature. The clamp is then removed, when the bulb is quickly filled with blood. Replace the clamp, remove the bulb, and seal in the gas flame. The loose ligature is now tightened and the wound closed. The glass bulb is put in a cool place until coagulation has occurred. The serum is withdrawn with a sterile pipette.

§ CIII. MEDIA FOR MOULD FUNGI.

1. Place some dry bread, finely grated, into test-tubes about 1½ inch high in each tube, or into Erlenmeyer flasks, about 1 inch in each.

2. Add enough water to convert the bread into a paste, and close the tubes or flasks with cotton plugs.

3. Sterilize in the steam sterilizer for fifteen minutes on each of three successive days.

The bread paste having an acid reaction cannot be employed for the cultivation of bacteria.

§ CIV. SABOURAUD'S MEDIUM FOR FAVUS, ETC.

Peptone . . .	0·8 gramme.
Mannite . . .	3·80 grammes.
Aqua distillata . .	100·0 c.c.
Agar-agar . . .	1·40 gramme.

Prepare in the ordinary manner, make slightly alkaline, fill in tubes, and sterilize fifteen minutes on each of three successive days.

MEDIA FOR NITRIFYING BACTERIA.

§ CV. WINOGRADSKY'S SOLUTION.

Ammonia sulphate . .	1 gramme.
Potassium phosphate . .	1 „
Pure water . . .	1000 c.c.

Place 100 c.c. of the above solution in each flask, and add to each 0·5 to 1·0 gramme of basic magnesium carbonate, suspended in distilled water, and sterilize by boiling.

§ CVI. PREPARATION OF TUBES, FLASKS, ETC., FOR THE PRESERVATION OF CULTURE MEDIA.

Both new tubes and those previously used should be boiled for an hour in a 2 to 3 per cent. solution of common soda, and carefully

swabbed out with a test-tube brush, using the cane handle form, as wire handles are apt to go through the bottom of the tube. When thoroughly cleansed, rinse with a warm 1 per cent. solution of commercial hydrochloric acid (this is to remove the alkali), rinse thoroughly in clean running water, and stand top down until the water has drained from them.

Fill the desired amount of media into the tubes from a small Erlenmeyer flask, or use a funnel with a pinch-cock, prepared for the purpose when exactitude is necessary, but for all practical purposes an Erlenmeyer flask is sufficient. Care must be taken that none of the material is dropped in the inside of the mouth of the test-tube, as it will cause the cotton plugs to adhere. The filled tubes are plugged with cotton wadding plugs, carefully rolled together before insertion, and the tubes sterilized in the steam sterilizer for fifteen to twenty minutes on each of three successive days. The old method of sterilizing the tubes and cotton wadding before filling is no longer in vogue, the discontinuous sterilization being sufficient.

§ CVII. METHODS OF CULTIVATING BACTERIA.

1. The platinum wires and loops, both before and after use, are sterilized in the Bunsen flame, from above downwards, the wire being held almost perpendicular in the flame, and the upper portion of the glass or metal rod it is attached to passes several times directly through the centre of the flame.

2. Fluid cultures are inoculated with one platinum loop-full of a pure culture.

3. Gelatine and agar-agar stab cultures are made with the platinum wire, making only one stab in each tube, reaching almost to the bottom of the medium.

4. Agar-gelatine and potato contact cultures are made with the platinum loop, the material being spread over the surface of the media. The agar and gelatine media being solidified obliquely, whilst the potato surface is either oblique or flat, when the material can be further spread with a potato-knife.

§ CVIII. KOCH'S ORIGINAL PLATE CULTURE METHOD.

For the Isolation of Germs in a Pure Culture.

1. Take three tubes containing 10 c.c. of sterilized nutrient gelatine, and melt the medium by heating in the water-bath at 40° C.

2. When the first tube has cooled to 30° C., remove the cotton

plug, and heat the mouth of the tube in the Bunsen flame, inoculate with a platinum loop of a liquid culture, or a trace of any semi-solid culture, or other desired material. After inoculation distribute the organisms evenly, allowing the fluid to flow gently backwards and forwards, avoiding contact with the cotton plug, as too much agitation causes air-bubbles to form. The tube is held between the thumb and first finger of the left hand, while the cotton plug is placed between the first joints of the first and second fingers of the same hand. This is now known as the original tube, and marked 'O.'

3. Take another tube of gelatine, remove the plug, placing it between the second and third fingers of the left hand, sterilize the mouth of the tube, place it alongside the original tube, and with a sterilized platinum loop transfer three loops of the gelatine in the original tube into the second tube. Replace the plug in the original tube and set on one side. The second tube is known as No. 1 Dilution, and marked 'I.'

4. The third sterilized tube of gelatine is inoculated with three platinum loops from the second tube, or No. 1 Dilution, in the same manner, and is known as No. 2 Dilution, and marked 'II.' (For marking the tubes for future identification, yellow or blue coloured pencils for writing on porcelain, metal, or glass, will be found very convenient.)

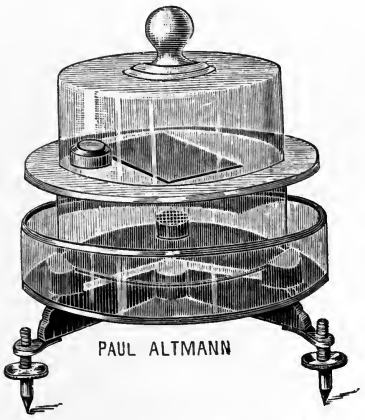


Fig. 11.—Koch's Plate Culture Apparatus.

5. Three sterilized glass plates are now removed from the plate box, placed one above the other on the ground glass plate of the plate culture apparatus (see Fig. 11), and the contents of the above tubes poured in order on the plates. When the gelatine is thoroughly set, the plates are placed on glass benches one above the other, the original plate being at the bottom in a plate culture dish. Instead of using plates, it is simpler to pour the gelatine into sterilized Petri-dishes. The prepared plates or Petri-dishes can be placed in the incubator at 22° C., or left to develop at room temperature; as a rule only two of the plates are fit for future observation—the original generally containing too many colonies. When finished, sterilize the platinum wires, and place the empty tubes in the disinfecting solution. A piece of filter paper, with the number and date, is sometimes placed on the glass benches below the plates.

§ CIX. METHOD FOR HARD SUBSTANCES, FAVUS CRUSTS, ETC.

1. Sterilize a large watch-glass in the open flame and cover it up.

2. Sterilize the bottom of an ordinary clean test-tube in the open flame.

3. Place a small quantity of sterilized bouillon on the watch-glass, add the material to be investigated, triturate until thoroughly reduced and mixed with the bouillon, and proceed as in process No. 2, previous method.

§ CX. AGAR-AGAR PLATE METHOD.

In making agar-agar plate cultures the fact must not be lost sight of that this medium solidifies at 39° C.

1. Melt three tubes of agar-agar at 90° C. (*freshly prepared tubes must not be used, as the water of condensation will cause all the developing colonies to run together*).

2. Place in a water-bath at 40° C., remove and inoculate quickly, making the necessary series, $O_{1\ 2}$.

3. Pour the contents of the tubes preferably into Petri-dishes, or if plates are used, without rims; place a spot of melted sealing-wax at each corner to prevent the agar-agar sliding off the plates.

§ CXI. METHOD WITH ORDINARY AGAR-AGAR IN TUBES.

1. Take three freshly prepared tubes of oblique surface agar-agar—usually called 'agar-slants'—with plenty of water of condensation in the bottom.

2. Inoculate only the water of condensation and transfer the bacterial suspension to the water contained in the series of tubes, making the usual number, $O_{1\ 2}$.

3. When the dilutions are completed, allow the water of condensation in each tube to flow gently over the oblique surface of the agar medium, and place in the incubator at 37° C.

§ CXII. BLOOD SERUM—AGAR PLATE METHOD.

1. Take three tubes of sterilized agar-agar, melt, retain fluid at 40° C. in a water-bath, inoculate, and make the usual dilutions, $O_{1\ 2}$.

2. Pour the contents of the tubes into Petri-dishes, and add sterilized liquefied blood serum heated to 40° C., mixing it thoroughly with the agar medium. (This method is specially adapted for the growth of Diphtheria bacilli and gonococci.)

§ CXIII. AGAR STROKE CULTURE PLATE METHOD.

1. Liquefy some tubes of agar-agar, pour into Petri-dishes, and allow to solidify.

2. Dip a sterilized platinum wire in the suspected material, raise the lid of the Petri-dish obliquely, and make several strokes with the platinum wire across the surface of the agar-agar. Instead of using dishes, take six or eight tubes of sterilized oblique surface agar-agar, and make strokes on the surface of the medium. (*The above methods are only used when a quick diagnosis is necessary.*)

§ CXIV. ESMARCH'S ROLL CULTURE METHOD.

1. Take three large wide-mouthed test-tubes containing 10 c.c. of sterilized nutrient gelatine.

2. Liquefy the gelatine, and inoculate with the material under investigation, making the usual dilution, $O_{1\ 2}$.

3. Push the cotton plugs well down, cut off any ends, and put on an india-rubber cap.

4. Cool the gelatine by twisting the tube round and round on its own axis in ice-water, when the gelatine will adhere to the inner walls of the tube in a thin film. Before putting the tube in the ice-water, roll the gelatine round the periphery of the internal portion of the cotton plug; by this means the centre of the plug remains free from gelatine, otherwise an air-tight cavity results.

5. To remove a colony from a roll culture, place the tube under a low power lens (see special apparatus, Fig. 12), select the colony, colour the outside of the tube over the colony, and then remove to other media, etc., with a bent platinum wire, the stain outside acting as a guide.

§ CXV. BOOKER'S ROLL CULTURE METHOD.

A much better method than the preceding, though depending upon the same principles, is that recommended by Booker.

1. Place a block of ice of convenient size in a dish, resting upon a towel (which prevents it slipping).

2. Take a plugged test-tube filled with warm water, lay it upon the block of ice, and thereby melt a groove in the ice.

3. The test-tubes (best filled with 5 to 6 c.c. of medium) are placed in the groove after inoculation, and revolved rapidly with the fingers of the right hand in one direction only. The left hand holding the dish regulates the obliquity of the tube, which at the beginning of the rotation should have the cotton plug at a higher level. The media should not come in contact with the plug.

This method can be used also for agar, but the tubes must be kept slanted for about twenty-four hours, otherwise the agar will not keep its position in the tube. After a certain number of hours the agar in the vicinity of the cotton plugs dries and adheres to the glass; with gelatine no such difficulty occurs. Rubber caps are not necessary.

§ CXVI. METHOD OF COUNTING COLONIES IN ROLL CULTURES.

For counting colonies in roll cultures, an apparatus designed by Esmarch (see Fig. 12) may be used; it consists of a test-tube, holder,

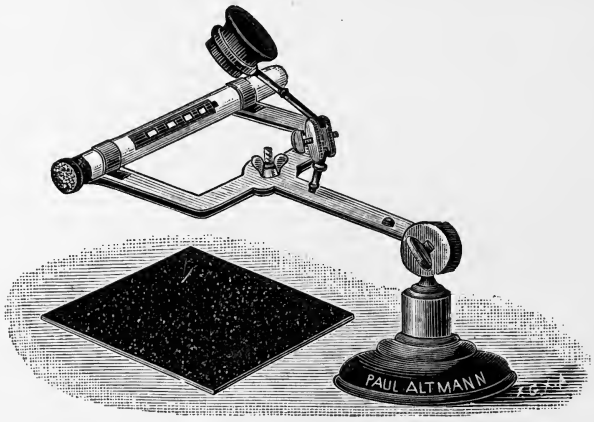


Fig. 12.—Esmarch's Apparatus for Counting Roll Culture Colonies.

and lens attached to a stand. The holder contains quadrangular apertures of different sizes, the number of colonies being counted in the larger or smaller apertures depending on the number of colonies in the culture. A simpler method consists in attaching a piece of paper, in which squares have been cut, to the tube by means of elastic bands, and using an ordinary hand or watchmaker's lens (Nuttall). The estimation of the total number is made, as with plate cultures, by

measuring the surface of the glass covered by the media, and multiplying by the number of colonies found in a certain square.

Gelatine plate or dish cultures are kept at ordinary room temperature or in the incubator at 22° C., and agar-agar plates at 37°. In twenty-four, forty-eight, or seventy-two hours they can be examined with the naked eye, hand lens, or microscopically with a low power lens.

The various kinds of characteristic growths are noted, and the number of colonies counted with the Wolffhügel apparatus (see Fig. 13).

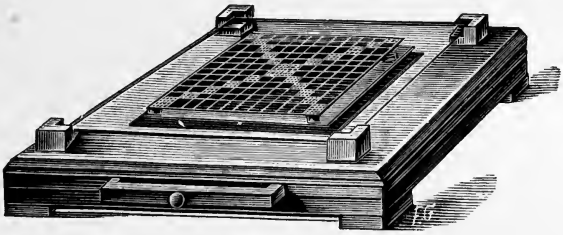


FIG. 13.—Wolffhügel's Counting Apparatus.

§ CXVII. METHOD OF OBTAINING A PURE CULTURE FROM A COLONY ON A PLATE OR DISH.

1. Examine the plate under the microscope with a low power, or use a hand lens, and find the desired colony, always selecting an isolated colony when possible.

2. Take a tube of sterilized nutrient gelatine, sterilize a platinum wire, and carefully remove a part of the desired colony on the point of the wire.

3. Remove the plug from the gelatine tube, holding the tube perpendicular, mouth downwards, in the left hand, and make a stab culture in the middle of the gelatine medium, extending almost to the bottom of the tube, heat the neck of the tube in the flame, and replace the plug; with liquid media the tube is held slanting.

METHOD OF KEEPING GROWING CULTURES PURE.

1. Inoculate the cultures into fresh medium every fourteen or twenty-one days.

2. Place the tube containing the original culture between the *thumb* and *first* finger of the *left* hand, and place the tube to be inoculated between the *first* and *second* fingers of the same hand.

3. Remove both cotton plugs, and hold between third and fourth and fourth and fifth fingers respectively of the left hand.

4. Pass a sterilized platinum loop into the original tube without touching the sides, and transfer one loop of material into the second tube, using similar precautions.

5. Heat the necks of both tubes in the flame, and scorch both plugs before returning them.

§ CXVIII. THE QUANTITATIVE PLATE CULTURE METHOD.

This method is used to determine the number of bacteria in a given quantity of *Material.* Fluids are examined as follows:—

1. Transfer with a sterilized capillary pipette, $\frac{1}{100}$ c.c. to 1 c.c. of the material into 10 c.c. of liquefied sterilized gelatine, and mix.

2. Another tube containing 10 c.c. of liquefied gelatine is inoculated in a similar manner.

3. Pour the contents of the tubes on plates, as in the ordinary plate method (§ 108), or into Petri-dishes previously sterilized, and set on one side for from twenty-four hours to seven days.

When the colonies have developed, the exact number is ascertained with the Wolffhügel's Counting Apparatus (see Fig. 13). '*Roll cultures can also be used.*'

'*Solid Material*' is first reduced in a sterile mortar (see Favus Crusts Method, § 109, process No. 2), with 5 or 10 c.c. of sterile bouillon, or physiological salt solution, and then tested as above.

§ CXIX. METHODS OF CULTIVATING ANAËROBIC BACTERIA.

Anaërobic organisms are characterised by their inability to grow in the presence of oxygen, and many devices are employed for the exclusion of oxygen from the cultures.

The preparation of suitable media and cultivation of anaërobic bacteria require skill and knowledge of bacteriological technique.

§ CXX. KOCH'S METHOD.

Inoculate a gelatine plate, and cover the inoculated surface with a thin piece of sterilized isinglass; organisms growing beneath this plate are supposed to grow without oxygen.

§ CXXI. HESSE'S METHOD.

Pour some sterilized oil on the surface of a gelatine stab culture, and the growth developing along the track of the needle is supposed to be of an anaërobic nature.

§ CXXII. LIBORIOUS'S METHODS.

a. Fill a test-tube three-quarters full of gelatine or agar-agar, sterilize, and place in a vessel of boiling water for ten minutes to expel all air from it. Cool the medium rapidly in ice-water, when between 30° and 40° C. and still fluid, inoculate, solidify rapidly, and seal up the tube in the flame. The anaërobic bacteria develop only in the lower layers of the medium.

FIG. 14.

Liborious's Tube for Anaërobic Cultures.

b. For this method the special Liborious tube is necessary (see Fig. 14). Hydrogen gas is passed through the side tube by means of the hydrogen apparatus, until all air is expelled (see Fig. 17, p. 67). The contracted parts at the neck and side of the tube are sealed in the flame, but owing to its small capacity give better results with fluid media. For the necessary precautions to be observed in using hydrogen, see § 123.

§ CXXIII. FRÆNKEL'S METHOD.

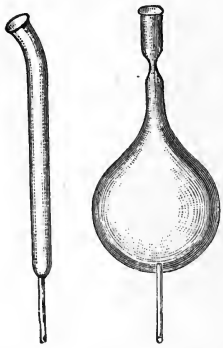
1. Prepare a tube in the same manner as for a plate or Esmarch roll culture.

2. Replace the cotton by a sterile rubber stopper with two glass tubes passing through it, and plugged with cotton wadding before sterilization. The tubes on the outside of the stopper are bent at right angles to the long axis of the test-tubes, and slightly drawn out in the flame. One of the tubes reaches within 0·5 c. of the bottom of the tube, while the other is cut off level with the inside of the stopper. The hydrogen apparatus (see Fig. 17, p. 67) is attached to the end of the longest tube, and hydrogen allowed to bubble through the tube until all air is expelled and its place taken by the hydrogen. The hydrogen must be passed through the gelatine at least five minutes, to ensure that all oxygen is expelled. The drawn out portions of the tubes are sealed in the flame, and the protruding end of the rubber stopper painted round with paraffin.

Before using the hydrogen apparatus test the hydrogen, and make sure it is free from oxygen, as follows :—

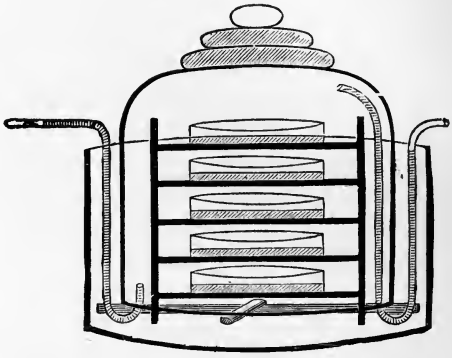
Fill an ordinary test-tube with water, close the mouth with the thumb, invert it, and place its mouth under water ; remove the thumb, and the water will be kept in by atmospheric pressure. Conduct the hydrogen into the test-tube by means of a piece of rubber tubing, when the water in the tube will be replaced by gas. Hold a flame near the mouth of the test-tube, and if no explosion occurs the hydrogen is safe to use.

Fig. 15.



Kitasato's Bottle for Anaërobic Cultures.

Fig. 16.



Botkin's Apparatus for Anaërobic Plate Cultures.

§ CXXIV. Kitasato devised a special flat bottle for the making of anaërobic cultures (see Fig. 15). Kitasato and Wiel also suggest the addition of

Formic acid, . . 0·3 to 0·5 per cent.
Glucose, . . . 1·5 to 2 „

or blue litmus tincture in 5 per cent. per volume to the culture medium in addition to an atmosphere free from oxygen.

§ CXXV. Botkin has devised a special apparatus for anaërobic plate cultures—see *Zeitschrift für Hygiene*, 1890, § 385 (see Fig. 16).

§ CXXVI. BUCHNER'S METHOD.

The bacteria are developed in an atmosphere robbed of its oxygen by pyrogallic acid. Either oblique, roll, or stab cultures are made in a test-tube which is placed inside a larger tube, having a brass support for the bottom of the smaller tube to fit into (see Fig. 18, p. 67). 1 gramme of pyrogallic acid and 10 c.c. of $\frac{1}{10}$th normal caustic

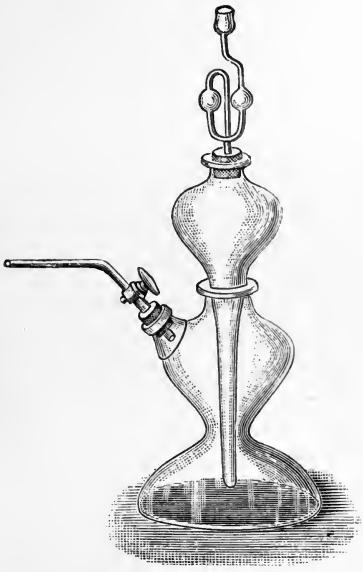
potash solution* are put in the larger tube, which is tightly plugged with an india-rubber stopper. The oxygen is quickly absorbed by the pyrogallic acid, and the organisms develop in the remaining constituents of the atmosphere—nitrogen, small amount of, Co_2, and a trace of NH_3.

§ CXXVII. KASPAREE'S METHOD FOR LIQUID MEDIA.

A special flask is necessary for this method, with a small tube terminating in a bulb blown into its neck about $\frac{2}{3}$ths of an inch above the top of the liquid medium, which is prepared as follows:—

1. Fill the flask with bouillon almost to the neck, then add 3 c.c.

Fig. 17.



Kipp's Hydrogen Apparatus.

Fig. 18.

Buchner's Tube for Anaërobic Cultures.

of liquid paraffin, and sterilize the whole in the steam sterilizer in the usual manner. This expands the bouillon, causing the paraffin to rise in the neck of the flask and overflow into the side tube and bulb. After sterilization is completed, only a thin layer of the paraffin remains on the top of the bouillon, and during heating a large portion of the air absorbed by the bouillon is driven out; its re-absorption while the flask is cooling is prevented by the thin paraffin film.

2. Before inoculation pierce the thin paraffin film on the top of

* A normal solution of caustic potash contains as many grammes to the litre as the number of its molecular weight—56·1 grammes to the litre of water.

the bouillon, and when the inoculation is completed, heat the side bulb gently to melt the paraffin, so as to allow it, by slightly inclining the flask, to pour on the film already formed above the bouillon. Upon hardening, this additional paraffin forms an almost air-tight layer, and when the flask is placed in the incubator, the heat causes the paraffin to be pressed upwards into the constricted neck of the flask; this closure is further completed by the gases generated in the culture.

§ CXXVIII. THE INCUBATOR.

Certain forms of bacteria develop at a higher temperature than others. Pathogenic or disease-producing organisms grow more luxuriantly at 37·5° C. than at lower temperatures, whereas the ordinary saprophytic forms develop almost at any temperature between 18° C. and 37·5° C. For the cultivation of pathogenic bacteria a special apparatus is used, known as a thermostat or incubator, made throughout of copper, with double walls, the space between being filled with water. It is provided with a closely-fitting double door with an inner door of glass, enabling the cultures, etc., in the chamber to be inspected without actually opening it. The regulation of the temperature within the incubator is maintained by an automatic regulator of sufficient delicacy to prevent a fluctuation of more than 0·2° C. in the temperature of the air within the chamber of the apparatus. A Koch's safety burner is generally used for heating; the safety attachment automatically turning off the gas supply, thus preventing accidents should the flame be extinguished when no one is near.

For illustration of a modern incubator and various attachments in position, see Fig. 19, p. 69.

§ CXXIX. SPECIAL REACTIONS PRODUCED BY BACTERIA DURING THEIR GROWTH.

The reactions produced by many species of bacteria in the course of their development in culture media are of great differential value. These changes in some instances are so great that they can be detected by simple reagents, whilst in others they are so slight as to require the most delicate test for their demonstration. Some bacteria produce at one period of their life an alkaline and at another an acid reaction. This is seen in cultures of Löffler's bacillus of diphtheria.

These reaction differences are best observed when a chemical substance, which does not interfere with the development of the

organism, is added to the media. In milk media, to which litmus tincture has been added (see § 85, page 51), organisms producing alkalies cause the blue colour to be intensified; others producing acids change it to red; while others bring about neither of

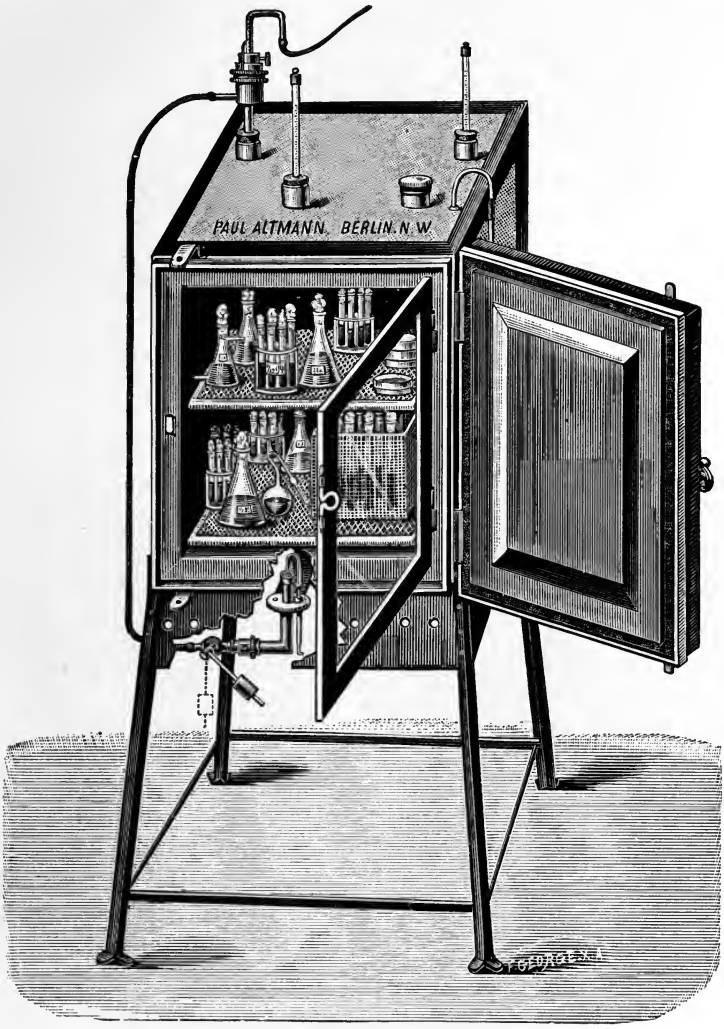


FIG. 19.—Incubator.

these changes. Tincture of litmus can also be added to gelatine and agar-agar for the same purpose.

In ordinary milk cultures coagula also appear, due to acids produced from milk sugar by bacterial action upon the casein of the

milk; again acids may be produced without coagulation being noticed.

Rosolic acid in alcoholic solutions (see § 83, page 51) is also added to culture media to test the above reactions.

§ CXXX. *Fermentation*, or the production of gas as an indication of fermentation, is associated with the growth of some bacteria, and is best seen in cultures grown in media containing 1 to 2 per cent. of grape sugar. Proceed as follows:—

1. Liquefy the medium, and reduce to the proper temperature.

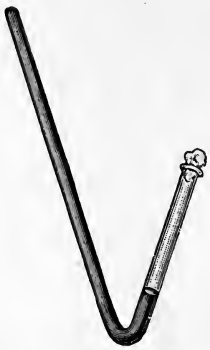
2. Place a small quantity of a pure culture of the organism under investigation in the liquid medium with a sterile platinum loop, and distribute it equally.

3. Place the tube in ice-water and solidify rapidly in a vertical position.

Fig. 20.

Smith's Fermentation Tube.

Fig. 21.



Dunbar's Fermentation Tube.

4. When solid, place in the incubator.

5. In twenty-four to thirty-six hours, if the organism causes fermentation of glucose, the medium will be dotted all over with small cavities containing the gas formed.

The property of fermentation with production of gas has now assumed such an important rôle as a means of differential diagnosis that not only the amount of gas or gases produced by an organism under consideration, but also the nature and quality, are determined. For this purpose special fermentation tubes are necessary (see Figs. 20 and 21.)

It is a tube bent at an acute angle, closed at one end, enlarged into a bulb at the other, and attached to a glass foot, so that it may stand upright. The tube is only used with fluid media as follows:—

1. Pour some 2 per cent. grape sugar bouillon, made just before using, into the bulb of the tube until it is half full.

2. Tilt the tube until the closed arm is nearly horizontal, so that the air in the arm may escape into the bulb and the fluid flow into the arm to take its place.

3. When the arm is completely filled, add enough of the liquid medium to cover the lowest expanded portion of the bulb, and close the opening of the bulb with cotton wadding.

4. Sterilize the tubes on three consecutive days by the usual method.

During the process of sterilization the tension of the water vapour in the arm forces most of the fluid into the bulb. When the tube cools, the fluid returns again into the arm, except in a small space at the top, occupied by air originally dissolved in the liquid, and which was driven out by the heat. The air bubble must be tilted out after each sterilization, and finally after the third sterilization the arm of the tube will be free from air.

After inoculation, the tubes are placed in the incubator, and the amount of gas collecting in the closed arm noted daily. The gas is usually found to consist of about one part by volume of carbonic acid and two parts by volume of an explosive gas, consisting largely of hydrogen.

§ CXXXI. METHOD OF DETERMINING THE NATURE AND QUANTITATIVE RELATIONS OF THESE GASES ACCORDING TO SMITH.

The bulb is completely filled with a 2 per cent. solution of sodium hydroxide (NaOH), and closed tightly with the thumb. The fluid is thoroughly shaken with the gas, and allowed to flow to and fro from bulb to closed branch several times to insure intimate contact of the CO_2 with the alkali. Lastly, *before removing the thumb all the gas is allowed to collect in the closed branch*, so that none may escape when the thumb is removed. If CO_2 is present, a partial vacuum in the closed branch causes the fluid to rise suddenly when the thumb is removed. After allowing the layer of foam to subside somewhat, the space occupied by gas is again measured, and the difference between this amount and that measured before shaking with the sodium hydroxide solution gives the proportion of CO_2 absorbed.

§ CXXXII. *Determine the explosive character of the residue as follows:—*

Replace the cotton plug, and allow the gas in the closed branch to flow into the bulb and mix with the air present there. The plug is removed, and a lighted match inserted into the mouth of the bulb. The intensity of the explosion varies with the amount of air present in the bulb.

§ CXXXIII. METHOD OF DETECTING INDOL IN CULTURES OF BACTERIA.

1. Cultivate the organism from twenty-four to forty-eight hours at 37° C. in Dunham's Peptone Solution (see § 82, p. 51), using four tubes kept under exactly the same conditions.

2. Apply the test as follows :—Take two tubes, each containing 7 c.c. of the peptone solution, but '*not inoculated*.' To one add 10 drops of concentrated sulphuric acid, to the other 1 c.c. of 0·01 per cent. solution of sodium nitrite, and afterwards 10 drops of concentrated sulphuric acid. In five or ten minutes if no rose colour appears, then indol is absent.

3. To two '*inoculated*' tubes add 10 drops of concentrated sulphuric acid, and in five or ten minutes, if no rose colour appears, add 1 c.c. of the sodium nitrite solution ; if no *rose colour* appears, then the '*indol reaction*' is absent.

4. When the *rose colour* appears with the addition of the concentrated sulphuric acid *alone*, then *indol* has been formed, and likewise a *reducing* body.

5. When the *rose colour* appears only with the addition of *both* the concentrated sulphuric acid and the nitrite solution, then *indol* has been formed during the growth of the organism, but no *nitrites*.

§ CXXXIV. TEST FOR NITRITES IN CULTURES.

Ilosvay's Modification of Griess's Method.

The following reagents are employed :—

A.

Naphthylamine . . .	0·1 gramme.
Aqua distillata . . .	20·0 c.c.
Acetic acid (25 per cent. solution) .	150·0 c.c.

Dissolve the naphthylamine in 20 c.c. of boiling water, allow it to cool, and mix with the acetic acid solution.

B.

Sulphanilic acid . . .	0·5 gramme.
Acetic acid (25 per cent. solution) .	150·0 c.c.

Mix solutions *A* and *B* together; the resulting mixture should be colourless. The solution is best when freshly prepared, but if kept in a close stoppered bottle retains its power for some time.

Add 1 volume of the above solution to 5 volumes of the culture, when if nitrites are present a *deep red* colour will appear in a few seconds; if no nitrites are present no changes take place.

§ CXXXV. REICHEL'S BACTERIA FILTER.

This apparatus consists of a glass flask and a porcelain filter which

Fig. 22.

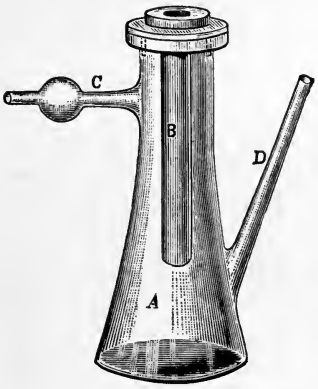
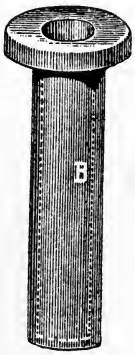


Fig. 23.



Reichel's Bacteria Filter.

fits in the flask, and is used for separating bacterial products out of culture media (see Fig. 22).

§ CXXXVI. METHOD OF USING THE FILTER.

1. Place some cotton wadding in the glass tube at C, and also at D.

2. The porcelain cylinder B is placed in the glass flask, and both sterilized in the hot-air sterilizer, a circular piece of asbestos being placed between the under ring of the porcelain and the upper surface of the neck of the glass flask.

3. When the apparatus is removed from the hot-air oven, a special perforated rubber cap is placed over the top of the filtering cylinder

and the glass flask, and the material to be filtered poured into the filter. It is necessary to attach a piece of rubber tubing with a pinch-cock at D, because the tube C is attached directly to the tube of a suction-pump, and filtration produced. It works well with a water force of $1\frac{1}{2}$ atmospheres, and a mercury vacuum meter of 200 mm.

§ CXXXVII. INOCULATION OF ANIMALS.

Inoculation is practised on animals to determine if an organism is pathogenic or disease-producing; and if so, the pathological results of its growth in the tissues of these animals, and the manner in which the organism gains entrance to the tissues in order to produce these results. The animals usually employed in laboratories for inoculation purposes are white mice, grey house mice, field mice, rats, guinea-pigs, rabbits, and pigeons. The inoculations are made subcutaneously, intravenously into the great serous cavities, or into the anterior chamber of the eye.

§ CXXXVIII. SUBCUTANEOUS METHOD.

1. Remove the hair or feathers, wash the skin with soap and water, and sterilize with corrosive sublimate solution 1 to 1000 (see § 66, p. 44).

2. For *liquids* use a sterilized hypodermic syringe. The skin is raised with a pair of forceps, and the point of the hypodermic needle inserted, and an incision made.

3. For *solid material*, a pocket is made in the skin as follows:— Take up the skin with the forceps, make a small incision with sterilized scissors, and cut a pocket under the skin. Hold the pocket open with sterilized forceps, and place the material as far back as possible without touching the edges of the wound with the platinum loop or *Koch's needle*. Earth can be introduced in this manner.

4. Pull the wound together and allow it to remain, or cover with a little iodoform collodion, or sear with a hot platinum needle. During the operation the animal must be held perfectly still. For the smaller animals many forms of holders are made, especially for mice, which are held in proper position for inoculation at the root of the tail. Guinea-pigs, rabbits, and pigeons are best held by an assistant. Pigeons are generally inoculated in the pectoral muscles, mice at the root of the tail, while the other animals are generally inoculated in the abdominal wall, either to the right or left of the median line.

§ CXXXIX. INTRAVENOUS METHOD.

In the rabbit this operation is generally performed in one of the veins of the ear, and the most suitable vein is the ramus lateralis posterior of the vena auricularis posterior, a very fine, delicate vessel running along the posterior margin of the ear; and being firmly fixed in the dense surrounding tissue, it does not roll about when you are inserting the needle. The largest branch of the vena auricularis posterior is the central branch, or ramus anterior, but the insertion of a needle into this vessel is accomplished with difficulty.

If there is only a little blood in the ear, pressure at the root of the ear will cause stasis of the blood and distension of the vessels, rendering them more visible. Another method of rendering a vein more prominent is to lightly press or gently prick with the point of a needle the skin over the vessel to be used. The injection is always made from the dorsal surface of the ear. Needles employed for intravenous injection must have a perfectly flat slanting surface free from curvature.

Care must be taken that no air is injected, and that the syringe and needles are sterilized, before use, in the steam sterilizer or in boiling water. The inoculated animal must be kept under close observation for an hour after the operation.

The syringes generally used are Koch's, Strohschein's, and Overlack's; but the latter is preferred, as the dose can be controlled with accuracy owing to the amount of air between the fluid and the piston being at a minimum.

§ CXL. INOCULATION INTO THE LYMPHATIC CIRCULATION.

Fluid cultures or suspensions of bacteria can be injected into the lymphatics by way of the testicles, by plunging the point of the needle into the substance of the testicle and injecting the desired amount of fluid.

§ CXLI. INOCULATION INTO THE GREAT SEROUS CAVITIES.

To inject fluid into the peritoneum, make a small nick through the skin, and plunge the needle direct into the peritoneal cavity. There is not much danger of wounding the intestines and other

viscera when the inoculation is made close to the median line, half-way between the sternum and the symphysis pubis, and a curved needle used, with an opening some distance from the point on the convex side.

When solid substances, bits of tissue, etc., are to be introduced, the operation must be conducted on the lines of a laparotomy, as follows:—

1. Shave the hair from a small area over the median line, wash the skin with soap and water, apply fresh water, rub with alcohol, and finally sterilize with corrosive sublimate solution 1 to 1000 (see § 66, p. 44).
2. Make a longitudinal incision about 2 c. long, close to the median line, through the skin and down to the fascia.
3. Two subcutaneous sutures, as employed by Halsted, are introduced transversely to the line of incision about 1 c. apart, and their ends left loose. These sutures do not pass through the skin proper, but are introduced into the subcutaneous tissues, passing into the abdominal cavity and out again, entering at one side of the line of incision and leaving at the other.
4. The remaining tissues are now cut through, and the bit of tissue deposited in the peritoneal cavity (under sterile precautions), the edges of the incision closed tightly and evenly by drawing and tying the sutures, and the line of incision dressed with iodoform collodion.

All instruments, sutures, ligatures, etc., used in the operation are previously carefully sterilized, either in the steam sterilizer, or boiled in a 2 per cent. solution of sodium carbonate for ten minutes, and the operator's hands cleansed with disinfecting solution (§ 66). The material placed in the abdominal cavity must also be handled with sterilized instruments.

§ CXLII. INOCULATION INTO THE PLEURAL CAVITY.

This is very seldom practised, as it is very difficult to enter the pleural cavity without injuring the lung.

§ CXLIII. INOCULATION INTO THE ANTERIOR CHAMBER OF THE EYE.

A puncture is made through the cornea just in front of its junction with the sclerotic, the knife passing into the anterior

chamber in a plane parallel to the plane of the iris, when the aqueous humour flows out. A few drops of a 2 per cent. solution of cocaine are placed in the eye previous to operating. Deposit the bit of tissue with fine sterilized forceps or a platinum loop through the opening upon the iris, where it remains, and its pathogenic properties upon the iris can be conveniently studied. The application of this mode of inoculation is very limited. Cohnheim employed this method in demonstrating the infectious nature of tuberculous tissues, tubercular iritis being a constant symptom when tubercular matter was introduced into the anterior chamber of the rabbit's eye.

§ CXLIV. METHOD OF INOCULATING RABBITS FOR THE DIAGNOSIS OF RABIES.

1. Remove the brain of the suspected animal, with antiseptic precautions, as soon as possible after death.

2. Place a small piece of the brain or spinal cord in a mortar, and thoroughly grind with a few c.c. of sterile water or bouillon.

3. The operator must disinfect the hands and sterilize all instruments.

4. Anæsthetize the rabbit with ether, clip the hair from the head between the eye and ear, wash the skin, and disinfect with ordinary sublimate solution (§ 66, p. 44).

5. A longitudinal incision is made through the skin and subcutaneous tissue in the median line, while a crucial incision is made through the periosteum on one side of the median line, thus avoiding hæmorrhage from the longitudinal sinus, and the four parts of the periosteum reflected or pushed back. Cut out a piece of bone about $\frac{1}{3}$ of an inch in diameter with a trephine and expose the dura mater.

6. Inject a drop or more of rabid material beneath the dura mater with a hypodermic syringe, replace the periosteum, suture the skin, disinfect, dry, and apply some iodoform collodion. Sometimes a piece of the suspected tissue may be introduced directly under the dura mater. The inoculation wound heals rapidly. Rabid symptoms may appear in fifteen to thirty days, sometimes earlier than fifteen days, and again from one to three months may intervene.

§ CXLV. OBSERVATION OF ANIMALS AFTER INOCULATION.

Inoculated animals must be kept under constant observation, and the following conditions noted :—

1. Temperature.
2. Loss of weight.
3. Peculiar position in cage.
4. Loss of appetite.
5. Condition of the coat or hair.
6. The condition of the secretions from the air passages, conjunctiva, kidneys, diarrhœa or hæmorrhage from the bowels.
7. The condition of the seat of inoculation. When an animal dies in two to four days after inoculation, evidence of either acute or toxic processes will be found.

When the inoculation produces chronic conditions the animal may be under supervision for weeks, and must be weighed daily at the same hour, the temperature taken at the rectum, the thermometer being pushed past the sphincter.

Too much stress must not be laid upon moderate and sudden daily fluctuations in either temperature or weight, as normal animals when confined in cages and fed regularly present striking temporary gains and losses in weight, and unexplainable rises and falls of temperature, often as much as a degree from one day to another.

§ CXLVI. POST-MORTEM EXAMINATION OF ANIMALS.

Perform the autopsy as soon as possible after death. When delay cannot be avoided, place the animal in the ice-chest until such time as is convenient.

A.

1. Sterilize a suitable post-mortem board with corrosive sublimate solution 1 to 1000, place the animal belly upwards, and tack the four legs fast to the board.
2. Wash the surface of the thorax and abdomen with corrosive sublimate solution, make an incision through the skin at the pubis, introducing one blade of the scissors, and extend the incision as far as the chin.
3. Carefully dissect the skin away from the abdomen, thorax, axillary, inguinal, and cervical regions, and fore and hind legs, and pin it to the board as far as possible from the thorax and abdomen. It is from the skin that the chances of contamination are greatest.

B.

All incisions from now on are made with sterilized instruments.

1. Take an ordinary potato-knife, heat it quite hot, and place it

in the abdomen in the region of the linea alba until the fascia begins to burn, when the knife is then held transverse to this line over the centre of the abdomen, making two sterilized tracks through which the abdomen may be opened by crucial incisions; two burned lines are also made along the sides of the thorax.

2. Make a central longitudinal incision from the sternum to the genitalia with heated scissors, the abdominal wall being held up with sterilized forceps, or a hook, to prevent the internal viscera being injured. A transverse incision is made in a similar manner.

3. Cut through the ribs with strong sterilized scissors along the sterilized tracks in the sides of the thorax, when the whole anterior wall of the thorax is easily lifted and entirely removed by severing the diaphragm connections.

4. When the thoracic and abdominal cavities are fully exposed a careful examination of the organs and surroundings is made without disturbing them.

5. Plate, Petri-dish, or roll cultures are prepared from the blood, liver, spleen, kidneys, and any exudates present.

The method is as follows :—

(1.) Heat a scalpel and scorch a small surface of the organ from which the cultures are to be made.

(2.) Heat the scalpel again and penetrate the capsule of the organ with the point, and through the opening insert a stout sterilized platinum loop, push it into the tissues, twist around, and obtain enough material from the centre of the organ to make the culture.

In making cultures from resisting tissues Nuttall's platinum spear can be used to advantage. It is a piece of heavy platinum with a spear head at one end perforated with a small hole, the other end being attached to either a metal or glass holder. When heated it can be readily thrust into the densest of the soft tissues, and when withdrawn after twisting, sufficient material will be found in the eye of the spear head for examination or the institution of cultures.

Cultures from blood are usually made from one of the heart cavities, the surface being seared with a hot knife before opening. As soon as the culture material is obtained, cover-glass specimens are prepared from each organ and existing exudates.

Small pieces of each organ are also preserved for future examination. (For method, see Examination of Organs and Tissues, § 31, p. 30.)

When the autopsy is finished the remainder of the animal should be burned and the instruments used sterilized by the process given under Sterilization (see p. 12). Wash the post-mortem board with

sublimate solution 1 to 1000, and sterilize in the steam sterilizer The cover-glasses and other material likely to contain infectious material must also be further sterilized when of no further use.

METHODS OF EXAMINING AIR, WATER, AND EARTH.

§ CXLVII. AIR—Ordinary Method.

1. Liquefy 10 c.c. of sterile gelatine, pour on a sterile plate or in a Petri-dish, and set aside to cool.

2. Remove the cover of the dish or plate, leaving the gelatine exposed for one hour.

3. Replace the cover, set aside, and examine any future development.

Agar can be used in place of gelatine. This method yields only qualitative results.

§ CXLVIII. AIR—Hesse's Method.

1. A sterilized glass cylinder, 70 c. long and 3 to 5 c. in diameter, containing 50 c.c. of sterile gelatine adhering to the inner surfaces, prepared according to the Roll Culture process (see § 114, p. 61), with one end of the cylinder closed by two elastic caps, the inner one having a central orifice, and the other with a cork and glass tube plugged with cotton wool.

2. The prepared apparatus is fitted on a tripod. The cotton wool plug is removed, and the small tube connected with an aspirator capable of drawing air through the cylinder at a velocity of half a litre per minute.

3. Before the aspirator is finally adjusted, the outer rubber cap at the opposite end of the cylinder is removed, when any germs passing into the cylinder adhere to the gelatine.

About 20 litres of air is the limit tested by this method. (For illustration of apparatus, see Fig. 24, p. 81.)

§ CXLIX. AIR—Petri's Method.

By this method a greater quantity of air can be tested than by Hesse's method.

1. Prepare a glass tube 9 c. long and 1·5 c. in diameter, containing

two filters of fine sand separated from each other by wire-gauze netting.

2. After the tube is sterilized and plugged, it is placed in a horizontal position, and the proximal end of the tube attached to an air-pump (see Fig. 25, page 82), capable of drawing from 5 to 10 litres of air per minute, and the plug at the distal end of the tube removed.

3. The air-pump is worked until the gauge registers 100 litres.

4. The sand filter at the distal end of the tube is removed and mixed with 10 c.c. of sterilized liquefied gelatine, which is poured into a sterile Petri-dish. The sand filter at the proximal end of the

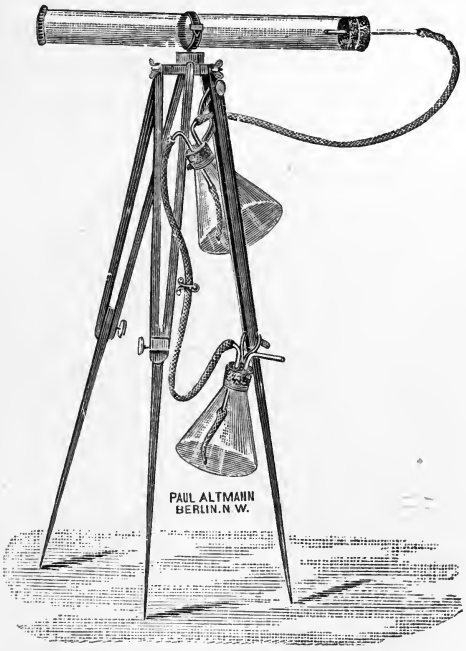


FIG. 24.—Hesse's Apparatus for Examining Air.

tube is used as a control, and must remain germ free when mixed with nutrient gelatine, and poured into a Petri-dish.

Powdered glass can be substituted for sand, and is more satisfactory, as any developing colonies can be readily observed.

§ CL. METHODS OF EXAMINING WATER.

Samples of water can be procured in sterilized Erlenmeyer flasks closed with cotton-wool plugs. They should be examined immediately, if possible, and never later than two hours after the sample is

obtained. When the examination must be delayed, then place the sample in the ice-box. Before testing a sample, shake the flask, as an equal distribution of the germs is necessary for an average result. Samples collected from a tap should not be drawn until the water has been flowing from fifteen to twenty minutes in full stream. When obtained from a stream or spring, take the sample about one foot beneath the surface.

Water analyses should always be made on the spot, when possible, as during transportation, unless packed in ice, a multiplication of the

FIG. 25.—Air-pump for use in Petri's Method.

organisms in the sample takes place. The following are the necessary articles for a *Transportation Case for Analyses on the Spot*:—

4 sterilized Erlenmeyer flasks to obtain the samples; 1 thermometer; 1 spirit lamp; 12 sterilized Petri-dishes in special box (see page 12); 12 tubes of sterile nutrient gelatine; 15 sterilized water pipettes in three cases; 1 folding tripod; 1 notebook; 1 pencil for writing on glass, etc.; and 1 towel.

The gelatine is melted, the inoculations made immediately, and poured into the Petri-dishes, allowed to solidify, and transported to the laboratory.

§ CLI. QUALITATIVE METHOD.

Transfer with a sterilized capillary pipette 1 c.c., $\frac{1}{2}$ c.c., or $\frac{1}{4}$ c.c. of the water to be examined into a tube containing 10 c.c. of liquefied sterilized nutrient gelatine. Mix, and pour the contents of the tube on a sterile glass plate, and proceed as at No. 3 process, '*Quantitative Plate Culture Method*,' § 118, p. 64. (The bottoms of Petri-dishes being uneven, plates are more reliable for water examination.)

§ CLII. KOCH'S METHOD FOR DEMONSTRATING THE VIBRIO CHOLERÆ ASIATICÆ IN WATER.

1. Take 100 c.c. of the suspected water, and mix with 5 c.c. of sterile 20 per cent. peptone chloride of sodium solution (§ 81, p. 50), make alkaline, and place in the incubator at 37° C.

2. When Cholera bacilli are present they develop, and are found in ten or twelve hours on the surface of the fluid, and can be further investigated and identified.

§ CLIII. ELSNER'S METHOD FOR BACILLUS TYPHI ABDOMINALIS.

Take some potato gelatine (see § 79, p. 50), and shortly before use add 1 per cent. of iodide of potash; after adding the suspected water, prepare plate cultures according to the ordinary method (§ 108, p. 58). This method is for the Bacillus typhi abdominalis, and the Bacterium coli communis, which grows the stronger of the two, and in forty-eight hours appears as dull brown colonies, while the Bacillus typhi abdominalis appears as bright watery drops. This method, however, is not absolutely reliable.

§ CLIV. QUICK METHOD OF DEMONSTRATING THE PRESENCE OF PATHOGENIC GERMS IN WATER.

1. Mix 100 c.c. of the suspected water with 5 c.c. of Koch's 20 per cent. peptone chloride of sodium solution (see § 81, p. 50), and place in the incubator twenty-four hours at 37° C.

2. Inoculate a guinea-pig with 1 c.c. of the mixture intraperitoneally, and if pathogenic organisms are present, the animal dies, and the organs, exudates, blood, etc., can be further examined.

3. If the water is pure, the guinea-pig remains alive.

§ CLV. SMITH'S METHOD OF ISOLATING CERTAIN ORGANISMS FROM A WATER SUPPLY, *i.e.*, INTESTINAL BACTERIA.

1. Add 1, 2, or 3 drops of the suspicious water to some fermentation tubes containing 2 per cent. grape sugar bouillon.

2. Place at 37° C., and if at the end of thirty-six or forty-eight hours gas accumulates in the tube, then intestinal bacteria are present, as ordinary water bacteria do not flourish in this medium.

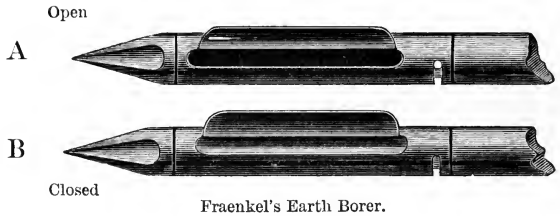
3. Isolate the gas-forming organisms by the ordinary plate culture method (see § 108, p. 58) for further identification.

When the number of colonies developed on a plate are too numerous to be counted with the Wolffhügel's apparatus (see Fig. 13, p. 63), the plate is examined with a low power and Ehrlich's eye-piece diaphragm.

§ CLVI. METHODS OF EXAMINING EARTH.

Fraenkel's Method.

1. Take an earth borer made according to Fraenkel's model (see Fig. 26, A open, B closed) when examining earth from the deeper strata. For superficial samples a borer is not necessary.



Fraenkel's Earth Borer.

2. Measure the desired quantity of earth in a platinum spoon, mix with 10 c.c. of sterilized nutrient gelatine, and proceed according to roll culture process (see § 114, p. 61).

The most important organisms found in soil are Bacillus tetani, Bacillus of symptomatic anthrax, Bacillus œdematis maligni. These organisms are anaërobic, and require special conditions for their development (see Cultivation of Anaërobic Bacteria, § 119, p. 64).

The nitrifying bacteria are also found in the most superficial layers of the soil. The best example of the activity of these organisms is the production of nitrates from the ammonia of the

fæcal evacuations of sea fowls in the saltpetre beds of Chili and Peru. It is largely by means of these organisms that growing vegetation obtains the nitrogen necessary for the nutrition of its tissues. Special cultivation media are necessary for the growth of these organisms.

Winogradsky's Method.

For preparation of the special media used, see Preparation of Media (§ 105, p. 57).

1. Inoculate one of the flasks with a little of the soil to be investigated.

2. In four or five days withdraw with a sterile pipette some of the solution from the above flask from over the surface of the layer of magnesia carbonate, and transfer to a second flask No. 2.

3. Change again some material from flask No. 2 in four or five days to a third flask, continue the changing process until ultimately the nitrifying organisms flourish as almost transparent films attached to the granulated magnesium carbonate at the bottom of the flask. The organism is known as the Nitromonas.

§ CLVII. POINTS TO BE OBSERVED IN DESCRIBING AN ORGANISM—(ABBOTT).

1. Its source—as air, water, or soil. If found in the animal body, is it normally present, or only in pathological conditions?

2. Its form, size, mode of development, occurrence of involution forms, or other variations in morphology. Grouping, as in pairs, chains, clumps, zoogloœa; presence of capsules; development and germination of spores; arrangement of flagella.

3. Staining peculiarities—especially its reactions with Gram's (or Weigert's fibrin) stain, and peculiar or irregular modes of staining.

4. Motility—to be determined on very fresh cultures and on cultures in different media.

5. Its relation to oxygen—Is it aërobic, anaërobic, or facultative? Does it develop in other gases, as carbonic acid, hydrogen, etc.

6. Both the macroscopic and microscopic appearance of its colonies on nutrient gelatine and on nutrient agar-agar.

7. The appearance of its growth in stab and slant cultures on gelatine, agar-agar, blood serum, and on potato.

8. The character of its growth in fluid media, as in bouillon, milk, litmus milk, rosolic acid, peptone solution, and in bouillon containing glucose.

9. Does it grow best in acid, alkaline, or neutral media?

10. Is the normal reaction of the medium altered by its growth? Is its growth accompanied by the production of indol? Is the indol associated with the coincident production of nitrites?

11. Is its growth accompanied by the production of gas, as evidenced by the appearance of gas bubbles in the media, both in media containing fermentable sugars and those from which these bodies are absent? When cultivated in sugar bouillon in the fermentation tube, what production of gas is evolved under known conditions? How much of this gas is carbonic acid and how much is explosive?

12. At what temperature does it thrive best, and the lowest and highest temperature at which it will develop? What is its thermal death-point, both by steam and dry-air methods of determining this point?

13. What is its behaviour when exposed to chemical disinfectants and antiseptics? Does it withstand drying and other injurious influences, both in the vegetative and spore stages? The germicidal value of the blood serum of different animals may also be tried upon it.

14. Its pathogenic powers—modes of inoculation by which these are demonstrated; quantity of material used in inoculation; duration of the disease and its symptoms; lesions produced, and distribution of the bacteria in the inoculated animal; which animals are susceptible and which immune, and the character of its pathogenic activities? Variations in virulence, and the probable cause to which they are due. Can they be produced artificially and at will?

15. The detection of specific, toxic, and immunizing products of growth.

16. Its behaviour when exposed to the influence of blood serum of animals immunized from it; also its behaviour when mixed with serum from an animal in the height of infection by it. Are the relations between the organism and the serum constant and specific?

PART III.

SPECIAL BACTERIOLOGY.

BACTERIA FOUND IN INFLAMMATION AND SUPPURATION.

Nearly all bacteria produce under certain conditions inflammation and suppuration. These changes can also be produced by chemical substances—acetic acid, ammonia, oil of turpentine, etc., but above all by the products (ptomaines, proteine, etc.) separated from bacteria. The bacteria mostly associated with ordinary inflammatory and suppurative processes are :—

1. The pyogenic cocci, staphylococci, streptococci, pneumococci, etc.
2. The Bacillus coli communis and allied members of that group.
3. The rarely present Friedlander's pneumobacillus.
4. Bacillus pyocyaneus.

STAPHYLOCOCCUS PYOGENES AUREUS.

Microscopical Appearances.—Micrococci from 0·7 to 1·2 μ in diameter, usually arranged together like bunches of grapes. (See Photomicrograph, Plate IV., Fig. 22.)

Motility.—Non-motile.

Staining Reactions.—They are easily stained with all the basic anilin dyes, and by the Gram and Cladius methods.

Biological Characters. — Facultative anaërobe, producing the yellow pigment only in the presence of oxygen. The minimum temperature for their growth is 6° C., maximum 44° C., optimum 34° to 38° C.

On Gelatine Plates. — Examined under a low power they form in the beginning round, coarsely granular colonies, of a greyish-white colour,

with sharply defined borders; later the colonies assume a yellow colour and liquefy the gelatine quickly.

In Gelatine Stab Cultures. — The development takes place along the whole length of the puncture, with accompanying liquefaction of the medium, the growth resembling a stocking.

On Agar Stroke Cultures. — It forms a moist, shiny, gold-coloured elevated growth; a similar growth occurring on potatoes.

Bouillon becomes densely clouded with a yellow sediment.

Milk is coagulated.

In milk and bouillon lactic acid is chiefly formed.

Vitality.—Cultures remain alive for one year, and are killed in a short time in the steam sterilizer. On silk threads saturated with staphylococci pus, and dried, the cocci are killed by 2 to 3 per cent. carbolic acid in five minutes.

Pathogenesis.—Cutaneous inoculation is negative, but subcutaneous injection causes a local abscess in mice, guinea-pigs, and rabbits; and intravenous injection in rabbits sometimes causes pyæmia.

Staphylococcus pyogenes albus.—This coccus is identical with the aureus, excepting the absence of the golden pigment.

Staphylococcus pyogenes citreus.—This coccus produces a citron-yellow pigment, but in other respects resembles the aureus.

Staphylococcus cereus albus and **cereus flavus** are seldom found, but are characterised by not liquefying gelatine media; the one exhibits a waxy white pigment, the other a waxy yellow pigment.

DIFFERENTIAL TABLE.

A. The Gelatine is liquefied.

1. Staphylococcus pyogenes aureus.
2. „ „ citreus.
3. „ „ rosaceus.
4. „ „ albus.

B. The Gelatine is not liquefied.

1. Staphylococcus cereus flavus.
2. „ „ griseus.
3. „ „ albus.

The Staphylococcus pyogenes aureus is found in furunculus, carbuncles, acute abscesses, circumscribed phlegmons of the skin, impetigo, sycosis, blepharo-adenitis, conjunctivitis phlyctenulosa, acute infectious osteomyelitis, suppuration of lymph-glands, empyema, articular and bursal suppuration, tonsillar abscesses, mammary

abscesses, suppuration of the parotid, idiopathic cerebro-spinal meningitis, strumitis, and suppurative peripleuritis. The albus is also often present in these infections—mixed infection being frequent.

STREPTOCOCCUS PYOGENES.

Microscopical Appearances.—Micrococci from 0·3 to 1 μ in diameter, arranged in more or less long chains.

Motility.—Non-motile.

Staining Reactions.—Easily stained with all the basic anilin dyes, and by the Gram and Cladius methods.

Biological Characters.—Facultative anaërobe, the optimum temperature for its growth being 30° to 37° C.; it also grows at room temperature.

On Gelatine Plates it develops in the form of small white granular colonies which do not liquefy the gelatine. Under a high power chains can be observed projecting from the sides of the discs.

In Gelatine Stab Cultures the growth is not confluent, but individual colonies are arranged next each other along the track of the needle. A similar growth occurs in agar stab cultures.

Bouillon, which is an excellent medium for streptococci, is not clouded throughout, but a flaky, creamy deposit is formed.

On Potatoes the growth is extremely scanty.

Milk is coagulated.

Vitality.—In cultures the streptococcus dies much sooner than the staphylococcus, only living about four months. In order to keep streptococci cultures virulent, Petruschky uses gelatine cultures, and renews them every five days, and keeps them in the ice-chest.

Pathogenesis.—Material containing streptococci, when rubbed on an abrased surface on a rabbit's ear, causes an erysipelatous inflammation; when introduced into mice or rabbits a septicæmia results, with or without a local abscess. Intravenous injection causes septicæmia. Many suppurative processes spread by means of the lymphatics, causing lymphangitis and lymphadenitis. It was in these suppurative changes that Rosenbach first obtained a pure culture of the Streptococcus pyogenes. It is also found in pyæmia. In puerperal pyæmia the streptococci are found in emboli in the bloodvessels of the kidneys. They are also found in severe forms of arthritis, acute endocarditis, in many cases of *secondary infection* following scarlet fever, and in diphtheritic false membranes.

THE STREPTOCOCCI OF ERYSIPELAS.

The presence of streptococci in erysipelatous inflammations of the skin was first observed by Koch. Fehleisen cultivated the cocci artificially, and proved their pathogenic properties. According to the above authorities they are more numerous upon the margins of the erysipelatous area, and may even be seen in the lymph channels a little beyond the red margin which marks the line of progress of the infection.

Microscopical Appearances. — Micrococci arranged in chains, consisting of either a few or many individual cocci.

Motility.—Non-motile.

Staining Reactions.—Any of the watery solutions of the aniline dyes can be used. It is also stained by the Gram and Cladius methods.

Biological Characters—

On Gelatine Plates small dots like greyish-white colonies form, which appear macroscopically opaque and coarse-grained, and never attain a very great circumference.

In Gelatine Stab Cultures small white round colonies form along the inoculation track.

On Agar Plates kept at incubator temperature point-like colonies develop which do not attain any great size.

In Stroke Cultures on Nutrient Gelatine or Agar small round transparent fine dew-drops develop along the inoculated part, which remain isolated.

In Bouillon the growth is better than in the solid media ; it forms a ropy sediment, which, when the tube is shaken, rises in the fluid. Microscopically examined, the sediment is found to consist of long chains. (See Photomicrograph, Fig. 27.)

On Potatoes they may grow, according to some authorities, but others agree that no growth occurs.

Pathogenesis.—Rabbits inoculated in the ear exhibit an erysipelatous inflammation extending from the point of inoculation to the head and neck. The temperature rises, and reaches its height in from eight to ten days, ending in recovery.

Fehleisen inoculated cultures obtained from the skin of patients with erysipelas into patients suffering from lupus and carcinoma, and has obtained positive results, a typical erysipelatous inflammation having developed around the point of inoculation, after a period of incubation of from fifteen to sixty hours. This was accom-

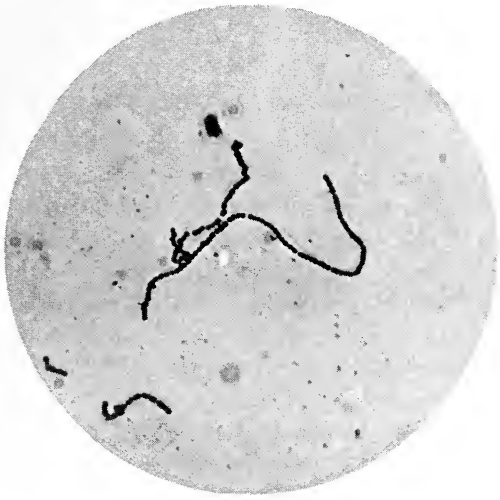


FIG. 27.—Streptococcus of Erysipelas. Cover-glass specimen from bouillon culture. Cladius stain. × 1000.

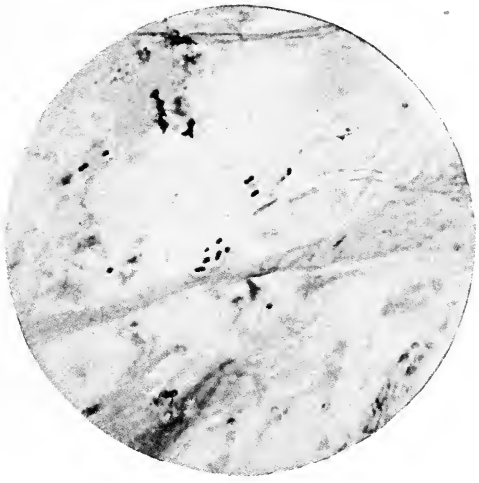


FIG. 28.—Diplococcus Pneumoniæ (Fraenkel). Cover-glass specimen from pneumonic sputum, showing capsules. Johne's method. × 1000.

[*T. Borthill, F.R.C.V.S., Photo., Edinburgh*, 1898.

panied by chilly sensations and an elevation of temperature. Persons who had recently recovered from an attack of erysipelas proved to be immune.

STREPTOCOCCUS PERNICIOSUS PSITTACORUM.

Found by Eberth and Wolff in parrots imported into Europe, the mortality being very great. Nodules were present on the surface of the lungs, spleen, and kidneys. In the bloodvessels of the nodules, and in the heart's blood, medium-sized cocci were found with a tendency to form chains (it must, however, be noted that parrots often die of *bird typhoid* or chicken cholera) which, with an inferior lens, might be easily mistaken for cocci. Further, parrots are also often affected with tuberculosis, and during the course of that disease mixed infection with streptococci might occur (Hirsch and Kolle).

The disease produced by this organism is of considerable importance, as being probably the source of some obscure forms of lung infection in man, which have been traced to diseased parrots kept as pets.

DIPLOCOCCUS OF PNEUMONIA (FRAENKEL).

Streptococcus Lanceolatus (Pasteur).

This organism occurs frequently in the exudate in pneumonia and secondary affections associated with that disease (pleuritis, pericarditis, peritonitis, meningitis, endocarditis, etc.). It is usually present in the sputum of pneumonic patients, and it is also found in normal sputum of healthy individuals.

Microscopical Appearances. — Spherical or oval cocci, usually occurring in pairs, but sometimes forming chains of three or four elements. In stained specimens from the fibrinous exudates of croupous pneumonia, and from the blood of inoculated animals, a capsule is visible surrounding the cocci. It is also occasionally seen on stained preparations from the surface of cultures or blood serum. (See Photomicrograph, Plate IV., Fig. 21.)

Motility.—Non-motile.

Staining Reactions.—The diplococci stain readily with the usual aniline stains, and by the Gram method, which distinguishes it from Friedlander's bacillus of pneumonia, the latter being decolorized.

To demonstrate the capsules in cover-glass specimens, place the specimen in 1 per cent. acetic acid for one minute, dry, and stain with Ehrlich's anilin water gentian violet, or stain by Johne's method. See Technique, § 22. (See Photomicrograph, Fig. 18, stained by Johne's method.)

Biological Characters.—Grows in the presence of oxygen, *aërobic*, but is also a *facultative anaërobe*. Under the latter conditions it retains its vitality and virulence much longer. The minimum temperature for its growth is 22° C., maximum 39·5° C. for cultures on solid media, and 42·5° C. for those on liquid media, while the optimum temperature is 35 to 37° C.

On Gelatine at 25° it develops fine delicate colonies. *The gelatine is not liquefied.*

On Oblique surface agar (*which must be only slightly alkaline*). *On Agar Plates* and blood serum the diplococci grow in small, fairly granular dewdrop-like colonies.

In Bouillon the growth exhibits nothing characteristic.

Milk is a favourable medium, and in some cultures coagulation results.

The diplococci grow best on media containing blood.

Vitality. — Pneumonic sputum attached to cloths, air-dried, and exposed to diffuse daylight, retained its virulence for rabbits in one series of experiments for a period of 19 days, and in another series for 55 days. Exposed to direct sunlight, the same material retained its virulence after twelve hours' exposure. In agar cultures the diplococci do not live long (four or five days), but in bouillon their vitality is more prolonged. The cause of the cultures dying is the formation of lactic and formic acids. Neutralizing the cultures with calcium carbonate causes them to retain their vitality for months. Exposure for ten minutes at 52° C. is sufficient for their destruction, and they exhibit very slight resistance to the ordinary germicides.

Pathogenesis.—The diplococci of pneumonia are pathogenic for rabbits, guinea-pigs, and mice. Rabbits infected subcutaneously with a fresh virulent bouillon culture die in one to two days of a typical septicæmia; rats are less susceptible to infection, and chickens and pigeons are immune. Kruse and Pansini also found a sheep and a horse immune.

BACILLUS OF PNEUMONIA (FRIEDLANDER).

This organism is seldom found in pneumonic patients. It occurs either alone or associated with other organisms, and is frequently found in the nasal discharge in *catarrh*, and in *otitis media acuta*.

Microscopical Appearances.—The bacilli are much larger than the diplococcus of pneumonia, the minimum size being 1 μ. They are arranged in diplo-formation or in chains. A capsule is present in specimens from sputum and inoculated animals, and it can also sometimes be observed in specimens prepared from cultures. (See Photomicrograph, Fig. 29.)

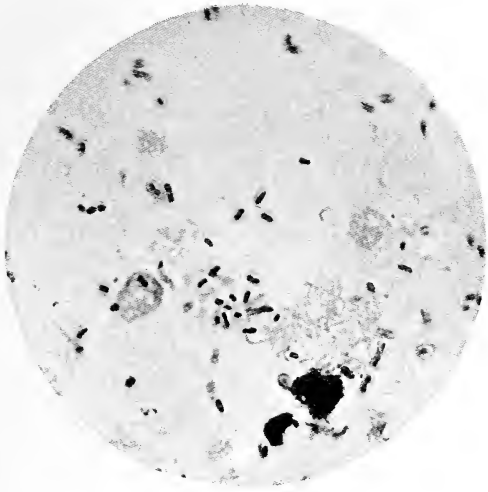


FIG. 29.—B. Pneumoniæ (Friedlander). Cover-glass specimen, showing capsules, from inoculated mouse. Johne's method. × 1000.

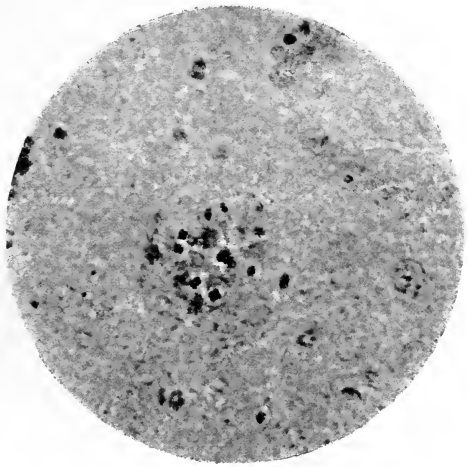


FIG. 30.—M. Tetragenus in section of spleen of inoculated mouse. Gram-Günther method. × 500.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

Motility.—Non-motile.

Staining Reactions.—It stains easily with the ordinary aniline dyes, but not by the Gram method.

Biological Characters.—It is aërobic and facultative anaërobic, growing both at ordinary room and incubator temperatures.

On Gelatine Plates it forms small elevated porcelain-like clusters; the gelatine is not liquefied, but eventually acquires a brownish colour.

In Gelatine Stab Cultures a typical nail-shaped growth occurs.

On Agar Media it forms a whitish coating.

On Potatoes it develops a yellowish-white coating which contains gas bubbles at incubator temperature.

Media containing grape sugar undergo fermentation, Co_2, H., ethylic alcohol, and acetic acid being formed.

Milk is not coagulated.

Vitality.—At 40° C. development ceases, the thermal death-point being about 56° C. The bacilli retain their vitality in ordinary culture media for a long time, living for several months.

Pathogenesis.—The bacillus of pneumonia is pathogenic for mice and dogs, and slightly so for guinea-pigs. It is distinguished from the diplococcus of pneumonia by rabbits being immune. Susceptible animals are inoculated direct into the pleural and abdominal cavities. They can also be affected by inhalation of dried pulverized cultures. In some cases pneumonic lesions are formed.

MICROCOCCUS TETRAGENUS.

This micrococcus was discovered by Koch in 1884 in a phthisical lung cavity. Gaffky made a further study, and described its pathogenic peculiarities for various experiment animals. Biondi also found it in human saliva.

Microscopical Appearances.—When obtained from the animal body it occurs mostly in groups of four surrounded by a capsule.

Motility.—Non-motile.

Staining Reactions.—It stains by the Gram method, the protoplasm remaining stained while the capsule is decolorized; also with the ordinary aniline dyes.

Biological Characters.—It grows best in the presence of oxygen on the usual media at from 35° to 38° C., and also at 20° C.

On Gelatine Plates white, shiny, prominent, round colonies develop.

In Gelatine Stab Cultures it grows on the surface as well as along the track of the needle; on the surface it forms a white, shiny exuberance. The gelatine is not liquefied.

On Agar it forms a white, moist, irregularly outlined covering.
On Potatoes, a shiny, thick, irregular patch is formed.

Pathogenesis.—In white mice and guinea-pigs an abscess sometimes forms instead of general septicæmia. Grey mice, dogs, and rabbits are not susceptible. Macroscopically, no alteration can be observed in the organs of dead animals, but microscopical examination will reveal the presence of the organism. In sections the organisms will always be found within the capillaries. (See Photomicrograph, Fig. 30.)

MICROCOCCUS GONORRHŒA.

(Gonococcus—Neisser).

This organism was discovered by Neisser in 1879 in gonorrhœal pus, and described by him as a 'gonococcus.' It was cultivated by Bumm in 1885, and its infective influence proved by inoculation into men. It is constantly present in virulent gonorrhœal discharges, generally in the interior of the pus cells, or attached to the surface of the epithelial cells.

Microscopical Appearances. — Cocci usually jointed in pairs. They are shaped like a pair of kidneys placed with the hilum in apposition, or like a coffee bean, and are separated by a distinct interspace. The length of the gonococci is 0·8 μ to 1·6 μ, diameter 0·6 to 0·8 μ.

Staining Reactions.—It stains quickly with methyl violet, gentian violet fuchsin, not so quickly with methylene blue, which is, however, the best stain for demonstrating its presence in pus. (For special staining methods see Technique, §§ 16, 17.) The results with the Gram method are negative, which enables it to be distinguished from other common pus cocci. Though, according to Bumm, other diplococci sometimes occur in gonorrhœal pus that do not stain by the Gram method, the most trustworthy diagnostic character is that the gonococci are found within the pus cells sometimes in one or two pairs only, frequently in considerable numbers, and sometimes almost filling the cells. (See Photomicrograph, Plate IV., Fig. 24.)

Biological Characters.—The gonococcus grows only at 37° C.

Plate Culture Method. — The gonorrhœal discharge is placed in a tube with human blood serum at 40° C., and two reductions made in the usual manner (see Technique, § 112) into two other blood serum tubes at 40° C. In these three tubes an equal quantity of 2 per cent. peptone agar, previously dissolved and cooled down to 40° C., is added, and three plate cultures are prepared and placed immediately in the incubator.

Instead of plates, Petri-dishes can be used. In twenty-four hours isolated gonococci colonies appear. The superficial colonies exhibit a dark punctiform centre, from which a delicate, finely granular coating extends round about the colony; the deeper colonies are greyish-white in colour, and possess an uneven appearance, and in two to three days acquire the shape of a blackberry. In re-inoculating from the colonies they are found to consist of a shiny, tenacious, compact mass.

Stroke Cultures on oblique solidified Blood Serum Agar are prepared as follows:—1 part of fluid human blood serum at 40° C. is mixed with 3 parts of melted agar-agar, also at 40° C., and placed in an oblique position to solidify. The growth on this medium is luxuriant; at first isolated grey colonies appear, which later become moist, shiny, tenacious slimy tufts, and from the margins a thin film-like coating extends.

A good liquid medium is prepared by mixing 1 part of human blood serum with 2 parts of peptone bouillon. In this medium the gonococcus forms a membrane on the surface, while the medium itself remains almost entirely clear.

In preparing the culture media animal blood serum can be used instead of human serum, although they do not grow so well, but nevertheless the gonococci grow very well on swine blood serum.

Pathogenesis.—Bumm made inoculations into the healthy urethra, and in two cases, once with a third generation culture and once with culture transferred for twenty successive generations, and in both cases a typical gonorrhœa developed as the result of the inoculation. The mucous membranes of man liable to gonorrhœal infection are those of the urethra, conjunctiva, the cervix uteri, and the vagina in children. Inoculations of gonorrhœal pus into the vagina or conjunctival sac of the lower animals, dogs, rabbits, horses, apes, are without result.

In *Blenorrhœa neonatorum*, according to Bumm, after infection the presence of gonococci may be demonstrated in the superficial epithelial cells of the mucous membrane and between them; that they soon penetrate to the deeper layers; and that by the end of forty-eight hours the entire epithelial layer is invaded by the diplococci, which penetrate by way of the connecting material, 'Kitt substance,' between the cells. They also multiply in the superficial layers of the connective tissue, and give rise to an inflammatory reaction, which is shown by an abundant escape of leucocytes from the capillary network.

Bacteriological Diagnosis.—The microscopical examination of the urethral discharge is of the greatest importance. Cover-glass specimens are prepared from the suspected discharge, air-dried, fixed in the flame, and stained with a watery solution of methylene blue. The cocci and nuclei of the pus cells are stained blue by this method, the cocci more intensely than the nuclei. The characteristic form of

the cocci, their position in the pus cells, and their negative reaction with the Gram staining method, allow the diagnosis of gonorrhœa to be accomplished with positive certainty. The absence of the gonococci in microscopical specimens of urethral discharge must be accepted with caution, as the gonococci are sometimes situated deep in the mucosa of the urethra, and often absent in the superficial discharge. It is therefore necessary in doubtful cases to irritate the urethra and stimulate the discharge. If the secretion after repeated examination, also with previous irritation of urethra, is found free from gonococci (numerous other cocci are usually present), then the gonorrhœa can be considered terminated, and only a *urethritis catarrhalis* existent.

BACILLUS PYOCYANEUS.

(*Bacillus of green pus. Microbe du pus bleu. Bact. Aeruginosum.*)

This bacillus is found in green or blue-coloured pus, especially in green-coloured bandages, from which pure cultures are easily obtained with plate cultures.

Microscopical Appearances.—Small thin bacilli about the length of the bacillus of mouse septicæmia, but a little thicker, and in cultures often form small chains, occasionally growing into filaments.

Motility. — Strongly motile, possessing only one flagellum (monotricha).

Spore Formation does not exist.

Staining Reactions.—It stains readily with the ordinary aniline dyes, but not by the Gram method.

Biological Characters. — This organism is a *facultative anaërobe*, growing both at room and incubator temperature.

On Gelatine Plates, flat, irregular, circumscribed colonies develop with radiating borders; the gelatine is liquefied quickly, the surrounding media exhibiting a green fluorescence.

In Gelatine Stab Cultures the medium is liquefied quickly.

On Oblique Surface Agar a whitish coating is formed, and the underlying medium is coloured green.

On Glycerine Agar the inoculated medium exhibits at first a blue colour, which gradually becomes darker.

On Potatoes a greenish-yellow or brownish growth takes place, the surrounding surface being coloured green.

Bouillon is clouded.

Milk is coagulated and peptonized.

This organism produces pyocyanin and a fluorescent green colouring

matter, formed only in the presence of oxygen, which is soluble in chloroform.

Pathogenesis.—This bacillus is pathogenic for guinea-pigs and rabbits—1 c.c. of a bouillon culture causing the death of the animal in from 12 to 36 hours. Smaller amounts do not kill the animals, but render them immune to doses fatal to animals not previously protected. In rabbits inoculated with a culture of the Bacillus anthrax a fatal result may be prevented by soon after inoculating the animal with a pure culture of the Bacillus pyocyaneus. Woodhead's experiments indicate that the antidotal effect is due to the chemical products of the growth of the bacillus and not to an antagonism of the living bacterial cells.

STREPTOCOCCUS OF STRANGLES OF THE HORSE (SCHÜTZ)

(*Ger.* Druse der pferde; *Fr.* Gourme.)

This is an infectious catarrh of the upper passages of the horse, with suppurative inflammation of the neighbouring lymph-glands, generally forming an abscess. The disease is often complicated with metastatic abscesses in other distant organs and lymph-glands, the virus being carried by means of the blood and lymph circulation. One attack gives immunity for years, perhaps for a lifetime.

Microscopical Appearances.—The Schütz streptococcus is found in the pus obtained from the lymph-gland abscesses in more or less long bundles of chains. The most significant formation is when the cocci are arranged in threads resembling a wreath of roses, lying either slightly bent or undulating between the pus corpuscles (see Photomicrograph, Plate IV., Fig. 23). Individual cocci in the chain sometimes appear larger than the others.

Staining Reactions.—The streptococci stain very well with fuchsin and gentian violet. For pus preparations the Cladius method gives good results, also the Gram method, the decolorizing being accomplished with a saturated alcoholic solution of fluorescin according to Kühne's modification.

Biological Characters.—*On Gelatine* the growth is weak, white colonies forming on the inoculation stab. The gelatine is not liquefied.

On Agar-Agar, inoculated with a drop of pus, numerous visible transparent colonies about the size of a pin-head develop. In stab cultures at 37° C., they form a greyish-white zone with wing-like projections.

On Solid Blood Serum at 37° the growth is most luxuriant. The

colonies appear at first as shiny grey drops; and after this a dry iridescent coating forms.

In Bouillon a flocculent white mass develops, finally forming a sediment at the bottom of the tube.

Pathogenesis.—Horses can be inoculated with pure cultures, abscesses forming at the point of inoculation, due to necrosis of the tissues. Inoculation into the mucous membrane of the nose causes typical purulent nasal catarrh, with accompanying inflammation and enlargement of the lymph-glands. Mice are also susceptible to infection when inoculated, an abscess forming at the point of inoculation, accompanied with metastatic suppuration throughout the course of the lymphatic and blood circulations.

Differential Diagnosis.—The disease is differentiated from glanders by experimentally inoculating field-mice, which are highly susceptible to glanders, but immune to strangles.

The streptococcus of strangles is not a very resistant organism, as white mice inoculated with dried pus remain unaffected.

STREPTOCOCCUS OF CONTAGIOUS MAMMITIS OF MILCH COWS.

This is a special form of mammitis occurring in milch cows, described by Nocard and Mollerau. It passes rapidly from one animal to the other. The disease commences at the base of the teats in the form of indurated lumps, which sooner or later invade the whole organ.

Microscopical Appearances.—Round or ovoid micrococci, 1·25 μ long and 1 μ broad, occurring in long straight or undulating chains. These characteristic chains are found in the milk and in the walls of the excretory ducts. They can be stained by the ordinary dyes, but not by the Gram method. The growth of this organism in cultures is checked by a trace of boric acid. By means of injections of 100 grains of 4 per cent. tepid solutions of boric acid into the teats of affected udders, Nocard and Mollerau succeeded in arresting the extension of the disease. The organism is also destroyed by a 3 per cent. solution of carbolic acid.

Biological Characters.—*On Gelatine Plates.*—Small, round, non-liquefying granular colonies of slow growth.

In Gelatine Stab Cultures.—In the form of a nail.

On Agar-Agar it grows badly.

Bouillon is very soon clouded.

Milk becomes acid and is coagulated.

Pathogenesis.—Pure cultures introduced into the teats produced the disease in the cow and goat. The dog, cat, rabbit, and guinea-pig remained unaffected by both intravenous and intraperitoneal injections.

The disease is communicated from the diseased to healthy cows by the hands of the milkers, which can be prevented by disinfection of the hands with 3 per cent. solution of carbolic acid. Milk from a diseased cow also infects the milk from the healthy animals, rendering *both unfit for human consumption.*

MICROCOCCUS OF GANGRENOUS MAMMITIS OF MILCH EWES.

This disease, also called *Mal de pis araignée*, causes great mortality in affected ewes.

Microscopical Appearances.—According to Nocard, a very fine micrococcus 0·2 μ in diameter, associated in groups of four or more, never in chains.

Staining Reactions.—Stains by the Gram method.

Biological Characters.—It is a facultative anaërobic organism.

On Gelatine Plates.—Grows on the surface in round, white, liquefying colonies.

In Gelatine Stab Cultures a funnel-shaped growth occurs.

On Agar-Agar an abundant yellowish-white growth develops.

Blood Serum is liquefied.

Milk becomes acid and is coagulated in twenty-four hours.

Cultures do not remain virulent unless renewed daily.

Pathogenesis.—Cultures inoculated into the teats of a ewe produce a rapidly fatal mammitis. The goat is refractory.

Inoculations into the ordinary experiment animals cause only a slight œdema, while in the rabbit an abscess forms, from which it recovers.

DIPLOCOCCUS OF PLEURO-PNEUMONIA CONTAGIOSA OF THE HORSE (SCHÜTZ).

(*Ger.* Breustseuche der Pferde.)

The Schütz bacterium is a small, slightly ovoid organism, sometimes possessing a capsule, found in the pulmonary tissue, and exudative pleuritis generally present in contagious pleuro-pneumonia of the horse.

Staining Reactions.—The organism stains easily with gentian violet or methylene blue. The Gram stain only gives positive results

when the discolorization in absolute alcohol is not continued for more than fifteen or twenty seconds, and in two minutes the organism is entirely decolorized. The above reaction with the Gram method differentiates this organism from Fraenkel's pneumococcus, which stains readily by the Gram method.

Biological Characters.—The diplococcus grows on gelatine and agar media at room temperature; the gelatine is not liquefied, and the growth in both media exhibits nothing characteristic.

Pathogenesis.—Affect mice, guinea-pigs, pigeons, and rabbits, but not fowls or swine. The etiological importance of this organism is established by its constant and characteristic presence in the pulmonary tissues and effusions of affected horses, and by the fact that Schütz produced typical cases of the disease by injecting healthy horses intrapulmonarily with the diplococcus. The bacteria, according to Von Rust, are present in the nasal discharges of affected horses. Fiedler isolated the diplococcus from the blood of affected animals, and produced the disease on healthy animals by intrapulmonary injection.

BOTRIOMYCOSIS.

Botryomyces (Bollinger); Discomyces (Rivolta); Botryococcus ascoformans (Kitt); Mykodermoid (Johne).

This parasite is found in the indurated tissue of scirrhus cord of castrated horses, and also in some form of fistulous withers, tumours at the point of the shoulder, and other indurations of the skin and subcutaneous tissue, also in the connective tissue of the pelvic cavity. In the pus of chronic mammitis of the cow (Czoker), and in the lungs, ribs, and pleural cavity; also in the spermatic cord of swine. MM. Porcat and D'or record four cases in man. Microscopically the parasite appears in grape or mulberry-shaped masses of pale greyish-yellow bodies, about the size of small grains of sand.

Microscopical Appearances.—The individual colonies are formed of granular clusters of symmetrical grains, united by a gelatinous substance, and enveloped within a close-fitting, transparent, colourless membrane, which holds them together.

Staining Reactions.—The parasite stains by the Gram method, and when eosin is used for a contrast, it stains the gelatinous substance. Picric acid has a similar action.

Biological Characters.—When grown on gelatine and potato media, the conglomerated and the envelop formation no longer exist. Gelatine is slowly liquefied; the developing colonies present first a grey

colour, finally becoming yellow, and consist of distinct individual cocci. (Kitt considers the Botryomyces to be a variety of the Staphylococcus pyogenes aureus.)

In Gelatine Stab Cultures it forms greyish-white threads, with slow liquefaction commencing at the periphery.

On Potatoes it forms a flat yellowish coating.

Pathogenesis.—Rabe and Kitt inoculated horses with pure cultures, and in four to six weeks genuine fibromas appeared. Mice are immune. Sheep and goats exhibit inflammation of the skin; later, necrosis accompanied with œdema. Guinea-pigs die from septicæmic changes.

The parasite generally confines its ravages to its primary seat. It may, however, migrate toward the lymph glands and into the blood, when metastatic foci appear, usually localized in the lungs, skin, etc.

BACILLUS ACNES CONTAGIOSÆ OF THE HORSE (DIECKERHOFF AND GRAWITZ).

This disease is a pustular dermatitis known as contagious acne of the horse, and is very readily transmitted to other animals. In severe cases ulcerations and inflammation of the lymph vessels and glands occur. It is easily distinguished from farcy by the cicatrization of the ulcers, and, moreover, the pustules do not occur in farcy. It is distinguished from horse-pox or variola of the horse by the fact that in variola the eruptions are always localized in the lips, nostrils, and pasterns. It is further identified by the presence of the bacillus discovered by Dieckerhoff and Grawitz in the pustules.

Microscopical Appearances.—Very small ovoid bacilli, 2 μ long, occurring singly and also forming small chains.

Motility.—Non-motile.

Staining Reactions.—Somewhat refractory with ordinary stains, but stains by the Gram method.

Spore Formation.—Absent.

Biological Characters.—*In Gelatine Stab Cultures* white colonies about the size of a millet-seed develop along the course of the needle.

On Agar-Agar, white colonies develop very slowly.

On Blood Serum, especially from horses and cattle (37° C.), in twenty-four hours small white colonies develop on the surface of the medium, a granular deposit being formed in the water of condensation.

On Potatoes it exhibits no growth.

Pathogenesis.—Affects the horse, rabbit, guinea-pig, ox, sheep, dog, and mice. The disease can be produced in horses by rubbing the acne scabs or a pure culture of the bacillus into the skin. Guinea-pigs subjected to similar treatment succumb in twenty-four hours. Subcutaneous injection in dogs and rabbits produces toxic symptoms and death, but the bacilli do not spread over the body. Mice and field-mice are not affected by rubbing, but by subcutaneous inoculation, die in one to ten days with the formation of abscesses, the bacilli being found in clusters in the organs. The natural disease is transmitted by means of grooming utensils, harness, blankets, etc., and is often localized in the regions covered by the saddle and the girth.

Tizzoni and Giovannini mention a case of acne contagiosa in man which proved fatal in thirteen days, and from the blood and skin they isolated a bacillus morphologically, and in cultures, resembling the bacillus of mouse septicæmia, but it was not pathogenic for mice, while it produced fatal results with rabbits and guinea-pigs, the conditions being similar to those found in the man. They considered this organism as only of secondary importance, and that the Staphylococcus pyogenes was the cause of the acne.

THE STREPTOTHRICES.

The organisms belonging to this subdivision resemble in their structure at one time the *thread fungi*, and at other times the bacteria. Like the mould fungi, they form cylindrical threads out of round cells which branch dichotomously, finally becoming visible to the naked eye as irregular radiating thread masses or mycelia. Single threads or fruit hyphæ grow upwards out of the substance, free in the air, and break up into chains of round germ cells (spores or conidia), which detaching from the plant are carried by the air, and the preservation of the species is thus assured.

STREPTOTHRIX ACTINOMYCES BOVIS.

The true nature of *Actinomyces bovis* was first recognised by Bollinger in cattle in 1877. The disease is characterised by the formation of specific tumours tending to suppuration, the lesions being frequently located in the jaw bones and subcutaneous connective tissue of the maxillary region, and are commonly known as wen, osteosarcoma, lumpy-jaw, etc., and when affecting the tongue as

wooden tongue, owing to the indurated condition of that organ. It also occurs in the retropharyngeal lymph-glands (Clyers).

The author recorded in the *Veterinary Journal* a case in the parotid gland of a young cow in California, which finally obliterated all the bloodvessels, the diseased gland being successfully extirpated. The disease has also been found in the liver, nasal cavities, larynx, lungs, and vertebræ. In the pig the muscles, lungs, mammæ, and bones of the cervical and dorsal vertebræ are affected; a case has also been recorded in the dog; and cases of actinomycosis of the tongue in the horse are also mentioned.

Section of a specific tumour reveals an abundance of granulation tissue, studded with soft parts or nodules of various sizes, containing numerous yellow or occasionally colourless grains, the smallest appearing about the size of a grain of sand; the larger, due to the union of the smaller grains, are of different forms. The grains vary in size from 0·1 to 1 mm. or more, being frequently cretaceous. The above-mentioned yellow granules are characteristic of an actinomycotic tumour. Ponfick transmitted the disease to other animals by means of those granules.

Microscopical Appearances.—Examined under a low power, the unstained granules appear as dark, finely granular, round or irregular balls. Under a high power and after the specimen is stained, the organism is found to consist of a central zone of very fine filaments, ramified and intermingled in a close network, with a few cocci arranged in the centre, and a peripheral zone consisting of radiating pyriform elements with large swollen or club-shaped extremities, which are either simple or branching. The branches are given off from either the pedicle or the club, and sometimes by subdividing themselves, the whole resembling the capitulum of a daisy (see Photomicrograph, Fig. 32).

There are other forms of actinomyces which are quite small, the club-like enlargements being absent, and *Cornil* and *Babes* describe a special condition of the filaments of the periphery terminating in various slight enlargements bearing conidia. The threads sometimes undergo segmentation, and resemble threads of bacilli. Finally, an agglomeration of club forms, consisting of masses of cocci, are sometimes observed.

Unstained specimens are prepared by squeezing the material under the cover-glass.

Staining Reactions.—It stains best by the Gram method, especially by Günther's modification (see Technique, § 38), also with heated carbol fuchsin. Double staining can be obtained with the Gram method and picro-carmine or saffronine, the threads of the fungi being stained a blue-black colour by the Gram method, while the clubs are stained red with carmine.

Biological Characters.—The actinomyces is a typical streptothrix possessing all the characteristics peculiar to that species. It is anaërobic and facultatively aërobic. Some authorities state that the growth is best under anaërobic conditions when first obtained from the animal body, while others consider it grows best aërobic.

On Gelatine Plates, in six days a very limited greyish-yellow growth develops, sometimes on the surface and sometimes in the depth of the medium.

In Gelatine Stab Cultures.—At the commencement a dull yellowish-white, elevated, shining compact growth develops on the surface of the medium, sinking later from slight liquefaction of the gelatine; along the track of the needle small yellowish-white knots develop at first, bristly outgrowths appearing later.

On Plain and Glycerine Agar opaque nodules about the size of a pin-head, which remain isolated for weeks or months, develop, the peripheries being formed of a fine delicate network. Large white nodules about the size of a lentil (rosette forms) sometimes also develop (see Photograph, Fig. 31).

On the oblique surface of Blood Serum the individual granules exhibit a yellowish brick or red-rose colour, covered with whitish downy filaments or threads.

In Bouillon the medium is not clouded, but round masses are formed in the bottom of the tube, which are separated with difficulty by shaking.

In Milk a granular growth takes place, followed by gradual peptonising.

On Potatoes the growth presents a yellowish-red colour, covered with a downy mass of threads.

In Eggs.—It grows well in pigeon and hen's eggs, either raw or when cooked for three or four minutes (prepare the egg and inoculate according to Günther's method—see Technique, page 52). The prepared egg is placed in the incubator with the inoculated end uppermost, and examined in from nine to twenty-eight days; if no putrefaction has taken place or decolorization, the growth appears in raw eggs both in the white and in the yelk, and cloudy masses of slime resembling nasal mucus develop in the albumen. In cooked eggs opaque white spots of the size of pin-heads develop between the yelk and white, and finally a mass is formed in the track of inoculation and on the surface of the coagulated albumen.

Microscopical Examination of Cultures.—The growths on agar media consist mostly of short rods mostly straight, but often comma shaped, or sometimes further bent. The dimensions of the rods vary; they may appear plump and thick, very slender and short, or in long thick rods, and sometimes club or olive-shaped at the ends (see Photo-

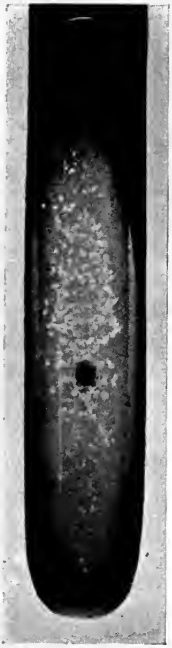


FIG. 31.—Actinomyces Bovis. Glycerine-agar culture. 'Rosette' form.

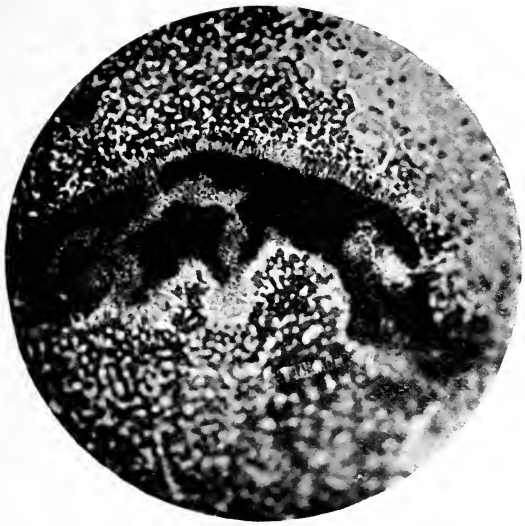


FIG. 32.—Actinomyces Bovis. Section of a tumour from the jaw. Stained by the Gram-Günther method. × 450.

FIG. 33.—Actinomyces Bovis. Cover-glass specimen from agar culture, 'showing clubs.' Fuchsin. × 1000.

[*T. Borthill, F.R.C.V.S., Photo., Edinburgh*, 1898.

micrograph, Fig. 33). Undulating or spiral rods are sometimes observed, but rarely in agar cultures.

In Egg Cultures beautiful net-like masses of threads occur; the threads at the periphery of the mass radiating, the ends being sometimes swollen and club-shaped.

The threads and also the short rods stain both by the Gram method and with fuchsin. When the threads are stained one hour in water fuchsin segments are sometimes observed, consisting of long and short rods, and short coccoid-like bodies arranged in an irregular manner, and separated by unstained interspaces of various breadths. Finally, micrococcus-like bodies are also found in agar and egg cultures, isolated in clusters of various sizes. They are sometimes clubbed or oval, sometimes more irregular and angular, staining intensely with gentian violet and by the Gram method. Inoculated in fresh media they give rise to fresh rods and threads.

Vitality.—It remains living in cultures from nine to twelve months. It is killed by heating five minutes at 75° C.

Pathogenesis.—Johne transmitted the disease to the cow and the calf by subcutaneous intraperitoneal and mammary inoculation. Ponfick and Israel also transmitted it to the calf and to the rabbit. Israel made his inoculation with the actinomycosis of man, the results being the same as those obtained with tumours from cattle. Accidental contamination is also recorded in persons attending affected animals.

The disease is supposed to be communicated to susceptible animals (Omnivora and Herbivora) by means of their food, especially straw and barley husks. Johne found an identical fungus on the surface of husks of barley, arrested on the tonsils of a healthy pig. Piana also discovered vegetable débris accompanying the actinomyces in a tumour in the tongue. In a case of abdominal actinomyces in the human subject, Ammentorp, in opening one of the abscesses, found in its centre a pin-sized concretion, in the middle of which a piece of barley awn was visible about 2 cm. long. A similar case was also observed in the Clinic at Vienna. In this instance the patient also suffered from abdominal actinomycosis, and a fæcal stone which was found in the patient contained at its centre a barley awn. Nocard records a case in a stableman kicked by a horse where a phlegmon developed in the upper part of the thigh, in which microscopic examination revealed the characteristic tufts of the actinomyces. The actinomyces affecting man is distinguished from that of cattle by its tendency to form tumours, and the slow manner in which the disease spreads in the surrounding structures, the newly formed

granulation tissue degenerating more quickly; suppuration also being more pronounced, accompanied by the formation of fistulæ which undermine the skin, passing through the muscular tissue, and by this means the fungus spreads from the jaws or neck to the pleura and ungs, and through the diaphragm into the peritoneal cavity.

ACTINOMYCES MUSCULORUM SUIS.

This organism was discovered by Düncker in 1884, in the muscles of swine. Although resembling the Actinomyces bovis, it is not identical (Günther).

STREPTOTHRIX HOFFMANI.

This is another organism very similar in its growth to the Actinomyces bovis. It is found in the air.

Microscopical Appearances.—It occurs as a branched mycelium, with swollen, club-like ends.

Staining Reactions.—It is stained by the Gram method.

Biological Characters.—It is aërobic, growing only at a temperature of over 22° C.

On Agar Media it grows in light brown warty colonies, which very soon coalesce.

Bouillon remains clear; a thick sediment is formed.

Potatoes.—There is no growth.

Pathogenesis.—For guinea-pigs and rabbits, only by subcutaneous injections; a local abscess being formed, and in the pus actinomycotic-like tufts with club-shaped ends are sometimes present, *distinguished from the Actinomyces bovis by the clubs staining the same as the threads.*

STREPTOTHRIX ACTINOMYCES HOMINIS (ISRAEL).

This organism was discovered by Israel and Wolff in two cases of human actinomycosis, and in its macroscopical, microscopical, and staining reactions resembles the Actinomyces bovis.

The differentiation in cultures is, however, well marked, as it only grows luxuriantly under anaërobic conditions, and under aërobic slightly or not at all. The optimum temperature is 37°, and the development is very slow.

On Agar-Agar irregular opaque colonies are formed, which in seven days attain the size of a pin-head; usually coalescing. To obtain pure

cultures, remove the pus under sterile precautions, and wash the small nodules or grains it contains in sterile water, and place them deep down in a tube of agar. As it develops, a thin film forms round the periphery, from which further nodules sometimes develop. The nodules sometimes attain the size of a lentil, and rosette forms appear.

Bouillon remains clear; a sediment consisting of white scales forms.

In Raw Eggs a cloudy slime forms.

In Cooked Eggs a greasy, granular mass.

In the cultures, usually straight, sometimes bent, rods with slightly swollen ends (somewhat like the bacillus of diphtheria) are found. The filament masses are present, as a rule, only in egg cultures.

Pathogenesis.—Affects guinea-pigs and rabbits by intraperitoneal inoculation; a genuine actinomycosis resulting, with the formation of typical tumours.

The differential diagnosis between this and Actinomyces bovis seems to exist only in culture peculiarities.

STREPTOTHRIX MADURÆ (VINCENT)

Found in madura foot, an ulcerative affection of the feet, rarely of the hands, in the East Indies; also in America, Morocco, and Italy.

Microscopical Appearances.—The parasite consists of branched threads, 1 to 1·5 μ thick, which on the surface of some media present filaments growing upwards, but spores are also formed in the substance of the medium.

Staining Reactions.—The threads and spores stain with the usual aniline dyes and by the Gram method.

Vitality.—The threads are destroyed by heating for three to five minutes at 60° C., the spores at 85° C.

Biological Characters. — Strongly aërobic; grows at ordinary temperature, but best at 37° C.

On Agar the growth is limited, while *on Glycerine Agar* it is luxuriant.

Gelatine is not liquefied.

The developing colonies are nodular, hard, yellowish-white in colour, later becoming reddish.

There is no growth on serum or in eggs.

In Bouillon the growth is limited, granules developing slowly.

On Acid Potatoes.—Warty, at first white, later red or orange coloured exuberances, studded with white threads.

Milk is peptonised slowly.

The growth is also very luxuriant on slightly acid vegetable infusions.

Pathogenesis.—Inoculation of various animals has failed, only local reactions being manifested.

STREPTOTHRIX EPPINGER.

This organism was found by Eppinger in a brain abscess. It consists of a branched mycelium. Fruit hyphæ and spores are only found in potato cultures.

Staining Reactions.—It stains by the Gram method.

Biological Characters.—It is aërobic, growing best at 37° C.

On Gelatine, yellow warty-shaped elevated colonies develop, which do not liquefy the gelatine.

On Glucose Agar the growth is orange coloured.

On Potatoes it forms a yellowish-red coating.

Bouillon remains clear, but flaky lumps develop.

Pathogenesis.—Guinea-pigs and rabbits inoculated with this organism develop a pseudo-tuberculosis.

STREPTOTHRIX FARCINICA (ROSSI DORIA).

(*Fr.* Bacille du farcin du bœufs (Nocard); *Eng.* Bovine Farcy.)

This bovine malady was formerly very prevalent in France, and exists also in Guadeloupe. The lesions are usually located in the limbs, and consist of swelling of the lymph vessels, terminating at the corresponding lymph-glands, those most usually affected being the brachial, pectoral, and prepectoral groups, which suppurate slowly. When the abscesses are opened the animal seems to recover, but other tumours appear, the animal pining and dying of general marasmus. The autopsy shows pseudo-tubercular lesions with purulent centres in the lungs, liver, spleen, and lymph-glands.

Microscopical Appearances.—Small, interwoven masses of threads, about 0·25 μ thick, arranged in tufts, are present in the pus from the abscesses.

Staining Reactions.—It stains by the Gram method, but is decolorized when the contact with the alcohol is prolonged; it also stains with Weigert's double stain. The spores stain with difficulty.

Biological Characters.—It is a purely aërobic organism, growing best between 30° to 40° C.

In Bouillon it forms round pellicles of a dull grey colour and oily appearance, floating in the liquid with an abundant film on the surface.

On Agar and Gelatine, small, more or less rounded opaque masses, thicker at the periphery than in the centre, develop.

In Milk it grows without changing the reaction or causing coagulation.

On Potatoes the growth is rapid.

Pathogenesis.—Pure cultures or pus injected into the peritoneal cavity of a guinea-pig cause pseudo-tuberculosis of the peritoneum in nine to twenty days, the characteristic tufted organisms being present in the centre of the nodules. *Intravenous* inoculation causes a generalized pseudo-tuberculosis. Intravenous inoculation of cattle and sheep causes a slow-forming pseudo-tuberculosis.

Subcutaneous inoculation in refractory animals causes an abscess which heals quickly.

BACILLUS ANTHRACIS.

(*Ger.* Milzbrand bacillus; *Fr.* Bactéridie du charbon).

This organism is always present in the blood of animals affected with anthrax, and can be isolated in pure cultures on artificial media. When susceptible animals are inoculated with portions of pure cultures, conditions similar to those found in the animal from which the original cultures was obtained are produced.

Microscopical Appearances.—In the blood of animals recently dead, the bacilli occur as large rods of variable size, from 3 to 10 μ long and 1 to $1\frac{1}{2}$ μ broad, often arranged in threads formed by several rods jointed together (see Photomicrograph, Fig. 34). In unstained specimens examined by means of a *hanging drop,* the ends of the rods appear round; while in stained specimens the ends of the rods are *square.* Under a high magnification the ends are found to be a trifle thicker than the body of the bacillus, and occasionally somewhat indented and concave—compared by Fraenkel, when stained with methylene blue, to small pieces of bamboo cane.

Bacilli obtained from the blood of affected animals possess *capsules* (see Photomicrograph, Plate I., Fig. 1, also Fig. 35). To demonstrate the capsules, stain the specimens by Johne's special process (see Technique, § 22). Capsules can also be demonstrated when the bacilli are cultivated in liquid blood serum medium. Günther has also found the capsules present in bacilli in sections stained with methylene blue. The square ends and the presence of capsules help to distinguish the Bacillus anthracis from certain other organisms that resemble it morphologically, especially putrefactive bacteria.

After death, the bacilli are only to be found in bloodvessels. In

sections of organs stained by the Gram or Cladius methods they are easily demonstrated (see Photomicrograph, Plate I., Fig. 3), showing a section of a mouse's lung with the capillaries filled with bacilli.

On artificial media the bacilli grow in long, parallel, or somewhat twisted and interlaced threads, which either form spores or degenerate into the so-called involution forms (see Photomicrograph, Fig. 37).

Motility.—Non-motile.

Staining Reactions.—The bacilli stain easily with all the ordinary aniline dyes, with hæmatoxylin, and by the Gram and Cladius methods.

Spore Formation.—The bacilli form spores under aërobic conditions at a temperature of 15° to as high as 37° C. Günther considers 28° C. the optimum, and that at a higher temperature the formation is somewhat irregular.

Spores are never formed in the living animal or in unopened carcases. The latter is most important from a sanitary point of view, in regard to the disposal of the carcases of animals dead from anthrax. The spores are ovoid, and one to two times as long as broad, one spore being present in nearly every bacillus, giving the thread the appearance of a chain of beads (see Photomicrograph, Plate I., Fig. 2). For the special method of staining the spores of the Bacillus anthracis, see Technique, § 27.

Vitality.—The resistance of anthrax spores to outside influences is not always constant. Some spores are killed by exposure to 5 per cent. carbolic acid in two days, and to steam at 100° C. in three minutes, while others resist 5 per cent. carbolic acid over forty days, and steam at 100° C. for more than twelve minutes. In a dry state the spores are destroyed instantly at 160° C.

When the bacilli are cultivated in bouillon to which $\frac{1}{2000}$ to $\frac{1}{5000}$ bichromate of potash is added, they lose the faculty of forming spores without losing their virulence. The power of forming spores is also lost when the bacilli are cultivated for many generations on gelatine media.

Biological Characters.—The Bacillus anthracis is a facultative aërobic organism growing best in the presence of oxygen, and in its absence slowly without liquefying the media. Exhibits no growth in CO_2. It grows quickly at 37° C., and ceases to grow under 12° and above 45° C.

On Gelatine Plates.—On the surface small whitish colonies appear, while those deeper in the medium are of a greenish-black colour. Under a low power the colonies exhibit a characteristic tangled mass of single threads projecting beyond the edges of the colonies in curly hairy tufts. The colonies begin to liquefy in three or four days.

In Gelatine Stab Cultures.—In twelve to twenty hours a thick, white central thread appears, from which white threads and branching rootlets radiate (see Photo., Fig. 36). After two days, liquefaction commences on

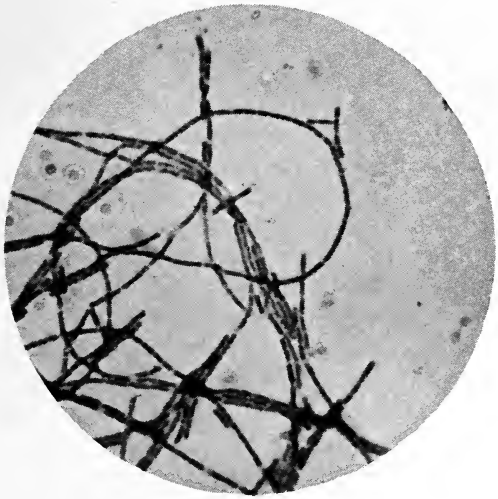


FIG. 34.—B. Anthracis. Leptothrix formation in a virulent bouillon culture. Methylene blue. × 1000.

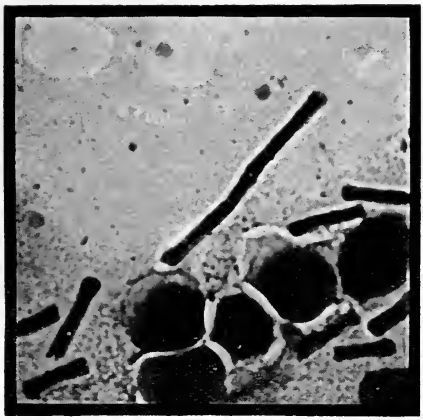


FIG. 35.—B. Anthracis, with capsules in mouse's blood. Stained by Johne's method. × 1500.

FIG. 36.—B. Anthracis. Stab culture in gelatine.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

the surface of the medium, spreading outwards and downwards until the whole medium is eventually liquefied, and the bacterial mass sinks to the bottom of the tube.

On Agar Plates the growth is similar to that on gelatine plates, but the colonies are not so compact, and consist of masses of long threads matted together, the growth continuing upwards upon the surface of the medium.

On oblique surface Agar a greyish-white tenacious coating is formed with thready edges, the condensation water remains clear, or is only slightly clouded.

Blood Serum is liquefied slowly.

Bouillon remains clear, but a cloudy sediment is formed.

Milk is coagulated and afterwards peptonized.

On Potato it develops as a dry granular whitish covering, more or less limited to the point of inoculation.

Pathogenesis.—Affects man, cattle, sheep, horses, guinea-pigs, rabbits, mice, and swine, and dogs under exceptional circumstances, the primary localization usually being in the throat; rats are also difficult to infect. In man the disease is named according to the manner of infection.

1. *Pustula Maligna.* — This is the most common form, and is the result of accidental inoculation of a cutaneous wound.

2. *Pulmonary form.*—Woolsorters' and ragpickers' disease is the result of the inhalation of dust charged with anthrax spores.

3. *Bowel or Intestinal Anthrax.*—Due to the consumption of meat from anthrax carcases.

Inoculation into Animals.—Portions of a pure culture of the Bacillus anthracis, when introduced into the subcutaneous tissues of the abdominal wall of guinea-pigs or rabbits, cause the death of these animals in forty-eight hours. Little or no change can be observed at the point of inoculation, but the subcutaneous tissue for some distance over the abdomen and thorax will be found œdematous, with small ecchymoses scattered throughout the œdematous portion; the underlying muscles are pale in colour. The internal viscera show no marked macroscopical changes, except the spleen, which is enlarged, dark-coloured, and soft. The liver may present the appearance of cloudy swelling. The lungs are red or pale red in colour, while the heart is usually filled with blood. The disease is a true septicæmia, and after death the capillaries throughout the body always contain the typical rod-shaped organisms in larger or smaller numbers.

Protective Inoculation against anthrax is practised in animals, according to Pasteur's method, with two vaccines prepared from virulent cultures attenuated by cultivation between 42° and 43° C.—

No. I. Vaccine grown fifteen to twenty days at 42° to 43° C.
No. II. „ ten to twelve „ „

In the preliminary tests vaccine No. I. killed only mice, while vaccine No. II. killed both mice and guinea-pigs, but not rabbits.

Cattle and sheep receive an injection of 0·33 c.c. of a four days' old bouillon culture of No. I., and the same dose of vaccine No. II. in ten or twelve days. The effects of the inoculation with vaccine No. I. should be scarcely noticeable, causing neither constitutional nor local symptoms, while vaccine No. II. may or may not cause some constitutional disturbance, and when it does the symptoms are rarely of an exaggerated nature, and disappear in a short time if the vaccines have been properly prepared and tested before use.

The above vaccines render sheep and cattle immune to inoculated anthrax, but, according to Koch, against natural infection by means of the intestinal tract, *i.e.*, bowel anthrax. Pasteur's vaccines cannot be employed with certainty, and furthermore, a great many unsatisfactory results have occurred, showing that the strength of the vaccines cannot be regulated with absolute certainty.

The part played by insects in conveying anthrax has recently been investigated by Dr Nuttall. He shows that bed-bugs and fleas may be gorged on anthrax victims, and then placed on sound animals, which, on being bitten, show no disease, while at the same time cultivation and inoculation experiments made from the above insects, as well as microscopical examination, reveal the fact that the B. anthracis, during its sojourn in the body of the insect, degenerates. Finally, the doctor states that it is conceivable to have an inoculation in an abrased skin surface by violently crushing the surfeited bloodsucker against the wound.

Bacteriological Diagnosis. — Remove some of the effusion from the deeper portions of the suspected pustule, and institute plate cultures (for special methods see Technique, page 58), and if typical colonies develop, then pure cultures are made, and animals inoculated. In cases of suspected *abdominal anthrax*, the fæces and vomit must be examined. In cases of *lung anthrax* the bacillus is sometimes found in the copious expectoration. Examination of the blood reveals whether general infection exists or not, and is of great account regarding the prognosis of the case, but it must also be remembered that the bacilli are principally localized in the capillaries.

In animals it is often necessary to decide if an animal died of anthrax or not. If shortly after death a microscopical examination is made of the blood from a foot, ear, tail, or the spleen (but it is considered advisable never to cut open a suspected anthrax carcase, as it only favours the development of spores, which are never formed in the

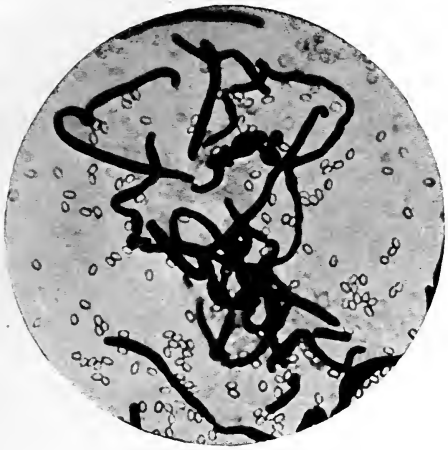


FIG. 37.—B. Anthracis, showing commencing involution forms and free spores. Fuchsin. × 1000.

FIG. 38.—B. Œdematis Maligni. Stab culture in gelatine.

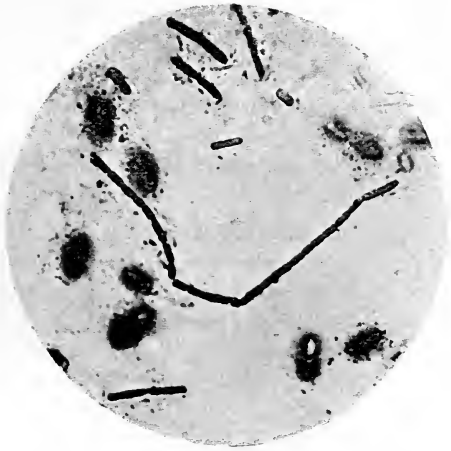


FIG. 39.—B. Œdematis Maligni. Cover-glass specimen from inoculated mouse. Cladius stain. × 1000.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

living animals or unopened carcases), and bacilli are detected exhibiting capsules when stained by Johne's method (see Technique, § 22), then the diagnosis is assured.

When an animal has been dead for several days, bacilli develop in the cadaver somewhat difficult to differentiate from the Bacillus anthracis. In such cases gelatine plate cultures must be instituted, and mice and guinea-pigs inoculated. In the inoculation of animals, the presence of the bacillus of malignant œdema must be guarded against, as it is often present, the inoculated animal dying of malignant œdema in spite of the presence of the Bacillus anthracis. This error can be prevented if the animals are 'inoculated cutaneously' with the suspected material, when only the Bacillus anthracis develops, and a false diagnosis is avoided. When putrefaction is too far advanced in a carcase under examination, the anthrax bacilli are sometimes completely annihilated by the concurrence of other species, so that a diagnosis is no longer possible.

MALIGNANT ŒDEMA.

(*Ger.* Bacillus Œdematis Maligni. *Fr.* Vibrion Septique.)

This organism is very widely distributed in nature; it is found in manured garden earth, in filth and dust, in house drains, and also in the intestines of animals.

Microscopical Appearances.—The bacilli are somewhat narrower than the Bacillus anthracis, and about the same length, but differ in the ends of the rods being rounded, and not squarely cut across. The peculiar bamboo rod shape found in anthrax bacilli is also absent.

Motility.—Motile, possessing 3 to 12 flagella, which are attached at the ends as well as the sides of the rods (see Photomicrograph, Plate I., Fig. 5); the motility is somewhat weak. For special methods of staining flagella, see Technique, §§ 23, 24.

Staining Reactions.—The bacilli obtained from the animal body, and from young cultures, stain easily with the ordinary aniline dyes.

By the Gram method positive results are only obtained when the specimen is stained for twenty-four hours at 37°, or with equal parts of anilin water, alcohol, and 5 per cent. carbolic acid solution of gentian violet for fifteen minutes. With the Cladius method the bacilli are easily stained in a few minutes.

Spore Formation.—The spores are formed generally in the middle of the rods, and are sometimes broader than the bacillus, forming a spindle or clostridium.

Biological Characters.—The bacillus is strongly anaërobic, grow-

ing in the ordinary media at room and incubator temperature, but only in the absence of oxygen.

On Gelatine Plates the growth is similar to the Bacillus subtilis.

On Agar Plates it forms a thick network of threads.

In Stab Cultures in grape sugar gelatine the growth begins about $\frac{1}{2}$ to $\frac{3}{4}$ of an inch below the surface of the medium in the form of a white strip, with side branches sprouting out (see Photograph, Fig. 38).

On Agar Stab Cultures the growth exhibits nothing characteristic.

Bouillon becomes cloudy from the formation of gas.

Growing cultures have a most unpleasant smell.

In Milk a part of the casein is precipitated.

Pathogenesis.—Affects guinea-pigs, rabbits, and mice. The strength of pure cultures when inoculated into susceptible animals is very variable. When a small pocket is made in the skin of a mouse and as much malignant œdema containing earth as will cover the point of a knife introduced, the animal will die in one to two days. Examination shows a general subcutaneous œdema, which contains large numbers of the bacilli (see Photomicrograph, Fig. 39). The bacilli do not pass into the internal organs unless the animal is left lying some time after it is dead.

Two cases of malignant œdema are recorded in man. They were sick with typhoid fever, and died three days after receiving a musk injection, by which means some malignant œdema colonies were introduced into the subcutaneous tissue. The infection of a healthy man has not been observed. Koch mentions a case of mixed infection of malignant œdema and anthrax in a guinea-pig. The following other animals are also susceptible :—Goats, calves, sheep, horses, swine, cats, dogs, chickens, pigeons, and ducks ; while cattle are immune.

The virus is weakened when passed through the body of a white rat.

Immunity. — Chamberland and Roux rendered guinea-pigs immune by injecting intraperitoneally bouillon cultures which had been sterilized in an autoclave ten minutes at 105° to 110° C.

BACILLUS ANTHRACIS SYMPTOMATICI.

(*Ger.* Bact. des Rauschbrands ; *Fr.* Bact. du Charbon Symptomatique ; *Eng.* Symptomatic Anthrax, Black Quarter, etc.)

This organism was first discovered by Bollinger in cattle affected with symptomatic anthrax, and further studied by MM. Arloing, Cornevin, and Thomas,—Kitasato being the first to obtain cultures on solid media.

The characteristic lesions produced by this organism are emphysematous swellings of the muscular and subcutaneous tissues of the

leg and the quarter, accompanied with the formation of gases with a strong odour, the following analysis being given by Kitt: Co_2 13 per cent., H. 76 per cent., N. 10 per cent. On section of the affected parts the muscles and cellular tissue are found saturated with bloody serum, while the muscular tissue is dark, almost black, in colour. In the affected areas, in the gall, and after death in the internal organs, the above organism can always be detected. It is also found in the soil in some localities.

Microscopical Appearances.—The bacilli are actively motile rods, 3 to 5 μ long, and 0·5 to 0·6 μ thick, with rounded ends, usually occurring singly, but sometimes forming short threads. The flagella are attached around the periphery of the organism, peritricha (see Photomicrograph, Plate I., Fig. 4). The bacillus forms spores, situated either in the middle or at the end of the rods (see Photomicrograph, Fig. 41). The organism commences to grow at 16° to 18° C., but spore formation takes place best at 37° C., and during this stage the organism becomes motionless, being only motile during the vegetative stage. The organism also undergoes degenerative changes, and involution forms are often present, not only in cultures but in the tissues of affected animals.

Staining Reactions.—This organism stains by the ordinary aniline dyes, and by the Gram method only when the staining process is prolonged. It stains easily by the Cladius method. The spores can also be stained by the ordinary methods (see Technique, p. 27).

Biological Characters. — The organism is strictly anaërobic, growing best in an atmosphere of hydogen, but not in carbon dioxide.

On Gelatine Plates in an atmosphere of hydrogen the colonies appear as irregular, slightly lobulated masses. The gelatine liquefies in a short time, the colony then presenting a dark lobulated centre surrounded by a delicate fringed-like zone.

In Gelatine Stab Cultures a radiating cloudiness appears, which increases in size as the gelatine softens, until finally the growth resembles a caterpillar (see Photograph of Culture, Fig. 40), where the characteristics of the growth correspond to those described by Sanfelice.

In deep stab cultures in grape sugar agar-agar at 37° C. the growth commences in twenty-four to forty-eight hours, accompanied by gas formation, which is considerably more than is produced by the bacillus of malignant œdema. The gaseous products have a putrid odour, like that produced by rancid butter.

Milk is coagulated by the formation of an acid.

Differential Diagnosis.—This organism is distinguished from the bacillus of malignant œdema as follows:—It is smaller and does not develop in long threads in the animal tissues, is more actively motile, and forms spores more readily in the living tissues than does the bacillus

of malignant œdema. It also differs in its reaction towards animals. Cattle, while susceptible to symptomatic anthrax, are practically immune to malignant œdema. Swine, dogs, rabbits, pigeons, and chickens are readily affected by malignant œdema, but not as a rule by symptomatic anthrax. According to Arloing, frogs can be inoculated if kept at a temperature of 22° C. Horses are affected only locally and not seriously with symptomatic anthrax, but are conspicuously susceptible to both artificial inoculation and material infection by the bacillus of malignant œdema. The distribution of the organisms over the earth's surface is also quite different, malignant œdema being present in almost all soils, while symptomatic anthrax appears confined to certain localities, especially places where infected herds have been pastured. Natural infection occurs principally in young cattle; next in order being sheep and goats; whilst in mankind a genuine case is not recorded.

The ordinary manner of infection is by wounds which not only tear the skin, but penetrate the subcutaneous tissue. The disease is also produced by the ingestion of forage soiled by very active virulent matters, and by the inhalation of dust charged with dried virus.

Vaccination.—Animals sometimes inoculate themselves accidentally, and as small doses cause immunity—this immunity being transmitted from the mother to the fœtus—the result is that part of the animals exposed to contagion escape its fatal effects. The French recommend the successive employment of two vaccines prepared from material from a fresh lesion spread out thinly, and dried at 35° C., and then a sufficient quantity of this powder is triturated in a mortar with 2 parts of water, and exposed to 100° to 104° C. for the first vaccine, and 90° to 94° C. for the second, during seven hours; when the dry vaccines are taken from the oven 1 centigram of the powder is diluted in $\frac{1}{2}$ c.c. of water for each animal. The vaccine prepared at 100° is used first, and the second in eight days. The inoculation is made in the cellular tissue of the ear, or on the internal face of the end of the tail, the second inoculation a little above the first. Kitt recommends a single vaccine from infected flesh heated six hours at 100° C., given in decigram doses, injected in the subcutaneous tissue near the elbow. Animals can also be vaccinated with natural virus either in the cellular tissue or intravenously; when the latter method is adopted great care must be taken to avoid inoculation of the surrounding tissue. Attenuated viruses are therefore generally preferred for the production of immunity.

TETANUS.

(*Eng.* Lockjaw; *Ger.* Wundstarrkrampf; *Fr.* Tetanos.)

This disease occurs in all the domestic animals and in man. The

FIG. 40. — B. Anthracis Symptomatici. Stab culture in grape sugar gelatine. (Caterpillar form.)

FIG. 41.—B. Anthracis Symptomatici and spores. Cover-glass specimen from grape sugar agar culture. Cladius stain. × 1000.

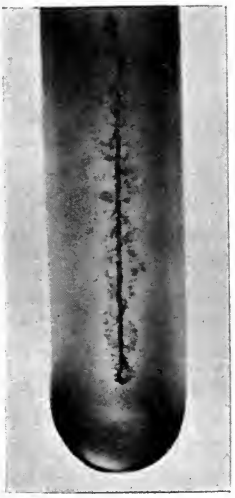


FIG. 42.—B. Tetani. Stab culture in grape sugar agar. (Pine tree growth.)

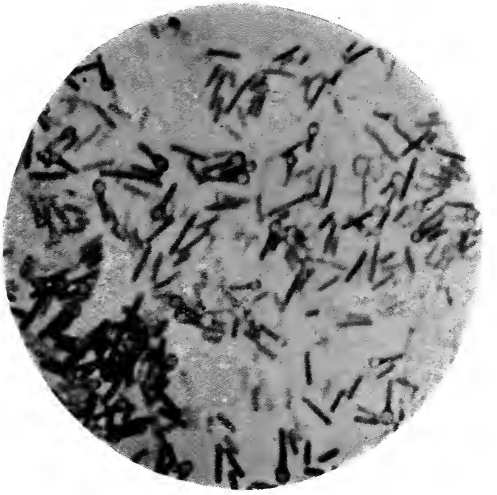


FIG. 43.—B. Tetani and spores. Cover-glass specimen from grape sugar agar culture. Fuchsin. × 1300.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

horse, ox, sheep, and goat are the most susceptible, and it has also been observed in the pig and dog. Chickens are immune. Nicolier produced tetanus in mice and rabbits by subcutaneous inoculation with particles of garden earth in 1884; and Kitasato cultivated the bacillus of tetanus in pure cultures in 1889.

Microscopical Appearances.—The tetanus bacillus is a slender rod with rounded ends 3 to 5 μ long, and 0·3 to 0·5 μ wide, and may appear as single rods, or in cultures as long threads.

Motility.—Motile, although not actively so; the flagella are attached somewhat similarly to those of the bacillus of malignant œdema (see Photomicrograph, Plate I., Fig. 6).

Staining Reactions.—It stains with the ordinary aniline reagents, and by the Gram and Cladius methods.

Spore Formation.—At 37° C. spores are formed in thirty hours, and at room temperature in about a week. The spores are situated at one end of the rod, and have a diameter of 1 to 1·5 μ, giving the rod the appearance of a drumstick (see Photomicrograph, Fig. 43, × 1300).

Excluded from air and light, the spores in a culture remain living and virulent for over a year. They can also resist heating to 80° C., while exposure to steam at 100° C. kills them in five to eight minutes. They resist the action of 5 per cent. carbolic acid for ten hours, but succumb when exposed for fifteen hours; but if 0·5 per cent. hydrochloric acid is added they are no longer active in two hours. Corrosive sublimate 1 to 1000 kills them in three hours, and when 0·5 per cent. hydrochloric acid is added they are killed in thirty minutes.

Biological Characters.—The tetanus bacillus is anaërobic. Recent observations, however, point to the possibility of it having an aërobic existence (Flugge). It grows well in an atmosphere of hydrogen, but not in carbonic acid.

Kitasato was the first to isolate the bacillus in pure cultures, and the following is the method he adopted.

Method.—Inoculate several white mice from the secretions from a wound in a typical case of tetanus. The material usually contains other organisms besides tetanus bacilli, causing more or less suppuration at the seat of inoculation in the mice.

To separate the tetanus bacillus from others present, smear the pus upon several oblique serum and agar-agar tubes, and place at 37° to 38° C. After twenty-four hours all the organisms will have developed, and microscopic examination will reveal the presence of a few tetanus bacilli, recognisable by their shape, like a small pin, the spore representing the head. After the culture has remained forty-eight hours at 38° C., it is subjected to a temperature of 80° C. in a water-bath for from three-

quarters to one hour. A series of cultures are now instituted in media specially prepared for the growth of anaërobic organisms by the addition of 1·5 to 2·0 per cent. of glucose.

Kitt obtained pure cultures without using the heating process.

The original material was reduced with sterile water, and the liquid inoculated by means of stroke cultures on the surface of horse or sheep serum media, and the cultures placed in an atmosphere deprived of its oxygen by Buchner's method (see page 66).

On Gelatine the colonies grow slowly, the central portion being of a golden yellow colour, with numerous threads radiating from the centre.

On Agar-Agar the colonies are very characteristic, the naked-eye appearance being that of fine, fleecy clouds, which under the microscope resemble a tangled mass of fine threads. The extraordinary fineness of the latter enables the colonies to be distinguished from other anaërobic organisms.

In Gelatine Stab Cultures the growth has the appearance of a cloudy, linear mass with outgrowths radiating into the gelatine from all sides; liquefaction follows slowly, with a coincident production of gas with an unpleasant empyreumatic smell.

In Agar Stab Cultures the growth has a very characteristic appearance, resembling a fir-tree (see Photograph, Fig. 42).

Bouillon is densely clouded.

In Milk the bacilli grow without causing any changes.

On Potatoes a moist invisible growth similar to that of the Bacillus typhi abdominalis occurs.

Pathogenesis.—Mice inoculated with a minute portion of a pure culture of the tetanus bacillus develop tetanic symptoms in twenty-four hours, which end fatally in from two to three days. Rats, guinea-pigs, and rabbits are similarly affected, but require larger doses than mice. A fatal dose for a rabbit is 0·3 to 0·5 c.c. of a well-developed bouillon culture. The period of incubation for rats and guinea-pigs is twenty-four to thirty hours, and for rabbits two to three days. Pigeons are but slightly if at all susceptible.

The tetanic convulsions appear first in the neighbourhood of the point of inoculation, finally becoming generalized.

At the autopsy there is usually only a small hæmorrhagic spot at the point of inoculation. No other changes are present, and the bacilli are only found at the point of inoculation (see Photomicrograph, Fig. 44), although, according to Schnitzler, they are sometimes found in the lymph-glands in direct relation with the inoculated part. Buedinger found that tetanus could be conveyed from an animal dead of it to a healthy animal by transplanting from one to the other portions of the lymph-glands associated with the seat of infection.

Death results from the absorption of a soluble poison which has been

isolated and studied; possessing, according to Kitasato, the following peculiarities. When cultures of the tetanus bacillus are filtered through porcelain, the filtrate contains the soluble poison, which when injected into animals causes tetanus. Animals inoculated with pieces of the organs of animals dead from the action of the tetanus poison are unaffected; but inoculation with the blood or pleural exudates produces positive results. The poison is therefore largely present in the circulating fluids.

Vitality.—The greatest amount of poison is produced in fresh, neutral bouillon very slightly alkaline in reaction. The poison loses its activity when exposed for 1½ hours to 55° C., twenty minutes to 60° C., and five minutes to 65° C. When dried at the temperature of the body with access of air, the poison is destroyed; but dried at ordinary room temperature, or at a similar temperature in the desiccator over sulphuric acid, it is not destroyed. Diffuse daylight diminishes the virulence of the poison. Its intensity is preserved for a much longer time when kept in the dark.

Direct sunlight destroys its poisonous properties in from fifteen to eighteen hours.

When diluted with a fixed amount of water or bouillon its activity is not diminished.

Mineral acids and strong alkalies lessen its intensity.

In man tetanus is a toxic disease the same as in animals, the bacilli never being found in the blood or organs, but localised at the point of inoculation, and lymph-glands associated with the seat of infection. The period of incubation ranges between one and twenty-two days; in a case of wound infection at a laboratory the incubation period was four days. The shorter the time between the infection and the appearance of tetanus, the more pronounced is the course of the disease, and the worse the prognosis.

In cases where the incubation period of the disease was one to ten days, only about 3 per cent. recovered. When the period of incubation was ten to twenty-two days 25 per cent. recovered, and by longer periods of incubation as high as 50 per cent. recovered.

Immunity and Cure of Tetanus with Antitetanic Serum in Animals.—Behring produced immunity in mice with bouillon cultures of tetanus bacilli weakened by adding trichloride of iodine. Fedorf also prepared a tetanus antitoxin in a dry form from blood serum. Antitetanic serum can also be obtained from horses immunised against tetanus in a similar manner to that employed in the production of diphtheria antitoxin. The animal selected is a young horse in good condition, which is first tested with mallein and then with tuberculin to ensure its freedom from glanders and tuberculosis.

'The tetanus bacillus is grown in a bouillon culture for four or five weeks, filtered through porcelain, and a small quantity of the filtrate, about half a drop, injected into the horse subcutaneously; the dose is again repeated in three days, and if no signs of tetanus appear, in about three days 1 drop is again injected, and the process repeated for several months with continually increasing doses of the filtrate, until the horse's system becomes so resistant to the toxine, that large quantities can be injected without ill effects. Six months after the first injection, the horse will probably remain unaffected by a dose of 2 ounces, and at the end of twelve or eighteen months as much as a pint can be injected with impunity. At frequent intervals during this process, small quantities of blood are removed from the horse, and the potency of the serum tested experimentally on mice and guinea-pigs. When it is found to confer sufficient immunity, the horse is periodically bled by piercing the jugular vein with a trocar connected by sterilized tubing with a sterilized and sealed glass flask. The blood obtained is allowed to coagulate, and its serum is separated and filtered for use. When the blood of the horse once acquires a sufficient protective power to be serviceable, the animal will continue to supply potent serum for a long and varying period without further dosing; but the supply must be continually tested experimentally.'—(Sidney Villar, F.R.C.V.S., *Proceedings of Royal Counties Veterinary Association*, Nov. 1897).

There are other two preparations in the market at present, one being in a powder form which is dissolved in distilled water by heat not to exceed 40° C., and as a curative agent in horses it is injected intravenously, as it is claimed that injection direct into the circulation yields results twenty-four hours quicker than subcutaneously. The second preparation is delivered in solution, and is used as a prophylactic in man and animals to counteract tetanus in wounds, in which experience leads one to expect the disease to develop. The amount of the dose (0·5 to 5 c.c.) is regulated by the time that has elapsed since the wound occurred. In operations on animals often followed by tetanus, *e.g.*, before castration, docking, etc., 0·2 c.c. is a sufficient dose.

Nocard considers antitetanic serum valuable as a prophylactic.

In some parts of France where tetanus is very prevalent, Nocard distributed antitetanic serum to sixty-three veterinary surgeons, who treated for the prevention of tetanus 2727 animals with it. Only one animal became affected, and this horse was not inoculated until five days after being pricked in shoeing. Although the delay was too great to prevent the appearance of tetanus, yet the disease was of a

very mild nature. During the same period these veterinary surgeons saw 259 cases in animals that were not so inoculated.

M'Watt records a case in the *Brit. Med. Journal* of a boy affected with tetanus, whose recovery he attributed to the use of Tizzoni's antitetanic serum.

CEREBRAL TETANUS.

At the Ninth International Congress of Hygiene, held at Madrid, the result of the experiments of Dr Borrel and Dr Roux regarding the tetanic disease produced by the direct inoculation of tetanic toxin in the brain substance of susceptible animals, was communicated by Dr Borrel. The special malady—cerebral tetanus—produced is defined by a set of symptoms, including excitement, epileptiform convulsions, strange or manifold desires, and other symptoms, varying according to the portion of the brain in which the antitoxin is introduced. This tetanic malady is absolutely different from the ordinary tetanus, but is none the less specific. Dr Borrel and Dr Roux also discovered that an animal rendered immune against injections under the skin was not immune against injections into the substance of the brain, and from this they conclude that subcutaneous injections of antitoxin do not affect the nerve cells and do not protect them. The same occurs when for therapeutic purposes serum is injected into an animal which has commenced to manifest the symptoms of tetanus, the nerve substance being already attacked by the toxin. The serum does not reach the nerve cells, and therefore the toxin can continue its deadly effects undisturbed. This explains the many failures to treat tetanus by antitoxin. The same authorities then tried to treat the disease by conveying the antitoxin direct to the brain substance, and were thus able to cure rabbits, guinea-pigs, etc., even when the disease had prevailed for some hours, and this at a time when very large subcutaneous injections had proved of no use whatever. On the other hand, when injected into the brain matter very small quantities of serum were sufficient. These researches on cerebral tetanus demonstrated that with animals immunized passively or actively the nerve cells are not immunized. An immunized animal which resists a subcutaneous inoculation of the toxin will die if the smallest quantity of this toxin is introduced into the brain matter. Immunity is not therefore due to some new property acquired by the nerve cells, as they remain unaffected unless directly attacked.

⌜Differential Diagnosis Table

DIFFERENTIAL DIAGNOSIS TABLE.

	Anthrax.	Malignant Œdema.	Tetanus.	Symptomatic Anthrax.
(1) Where present in the animal tissues	In the blood and organs in large numbers	Particularly near the point of infection and in the bloody œdema	Scanty in the wound secretion; never in the blood; sometimes in the nearest lymph-glands to the point of infection	In the bloody serous exudations at the point of infection and in the carcase
(2) Characteristic appearance of the organism in the tissues, blood, etc.	Rods always without spores; capsules can be demonstrated in cover-glass specimens	In single rods with rounded ends, and sometimes in long jointed threads	Forms spores in the body, and occurs in rods and threads with the peculiar end spore formation or *drumstick*	Forms spores in the body, which are situated either at the end or middle of the rod, giving it a club shape; sometimes also forms short threads
(3) Motility	*Non-motile*	Motile, *but not always*	Motile, principally vegetative forms, without spores	Motile, principally vegetative forms, without spores
(4) Growth	Aërobic	Anaërobic	Anaërobic	Anaërobic
(5) Stab Cultures	Central growth, seldom without hair-like formations	Mostly without hair-like formations, also in isolated ovoid zones	Generally like a *fir tree*	Grows in gelatine with a formation like a caterpillar
(6) In milk	Coagulation	Coagulated slowly	*Coagulation absent*	*Coagulated quickly*
(7) Fermentation	Negative	Present	Present	Present
(8) Reaction towards the Gram stain	*Good*	Only by a prolonged exposure to the staining reagents	*Good*	By prolonged exposure to the staining reagents
(9) Pathogenic for the following experimental animals	Mice, guinea-pigs, and rabbits	Mice, guinea-pigs, and rabbits	Mice, guinea-pigs, and rabbits	*Not for rabbits*

Bacteria associated with Meat-Poisoning.

BOTULISMUS (VAN ERMENGEN).

Notwithstanding numerous investigations as to the cause of meat poisoning, the question still remained somewhat obscure until Van Ermengen discovered a specific anaërobic organism, the Bacillus botulinus, during an epidemic at Ellezelles, Belgium. Under the term 'meat-poisoning' two complex set of symptoms, with different clinical manifestations, are included, which are differentiated by the prevailing symptoms. The one form, described as gastro-intestinal, appears as a cholera nostras, a simple or hæmorrhagic gastro-enteritis,

accompanied with fever, albuminuria, and skin eruptions of various form and intensity. The gastro-intestinal symptoms occur after eating tainted meat, or meat from animals slaughtered while suffering from pyæmia, septicæmia, and puerperal fever. The organisms mostly identified as the cause of these symptoms belong to the coli group, and more rarely in some cases to the proteus group of organisms.

The second form of symptoms are analogous to those of the so-called sausage-poisoning, with pronounced nervous symptoms of central origin, secretory and motor disturbances, suspension of salivary secretion, dryness and redness of the mouth and pharyngeal mucous membrane, difficulty in swallowing, hoarseness, mydriasis, ptosis, etc. This form is now known as botulismus, and appears after the consumption of certain kinds of blood and liver sausages. It is also caused by using decayed salt fish, smoked meat, hams, preserved meats, etc.

Microscopical Appearances of the Bacillus botulinus.—Large rods 4 to 6 μ long, 0·9 to 1·2 μ broad, with slightly rounded ends. The formation of threads was seldom observed, but involution forms were frequent. They are rarely found in the blood and organs of infected animals, being mostly situated at the point of inoculation.

Motility.—Slightly motile, possessing four to eight flagella.

Staining Reactions.—The staining is easy, and positive results are obtained with the Gram method when the alcohol is not allowed to work too long during the process of decolorizing.

Spore Formation.—Spores are formed in the cultures, and in organical structure they are ovoid in shape, and situated usually in the end, very seldom in the centre of the rod.

Vitality.—They are destroyed by a temperature of 85° C. in fifteen minutes, and at 80° C. in an hour. Five per cent. carbolic acid destroys them in less than twenty-four hours. Dried spores exposed to diffuse daylight still produce cultivations in three months.

Biological Characters. — The Bacillus botulinus is strictly anaërobic, and grows best between 20° and 30° C.; at over 35° C. spores are no longer formed, the growth is not so luxuriant, and involution forms appear.

The culture media must be decidedly alkaline, and the addition of 2 per cent. grape sugar favours their growth.

On Gelatine Plates, in four to six days, round, transparent, brownish-yellow colonies develop, having a thick, lustrous, granular appearance, and surrounded by a small liquefied area; later the margins of the

colony become irregular and radiating, finally giving off variously shaped processes.

In Gelatine Stab Cultures round whitish growths occur along the course of the needle, from which processes sometimes extend into the surrounding medium, the gelatine is liquefied, and gas is quickly formed.

Grape Sugar Bouillon is densely clouded.

In Milk there is a slight growth, without any alteration of the medium.

On Potatoes there is no growth. The cultures give off a smell of butyric acid; on media containing sugar, the bacilli also form butyric alcohol, hydrogen, carbon dioxide, and methan.

Pathogenesis.—Affects guinea-pigs, mice, and monkeys. One or two drops of a liquefied gelatine culture, given on a piece of bread or in milk, is sufficient to kill the animals. Cats can be fed with large doses without being affected, but when inoculated with large doses (5 to 10 c.c.) they die in one to two days, and with small doses (1 to 2 c.c.) in eight to twelve days.

The incubation period is about thirty-six hours, when the animals become depressed, do not care to move, refuse their food, and on the third day appear stupid, the eyes almost motionless, and the pupils greatly dilated. The enlargement of the pupil increases to an enormous extent in the next few days. The tongue hangs out of the mouth, and finally the animal cannot retract it; the fæces and urine are withheld, death usually occurring from paralysis of the respiratory and circulatory organs. Very small doses of the bacilli cause marasmus, the cats dying in several weeks with symptoms of paralysis and degeneration of the internal organs. Pigeons receiving 1 to 2 c.c. of a culture exhibit first, paralysis of the wings, and finally general paralysis.

The pathological anatomical changes usually present are, a more or less well-marked hyperæmia of most of the organs; in some instances acute, sometimes interstitial, and sometimes parenchymatous hepatitis, with fatty degeneration, and desquamative parenchymatous nephritis, fatty degeneration of the heart muscle, and also of the muscle of the eye. The degenerative changes found in the central nervous system are of especial interest. In the spinal cord the changes are confined almost entirely to the grey substance of the cord along the anterior cornu. In the medulla oblongata the ganglion of the hypoglossal nerve, the dorsal ganglion of the vagus, the middle small-celled ganglion of the motores oculorum, and of other cranial nerves, are affected. Cultures obtained from fresh organs of animals inoculated intravenously do not exhibit a very pronounced growth, but if the organs are previously placed in the incubator at 30° C. for twelve to twenty-four hours, numerous bacilli can be isolated. The Bacillus botulinus does

not cause a genuine infection, but an intoxication, and can be placed in the same category as tetanus and diphtheria. The toxin can be precipitated with alcohol, tannic acid, and neutral salts. Other organisms found associated with the Bacillus botulinus appear neither to favour or retard its poisonous products.

Bacteriological Diagnosis. — In one of Van Ermengen's cases the spores of the Bacillus botulinus were found in a ham, mostly in the red or lean part, seldom in the fat. For animal experiments take four parts of the suspected ham and cut it into small pieces, and add five parts of sterilized water, and inject a minute quantity of the infusion. Van Ermengen was able to isolate the Bacillus botulinus out of the spleen, stomach, and intestinal contents of a man dead from meat-poisoning.

Immunity.—Kempner produced immunity in animals with the filtrate of a bouillon culture. The serum of the immunized animals was highly antitoxic, and doses of 1 to 5 c.c. injected three to twenty-four hours after a guinea-pig was poisoned with the Bacillus botulinus resulted in the recovery of the affected animals. Kempner and Pollack's investigations show that after twenty hours the intoxication causes changes in the central nervous system, which again becomes normal when the serum is used.

BACILLUS ENTERITIDIS.

This bacillus was discovered by Gärtner in 1888 in a meat-poisoning epidemic, and since then has been observed in a number of similar cases.

Microscopical Appearances.—Small rods. Hanging-drop specimens from gelatine cultures show a difference between the centre and the ends or polar portions of the rods, the former appearing to consist of a less refractive substance. This peculiar difference appears only to exist in gelatine cultures.

Motility.—Motile, possessing 2 to 5 long flagella.

Staining Reactions.—When stained with the ordinary aniline dyes, the middle portion of the rod is strongly stained, while the ends or polar portions are either weakly stained or entirely uncoloured. The reaction with the Gram method is negative.

Biological Characters.—It grows at both room and incubator temperature; by the latter method the growth is quicker.

On Gelatine Plates the colonies developing on the surface of the medium appear as thin transparent films. The gelatine is not liquefied.

In Gelatine Stab Cultures the growth extends along the whole length

of the middle puncture, a film developing on the surface; old cultures give off a slight odour.

On the surface of Agar at 37° C. a copious grey transparent coating occurs, and a slight odour is given off.

On Potatoes at 37° C. in two days a somewhat copious greyish-white shiny coating develops.

Bouillon cultures exhibit strongly-marked cloudiness throughout the medium.

Grape Sugar Bouillon exhibits the same changes as occur with the Bacterium coli commune; the medium becomes strongly acid, and gas is formed, sometimes CO_2 and sometimes a combustible gas.

In Milk Sugar Bouillon no acid reaction occurs; the fluid remains alkaline, but sometimes, although not always, small gas bubbles are found consisting of CO_2 or a combustible gas.

Milk is not altered in chemical reaction nor coagulated.

Indol is not formed in twenty-four hours' old cultures at 37° C., and the *Nitroso-indol* reaction is absolutely negative.

The Bacillus enteritidis grows excellently under aërobic conditions in the ordinary nutrient media, but only under anaërobic conditions in the presence of grape sugar.

Pathogenesis.—Mice and guinea-pigs are very susceptible to subcutaneous inoculation, while rabbits are less susceptible to infection. Mice die in one to three days, guinea-pigs in about five days, and the inoculated bacteria are again found in the heart's blood and organs of these animals. Mice die in five to eight days when fed with food containing the bacteria, and the specific bacteria are found in their internal organs; guinea-pigs are also infected by way of the digestive tract.

In man, this bacillus causes gastro-enteric symptoms which may be more or less intense, occasionally causing death, specific bacteria being found in the organs. They appear to be introduced into the intestinal canal with the food; very probably such meat is obtained from animals in which the Bacillus enteritidis was already present during life. In what animal diseases this bacillus can possibly originate is not known.

BACILLUS MORBIFICANS BOVIS.

This organism was found by Basenau in a cow affected with puerperal fever.

Microscopical Appearances.—Rods about the same size as the typhoid bacillus, 0·3 to 0·4 μ wide, and 1 to 1·2 μ long, sometimes arranged in pairs.

Motility.—Strongly motile.

Spore Formation absent.

Staining Reactions.—Easily stained with the ordinary agents, but not by the Gram method.

Biological Characters.—The developing colonies resemble those of the Coli commune, but are more granular.

In Stab Cultures and on *Agar media* greyish-white tufts.

On Potatoes a moist yellow coating which never becomes brown.

Bouillon is clouded, a film forming on the surface.

Milk is not coagulated.

Grape Sugar is slightly fermented, but *Cane Sugar* remains unchanged.

Vitality.—Cultures are killed when heated one minute at 70° C.

Pathogenesis.—Mice, rats, guinea-pigs, and rabbits are very susceptible to infection by subcutaneous and intraperitoneal inoculation, also by feeding. Dogs and cats are immune. The above bacillus is also probably pathogenic for man, as many cases of sickness have been observed to follow the consumption of the flesh of animals affected with puerperal fever (Ostertag).

THE PROTEUS GROUP OF BACTERIA.

This group of organisms discovered by Hauser in 1885 consists of three species—

1. Proteus Vulgaris.
2. „ Mirabilis.
3. „ Zenkeri.

(1) Proteus Vulgaris.

Microscopical Appearances.—Small rods of various sizes, generally occurring in pairs, but sometimes arranged in filaments. Involution forms frequently occur, the most common being spherical bodies about 1·6 μ in diameter.

Motility.—Strongly motile, possessing numerous flagella (see Photomicrograph, Plate II., Fig. 12).

Spore Formation is absent, and the bacilli are killed by five minutes' exposure to a temperature of 55° C.

Staining Reactions.—The bacilli are easily stained with fuchsin, not so easily with the ordinary watery solutions of the dyes. By the Gram method the reaction is negative.

Biological Characters.—It is a facultative anaërobe, and at ordinary room and incubator temperature the growth is equally luxuriant, the optimum temperature being 20° to 25° C.

On Gelatine Plates small round yellowish colonies with thick centres

and irregular edges develop at first, from which brush-like offshoots are thrown out. Other colonies are surrounded by a zone of threads which, partly in circular, partly in irregular twisted forms, surround the central opaque mass. The gelatine is quickly liquefied. Straight and twisted offshoots, which frequently become detached from the mother-stem, grow into the surrounding medium, and continue moving in the somewhat softened gelatine. This condition is known as *Swarming Islands* (see Photomicrograph of same, Fig. 45), which is easily observed in cultures on 5 to 6 per cent. gelatine. Peculiar figures and designs also occur, whereby the proteus has been designated the *Bacillus figurans*.

Stab Cultures in gelatine liquefy very quickly.

On oblique surface Agar a rapidly extending moist grey layer is formed.

On Potatoes a dirty greyish coating develops.

Bouillon becomes uniformly clouded. Cultures on all the different media give off an abominable smell.

Pathogenesis.—When a considerable quantity of a proteus culture (3 c.c.) is injected intravenously or into the peritoneum of a rabbit or guinea-pig, the animals die of acute enteritis and peritonitis. The intravenous injection of 5 to 10 c.c. of a bouillon culture in dogs causes more typical symptoms and lesions. The symptoms produced are bloody vomiting and diarrhœa, combined with severe tenesmus and elevation of the temperature. At the autopsy an intense hæmorrhagic enteritis is found. The blood and internal organs either contain none or very few bacilli. With filtered cultures and with cultures containing the remains of dead bacilli, the same results are obtained. The proteus is also fatal to mice, and the bacilli can again be cultivated from their organs, and the more often the bacillus is passed through mice the more virulent it becomes.

The proteus bacteria are found in all putrefactive processes, and in the intestinal canal. In man the proteus forms a mixed infection with the ordinary exciters of inflammation. They cause the ichorous, putrid phlegmon sometimes observed in cases of cadaveric infection. The proteus, further, sometimes penetrates suppurating wounds, and increasing these, forms toxic products which, when absorbed, cause the so-called 'putrid intoxication.' According to H. Jäger, certain forms of icterus, accompanied with fever, pain in the muscles, and enlarged liver and spleen, known as 'Weil's disease,' are produced by the proteus. Jäger was able to cultivate from the urine, and after death, from the organs of individuals dead of Weil's disease, a fluorescent proteus. The infection resulted in these cases from bathing in river-water which was contaminated with the proteus. An outbreak of disease due to the proteus fluorescens also occurred among some poultry kept on the banks of a small stream.

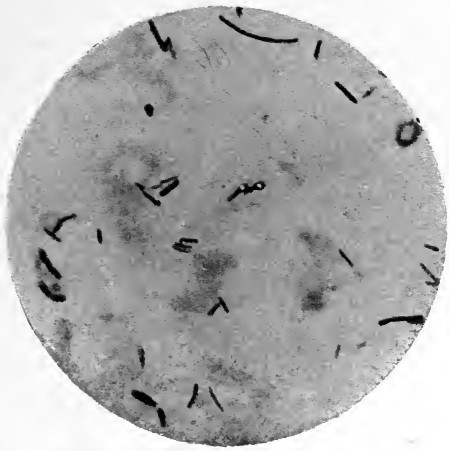


FIG. 44.—B. Tetani. Cover-glass specimen from point of inoculation. Cladius stain. × 1000.

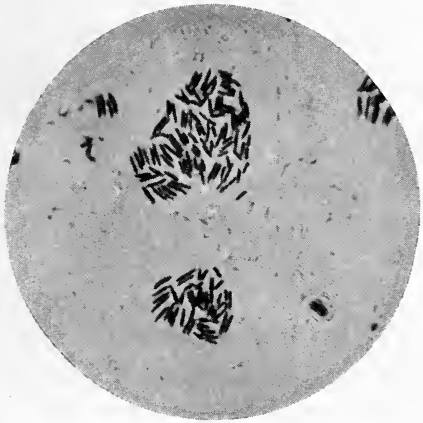


FIG. 45.—B. Proteus Vulgaris. Contact specimen from gelatine plate (swarming islands). Fuchsin. × 1000.

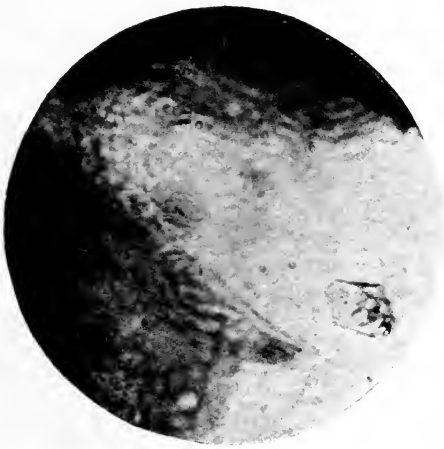


FIG. 46.*—Bacillus Mallei. Section of glanders nodule from horse, showing single bacillus. Stained by Löffler's method. × 1000.

* For the tissue from which this specimen was obtained, I am indebted to Wm. Hunting, F.R.C.V.S., Editor of *The Veterinary Record*.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

E. Levy found the proteus to be the cause of a hæmorrhagic gastro-enteritis which appeared in seventeen persons after partaking of decomposed meat.

According to Booker, the proteus vulgaris plays an important part in the production of the morbid symptoms characteristic of cholera infantum. It was found in the alvine discharges of affected infants, but not in those of healthy infants. The prominent symptoms in the cases where the proteus was found were drowsiness, stupor, emaciation, more or less collapse, frequent vomiting and purging, with watery and generally offensive stools.

Bacteriological Diagnosis.—Plate cultures are prepared from the pus, from the ichorous phlegmons, and also from the urine obtained under sterile precautions from the patients affected with Weil's disease.

(2) Proteus Mirabilis.

Microscopical Appearance.—Rods of various lengths, the smallest being about 0·6 μ in length.

Spore Formation absent.

Biological Characters.—*On Gelatine Plates* the deep-lying colonies exhibit curiously formed, twisted zoöglœa masses. The surface colonies occasionally form the *swarming islands* like the Proteus vulgaris. The gelatine is liquefied slowly.

(3) Proteus Zenkeri.

Microscopical Appearance.—Bacilli 0·4 μ broad, and averaging 1·6 μ long.

Spore Formation absent.

Biological Characters.—It occasionally forms *swarming islands,* like the Proteus mirabilis; but the gelatine is *not liquefied.*

BACILLUS MALLEI.

(The Glanders Bacillus; *Ger.* Rotzbacillus; *Fr.* Bac. de la Morve.)

This organism was discovered by Löffler and Schutz in 1882 in the diseased tissues of animals affected with glanders. It was isolated in pure cultures, which, when inoculated into susceptible animals, reproduced the disease with all its clinical and pathological manifestations.

Microscopical Appearances.—Short rods, 2 to 3 μ long, 0·2 to 0·4 μ thick, with rounded or slightly pointed ends, generally occurring in single rods, sometimes in pairs, and very seldom in long filaments.

Motility.—Non-motile.

Staining Reactions.—The bacilli stain readily in cover-glass specimens with the ordinary basic aniline dyes (see Photomicrograph, Plate III., Fig. 16). The staining is somewhat irregular, the bacilli presenting a granular aspect owing to alternating clear and uncoloured spaces. (For special staining methods, see Technique, § 15.) They are not stained by the Gram method.

The bacilli are always present in diseased tissues, although great difficulty is usually experienced in demonstrating them by staining methods. When properly stained, they are found most numerous in the centre of the nodules, becoming gradually less so towards the periphery. Their usual position is between the cells, but sometimes they almost fill some of the epithelial cells. They are always present in the nodules (see Photomicrograph, Fig. 46), rarely in the blood, and if so, only in small numbers. (For special methods of staining the bacilli in sections of tissues, see Technique, §§ 43, 44.)

Spore Formation.—It is still an open question if the glanders bacillus forms spores or not. Some observers claim that spores are present. The evidence of spore formation cannot be accepted when the organism is subjected to the following tests:—

(1) The Bacillus mallei does not at any stage of its growth resist exposure to 3 per cent. carbolic acid solution longer than five minutes, nor to 1 to 5000 corrosive sublimate solution for more than five minutes.

(2) It is destroyed in ten minutes in some experiments, and in five minutes in others, when exposed to a temperature of 55° C.

(3) When dried, according to various authorities, it loses its vitality in from thirty to forty days.

The conditions exhibited above are directly opposed to the existence of spores.

Biological Characters.—The Bacillus mallei is a facultative organism, growing both with and without oxygen. It develops on the ordinary nutrient media. Minimum temperature, 25° C.; optimum, 37° C.; maximum, 42° C. The best growth is exhibited on 5 per cent. glycerine agar medium.

On Agar Media, with or without the addition of 5 per cent. glycerine, it forms a moist, opaque, glazed layer, devoid of special characteristics. Sometimes no growth occurs on ordinary agar.

On Blood Serum it forms a moist, opaque layer of yellowish or dirty-brownish drops. The serum is not liquefied.

On Potatoes the Bacillus mallei exhibits a very characteristic growth, which is somewhat rapid, and in twenty-four to thirty-six hours at 37° C. a moist, amber-yellow, transparent deposit appears, becoming deeper in

colour and denser in consistency, until it finally presents a reddish-brown colour and the surrounding surface of the potato becomes darkened. According to Semner, the Bacillus mallei exhibits unusual pleomorphism on potatoes, often forming long felt-like interlaced filaments, not unlike the threads of the Bacillus anthracis, and, finally, blebby and club-like swellings.

In Bouillon a diffuse clouding takes place, a tenacious, ropy sediment being ultimately formed.

In Milk Media to which a little litmus is added, the blue colour becomes reddish in four or five days, and quite red in two weeks at 37° C., the milk being finally separated into a firm clot of casein and clear whey.

Vitality.—Cultures of the Bacillus mallei lose their virulence very quickly by a natural weakening as early as the fourth and fifth generations; therefore to retain the cultures virulent it is necessary after two or three culture generations to pass the virus through a susceptible animal. According to Löffler the Bacillus mallei lives three months in a dry condition, while other authors find that when spread out in a thin layer the bacilli die in ten days. When exposed to heat they are killed at 100° C. in two minutes, and at 80° C. in five minutes. Exposed to the action of corrosive sublimate 1 to 1000 they are killed in fifteen minutes, and in 5 per cent. carbolic acid in one hour; and they also lose their virulence quickly in distilled water (six days). The virulence is not destroyed by putrefaction; inoculations made with central portions of glanders lungs, exposed to the air for fifteen, eighteen, and twenty-six days, have given positive results.

Pathogenesis.—The ass, mule, horse, goat, cat, sheep, dog, pig, and mankind, are susceptible. Cattle are immune.

Among laboratory animals, the field mouse, wood mouse, and the guinea-pig are the most susceptible, the rabbit being less so; white mice and house mice are immune. Birds, with the exception of the pigeon, are refractory.

Field mice inoculated subcutaneously with a small quantity of a culture die very quickly—in three or four days. The spleen is found enlarged and generally studded with minute grey nodules, which are rarely present in the lungs, but frequently found in the liver. Pure cultures can be obtained from these nodules. The characteristic lesions are much more marked in the guinea-pig, which lives from six to eight weeks after inoculation. The specific inflammatory condition of the mucous membrane of the nostrils is almost always present; the joints are infiltrated and swollen. Orchitis and epididymitis are present in male animals, while the internal organs, lungs, kidneys, spleen and liver are generally the seat of the characteristic nodular formations. Pure cultures can be obtained from the diseased centres.

Modes of Infection and Course of the Disease.—According to M. Nocard, the only means of experimentally obtaining miliary tubercles in the lungs of horses identical with those found in cases of natural glanders, is to cause the animal to ingest virulent matter, cultures, or pus.

Prieur, according to Nocard, gives the most complete and most lucid dissertation of the actual state of our knowledge of the farco-glanderous affection (*Veterinary Record*, 505, 1898).

The author concludes thus:—

'1. The glanders virus commonly gains entrance into solipeds by means of the alimentary canal.

'2. Experimental glanders, determined in solipeds by the glanders virus, evolves exactly as clinical glanders.

'3. Translucid tubercles are of a glanderous nature.

'4. Certain forms of cutaneous glanders may in man be cured by the means of very energetic and rapidly instituted treatment.

'5. Certain cases of pulmonary glanders are in the horse capable of cure by the effect of the sole force of the economy aided by a special hygiene.

'6. The difference of curability in man and the horse results from the ordinary mode of contamination of each species. In man cutaneous glanders is an *incursive* glanders (inoculation), of which one may with success endeavour to arrest the invading march. Cutaneous glanders in the horse, on the contrary, is a *recursive* glanders infection, the manifestations of which indicate defect of the economy.

'7. The employment of mallein is the sole means of diagnosis which we have at our disposal in those cases of glanders exempt from clinical signs.

'8. An animal which having presented a *complete* reaction to mallein and does not react again after a variable number of injections of the reagent, may be considered as cured.

'9. Rigid application of the rules of sanitary police remains the most efficacious measure of opposing the development of glanders in man and animals.'

Schültz, in his recent work on the Experimental Pathology and Pathological Anatomy of Glanders, formulates the following conclusions (*Veterinary Record*, 502, 1898):—

'1. Primary pulmonary glanders is not developed in consequence of the introduction of the specific bacilli into the alimentary canal.

'2. The existence of primary pulmonary glanders has not yet been demonstrated.

'3. The grey and translucid granulations of the lungs of horses are not glanderous, but simply inflammatory lesions provoked by a parasite that can determine similar lesions in the kidneys.

'4. The equine pulmonary glanderous tubercle is a nodule hepatization which undergoes a special disaggregation (chromatotexis).

'5. Old glanderous granulations contain giant-cells.

'6. Glanderous granulations do not calcify, whereas parasitic granulations do.'

It is evident from the above extracts, and the contradictory nature of the same, that the question of the mode of the entry of the glanders virus in solipeds is still open.

In man, where the virus enters, a local swelling appears, which spreads quickly, accompanied with suppuration and cording of the neighbouring lymphatics. Multiple abscesses are next formed in the skin, muscles, and internal organs, and there are often suppurative changes in the joints; at this stage the disease resembles pyæmia. Characteristic glanders nodules appear in the mucous membranes, particularly in the nose, which soon disintegrate, forming ulcers. Death is caused by general infection, carried by means of the lymph circulation.

Heredity. — Löffler observed a female guinea-pig which resisted a glanders inoculation, and five months after being inoculated gave birth to one young one, which at birth seemed perfectly healthy, but died in a week from glanders of the viscera.

BACTERIOLOGICAL DIAGNOSIS OF GLANDERS— METHOD OF STRAUSS.

Inoculate a male guinea-pig in the peritoneal cavity with a solution of the suspected material or culture, making the inoculation direct in the middle line of the abdomen, otherwise other bacteria may be introduced into the vesiculæ seminalis, and cause orchitis, etc., or introduce a piece of the suspected tissue. If the material inoculated is from a genuine case of glanders, the testicles commence to swell in thirty hours, and the skin over them becomes hyperæmic, shiny, and finally desquamates, evidence of the formation of pus appears, and the purulent orchitis often breaks through the skin. The diagnostic symptom is the tumefaction of the testicles.

Mallein.—This consists of the filtered products of the glanders bacillus,—a group of compounds bearing a similar relation to glanders that tuberculin bears to tuberculosis. It is prepared from old glycerine bouillon cultures of the glanders bacillus by steaming them for several hours in the sterilizer, or in the autoclave for fifteen minutes at 115° C., and filtering through unglazed porcelain, the filtrate being concentrated one-sixth its volume, and mixed with an equal volume of $\frac{1}{2}$ per cent. solution of carbolic acid. This yields an active mallein, the dose being 1 c.c., and it gives good reactions.

The *Diagnosis of Glanders with Mallein,* according to Hunting, vide *Veterinary Record,* December 4th, 1897.—'*With mallein diagnosis is easy, and in ninety-nine out of a hundred cases is certain. An injection of mallein under the skin of a healthy horse has no effect, or at most it produces a swelling as big as a watch at the point of injection. An injection into a glandered horse produces two reactions,—a large and painful swelling at the point of injection, and a rise of temperature to* 104° *or even* 106° *Fahr. The indications of mallein are not always so exact that it can be used without brains. It will not do to say no horse is glandered until the temperature rises* 4° *and a swelling appears within twenty-four hours measuring five inches across. Sometimes the local swelling is less, and sometimes the temperature does not rise much. When the temperature is already* 103° *F. a rise may not take place at all; but in such a case a painful swelling at the point of injection is conclusive evidence. In hundreds of cases I have proved the trustworthiness of mallein, when no outward sign of disease existed, by post-mortem results. In hundreds of healthy horses I have known it used without one ill effect.*'

The above conclusions are of immense practical and diagnostic value owing to Hunting's extensive practical experience with glanders and the use of mallein in London.

M. Nocard recommends that only animals presenting clinical signs reacting to the mallein test should be slaughtered. The other animals should be isolated and submitted every month or two months to the mallein test, and when they have supported two tests without reacting, they can be placed at the free disposal of the owners, for they will then have completely and definitely recovered from the glanders lesions which they carried in their lungs. M. Nocard further states that recovery is an occurrence far from being rare. The glanders nodules found on post-mortem examination of such cases did not produce disease when inoculated into susceptible animals, nor could any diagnostic cultures be obtained.

BACILLUS ORCHITICUS.

Found by Kutscher in the nasal discharge of a horse affected with glanders.

Microscopical Appearances.—Similar to the glanders bacillus.

Motility.—Non-motile.

Staining Reactions.—With ordinary stains and by the Gram method.

Vitality.—Killed by exposure to 55° C. for five minutes.

Biological Characters.—It grows on all the ordinary media except milk.

On Gelatine Plates it forms colonies that resemble old colonies of the bacillus of Asiatic cholera; liquefaction occurs somewhat rapidly at 22° C.

On Agar, thick white tufts.

On Blood Serum an orange-yellow pigment is often formed; the medium is liquefied.

In Bouillon and *Peptone Solution* small flakes are formed, the medium very seldom becoming clouded.

Pathogenesis. — Guinea-pigs (male) when inoculated intraperitoneally with a small quantity of the virus, exhibit swelling of the testicles in forty-eight hours, and generally die in four to five days. The principal lesions are nodules in the mesentery and testicles (rarely in the abdominal organs). Large doses caused death in one to three days with more pronounced changes in the peritoneal cavity. Small doses introduced subcutaneously caused death in one to two days, with an extensive œdema affecting the whole abdominal wall. Guinea-pigs that recovered were found later to be immune to further infection. *Mice* inoculated subcutaneously with small doses die in four to seven days, an abscess developing, the surrounding tissue being œdematous, and infiltrated with small hæmorrhages. The bacilli were only present in the pus from the abscess, and frequently within the leucocytes. *Intraperitoneal* inoculation produced death in the same time, numerous nodules being formed on the peritoneum; the liver and spleen being seldom affected. Intrapulmonary injection caused the formation of a watery hæmorrhagic effusion into the serous cavities of the thorax, and the development of numerous grey nodules on the pleuræ, associated with small lobular pneumonic centres.

Rabbits are not so susceptible to infection, while chickens and pigeons are immune.

Differential Diagnosis.—With the Gram method of staining the bacillus of glanders gives *negative results,* while the Bacillus orchiticus yields *positive results.*

EPIZOÖTIC LYMPHANGITIS, OR AFRICAN FARCY.

An organism discribed as the '*Cryptococcus of Rivolta,*' a species of micrococcus measuring 3 to 4 μ in diameter, slightly ovoid and somewhat pointed at one of its extremities, has been found in the pus and lesions of this disease by Rivolta and Nocard. It stains by the Gram, Weigert, and Kühne methods.

The dimensions and refringence exhibited by the organism are such that it is impossible, even in unstained specimens, to mistake it for any other element (Nocard).

Ulcers resembling those of acute glanders have been found on the nasal mucosa of animals affected with epizoötic lymphangitis. Nocard, however, found the above cryptococcus in these lesions, and thus affirmed

their relation to lymphangitis and not to glanders, the bacillus of glanders being, moreover, absent.

TUBERCULOSIS.

The infectious nature of this disease was first demonstrated by Villemin in 1865, when he communicated the disease to healthy experiment animals with tuberculous material. Cohnheim confirmed these experiments by inoculations into the anterior chamber of the eyes of experiment animals. In 1882 Koch discovered the Bacillus tuberculosis, which is now known as the cause of mammalian tuberculosis.

Microscopical Appearances.—Koch's bacilli are small, thin rods, varying in size from 0·2 to 0·4 μ broad, to 3 to 4 μ long; they are slightly bent, generally occur singly, but in cultures sometimes form chains of four to six individuals; in rare cases they exhibit a club-like swelling at one end and branches, whereby a certain relationship with the actinomyces group has been ascribed to them.

Motility.—Non-motile.

Spore Formation.—The clear spaces present in stained specimens have been described by some authorities as spores, and by others as due to degenerative processes.

Staining Reactions.—The bacilli stain with difficulty, but once they are stained they retain the dye with great tenacity. The results with the Gram and Cladius staining methods are positive. For the special methods of preparing and staining cover-glass specimens and sections see Technique, §§ 13, 14, 40.

According to Koch's recent investigations, tubercle bacilli contain two solid fatty acids, one of which is soluble in reduced alcohol and saponified by caustic soda; the other is not saponified, and is only soluble in boiling absolute alcohol and ether. Both of these fatty acids are stained by the specific tubercle bacilli stains; but by the process of decolorization, the one soluble in alcohol gives up the stain, while the other retains it; thus the acid fixes the staining material and accounts for the staining reaction (see Photomicrograph of tubercle bacilli in sputum, Fig. 47). It is possible with a warm solution of caustic soda to remove the fatty acids out of the body of the bacilli, and observe by a microscopical examination how they pass out in the form of colourless drops, and coalesce into larger drops. According to Koch, these fatty acids form a continuous layer in the bodies of the bacilli, thus providing them with a protection against external influences.

This peculiar micro-chemical staining reaction found in the case of

the Bacillus tuberculosis is not confined to that organism alone, as other species of bacilli, when similarly treated, react in the same way, *e.g.* :—

1. *The smegma bacillus,* located in the smegma, often seen beneath the prepuce and upon the vulva, both normally and in disease.

2. *Lustgarten's bacillus of syphilis,* found principally in the primary sores associated with that disease.

3. The bacillus of leprosy.

4. *The acid-resisting bacteria* found in butter.

Hueppe differentiates the first three organisms and the Bacillus tuberculosis as follows :—

1. Treat the preparation, stained with carbol fuchsin, with sulphuric acid, and the syphilis bacillus is decolorized almost instantaneously.

2. If not at once decolorized, treat with alcohol, and if it is the smegma bacillus, it will lose its colour.

3. If it is still not decolorized, it is either the leprosy or tubercle bacillus. According to Baumgarten, the Lepra bacillus is stained by an exposure of six or seven minutes to a cold, saturated, watery solution of fuchsin, and retains the stain when subsequently treated with acid alcohol (nitric acid 1 part, alcohol 10). When treated for the same length of time, the bacillus of tuberculosis does not ordinarily become stained.

Biological Characters.—It is very difficult to obtain a pure culture of tubercle bacilli, because they grow extraordinarily slowly, and require for their development an incubator temperature of—minimum, 29° ; maximum, 41° ; and optimum, 37 to 38° C.

The Koch bacillus grows well on blood serum, 4 to 6 per cent. glycerine agar, and in glycerine bouillon. The isolation of the tubercle bacillus from the mixture of bacteria in tubercular sputum by means of glycerine agar plates is almost impossible ; on account of the tubercle bacilli growing so slowly, the other bacterial colonies outgrow and overwhelm them easily. It is only possible to obtain a pure culture when the material is quite pure at the commencement. The following is the method of procedure :—

Inoculate some guinea-pigs (which are very susceptible) with material containing tubercle bacilli. In about four weeks the first inoculated animal dies, the autopsy revealing a well-marked tuberculosis of the internal organs. One of the other guinea-pigs is now killed, the skin disinfected with warm water and sublimate solution (1 to 1000), and removed with a knife heated in the Bunsen flame. The peritoneum is opened with other sterilized instruments, and the spleen removed with forceps previously heated in the flame. By this mode of infection the spleen is usually the most strongly affected. A portion containing the tubercles is removed from the spleen with sterilized forceps, and pressed between two aseptic scalpels or glass slides in order to obtain material containing bacilli, which is conveyed by means of a stout sterile platinum needle

on to the surface of blood serum medium. The whole process must be accomplished quickly and with the greatest cleanliness, because if another germ enters the serum tube, it will soon outgrow the tubercle bacillus. As a greater precaution, several cultivations ought to be instituted at the same time. The cultures remaining a long time in the incubator at 37° to 38° C., they must be closed with indiarubber caps previously sterilized in sublimate solution, or the superfluous overhanging cotton-plug burnt off in the flame, and the tube closed with melted paraffin or sealing-wax. Without these precautions the water of condensation will be all absorbed and the culture medium dried up.

When the inoculation is successful, signs of growth are observed in fourteen days in the blood serum tubes. Small, grey, dry scales develop, which when examined under a low power appear composed of delicate twisted lines. The development goes on slowly, and in four to six weeks other tubes can be inoculated from the original culture. The growth of the second generation requires two weeks before it is distinct. Later generations, usually the fifth or sixth, grow more luxuriantly and quicker, especially when a special incubator is used, provided with an atmosphere of steam, so that the use of indiarubber caps, etc., can be dispensed with. Under such conditions, in seven to fourteen days the whole of the surface of the serum medium is covered with the characteristic dry scales. From the fifth serum generation it is easy to obtain cultures on glycerine agar. On this medium the development is much more abundant, the bacilli forming a greyish dry coating of brittle, curly, slightly elevated fragments (see Photograph, Fig. 48).

This coating growing downwards, covers any water of condensation present without clouding it, and when the culture is left long enough in the incubator it grows a considerable distance up the free sides of the test-tubes, where no nutrient medium is present. Veal bouillon, with 6 per cent. glycerine added, and filled in Erlenmeyer flasks, is the best fluid medium. When inoculating this bouillon it is necessary to place the dry scales in the fluid so that they swim on the surface, as the tubercle bacilli, being susceptible to oxygen, develop only in the upper portions of the medium where the air has sufficient access. On the surface of veal 6 per cent. glycerine bouillon the tubercle bacilli form a membranous surface growth which exhibits the same characteristics as the coating on glycerine agar. Under favourable circumstances, in a few weeks, sometimes in ten days, the growth extends a considerable distance up the walls of the tube; the lower portion of the bouillon *remains perfectly clear*, which is a characteristic of the growth of the tubercle bacillus.

On Potatoes, the under ends of which project into a solution containing 5 per cent. of glycerine and 5 per cent. of NaCl., placed in the bottom of the tube, the tubercle bacilli develop very well, forming on the surface of the potatoes thick warty tufts, the glycerine solution

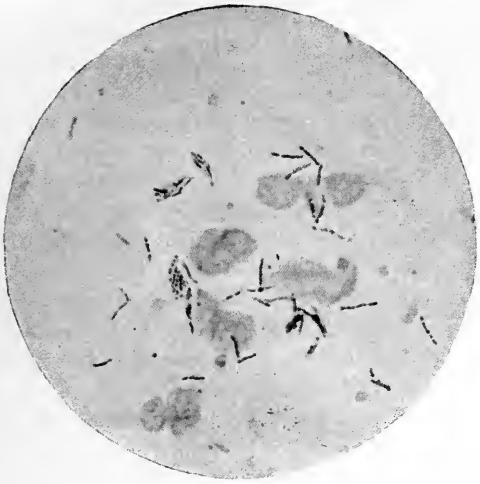


FIG. 47.—B. Tuberculosis. Cover-glass specimen prepared from sputum. Ehrlich's method. × 1000.

FIG. 48.—B. Tuberculosis. Glycerine agar culture.

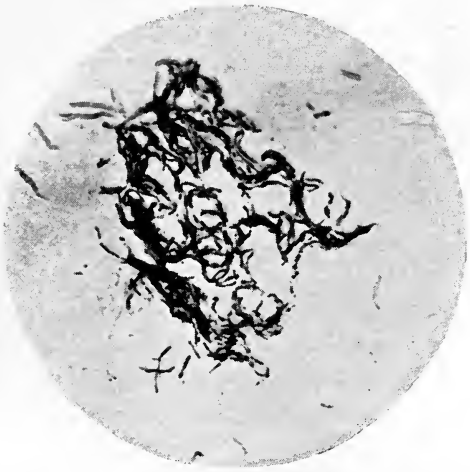


FIG. 49.—B. Tuberculosis. Cover-glass specimen from pure culture on glycerine agar. Ehrlich's method. × 1000.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

remaining clear. The potato culture medium is prepared according to Roux and Globig's method (see Technique, p. 49). In 1882 Koch and Kitasato devised a means of obtaining cultures of tubercle bacilli direct from the sputum of phthisical patients. They washed out the patient's mouth with an antiseptic gargle, and emptied the expectorate into a Petri-dish. One of the expectorated masses of sputum is washed in several changes of sterile water to free the exterior of bacteria; out of the middle of the washed sputum a portion is removed with the platinum needle, and stroke cultures prepared on the surface of blood serum. The developing cultures exhibit a somewhat different appearance to those cultivated from the bodies of animals; they appear as round, whitish, transparent colonies, but the later generations grow in the same manner as those previously described. The bacilli can be cultivated through many generations for several years without injuring their power of growth, although the older cultures become less virulent. (For Photomicrograph of specimen from pure culture, see Fig. 49.)

Vitality.—The Bacillus tuberculosis is destroyed by heating for ten minutes at 70° C., one minute at 95° C., one hour at 60° C., and four hours at 55° C. They only resist the action of direct sunlight for from some minutes to some hours, according to the thickness of the growth. Exposed to diffuse daylight they are killed in a week.

Pathogenesis.—None of the domestic animals are immune. The guinea-pig is the most susceptible of all the experimental animals, a very small quantity of tubercle bacilli being sufficient for their infection; next come the rabbit and the field mouse; less susceptible, and still far from immune, are white mice and dogs. Young animals exhibit a greater predisposition for tuberculosis than older animals. Typical tubercular lesions are produced in guinea-pigs, rabbits, and field mice by subcutaneous injection, inoculation into the anterior chamber of the eye, intraperitoneal and intravenous injection, or by inhalation of moist powdered tubercle bacilli.

Susceptible animals infected by feeding with tubercular matter die of abdominal tuberculosis.

Baumgarten inoculated animals in the anterior chamber of the eye, and in three days found the bacilli in the auricular lymph-glands. Intravenous injection produces a generalized miliary tuberculosis. The experiments of Schüller are of especial interest in relation to many localized tubercular diseases of man; he injected tubercular material in a suitable part of an animal, then produced an injury in the region of the knee-joint, and observed that the infection became localized at that point. Dead tubercle bacilli produce pyogenic results; they are *positive chemotactic*, drawing the leucocytes towards

them. When dead bacilli are injected intravenously into rabbits, in the animals killed, after some time, small tubercles are found throughout the lungs and liver, formed of round cells, epithelioid cells, giant cells, and dead tubercle bacilli, which cannot be distinguished from the genuine Bacillus tuberculosis. Baumgarten considers that the dead tubercle bacilli produce a tuberculosis similar to that produced by a foreign body (pseudo-tuberculosis).

Instances in which physicians and veterinary surgeons have contracted the disease, in making autopsies of diseased men or animals, are incontestable, although fortunately rare. The virus in such cases gains entrance by some cutaneous wound, causing at first a more or less limited cutaneous tuberculosis, which later may become generalized.

BOVINE TUBERCULOSIS.

According to most authorities, tuberculosis of the lungs (see Photomicrograph, Fig. 50) is the most frequent of all the primary forms of this disease in cattle, infection being caused by dried tubercular matter inhaled into the lungs. Primary tuberculosis also occurs in the lymph-glands of the head and neck, in the mesenteric glands, the intestines, the liver, the genital organs, and the udder. Bang is inclined to believe that the udder is now and then primarily affected in animals that are in very good condition. Eber reports a case of primary tuberculosis of the penis, and cases involving the vagina and vulva. Finally, *generalized* infection, due to the dissemination of tubercle bacilli through the blood, occurs in two forms: (1) The *acute* form, when large numbers of bacilli have escaped into the circulation, whereby numerous tubercular foci appear in various organs; (2) The *chronic* form, as seen in old cows affected with tuberculosis for many years. A few cases of congenital tuberculosis have been recorded in calves. Johne found tubercle bacilli twice in the organs of embryos. The writer found a case of tubercular meningitis in a calf in California. The mother of this calf was tested with tuberculin and reacted, the post-mortem revealing tubercular lesions, one ovary being affected.

King mentions a case of a cow giving birth to twin calves. The viscera of both, when submitted to examination, proved tubercular. The cow was not tested with tuberculin, being considered, from apparent symptoms, affected with generalized disease.—(*Proceedings of National Veterinary Association*, Leeds, 1898.)

The bacillus of Koch, according to Nocard, is only in very ex-

ceptional cases transmitted from mother to fœtus; the predisposition to receive and develop the germ is, however, hereditary. Infection rarely takes place while diseased and healthy cattle are pastured together in the open. Nocard and other observers frequently point to the fact of the animals being infected while standing in stalls adjacent to the coughing 'piner,' the disease often extending along one side of the byre, while the cows on the other side may remain sound, and it is also observed that the animals longest stabled furnish the largest proportion of cases. While the author was Inspector for the city and county of San Francisco, the tubercular seizures, with a few exceptions, were all dairy cows, tubercular lesions in range cattle being hardly ever observed.

Nocard is against the total seizure of the carcases of cattle which have localized tuberculosis, but are otherwise in good condition. He insists that the bacilli of Koch, the true infectious material, does not exist either in the blood or in the muscles, excepting for very brief periods, and in cases of advanced general tuberculosis. In some of Nocard's experiments with meat from cases of generalized tuberculosis, he found that although it had no bad effects when eaten, the expressed juice produced tuberculosis in one or two of the guinea-pigs when injected into the peritoneum. It is generally admitted that the milk of tuberculous cows is greatly more liable than their flesh to produce human tuberculosis. That this is so is very conclusively proved by the fact that abdominal tubercle is practically confined to children under five years whose food consists very largely of raw milk, while the incidence is greatest in cases of infants hand-fed. Nothing except milk infection can account for the terrible prevalence among children of tabes mesenterica and allied tubercular disease. The milk is not, however, always virulent. It is only so when the udder is infiltrated with tubercular nodules. Nocard states that in his experience he never found the milk virulent when the udder was free from tuberculous lesions, and out of fifty-four cows seized for general tuberculosis which he specially examined, only three had tubercular lesions in the udder. At Copenhagen the proportion is still lower. Bang estimates it at less than 3 per cent. of the number of tuberculous cows. However, the difficulty of deciding as to the non-existence of tubercle in the mammary gland justifies the milk from suspicious cases being excluded from consumption. (For the special methods of examining and staining tubercle bacilli in milk, see Technique, § 20.)

The question of living tubercle bacilli existing in ordinary market butter has led to considerable investigation since Obermüller stated

that in fourteen samples of butter he found genuine tubercle bacilli, capable of causing infection. Rabinowitsch, in eighty samples of butter that he examined, did not find the Koch bacilli once, and he considered that the positive results obtained by others were due to the acid-resisting tubercle-like bacilli previously discovered by him being mistaken for genuine tubercle bacilli.

Petri's results occupy an intermediate position between those of Obermüller and Rabinowitsch. He found genuine tubercle bacilli in 30 per cent., and the acid-resisting tubercle-like bacilli in 60 per cent. of the samples examined.

Horman and Morgenroth conducted a series of investigations recently, and in ten samples of market butter examined they found genuine tubercle bacilli in three of the samples (see Photomicrograph of same, Plate III., Fig. 18), and in some of the samples, Rabinowitsch's acid-resisting bacilli; and in one of the experiments the resisting bacteria and genuine tubercle bacilli were found associated together. Glycerine agar was of no use as a culture media; blood serum, with the addition of 5 per cent. of glycerine, being found the best medium in these investigations.

The acid-resisting tubercle-like bacilli found in butter are described by Horman and Morgenroth as follows:—

Microscopical Appearances.—Slender rods, similar to the Koch's bacillus of tuberculosis in form, slightly bent, and sometimes the end of the rod is thickened.

Motility.—Non-motile.

Staining Reactions.—When stained by Günther's method for tubercle bacilli, they are not decolorized, but their resistance is not so pronounced as with Koch's bacilli, as on the edges of the cover-glass and contact specimen of colonies many of the rods are stained slightly blue. In old cultures unstained portions in the stained rods were often observed. The reaction with the Gram method is positive.

Biological Characteristics. — *On Gelatine Plates* the growth is very slow. The deep-lying colonies are round, glistening, of a light-yellow colour, and finely granular throughout. The superficial colonies are also round, transparent, and possess irregular, finely serrated margins.

The gelatine is not liquefied.

In Gelatine Stab Cultures a growth occurs along the middle track, and after a long time a thick coating develops on the surface.

On Agar oblique surface Cultures in twenty-four hours, at incubator temperature, a white, creamy coating develops on the surface of the

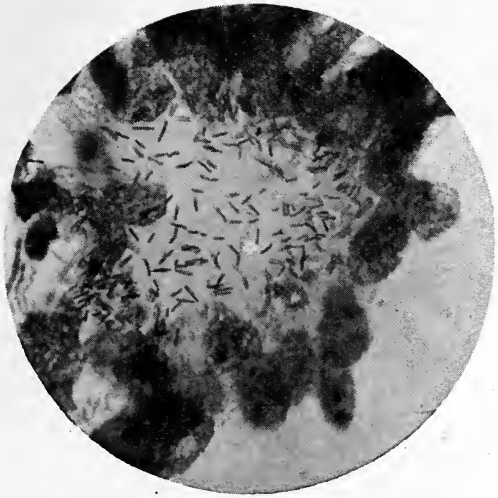


FIG. 50.—B. Tuberculosis. Cover-glass specimen, lung of a cow. Ehrlich's method. × 1000.

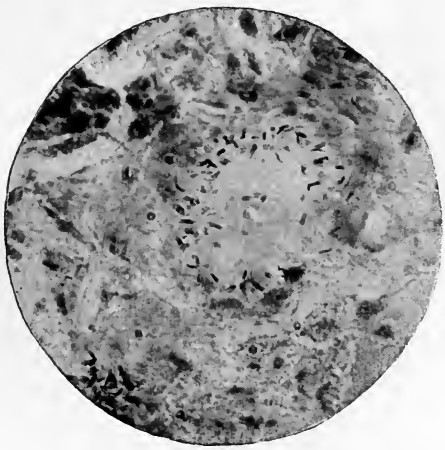


FIG. 51.—B. Tuberculosis. Section of mesenteric gland of the horse. Ehrlich's method. × 600.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

water of condensation, a thin membrane which passes on to the walls of the tube. In old cultures the coating is frequently very much corrugated, and sometimes also exhibits a yellow, orange, or light-brown colour.

Bouillon or *Glycerine Bouillon* becomes clouded with a sediment, and in forty-eight hours a membrane forms on the surface, which sinks very easily to the bottom of the medium, if shaken, after which another membrane forms, which passes up the sides of the tube. A slight formation of *indol* was observed in bouillon cultures.

On Potatoes the growth is rapid and plentiful, exhibiting a thick, greyish, moist coating in twenty-four hours.

Sterile Milk is not altered by the growth of the bacteria, but a yellowish-white membrane forms on the surface.

The morpho-biological characteristics above mentioned correspond to those of the acid-resisting bacteria discovered by Rabinowitsch.

Pathogenesis.—These acid-resisting bacteria produced disease processes in guinea-pigs when injected into the peritoneum.

The liver was the only organ in which the lesions bore a distinct resemblance to those of tuberculosis. The tubercles were of a greyish-white colour, and penetrated from the surface of the liver into the parenchyma, easily detached from the surrounding tissue. Small to pinhead-sized yellow spotted foci were also observed on the surface as well as in sections of the organs. The spleen, on the contrary, never exhibited the appearance of a genuine tuberculous spleen. The enlargement, dark colour, and characteristic marbling were awanting. In the most of cases extensive peritoneal adhesions were present. The mesenteric lymph-glands were not very much enlarged. In one case softening was observed; it was of a purulent nature, not caseous. These bacteria frequently cause peritoneal lesions which exhibit a decided tendency to recovery. In cases where reparative changes were observed, the acid-resisting bacteria appeared to die and disappear, because in cover-glass specimens and sections from some of the guinea-pigs experimented with, the bacteria could no longer be detected. Two chickens and one dog injected intraperitoneally with a pure culture remained unaffected. According to Rabinowitsch, rabbits are refractory. Injection into the anterior chamber of the rabbit's eye caused inflammatory changes which did not persist very long.

HORMAN AND MORGENRATH'S METHOD OF DEMONSTRATING TUBERCLE BACILLI IN BUTTER.

1. Inject 4 to 5 c.c. of butter melted at 37° C., and thoroughly mixed into the peritoneal cavity of three guinea-pigs. (Allowing the butter to

stand from twelve to twenty-four hours at 34° C., as recommended by Rabinowitsch, is unnecessary.)

2. From the organic lesions of the guinea-pig that die or are killed in four to six weeks cultures are instituted on, at the least, eight to ten tubes of blood serum, and at the same time pieces of the organs are inserted into the peritoneal cavities of two guinea-pigs and one rabbit.

3. These latter animals are killed not later than four weeks, and from any existing lesions blood serum cultures instituted.

Conclusions.—(1.) It has been observed that genuine Koch tubercle bacilli are often present in butter.

(2.) Acid-resisting forms of bacteria are also found in butter, which cause disease processes in guinea-pigs. These changes were, however in the above authors' experiments not such that they could not be distinguished from the changes produced by genuine tubercle bacilli.

EQUINE TUBERCULOSIS.

In the horse this disease manifests itself in two forms.

1. *The Abdominal Form,* which seems to be the more frequent, is characterised by confluent lesions in the spleen, sub-lumbar and mesenteric glands (for section of same showing tubercle bacilli, see Photomicrograph, Fig. 51), liver, and intestinal mucous membrane. According to Nocard, infection seems to take place by way of the alimentary canal. When the lungs are invaded as a sequel of the abdominal form, the lesions appear of recent origin, and consist of a diffuse infiltration of the interlobular connective tissue without apparent tubercles, caverns, or centres of softening, which, according to the above authority, accounts for the absence of cough and discharge or expectoration, and the non-transmission of the disease to other horses in the same stable.

Nocard also states that in advanced cases an abundant polyuria, lasting for several weeks, occurs. Koch's bacilli are extremely abundant in the lesions, and of very great length. Nocard considers that the bacillus of equine abdominal tuberculosis is more closely allied to the bacillus of avian tuberculosis than to the bacillus of mammalian tuberculosis.

2. *The Thoracic Form.*—In this type the disease seems to originate primarily in the lungs, because these organs and the bronchial glands are most severely affected. A genuine acute miliary tuberculosis sometimes occurs; at other times the parenchyma of the lungs is studded with small abscesses with a fibrous capsule, enclosing pus that is very rich in bacilli. Nocard claims that the distinction drawn between the two types of equine tuberculosis, on clinical and pathological grounds, is confirmed by the determination of the causal agent, for while the

thoracic form is referable to human tuberculosis, the abdominal form, as already mentioned, is more closely allied to the bacillus of avian tuberculosis.

CANINE TUBERCULOSIS.

In the dog the disease also occurs in two forms, the *abdominal* and the *thoracic*. Many cases have been recorded, the principal lesions mentioned occurring in the liver, hepatic and mesenteric glands, as well as generalized tuberculosis of both lungs and the bronchial glands.

TUBERCULOSIS OF SWINE.

The pig is very susceptible to experimental disease, and according to some authors, the scrofulous conditions (see Photograph, Fig. 53) occasionally observed in pigs are due to tuberculosis. According to Nocard the disease in the pig often develops with great rapidity and passes unperceived. In the chronic form the bacilli are rare, and appear to have lost part of their virulence; and when inoculated into guinea-pigs they produce a disease of slow course, but the period of incubation becomes shortened when the bacilli are inoculated from the first guinea-pig to others in series.

According to Nocard, nine out of every ten pigs are infected through the alimentary canal. Many investigators have produced the disease by feeding pigs with milk from cows with tuberculous udders. In 1877, pigs were kept under the shambles in San Francisco, and fed on the offal which fell down a shoot. The percentage of tuberculosis that occurred in those swine was beyond conception, the livers and spleen being studded with masses of tuberculous nodules. (For Photograph of a tuberculous spleen of pig, see Fig. 54, and for Photomicrograph of a section of a tubercle from same, see Fig. 52.)

Tuberculin.—The use of tuberculin as a curative agent has not fulfilled the expectations at first anticipated. When injected into healthy persons it has no reaction, but in tubercular patients a pronounced systemic reaction results. Koch has lately produced a new tuberculin, known as TO. and TR., prepared by triturating dried cultures of tubercle bacilli in a mortar without anything being added for a considerable time. The mass is then mixed with distilled water and placed in a centripetal machine, when an opalescent, transparent, whitish fluid is obtained free from bacilli. Trudeau and Baldwin have recently conducted experiments with this new preparation in New York, and found that it still contained living tubercle bacilli, capable of producing tuberculosis in guinea-pigs;

results since confirmed by other investigators. In preparing the original tuberculin, according to Kühne's investigations, the ordinarily prepared culture media contain so much peptone that there is always more or less albumose present, rendering it impossible to separate the true products of the bacillus from other substances present in the culture media, hence special culture media formulæ are used by some investigators. Koch in his original method, after testing the purity of six to eight weeks' old veal bouillon cultures of the tubercle bacilli by microscopical examination, poured them into a suitable vessel, and evaporated to one-tenth the original volume over a water-bath. The liquid was then filtered through porcelain. The crude tuberculin obtained by this process contains 40 to 50 per cent. of glycerine, is soluble in water, insoluble in alcohol, passes readily through dialyzing membranes, and is not destroyed by a boiling temperature, keeps well, and preserves its activity indefinitely.

The original tuberculin is very valuable as a diagnostic agent in bovine tuberculosis. For this purpose it is diluted with 9 parts of water containing $\frac{1}{2}$ per cent. of carbolic acid. About 3 c.c., or 60 minims, are injected subcutaneously; the point of injection is immaterial, but the side of the neck where the skin is thin is the most suitable place. It is also more practical to use a large-sized inoculating needle instead of the smaller varieties; the hair is clipped from the part selected, which is thoroughly cleansed and disinfected before the injection is made. Before the tuberculin test is applied, the temperature of the animals ought to be taken every two hours, at least six or seven times before the injections are made, as in many animals the variations are sufficiently constant to make their determination by precise measurements practically necessary in every tuberculin test from which reliable results are expected. After the injection is made the temperature ought to be taken again in eight to ten hours, and from then on every two hours, until a decided reaction, continuous during several hours, has occurred, or until eighteen to twenty hours have elapsed since the time of injection. The febrile reaction in tuberculous cattle, following the subcutaneous injection of tuberculin, begins six to ten hours after the injection, reaches the maximum in nine to fifteen hours, and returns to the normal in eighteen to twenty-six hours after the injection. The elevation in the temperature sufficient to constitute a reaction has variously been given from 0·5° to 1° C., but consideration of the number of degrees the temperature after the injection rises above the temperature before the injection is not alone sufficient—the height of the temperature and the duration of the reaction must also be taken into account; advanced cases of tuberculosis occasionally fail to react, while the reaction is frequently highest in young animals in the first stage of the disease. In fact, the variation of the temperature of an animal during the course of the day is frequently so great that if the variation is not determined, and the

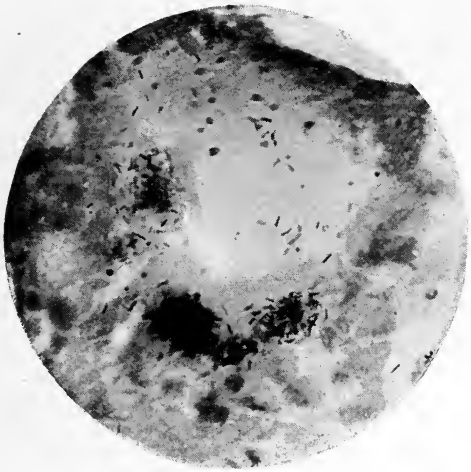


FIG. 52.—B. Tuberculosis. Section of tubercle from spleen of pig. Ehrlich's method. × 600.

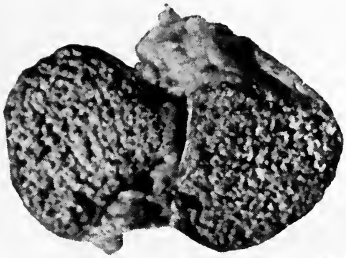


FIG. 53.—*Scrofulous gland.* Cut in two from neck of pig, filled with caseous and calcareous matter.

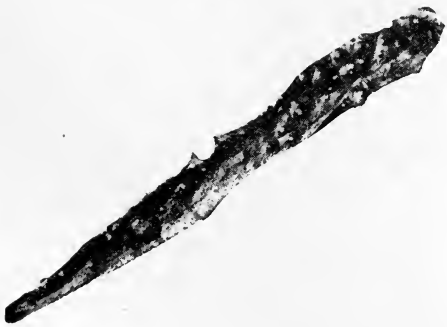


FIG. 54.—Spleen of a pig, showing tubercular nodules.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

temperature is taken only once previous to a tuberculin injection, it is merely a matter of chance if a high temperature, natural to the animal and independent of the action of the tuberculin injection, is not confounded with and erroneously taken for a tuberculin reaction. The range of the thermal reaction gives no indication of the extent of the tubercular lesions in the animal. Instances are recorded where a second injection of tuberculin has altogether failed to produce a reaction in animals which gave a very decided reaction after the first injection, notwithstanding that the two injections were separated by a very considerable period of time. This question of the non-reaction to a second injection is a matter for future investigation. Should the foregoing instances be correct, there is nothing to hinder unscrupulous persons to so prepare their animals, that when submitted to a *tuberculin* test the results are negative, and unhealthy stock left as centres of infection.

Immunity.—At the Ninth International Congress of Hygiene, Behring stated, '*The vaccine of tuberculosis has not yet been found*,' and that he had hoped to find an active antitoxin with a corresponding toxin, but so far he had been unsuccessful. He would even be sceptical as regards such a result had he not found that birds were much more suitable for this kind of research than mammals. With sodium and other reagents, mucin as well as other chemical substances could be extracted from tubercle bacilli. These bodies had nothing to do with the toxin of tuberculosis. If the bacilli were subjected to a temperature of 105° C., freed from fat, and treated with glycerinated water, insoluble albuminous bodies were obtained, which had a virulence twenty times greater. The toxin of tuberculosis was therefore not identical with the primitive substance. The chemical constitution of the toxin was not modified by the operations to which it was subjected. Behring stated the substance which he had isolated was eighty or even one hundred times more virulent than Koch's tuberculin. He had also obtained an antitoxin by passing the virus through a horse in the same manner as was done in the case of diphtheria and tetanus. There was, however, this difference, that in a phthisical patient more than 0·5 c.c. to 1 c.c. could be injected without producing injurious effects, general as well as local. Behring lays it down as a principle that the harmlessness of an antitoxin is an indispensable condition of its practical application, but there are many other difficulties which must be overcome before an antitoxin of tuberculosis can come into use in the treatment of human beings. In bovine animals it is already possible by its means to cure a declared tuberculosis, but even in them one gathers that the remedy at present is somewhat dangerous to life. This, however, as pointed

out by Behring, is important only from an economic point of view. If of the one hundred animals treated with the serum ninety are cured and ten are killed, the treatment should still be adopted. Experiments to determine the value of this treatment are to be conducted on an extensive scale in the Berlin Veterinary School.

AVIAN TUBERCULOSIS.

The bacillus of avian tuberculosis is closely allied to the bacillus of mammalian tuberculosis.

Microscopical Appearances.—It is thinner and more slender than the bacilli found in man and mammals, and club and branched forms are more frequent (see Photomicrograph, Fig. 55).

Staining Reactions.—It is easier stained than the bacilli of man and mammals, but exhibits a similar reaction towards decolorizing agents.

Biological Characters.—It is not so difficult to cultivate as the other forms, growing in ordinary agar and in ordinary bouillon; but the addition of glycerine to the media assists the growth to a great extent, which, moreover, is much quicker than the growth of the bacillus of mammalian tuberculosis. The cultures are not so dry, but much moister, forming a coherent coating; and on solid culture media the growth forms a film over the water of condensation. Old cultures exhibit a yellowish colour.

Differential Characteristics.—The bacillus of avian tuberculosis grows just as luxuriantly at 42, 43, 45° C. as at 37° C., a characteristic not exhibited by the bacillus of human tuberculosis, as it ceases to develop at this high temperature. Supposing both forms to be identical species, the effects of the high temperature on the cultures may be due to the avian bacilli having become adapted to a high temperature during their sojourn in the body of the bird, the normal temperature of same being 41° to 42° C. The bacilli of avian tuberculosis are more resistant towards heat than the bacilli of human tuberculosis, being first killed by exposure for fifteen minutes at 70° C.

The bacilli are found in the tuberculous lesions, which are characterised by tough masses of nodules, calcareous infiltration often occurring. Giant cells are very scarce. A few cases of avian tuberculosis have been observed in man and mammals.

Pathogenesis.—The most of birds are very susceptible, and can be infected by all the different methods of infection. According to Baumgarten, the spontaneous outbreaks are in most cases congenital. Guinea-pigs and dogs are somewhat refractory, without, however, possessing a

perfect immunity. The avian tuberculous bacilli usually develop badly in mammals; and, on the other hand, mammalian tubercle bacilli are acclimated with difficulty in birds.

At the recent Congress in Paris, M. Nocard communicated the following interesting facts regarding avian tuberculosis. The fowl cannot be infected by inoculation with tuberculosis from a human being any more than from a bovine. On the other hand, the dog and guinea-pig, although most susceptible to the action of both human and bovine tuberculosis, are very refractory to avian tuberculosis. If, however, a guinea-pig is inoculated intraperitoneally with avian tuberculous material, it often dies, showing a special kind of lesion. The sputum of a tuberculous patient will kill a rabbit by inoculation, but only very rarely a guinea-pig. M. Nocard enveloped a glycerinated bouillon culture of human tuberculosis in little sacs of collodion, and placed them in the peritoneal cavities of poultry. The sacs were removed in from five to eight months, and found to contain a sort of paste made up of bacilli. Cultures instituted from this paste grew extremely well, and the interesting fact was observed that the bacillus had lost its human characteristics and assumed those of the avian bacillus. It grew at high temperatures, and the cultures did not present the characteristic frayed-out lumps. The bacillus was not virulent enough to produce tuberculosis in fowls until it was passed through two or three fowls, and a period of four to six months had elapsed. In one case a fowl suddenly contracted tuberculosis eleven months after the introduction of the collodion sac. At the post-mortem examination it was found that the sac had burst, showing that development had proceeded far enough when the sac ruptured to produce tubercle in the fowl. Nocard considers that human and avian tuberculosis are only two different varieties of the same disease.

Diagnosis.—The examination of the tuberculous material for bacilli is conducted the same as the examination of human and mammalian material.

Nocard states that if, as appears probable from his investigations with equine tuberculosis, the human subject may contract tuberculosis from the fowl, the most elementary prudence requires that the sale of fowls coming from a place in which the disease exists ought to be interdicted, for the consumption of a fowl in the roasted condition involves the risk of the ingestion of a considerable number of living and virulent bacilli.

PSEUDO-TUBERCULOSIS.

The term 'pseudo-tuberculosis' is applied to certain pathological processes which resemble the genuine tubercle, but are dependant on

other causes other than Koch's bacillus of tuberculosis. The etiology of pseudo-tuberculosis is remarkably manifold. The following are known as causal factors of pseudo-tuberculosis :—

1. Inanimate foreign bodies.
2. Animal parasites.
3. Bacteria.
4. Vegetable parasites.

These pseudo-tuberculoses caused by foreign bodies can be produced easily with all kinds of substances, *but are not transmissible from animal to animal.*

The pseudo-tuberculosis produced by animal parasites is only found in the lower animals. In the cat it is caused by the *Ollulanus tricuspis ;* in the sheep by the *Pseudalius ovis pulmonalis ;* in the calf by the *Strongylus refuscens ;* in the dog by the *Strongylus vasorum.*

Muira records the only case in man, which occurred in a patient that died of beri-beri, a fibrous tubercle being found in the mesentery caused by distoma eggs.

Many cases of pseudo-tuberculosis in animals caused by bacteria are mentioned, the principal being zoöglœic tuberculosis, described by Malassez and Vignal.

Microscopical Appearances. — Thick, short rods, frequently exhibiting cocci in the form of chains in small groups or in zoöglœa. Spore formation is absent.

Staining Reactions.—The reaction with the Gram method is negative. Sections are best stained with Malassez's blue prepared as follows :—

Two per cent. solution of carbonate of soda, - -	10 c.c.
Saturated anilin water, - - - -	5 c.c.
Absolute alcohol, - - - - -	3 c.c.
Solution made with 9 vols. of distilled water and 1 vol. of concentrated solution of methylene blue in 90 per cent. alcohol, - - - - -	3 c.c.

Sections remain in this solution two or three days, and are then washed in water stained with methylene blue, and cleared in oil of bergamot or turpentine.

Biological Characters. — *On Gelatine Plates* colonies develop somewhat similar to those of typhus abdominalis. The medium is not liquefied.

In Gelatine Stab Cultures the growth resembles a flat nail.

On Agar, a greyish, fœtid growth.

On Potatoes, a yellowish coating.

In Bouillon a flaky cloudiness occurs at first, then a sediment is formed, the upper portions of the medium becoming clear.

Pathogenesis.—The bacillus of pseudo-tuberculosis is pathogenic for guinea-pigs, death occurring in five to six days, also for dogs and horses. The post-mortem lesions resemble those of genuine (Koch) tuberculosis, especially in the abdominal organs, which the pseudo-bacillus especially attacks. The differential diagnosis is, however, not difficult, the easy staining and the quick growth of the pseudo-bacillus yielding a distinction without difficulty.

Courmont also describes a bacillus found in tubercular lesions of the pleura of the ox (the bacilli of Koch being absent). This bacillus is short, with its substance condensed at both ends, and a clear, slightly constricted middle ; it does not form chains or diplococci. It is both aërobic and anaërobic, grows quickly, is easily cultivated in all kinds of media up to 46° C. It is pathogenic for guinea-pigs, which die in four to eight days with generalized tuberculosis, and for rabbits, in which disseminated and confluent tubercles are found in the spleen, liver, and lungs. The bacillus is also found in the blood of inoculated animals, and it also becomes generalized without affecting the lymphatic glands. The pseudo-tuberculosis caused by the higher organized vegetable parasites likewise manifests itself, especially in animals. Different forms of streptothrix and aspergilli, particularly the Aspergillus glaucus and fumigatus, require consideration. Pigeons often succumb from a miliary tuberculosis, the Aspergillus fumigatus being found present in the interior of the granulations. Lung affections are also sometimes observed in individuals engaged in the feeding of pigeons, which are apparently due to the same parasite—at least the Aspergillus fumigatus is found in the expectoration of these patients. It is probable that the parasite is conveyed with the grain used to feed the pigeons. Eppinger records a case of pseudo-tuberculosis in man due to the Streptothrix Eppinger, see page 108.

Valee has recently, in the *Recueil de Médecine Vétérinaire,* described a new form of pseudo-tuberculosis in calves due to an organism which is much smaller than that of swine erysipelas. It occurs either isolated or in small masses in the diseased tissues, and stains by the Gram-Nicolle process. When isolated and cultivated on the different media, it produces the original lesions in the various animals experimented upon. The principal lesions in the affected calves are in the liver, which is normal in size, but covered with fine granulations or tubercles of a greyish colour, in some cases confluent. There was also a slight perihepatitis, and the tissue of the gland was very friable.

BACILLUS LEPRÆ.

This organism, discovered by Hansen in 1879, is found chiefly in the interior of the peculiar round and oval cells found in leprous tubercles. The bacilli have also been found in the lymphatic glands, liver, spleen, testicles, and in the thickened portions of nerves involved in the anæsthetic form of the disease. According to some authorities they have also been found in the blood. The bacilli lie in the leprous cells in great numbers, and also in the lymph spaces outside of these cells. They are not found in the epidermal layers of the skin, but, according to Babes, they may penetrate the hair follicles.

Microscopical Appearances.—The Bacillus lepræ resembles the tubercle bacilli in form, but is more uniform in length and not so frequently bent or curved, and is from 4 to 6 μ in length, and less than 1 μ in width; the ends of the rods are pointed, and in stained specimens unstained spaces similar to those in the Bacillus tuberculosis are present.

Motility.—Non-motile.

Staining Reactions.—The bacilli stain readily with the aniline dyes, also by the Gram method and by the Cladius method (see Photomicrograph, Plate III., Fig. 17). For differential staining reactions see Tuberculosis, p. 136.

Biological Characters.—It has not yet been obtained in pure cultures, so that its etiological relation to the disease with which it is associated is based upon the demonstration of its constant presence in leprous tissues.

Pathogenesis. — Leprous tissues containing the bacillus are infectious, and may produce this disease. Arning inoculated a condemned criminal in the Sandwich Islands subcutaneously with fresh leprosy tubercles, who was under observation until his death occurred from leprosy at the end of five years. Positive results have also been obtained in the lower animals. Melcher and Ortman inoculated rabbits with pieces of fresh leprosy tubercles in the anterior chamber of the eye, the animals dying at the end of several months; the characteristic tubercles containing the bacilli being found distributed throughout the various organs.

BACILLUS SMEGMATIS.

Found in the smegma præputii between the scrotum and thigh and between the labiæ. It is also found in the cerumen, and occasionally on the skin.

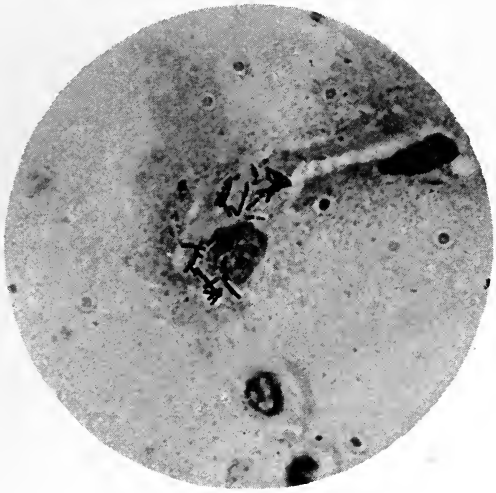


Fig. 55.—Bacillus of Avian Tuberculosis. 'Branched forms.' Cover-glass specimen from the liver of a chicken. Ehrlich's method. × 1000.

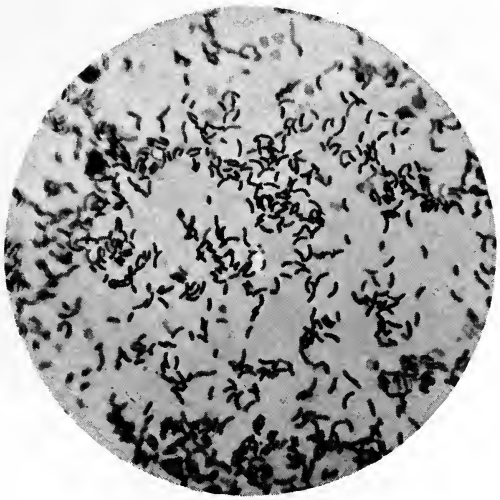


Fig. 56.—B. Choleræ Asiaticæ. Agar culture. Fuchsin. × 1000.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

Microscopical Appearances.—The bacilli lie in clusters either in or between the epithelial cells, the size and form of the rods being very similar to the Bacillus tuberculosis.

Staining Reactions.—They stain with difficulty, are acid-resisting when stained by the methods for tubercle bacilli (see Technique, §§ 13 and 14), but are decolorized when treated one minute with absolute alcohol. The reaction with the Gram method is positive.

Biological Characters.—Doutrelepont and Matterstock obtained a culture of a similar organism on coagulated hydrocele fluid, which coloured the medium brown; further cultivation was not successful.

Differential Diagnosis (see Bacillus Tuberculosis, p. 137).—It is most likely to be mistaken for the Bacillus tuberculosis in the examination of urine.

THE COMMA BACILLUS OF ASIATIC CHOLERA.

(Vibrio Choleræ Asiaticæ.)

In 1883 Koch discovered that in all cases of Asiatic cholera a particular form of bacterium was present, and that these bacteria were found exclusively in cases of genuine Asiatic cholera.

Microscopical Appearances.—Slightly curved rods with rounded ends from 0·8 to 2 μ in length, and about 0·3 to 0·4 μ in breadth. The rods are usually curved like a comma, but are occasionally in the form of a half circle, or two contact rods curved in opposite directions may form an S-shaped figure. The typical comma shape is best observed in specimens prepared from young cultures (see Photomicrograph, Fig. 56). When newly-developed individual bacilli remain attached together, they form long spirals. This condition is frequently observed in cultures, especially in old cultures or on the addition of weak antiseptics, *i.e.* alcohol. In human cholera dejections, the spirillum forms are extremely seldom observed. In the peritoneal exudate of inoculated guinea-pigs spirillum forms are especially frequent. The spirilla are considered to be *involution forms.*

Motility.—Strongly motile. When examined in a hanging-drop culture they resemble a swarm of gnats. The motility is due to one flagellum separated at the end of the rod (Monotricha). (See Photomicrograph, Plate II., Fig. 10, stained by the author's orcëin method).

Spore Formation.—Spores do not exist. The *arthrospores* described by Hueppe have not been confirmed by other investigators.

Staining Reactions.—The best results are obtained with a saturated watery solution of fuchsin or with carbol fuchsin. The stain must

be allowed to work a considerable time. The reaction with Gram's method is negative. Sections may be stained with Loffler's methylene blue solution.

Biological Characters.—The comma bacillus grows on all the ordinary nutrient media, also in the absence of oxygen (facultative anaërobe), but according to recent investigations the oxygen must not be completely absent. The culture media must possess a decided alkaline reaction, as the comma bacillus is very sensitive to the smallest quantity of acid.

The minimum temperature at which development takes place is 8° C., optimum 30° to 40° C.

On Gelatine Plates the development takes place best at 22° C., and in twenty-four to thirty hours, when examined under a low power, small yellowish-white granular knotty colonies with uneven rough edges are present, the surface looks as if it were covered with little fragments of broken glass; while the colony has a shining appearance when liquefaction commences. An ill-defined halo is first seen to surround the granular colony, which exhibits a peculiar roseate hue by transmitted light.

In Gelatine Stab Cultures development occurs along the line of inoculation, but the liquefaction occurs first only at the surface; on the second day, at 22° C., a short funnel is formed with a very narrow mouth, the upper portion of which contains air, and below this a whitish viscid mass. The funnel now increases in depth and diameter, and in four to six days may reach the edge of the test-tube; in eight to fourteen days the upper two-thirds of the gelatine is liquefied, and in a few weeks the gelatine is completely liquefied.

On Agar Plates the growth is not so characteristic as with gelatine media; the surface colonies exhibit a peculiar light greyish-brown transparent appearance.

On Agar Stroke Cultures a greyish-white, moist, shining coating develops.

Blood Serum is liquefied slowly.

On Potatoes placed in the incubator a thin semi-transparent brown or greyish-brown layer is developed. In some potatoes no growth takes place, but development takes place if the potatoes are rendered alkaline with a solution of soda, or cooked in a 3 per cent. solution of common salt.

Milk is a favourable culture medium.

Bouillon is clouded, and in the majority of cases, when placed in the incubator, a thin membrane forms on the surface of the medium.

Like most vibrios and spirilla the comma bacillus possesses the peculiarity of being able to multiply very energetically in bouillon reduced with six to eight parts of water. It also develops in 1 per cent.

watery solution of peptone, to which $\frac{1}{2}$ per cent. of chloride of sodium is added, and if the peptone used is not alkaline, then the medium must be rendered alkaline with a solution of soda.

Specific Reactions.—When a drop of pure reduced hydrochloric or sulphuric acid is added to cultures of cholera bacilli grown in peptone media, a rose or purple-red colour results, which is known as the nitroso-indol reaction. The comma bacilli possess the faculty of first forming indol, and then changing the traces of nitrates in the culture solution into nitrites. Other vibrios exhibit the nitroso-indol reaction besides the cholera vibrio, *i.e.*, vibrio Mentschnikoff—vibrio Berolinesis (see section on Water Bacteria, page 206). Finkler and Prior's vibrio and Denecker's cheese vibrio also form indol, but no nitrite, the addition of a pure acid that does not contain nitrous acid producing no red colour. In bouillon, under certain circumstances, the reaction fails when either too much or too little nitrates are present.

There are two methods whereby genuine cholera bacilli are differentiated from other similar vibrios, known as Pfeiffer and Gruber's reactions.

(1.) *Pfeiffer's Reaction* is produced as follows :—Some blood serum of a guinea-pig or other animal rendered immune to cholera is reduced with ordinary bouillon in the proportion of 1 to 100, and in 1 c.c. of the above mixture, a platinum loop (capable of holding about 2 mg.), full of the vibrio species under investigation, is added, and the inoculated mixture injected into the peritoneal cavity of a guinea-pig weighing about 200 grams. Every five minutes some of the peritoneal effusion forming is removed by means of fine glass capillary pipettes, and examined, both stained and unstained. If it is the genuine Koch's comma bacillus, they will be observed to become non-motile first, then transform themselves into small balls, which finally, in about twenty minutes, become quite loosened. When the above phenomena are absent, then the vibrio belongs to another species. To prevent an error arising, a control guinea-pig is inoculated intraperitoneally with 1 c.c. of normal serum bouillon mixture, 1 to 100, to which one loop of the suspected culture is added. If in twenty minutes the peritoneal effusion contains living motile bacilli (which were killed with immune serum), then the diagnosis of Asiatic cholera can be given with safety.

(2.) *Gruber's Reaction.*—A small portion of the vibrio culture under investigation is placed with the serum of an animal vaccinated against cholera in the proportions of 1 to 50, 1 to 100, and upwards. The mixture is examined at once with a high power. If the vibrio become non-motile, flock together in herds, and agglutinate, then the bacilli are without doubt genuine cholera vibrio.

A macroscopic agglutination can also be procured by inoculating bouillon with the suspected vibrio and adding cholera-immune serum in

the same proportions as above. If in sixteen to twenty-four hours the vibriones are rolled together in flakes at the bottom of the reagent glass, and the upper portions of the fluid remain clear, then the reaction is the same as that exhibited by the genuine cholera vibrio.

Vitality.—The comma bacilli do not exhibit much resistance. They are destroyed in water heated to 52° C. in four minutes. They withstand lower temperatures better; in ice they lose their vitality in a few days. The addition of 0·07 to 0·08 per cent. hydrochloric or nitric acid to neutral culture media prevents their growth; this explains why normal gastric juice, which contains about 0·2 per cent. of hydrochloric acid, exhibits an insurmountable obstacle to the cholera bacilli. When spread in a thin layer and dried they lose the faculty of further development in three hours. In moist surroundings under favourable circumstances Koch's bacilli live a long time—about nine months. In six months old agar and gelatine cultures they sometimes still retain their vitality. Weak solutions of the ordinary antiseptics kill the cholera bacillus very quickly; a $\frac{1}{2}$ per cent. solution of carbolic acid kills them in a few minutes. In the dejections of cholera patients the bacilli sometimes remain alive for weeks, but this occurs only under extremely favourable circumstances. The cholera bacillus multiplies to some extent in sterilized river or well water, preserving its vitality in such water for several months. In milk and water containing other bacteria it dies out in a few days. In greatly diluted bouillon media the cholera bacilli may take the precedence of the common saprophytic bacteria, forming upon the surface of the medium the characteristic film.

The comma bacilli only survive for a few days when mixed with normal fæces.

Pathogenesis.—The introduction of cholera bacilli into the stomach of man may cause no bad result, but sometimes a more or less intensive diarrhœa results (self-infection by Pettenkoffer and Emmerich), and in other cases genuine dangerous cholera with all the clinical symptoms. A young doctor died in Hamburg of typical cholera, caused by a drop of peritoneal exudate containing vibrio getting into his mouth during the demonstration of Pfeiffer's reaction. Subcutaneous injection with cholera bacilli in man only causes local symptoms and slight fever. According to Klemperer the blood thereby acquires immunizing properties.

A disease resembling cholera can be produced in guinea-pigs by direct introduction of the vibrio into the duodenum—evading the stomach and tying the gall-duct—or by introduction of the bacilli into the stomach, previously rendered alkaline with a solution of soda, and at the same time injecting 2 to 3 c.c. of tincture of opium into the peritoneal cavity. Tying the gall-duct and injecting tincture of opium into the peritoneal cavity interrupts the peristaltic action of the bowels.

Bacteriological Diagnosis.—(1.) *Microscopical Examination.*—

Cover-glass specimens are prepared from the mucus in the fæces, stained with a reduced solution of carbol fuchsin. The diagnosis of Asiatic cholera is very probable when the individual bacilli appear to lie behind each other in a direction like a small swarm of fish in a slowly running stream, but, nevertheless, culture experiments must be instituted.

(2.) *Examination by Cultures.* — Gelatine plate cultures are instituted from the fæces, when possible from a flake of mucus. The usual reductions are made (see Technique, § 108) for the special characteristic growth on gelatine of the colonies of Koch's bacillus (see Biological Characters, p. 154).

(3.) *The Peptone Water Culture Method of Koch and Schotellius.* —Besides the institution of plate cultures, peptone water cultures must be prepared, because in cases where the cholera bacilli are not numerous they do not develop, being overcome by the ordinary fæces bacteria.

An Erlenmeyer flask containing 1 per cent. peptone and ½ per cent. common salt solution is inoculated with a platinum loop of the suspected fæces or mucus and placed in the incubator at 37° C. As soon as the fluid exhibits the slightest trace of turbidity, which generally occurs in six to ten or twelve hours, a portion is removed from the surface and examined in a *hanging-drop*, and cover-glass specimens prepared. If a pure culture is obtained, then the diagnosis is assured. It is not always so simple, as the surface growth is sometimes contaminated with other bacteria, most frequently the Bacterium coli commune, and it is therefore necessary to make plate cultures, and by this means isolate the Koch bacillus, which has now increased in the peptone solution, and numerous colonies develop in the Petri-dishes.

Pure cultures are now prepared from the plate cultures, and tested by the nitroso-indol reaction, as well as by the previously mentioned Gruber and Pfeiffer reaction. Animal experiments are also instituted. Should all these tests yield positive results, then the diagnosis is conclusive and certain. Agar plates can be used instead of gelatine plates, and possess the advantage that they can be placed in the incubator at 37° C. and examined in eight to ten hours.

THE EXAMINATION OF WATER FOR CHOLERA BACILLI.

It is necessary, to obtain satisfactory results, to use enormous quantities of the suspected water. About 100 to 1000 c.c. of the

suspected water is placed in sterile flasks, and to each sample 1 per cent. alkali peptone (Witte's peptone is the best) and ½ per cent. of common salt is added. The peptone and salt are kept ready for use in sterile solutions. The mixture, after its alkalinity is tested, is placed in the incubator and examined and tested in the same manner as the previously mentioned peptone water-culture method of Koch and Schotellius.

SPIRILLUM OF FINKLER AND PRIOR.

(Vibrio Proteus.)

This vibrio was isolated from the dejections of patients with *cholera nostras* which had been allowed to stand for some days, but it has since been proven to possess no etiological significance in that disease. To-day it is only of historical interest, the cultures being transferred from tube to tube in the laboratory.

Microscopical Appearances.—It bears a great resemblance to the vibrio of the Asiatic cholera, but the curved segments are somewhat longer and thicker, and not so uniform in diameter, being often thicker in the middle than at the poles. The spiral filaments are not so numerous, and also shorter than those formed by the cholera vibrio. In unfavourable media involution forms are common.

Motility.—Strongly motile, possesses a single flagellum at one end (Monotricha). (See Photomicrograph, Plate II., Fig. 9, stained by the author's orcëin method).

Staining Reactions.—Stains with the ordinary aniline dyes, best with an aqueous solution of fuchsin.

Biological Characters.—Aërobic and facultative anaërobic liquefying vibrio ; grows at ordinary room temperature.

On Gelatine Plates small white punctiform colonies develop in twenty-four hours, which under microscopic examination are seen to be finely granular and yellowish or yellowish-brown in colour ; the gelatine around the colonies liquefies rapidly, and when the organism is abundant, liquefaction is usually complete in twenty-four hours. Isolated colonies on the second day form saucer-shaped depressions in the gelatine.

In Stab Cultures liquefaction progresses much more rapidly than with the cholera vibrio, a stocking-shaped pouch of liquefied gelatine appearing in two days, the whole medium being liquefied in about a week ; a whitish film forms on the surface of the liquefied medium.

On Agar Media a moist shiny layer covering the entire surface is quickly developed.

On Blood Serum the growth is rapid and causes liquefaction of the medium.

On Potato at room temperature a shiny, greyish-yellow, glistening layer occurs, soon spreading over the surface of the potato. (*The vibrio of Asiatic cholera exhibits no growth on potato at room temperature.*) The cultures give off a strong putrefactive odour, and in media containing sugar produce an acid reaction.

Pathogenesis.—Pathogenic for guinea-pigs when injected into the stomach previously rendered alkaline with soda.

SPIRILLUM TYROGENUM.

(Vibrio Deneke.)

This vibrio was obtained by Deneke from old cheese.

Microscopical Appearances.—Curved rods and long spiral filaments resembling the cholera vibrio, the diameter of the commas being uniform throughout, so that it more closely resembles the cholera vibrio than does that of Finkler and Prior.

Motility.—Strongly motile, possessing a single flagellum at one end (Monotricha).

Staining Reactions.—Stains with the usual aniline dyes, best with an aqueous solution of fuchsin.

Biological Characters.—Aërobic and facultative anaërobic liquefying vibrio, growing at ordinary room temperature more rapid than the cholera vibrio, but not so rapid as the vibrio proteus of Finkler and Prior.

On Gelatine Plates small punctiform colonies develop, which on the second day are about the size of a pinhead and have a yellowish colour; examined under a low power they appear coarsely granular, yellowish-green coloured in the centre and paler towards the margins; funnel-shaped cavities are formed when liquefaction commences.

In Gelatine Stab Cultures liquefaction takes place along the inoculation track, the vibrios sinking to the bottom of the medium in a mass, while a thin yellowish layer forms upon the surface; complete liquefaction takes place in about two weeks.

On Agar a yellowish-white coating is formed on the surface.

Blood Serum is quickly liquefied.

In Bouillon or Peptone Solution the nitroso-indol reaction is awanting.

Pathogenesis.—Fifteen guinea-pigs were treated with soda and tincture of opium, the same as Koch's method with Asiatic cholera, and the vibrio introduced into the intestines, but only three of the infected animals succumbed.

MILLER'S SPIRILLUM.

This spirillum was obtained by Miller from a carious tooth. *Morphologically* it is indistinguishable from the spirillum of Finkler and Prior.

Motility.—Non-motile.

Staining Reactions.—Same as the vibrio proteus.

Biological Characters.—*On Gelatine Plates* small transparent pits of liquefaction appear in twenty-four hours; in the centre of the colony a minute white speck can be seen. Examined under a low power, the larger colonies are granular and regularly round, and usually surrounded by a peripheral zone somewhat darker than the central portion of the colony. On close examination the circumference can be observed fringed with short cilia-like growths usually twisted in all directions. *The deeper colonies* are round, sharply circumscribed, of a pale yellowish or greenish-yellow colour, and marked by delicate irregular lines or ridges. In forty-eight hours the plate, containing many colonies, is liquefied.

In Stab Cultures the gelatine is liquefied very quickly along the inoculation track.

On Agar the growth exhibits nothing characteristic.

On Potato, like the vibrio of cholera, at 37° C. it forms a dry white patch on the surface, often only visible when the tube is held to the light in a special way.

In Bouillon it forms no pellicle.

Solid Blood Serum and *Egg Albumen* are liquefied.

Glucose is not fermented. Indol is not produced.

Milk containing blue litmus tincture is almost completely decolorized in from three to four days at 37° C., with coincident coagulation of the casein and the formation of a layer of whey about it.

Pathogenesis.—Out of twenty-one animals previously treated by Koch's method with soda and tincture of opium before infection, only four died.

(*The other vibrios are to be found in the section on Water Bacteria.*)

SPIRILLUM OF RELAPSING FEVER.

(Spirochæte Obermeieri).

Found by Von Obermeier in the blood of a febris recurrens patient.

Microscopical Appearances.—Long, wavy, flexible threads, with

ten to twenty 'throws' in its length. The length varies from 16 to 40 μ, and the diameter about $\frac{1}{3}$ to $\frac{1}{4}$ of the comma bacillus.

Motility.—Strongly motile, exhibiting undulations which pass along the threads like a wave.

Staining Reactions.—Somewhat easily stained with fuchsin, alkaline, methylene blue, and Bismarck brown. Some spirilla, owing to their fineness, are only visible with a high power and strong illumination, while, when present in masses in the blood, they are easily seen in both unstained and stained preparations.

Biological Characters.—Outside the body, in blood serum and $\frac{1}{2}$ per cent. chloride of sodium solution, they retain their motility for a long time. They have, however, never been cultivated on artificial media.

Pathogenesis.—Monkeys are susceptible when inoculated with human blood containing the spirillum, a typical fever being produced during the height of the same. Great numbers of the spirilla were present in the blood, but were not either before or after. They were also found in the organs of animals killed during the height of the fever.

The disease could be conveyed from one monkey to another, but only with blood containing spirilla. The typical fever was again produced in a monkey which received a second injection in a few days or weeks after recovery from the first injection (Koch and Carter).

VIBRIO RUGULA.

Found in fæces, water, and deposit on the teeth.

Microscopical Appearances.—Slightly bent, finely granular, motile rods, with flagella (see Photomicrograph, Fig. 57) situated at the end of the organism in bundles (Lophotricha).

Biological Characters.—Optimum temperature about 37° C.

On Gelatine Plates it forms irregular white colonies, the surface colonies consisting of delicate tufts.

On Agar the growth on the surface of the medium does not possess great vitality, the development in the condensation water being much stronger.

On Blood Serum, which is not liquefied, the growth is luxuriant.

Sterile Milk is not altered.

Indol formation has not been observed.

It is *non-pathogenic.*

SPIRILLUM UNDULA.

Found in putrid fluids, especially in infusions of straw. Thick, strongly-motile spirilla, the flagella arranged in a bundle at one pole (Lophotricha). (See Photomicrograph, Fig. 58.)

Biological Characters.—The optimum temperature appears to be between 22° and 27° C.

On Gelatine Plates it forms sharply defined, granular, greenish-yellow colonies, while the medium appears to be slightly liquefied.

In Gelatine Stab Cultures, on the upper portion of the puncture, a veil-like clouding of the medium occurs, while round the opening of the puncture a whitish growth with ragged tufted edges develops.

On Agar Media the water of condensation is clouded, but no film is formed. Kutscher distinguishes two forms—Spirillum undula majus, and Spirillum undula minus.

BACILLUS DIPHTHERIÆ.

This bacillus was first observed by Klebs (1883) in diphtheritic false membranes. It was cultivated in pure cultures, and its pathogenic properties demonstrated by Löffler in 1884.

Microscopical Appearances.—Somewhat plump rods of variable sizes, 1 to 6 μ long, and 0·5 to 1 μ broad, either straight or slightly curved, with rounded ends. Irregular forms are very common, and indeed are characteristic of this bacillus. In the same culture and in unfavourable media great differences in form and dimensions occur; one or both ends may appear swollen (see Photomicrograph, Plate IV., Fig. 19, in the centre of the field), or the central portion may be thicker than the extremities, or the rod may consist of irregular, spherical, or ovoid segments. The rods sometimes also lie in clusters alongside of each other in a characteristic manner, like a bundle of faggots or a spilled box of matches. They also occur in branched forms, but this condition is comparatively rare.

Spore Formation absent, but the cultures remain alive for five months.

Motility.—Non-motile.

Staining Reactions.—The best results are obtained with methylene blue, or a weak solution of carbol fuchsin; gentian violet stains too intensely, obscuring the structure of the organism. The reaction with the Gram and Cladius methods is positive. Roux's double stain (see Technique, p. 41) also stains the bacilli very well. Neisser has recently

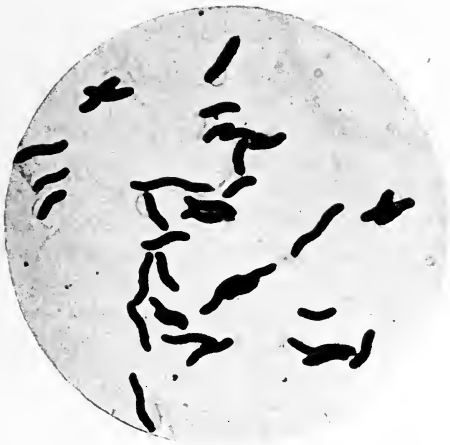


Fig. 57.—Vibrio Rugula, with Flagella. Agar culture. Stained with Orcein solution. × 1000.

Fig. 58.*—Spirillum Undula, with Flagella. × 1000.

* I am indebted to Herr Hänsel, 29 Dorothean Strasse, Berlin, for this specimen.

described a double stain (see Technique, p. 23) which can be used as a means of differential diagnosis. In sections the bacilli can be stained by Löffler's method, also by the Gram and Cladius methods.

Biological Characters.—*Aërobic* and *non-liquefying*, growing most freely in the presence of oxygen, but is also a *facultative anaërobe*. The growth takes place only between 20° and 42° C. on all slightly alkaline media; the optimum temperature is about 35° C.

On Gelatine Plates.—Small, round, white colonies develop, which under a low power appear yellowish-brown in colour, and granular, with irregular borders, rarely reaching a diameter of over 1·5 mm.; the development is very slow, nothing very characteristic being observed in less than seventy-two hours.

In Gelatine Stab Cultures.—The same above mentioned; small, white, round colonies, not exceeding the above size, develop along the inoculation track. At 24° C. surface growth and indications of a nail-shaped growth appear. The gelatine is not liquefied.

On Agar.—Best on *glycerine agar* plates; in twenty-four to forty-eight hours small, greyish-white, shining colonies develop, which macroscopically often exhibit a stratified appearance; and under a low power appear granular, with irregular borders.

On Agar Stroke Cultures.—In twenty-four hours small, transparent, slightly elevated colonies appear; the further growth is very scanty, and does not extend far from the inoculation track.

In Agar Stab Cultures.—Colonies develop along the inoculation track with a minimal amount of surface growth.

In a mixture of *glycerine agar* and *human blood serum* the growth is much more pronounced and extensive (see Photograph of Culture growing in this medium, Fig. 59).

On Löffler's Serum (for preparation of same see Technique, p. 56).—In twenty-four hours somewhat large, whitish opaque colonies of firm consistence develop, which only increase slightly in size during the next few days. This medium is the best for diphtheria bacilli, and is always used for differential diagnosis.

Bouillon.—In this medium the bacillus grows in fine clumps, which fall to the bottom of the tube, or are deposited on the sides without causing any clouding of the medium. The bouillon may appear diffusely clouded to the naked eye, but when examined microscopically in a hanging-drop the clumpy arrangement is easily observed. In bouillon kept at 35° C. for some time a whitish film often forms over part of the surface. The reaction of the bouillon is subject to changes—frequently at first it is acid, and subsequently again alkaline. These changes can be observed in the medium when a little rosolic acid is added. These reactions are attributed to the primary fermentation of muscle sugar often present in the bouillon.

On Potato, which is rendered alkaline, a delicate coating forms.

Milk is a favourable medium, and is not coagulated. Owing to the fact that diphtheria epidemics appear sometimes to be spread by the milk supply, Schotellius instituted the following experiments :—1 c.c. of a bouillon culture of the Bacillus diphtheriæ was added respectively to 20 c.c. bouillon, 20 c.c. fresh milk, and 20 c.c. boiled milk. The fresh milk was obtained direct from the cow's udder, which was previously cleansed. According to the calculation of this author, 1 c.c. of each of the three culture fluids yielded the following number of colonies, as estimated by plate culture methods after six hours' cultivation at ordinary temperature :—

Fresh milk	=	21,280·000	Diphtheria colonies.
Sterilized milk	=	2,280·000	,, ,,
Bouillon	=	7,600·000	,, ,,

After longer incubation at 37° C. :—

Fresh milk	=	50,160·000	Diphtheria colonies.
Sterilized milk	=	6,080·000	,, ,,
Bouillon	=	18,240·000	,, ,,

In cooked and *raw eggs* the diphtheria bacillus develops in both the white and the yelk very well, also on solid egg albumen, when it sometimes exhibits branched forms.

Vitality.—Corrosive sublimate 1 to 1000 kills cultures in thick layers within twenty seconds ; 5 per cent. solution of permanganate of potash, 5 per cent. solution of carbolic acid, and 3 per cent. carbolic acid in 30 per cent. alcohol, in the same space of time. The pure juice of a lemon kills the bacilli very quickly. They are destroyed when heated at 60° C. for ten minutes.

In thick layers they resist drying for some months ; when in a dry state they die very quickly. They stand cold well, but in the ice-chest they rapidly lose their power of producing a toxine. According to Löffler the bacilli remain alive in gelatine cultures for 331 days. In a box of wooden bricks with which a child suffering from diphtheria had been playing itself, Abel found Löffler's bacillus six months after. It has also been found in soiled linen, hair, drinking glasses, etc. Diphtheritic membranes dried and kept in the dark are, months later, capable of producing cultures.

Pathogenesis.—Under natural conditions diphtheria does not occur in animals ; the so-called spontaneous chicken and pigeon diphtheria are etiologically different diseases. By inoculation into the trachea in cats and rabbits you get true diphtheritic symptoms—general toxæmia and death from absorption of the toxines formed at the seat of disease. Guinea-pigs inoculated subcutaneously with 0·1 to 0·5 c.c. of a bouillon culture die in from four to five days, showing the following post-mortem appearances :—Extensive œdema, hyperæmia, and ecchymosis at seat of

inoculation, lymphatic glands congested, exudation into the pleuræ, peritoneum, and pericardium. Suprarenal capsules are enlarged and show hæmorrhagic infiltration; spleen is sometimes enlarged; also fatty degeneration of liver and kidneys. Rabbits are not so susceptible, and generally recover after a small injection. When death does not follow inoculation rapidly, the visceral changes are less marked, and we often get nervous symptoms with paralysis progressing from behind forward. Among common animals rats and mice alone are immune, but MM. Borrel and Roux found that though rats were not affected by inoculation, yet if they injected the toxine into the brain the animal died from diphtheritic paralysis.

Differential Diagnosis.—In diphtheria we are specially liable to get cases of mixed infection and pseudo-diphtheria. In the former we may have streptococci, staphylococci, pneumococci, and B. coli communis.

Pseudo-diphtheria is due to bacilli which resemble very closely in their morphology the true Löffler's bacillus, but are distinguished as follows:—They are non-pathogenic for experimental animals, and when grown in alkaline bouillon do not change the reaction of the medium. The genuine bacillus changes slightly alkaline bouillon to acid, which later (after some months) again becomes alkaline.

Immunity.—Fraenkel was the first to immunize guinea-pigs against diphtheria by infecting them with a toxin modified by exposure to a temperature of 70° C.; but to Behring belongs the credit of the fundamental discovery that the blood of an animal immunised for a certain infectious disease may be employed for protective inoculation, and even in larger quantity exercise a curative influence after infection has occurred. This is one of the greatest discoveries of recent years in scientific medicine, even if the practical results attained in human infectious diseases do not justify all the expectations that were entertained regarding it. The above discovery has been specially applied to prevent and mitigate the ravages of diphtheria. A disease that can attack the same child more than once, therefore, does not belong to the class of diseases producing a permanent immunity after recovery. It is, however, well known that after recovery from diphtheria a certain temporary immunity is conferred, as the blood serum of children during convalescence has been found to possess immunizing properties.

The diphtheria toxin used for immunizing animals in the preparation of the antitoxin is obtained by cultivating the virulent diphtheria bacillus in bouillon exposed to the air, or by making cultivations in a current of moist air in 2 per cent. peptone alkaline bouillon placed in flat-bottomed Fernbach flasks, and sterilized previous to inoculation. In three weeks, or longer, the culture is rich enough in toxins to be employed. The culture is next filtered through a Chamberland filter, and the clear filtrate preserved in vessels well filled, and protected from

the light and kept at ordinary temperature. One-tenth c.c. of a toxin so prepared is usually fatal in forty-eight hours to a guinea-pig weighing 500 grammes. It loses its activity after a time, though very slowly if kept in the manner above mentioned.

There is considerable variance in the degrees of virulence of different cultures of diphtheria bacilli. This varying virulence tends to explain the protean nature of the disease and the differing character of the various epidemics. It also helps to explain the occurrence of diphtheria bacilli in 'rhinitis fibrinosa' and other benign affections.

Experiment animals are easily immunized. Behring, and later Roux, immunized horses with diphtheria toxin which had its poisonous properties weakened by the addition of a solution of trichloride of iodine, or iodide of potassium.

A serum possessing very high immunizing properties is obtained from the horse by introducing into that animal large quantities of diphtheria toxin as follows:—

ROUX'S METHOD OF IMMUNIZING A HORSE.

Subject.—Seven-year old horse, weighing about 400 kilogrammes. The toxin used was very active; $\frac{1}{10}$ c.c. killed in forty-eight hours a guinea-pig weighing 500 grammes. The point of injection was under the skin of the neck or behind the shoulders.

Days of Injection.	Injection of	Toxin with Iodide of Potash.	Reaction.
1	$\frac{1}{4}$ c.c.	1—10	No reaction.
2	$\frac{1}{2}$,,	1—10	do.
4, 6, 8	$\frac{1}{2}$,,	1—10	do.
13, 14	1 ,,	1—10	do.
17	$\frac{1}{4}$,,	Pure toxin.	Slight œdema, no fever.
22	1 ,,	do.	do.
23	2 ,,	do.	do.
25	3 ,,	do.	do.
28	5 ,,	do.	do.
30, 32, 36	5 ,,	do.	do.
39, 41	10 ,,	do.	do.
43, 46, 48, 50	30 ,,	do.	Well-marked œdema, disappearing in twenty-four hours.
53	60 ,,	do.	do.
57, 63, 65, 67	60 ,,	do.	do.
72	90 ,,	do.	do.
80	250 ,,	do.	do.

In eight to ten days after the last injection 5 to 6 litres of blood are taken from the jugular vein with a sterilized trocar, and placed in the ice-chest, when a clear serum forms.

Behring preserves the serum thus obtained by adding 0·5 per cent. of carbolic acid. Schering's serum is preserved with 0·4 per cent. of

trikresol which is considered to possess about twice the antiseptic power of carbolic acid, and is only half as poisonous, while in the Pasteur Institute they use a piece of camphor. Serum desiccated *in vacuo* is convenient to send to a distance, and reacquires its preventive properties when dissolved again in eight or ten times its weight of pure water.

Instructions for the use of Schering's Diphtheria Antitoxin.—A hypodermic syringe is used, holding 10 to 12 c.c., which, before use, must be thoroughly asepticized with alcohol and a 1 per cent. solution of trikresol.

The injection is made deep into the subcutaneous connective tissue of the skin, the back, between the shoulder blades, or the thighs, being the points of election. Before the injection is made, the skin is scrubbed with soap and water, and then with ether. After the injection the puncture may be immediately sealed with rubber plaster, or cotton and iodoform collodion, to prevent any loss of fluid through the dermal puncture.

For the immunization of children and adults in families where diphtheria has occurred, isolation being unnecessary, and also for general use among those who have been exposed in epidemics, the dose is as follows :—

Up to two years of age, 0·5 c.c. (8 minims).
From two to ten years of age, 1 c.c. (16 do.).
Over ten years, and for adults, 2 c.c. (32 do.).

If in four to six weeks the epidemic has not ended, it is well to repeat the injection.

For the Cure of Diphtheria extensive practice has shown that a single injection of 5 c.c. (80 minims) of the solution at present supplied is *certainly curative if used at the beginning of the disease.* In severer cases that have lasted some time, two injections must be given in the course of twelve to twenty-four hours, of 5·20 c.c. (80 minims to $5\frac{1}{2}$ fluid drachms), according to the body-weight. In some cases still larger doses have been successfully employed. The further repetition of the injection depends upon the following factors :—

a. The course and increase of the exudation, and the condition of the glands.
b. The course of the temperature.
c. The condition of the kidneys.
d. The condition of the pulse and the heart sounds.

BACILLUS DIPHTHERIÆ COLUMBARUM.

Obtained by Löffler in 1884 from the false membranes in the mouths of pigeons. Chickens are also affected.

Symptoms.—In pigeons, reddened patches on mucous membrane of mouth and fauces, which are covered later with a layer of thick yellow fibrinous exudation, the back part of tongue, fauces, and corners of the mouth being specially affected. In chickens the tongue, gums, nares, larynx, and conjunctival mucous membranes are the parts affected (see Photo., Fig. 60, of two Plymouth Rock chickens suffering from this disease). The disease is very fatal to young fowls, the choice varieties being most susceptible.

Microscopical Appearances.—Short bacilli with rounded ends usually grouped together. They are longer and narrower than the bacillus of chicken cholera. Sections of liver show them in irregular masses in the interior of the vessels.

Motility.—Non-motile.

Spore Formation absent.

Staining Reactions.—Stain with ordinary aniline dyes. Gram's reaction negative.

Biological Characters.—Aërobic non-liquefying bacillus.

On Gelatine Plates greyish white colonies, which under a low power resemble the typhoid bacillus.

In Gelatine Stab Cultures, grows like a nail, with a whitish head.

On Egg, Agar-Agar, Potato and *Blood Serum* it forms a greyish covering.

Bouillon is clouded, but there is no *indol* reaction.

Pathogenic for rabbits, mice, small birds, pigeons, and chickens. Rats are only slightly affected.

There are white masses of necrosed liver tissue in the livers of mice in whom the disease has been inoculated containing large numbers of bacilli in the interior of the vessels. This characteristic is considered by Löffler to be the best method of identifying the bacillus.

DIPHTHERIA VITULORUM.

Obtained by Löffler in 1884 in false membranes from the mouths of calves suffering from an infectious form of diphtheria.

Symptoms.—Yellow patches on mucous membrane of cheeks, gums, tongue, sometimes of larynx and nares, yellow discharge from the nose, excessive salivation, occasional coughing, and diarrhœa. The animal may die in four or five days, or may survive for several weeks. Diphtheritic patches like those in mouth, etc., occur in the large intestine, and sometimes abscesses are found in the lungs.

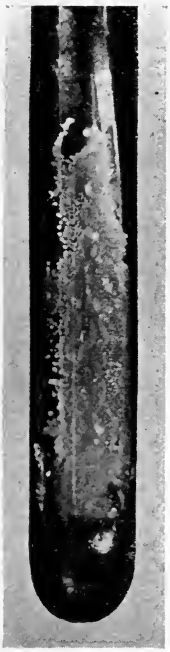


FIG. 59.—B. Diphtheriæ. Human blood serum agar culture.

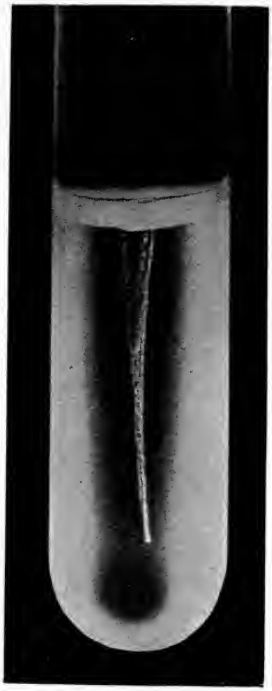


FIG. 61.—B. Typhi Abdominalis. Gelatine stab culture.

FIG. 60.—*Avian Diphtheria.* Chickens in the last stages of the disease.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh,* 1898.

Microscopical Appearances.—Bacilli five or six times as long as broad, usually in filaments.

Biological Characteristics.—This bacillus does not grow in nutrient gelatine blood serum from sheep and other usual media, but in the blood serum of calves pieces of the affected tissue gave a whitish growth of the bacillus, which, however, did not grow when transferred afresh to serum.

Pathogenesis.—Fatal for mice in from seven to thirty days when inoculated subcutaneously, the autopsy revealing extensive infiltration of abdominal walls, which often spreads into the peritoneal cavity, enveloping the viscera in a yellowish exudation. The bacilli are formed in this exudation, and mice inoculated with some of the fluid die similarly. Non-pathogenic for rabbits and guinea-pigs.

BACILLUS TYPHI ABDOMINALIS.

(Bacillus of Typhoid Fever.)

This organism was first observed by Eberth in the internal organs of typhoid cadavera. Koch also saw the bacillus about the same time, and photographed it. It was first obtained in pure cultures by Gaffky, and has also been found during life in the blood, urine, and fæces of typhoid patients.

Microscopical Appearances. — Short, plump rods with rounded ends, 1 to 3 μ long, and 0·5 to 0·9 μ broad, which in sections of tissue are usually found singly, but in cultures often found in long threads. (See Photomicrograph, Fig. 62.) In agar cultures at 37° C., in the bodies of animals, and in human tissues, the rods are more plump, and smaller in all directions than on gelatine and potato media, and the long threads occur more frequently at lower temperatures.

Motility.—Actively motile, each bacillus possessing eight to eighteen flagella situated along the sides and ends (Peritricha). (See Photomicrograph, Plate II., Fig. 7, stained by the author's orcëin method; also the same preparation × 1500 diameters, Plate II., Fig. 8.)

Spore Formation does not exist. The so-called spores of Gaffky are, according to later investigations, involution forms.

Staining Reactions.—With the ordinary aniline dyes the rods do not stain so readily as most other organisms. Watery solutions of the dyes and a weak solution of carbol fuchsin give the best results when slightly heated during the staining process. They do not stain by the Gram method. Small vacuoles are sometimes present in the rods, due to retraction of the protoplasm from the cell envelope of the bacilli.

The bacilli are easily detected in the tissues, especially in pieces of the liver or spleen, where they can be observed massed together in characteristic clumps when stained with carbol fuchsin at the ordinary room temperature, or at 40° to 45° C., washed in absolute alcohol, cleaned in xylol, and mounted in xylol balsam. Alkaline methylene blue can also be used. The characteristic clumps in the tissues consist of small ovals or rods closely packed together, individual bacilli being often only visible at the periphery of the mass, usually in the neighbourhood of a capillary.

Biological Characters.—It grows at any temperature between 20° and 38° C. on the ordinary nutrient media. The growth is most luxurious at incubator temperature, while at ordinary room temperature the development is very slow. It also grows both with and without oxygen (facultative anaërobe), and fairly well in CO_2; and as a contrast to most other pathogenic bacteria, grows luxuriantly on slightly acid media.

On Gelatine Plates the deep colonies are small, punctiform, and sharply circumscribed; under a low power they exhibit a brownish colour. The superficial colonies are much larger, forming a bluish-white, iridescent, fine coating with irregular borders, denser in the centre than at the periphery, and under a low power exhibit a brownish colour and wrinkled appearance. The gelatine is not liquefied.

In Gelatine Stab Cultures the growth is mostly limited to the surface, with limited, thready, granular growths down the track of the needle, often of a yellow or yellowish-brown colour (see Photograph, Fig. 61).

On Gelatine Stroke Cultures a fine, iridescent, bluish growth extends from the centre, and soon covers the whole surface of the gelatine. The gelatine is not liquefied.

On Agar and *Blood Serum Media* an extrusive, thick coating develops which presents no typical characteristics.

On Potato.—The growth upon the surface of a cut potato appears as if nothing had developed; but if examined, it will be found that the whole surface of the potato is covered with tufts, which, when examined microscopically, will be found to consist of numerous motile rods. This peculiarity of growth occurs, as far as is known at present, exclusively in this bacillus. Sometimes, though rarely, the growth is visible, for there are some kinds of potatoes upon which the bacilli developed a raised circumscribed tuft of a yellowish or brownish colour. These potatoes possess either a neutral or alkaline reaction, while the typical growth is confined to those exhibiting an acid reaction.

In Milk Media an acid reaction results from the growth without causing coagulation, whereas the coli commune causes an acid reaction and coagulation in twenty-four to forty-eight hours at 37° C.

Bouillon is clouded, with a quantity of sediment and slightly acid reaction.

In Grape, Milk, and Cane Sugar Media no fermentation takes place, and according to Hellstrom, in media containing 4 per cent. milk sugar and 1 to 4 per cent. peptone, inoculated with the typhus bacillus, and kept at 37° C. for twenty-four hours, the colonies developing are much smaller than those of the coli commune under similar conditions.

On Halz's Potato Gelatine (for preparation of same, see Technique, § 79) the growth of this bacillus and the coli commune is more pronounced than that of other ordinary bacteria. To this medium Elsner added 1 per cent. iodide of potassium (see Technique, § 79). On this medium the coli commune grows more energetically than the typhoid bacillus, exhibiting dark brown colonies in forty-eight hours, whereas the colonies of the typhoid bacillus appear as clear, watery drops. This growth is however not absolutely constant, further identification with other culture methods being necessary.

Vitality.—In sterilized water the typhoid bacilli live as long as three months, and increase in numbers at first; in ordinary water they are destroyed by the concurrence of the ordinary water bacteria in about fourteen days; in running water this takes place more quickly. Under favourable circumstances, protected from light and drying, they live a long time. In fæces they appear to live three months or more, depending upon the number of putrefactive organisms present. They can withstand cold very well; freezing and thawing two or three times does them no harm. They are not so resistant to heat, being destroyed with certainty in ten minutes at 60° C., and in a shorter time at higher temperatures.

Specific Reactions.—1. The indol reaction does not exist except by Peckham's method of repeated transplantation at short intervals into either Dunham's peptone solution, or freshly prepared alkali tryptone solution. Chantemasse has also seen a red colour produced in old cultures with the addition of the indol reagents.

2. Produces no formation of gas in the culture media. This test is made with grape sugar bouillon and a fermentation tube at 37° C.

3. On lactose-litmus-agar, pale blue colonies develop with no reddening of the surrounding medium; but if glucose is used instead of lactose, both the colonies and the surrounding medium become red.

4. *Gruber's Reaction.*—When immune serum is added to a bouillon culture of the suspected bacillus in the proportion of one to forty, in twenty-four hours, if the organism is the typhoid bacillus, appearances of agglutination are evident; the bacteria form granular masses at the bottom of the test-tube, while the upper portions of the medium remain clear. These phenomena can be further examined microscopically, and the agglutination and loss of motility of the organisms confirmed.

Widal's Reaction.—The following is the method of making the test:—A drop of blood is taken from the ear or finger of the suspected typhoid

in a U-shaped tube and centrifugalised, the tube is broken off at the junction of the serum and corpuscles, and the drop of serum blown on to a glass slide. The necessary quantity is sucked up to the first mark on a special straight capillary tube, with another mark corresponding to sixteen times the volume at the first mark. Bouillon is then sucked up with the serum until the second mark is reached, and the whole blown on to a glass slide, mixed, and again sucked up; and the process repeated two or three times to ensure thorough mixing. The emulsion of typhoid fever bacilli is prepared by taking a small platinum loop full of a culture not more than twenty-four hours old, grown on rather dry agar, and carefully rubbing it up against the side of the glass tube containing 1 c.c. of bouillon with 1 drop of the bouillon, and subsequently mixing it with the whole quantity. A control preparation is examined microscopically to make sure there are not any, or very few pre-existent clumps in the emulsion. A small drop of the diluted serum is then placed on a cover-glass, and a drop, as near the same size as possible, from the typhoid emulsion mixed with it, and a hanging-drop specimen prepared in the usual manner. Microscopically examined, the bacilli will be observed to gradually form groups of three or four, which, with the addition of other bacilli, constantly increase in size, until the majority are in 'clumps' with impaired or lost motility. If the reaction is marked within thirty minutes the case is one of enteric fever, but without great experience it is impossible to say that the absence of this reaction negatives such a diagnosis. In negative results more than one examination should be made, for it occasionally, although rarely, occurs that probably from experimental errors, such as varying quality of bouillon, etc., that the reaction is seen on one day and not on another.

Wyatt Johnston has published results got by using a watery solution of long-dried blood serum from typhoid patients. The crust of blood is covered with a drop of water, and on standing for one or two minutes a drop of this is mixed with one loopful of a typhoid culture, a second loopful being added later. The agglutinative action is seen as in the ordinary way of doing Widal's reaction.

In 1897, the author, whilst working this test in California with Prof. Kerr of the California University, found that quite good results were got by using filter paper to absorb the blood, and then making a watery solution as above. This method was much more convenient for most ordinary cases than the capillary tubes with centrifugalisation.

The Investigation of Water for Typhoid Bacilli.—This is essential in all outbreaks of typhoid fever, for water has been repeatedly shown to be the vehicle of infection. For this examination, carbolic acid is added to the water in the proportion of 0·05 to 0·25 per cent., as this addition serves to inhibit the development of the ordinary liquefying water bacteria, while the typhoid and some allied forms grow in presence of a

small amount of phenol. Plate cultures are then instituted with the carbolized water after the method of Elsner.

In this examination it is apparent that to get positive results large quantities of the water may have to be examined, and this is best done thus:—An alkaline sterilized solution made with peptone and common salt, containing 1 gramme of each in a given volume, is taken. With this you mix 100 c.c. of the carbolized water supply, and place in sterile Erlenmeyer flasks, and put in the incubator for eighteen to twenty-four hours. Under these conditions the growth of any typhoid bacilli is allowed to go on practically without competition, and the bulk of the mixture gives greater chances of their appearing in plate cultures.

This system enables one to separate out chiefly typhoid and pseudo-typhoid forms as well as coli commune, which is really more resistant to phenol than is typhoid itself, and further researches must be instituted with the individual colonies to get absolute differentiation.

Pathogenesis.—When some virulent culture is introduced in mice, guinea-pigs, rabbits, and goats, death occurs with the following symptoms:—Spasms, falling temperature, and diarrhœa. By subcutaneous injection large quantities of the culture are necessary, but by intraperitoneal and intravenous methods a small quantity is sufficient. Experimental results in the lower animals show that in most cases death occurs without the appearance of typical pathological changes, the fatal result in most cases being due to toxic rather than to infective action of the virus. Cygnaeus introduced typhoid bacilli into the tissues of dogs, rabbits, and mice, and produced changes in the small intestines, histologically and macroscopically analogous to those found in the human subject. Abbot obtained only one positive result out of a large number of experiments, producing an ulcer in the ileum of a rabbit, macro- and microscopically identical with that found in man. Cultivations were obtained from the spleen, and the typical bacilli demonstrated in characteristic clumps in sections of the same. Sanarelli found that rabbits, guinea-pigs, and mice were rendered susceptible to infection when first inoculated with the products of the growth of certain saprophytes, Proteus vulgaris, Bacillus prodigiosus, and Bacillus coli communis, and when subsequently fresh cultures of the typhoid bacillus were introduced intravenously or into the peritoneal cavity, death resulted in twelve to forty-eight hours, with well-marked pathological changes in the digestive tract, especially in the small intestines. The infection is general in those cases, and the bacilli can be recovered from the blood and internal organs. Sanarelli considers that the toxic condition produced by the absorption of the products of the saprophytes may be analogous to a similar condition

occurring in man from absorption of abnormal products of fermentation from the intestinal canal. An auto-intoxication whereby the resistance of the individual to infection by the typhoid bacillus, should it gain access to the alimentary tract, is reduced.

BACTERIUM COLI COMMUNIS.

(Bacillus Neapolitanus. The Colon Bacillus.)

This bacillus is a normal inhabitant of the intestines of man, cattle, swine, and dogs. It is also found associated with diseased conditions, such as inflammatory and suppurating processes in the peritoneal cavity, infectious enteritis, affections of the liver, puerperal fever, broncho-pneumonia, empyema, endocarditis, meningitis, cystitis, and pyelo-nephritis.

Microscopical Appearances.—Occurs in short motile rods, mostly in pairs (see Photomicrograph, Fig. 64), sometimes in threads, and vacuoles are frequently present in the middle of the rod as unstained spots.

Motility.—The rods possess numerous long flagella (see Photomicrograph, Plate II., Fig. 11).

Spore Formation is absent.

Staining Reactions.—Stains with the ordinary aniline dyes, but not by either the Gram or Cladius method.

Cultivation.—In cultures the organism grows under both aërobic and anaërobic conditions, and in the latter condition forms gas in the media.

On Gelatine Plates it forms iridescent colonies with wavy bent borders on the surface, while the deeper colonies are round and of a brownish colour; the gelatine is not liquefied.

In Gelatine Stab Cultures it grows in the form of a nail with limited growth along the track of the needle (see Photograph of Culture, Fig. 63).

On Agar-Agar it forms a grey coating.

On Potato the growth is of a brownish colour.

In Bouillon it causes diffuse clouding.

In Milk Media it causes coagulation, which generally takes place in about thirty-six hours.

Special Reactions.—Indol is formed by adding 1 c.c. of a 2 per cent. solution of potassium nitrite and a little sulphuric acid to 10 c.c. of a bouillon culture, when a rose-red colour results. It also produces indol in Dunham's peptone solution in forty-eight to seventy-two hours. This organism also causes fermentation in milk sugar, grape sugar, and glycerine media.

On Lactose-Litmus-Agar, the colour of the colonies is pink, and that of the surrounding medium blue-red.

Pathogenesis.—Affects mice, guinea-pigs, and rabbits, the strength

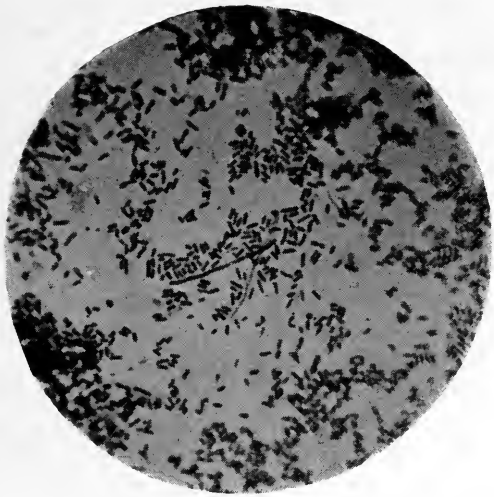


FIG. 62.—B. Typhi Abdominalis. Agar culture. Fuchsin. × 1000.

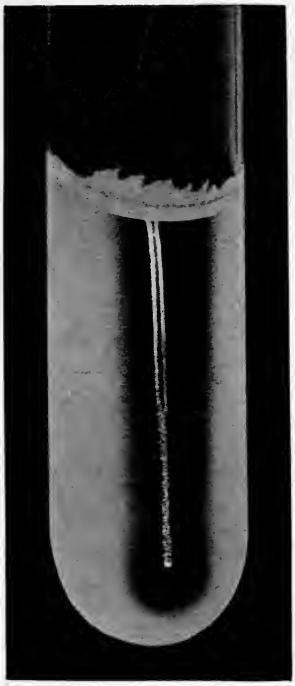


FIG. 63.—B. Coli Communis. Gelatine stab culture.

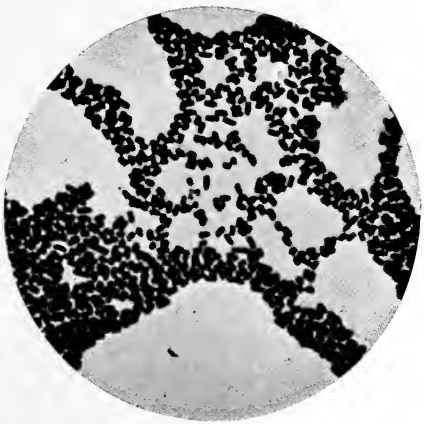


FIG. 64. B. Coli Communis. Agar culture. Fuchsin. × 1200.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

of the virus varying according to the severity of the processes from which it was originally isolated.

For the differentiation of the Bacillus coli communis and Bacillus typhi abdominalis, Piorkowski institutes cultures in bouillon, gelatine, and agar, prepared with urine, to which the desired quantity of peptone, gelatine, and agar is added; sterilization, etc., being accomplished in the ordinary manner.

Cesaris-Demel (*Giornale della R. Accad. di Medicina di Torino*, 1898, No. 3) describes a new method for the differential diagnosis of the typhus bacillus and the B. coli communis as follows:—

Cultures of the organisms are instituted in bouillon prepared from a calf's liver. The sugar present in the liver will be fermented by the B. coli communis, and it will exhibit a quick luxurious growth, while on this medium the growth of the typhus bacillus is confined to very narrow limits.

DIFFERENTIAL DIAGNOSIS TABLE.

	Bacillus Typhi Abdominalis	Bacterium Coli Communis
(1) Motility	Usually very pronounced; large numbers of flagella, staining without much difficulty with orcëin	As a rule not very pronounced, sometimes absent; flagella demonstrated with difficulty, fewer and shorter
(2) Cultures	On gelatine plates the typhoid colonies develop somewhat slowly, while on potatoes they are as a rule invisible (not always) Does not coagulate milk with an acid reaction	On gelatine the colon bacillus develops more rapidly than the typhoid, and on potatoes it grows luxuriantly and is always visible Coagulates milk with acid reaction in thirty-six to forty-eight hours in the incubator
(3) Fermentation	Causes no gas formation in media containing glucose, lactose, or saccharose	Forms gas in glucose, lactose, and saccharose media
(4) Changes observed in agar or gelatine medium containing lactose and litmus tincture of slightly alkaline reaction	The colonies are of a pale blue colour, and there is no reddening of the surrounding medium	The colonies are pink, and the surrounding medium red
(5) Indol test	Does not *as a rule* produce indol in solutions of peptone	Produces indol in solutions in forty-eight to seventy-two hours at 37-38° C.
(6) Widal's reaction	When a twenty-four-hour-old bouillon culture is placed in contact with a genuine case of typhoid fever, after the fifth day of the disease the characteristic agglutination or clumping occurs	The reactions toward this test *are negative*
(7) Pfeiffer's reaction with typhoid serum	*Positive*	*Negative*

Kashida has recently observed that the acid produced by the colon bacilli in cultures becomes neutralised in a little time, an alkaline reaction soon appearing, which was so strong that colonies on litmus milk sugar agar plates were coloured an intense blue, and when rosolic acid was used as a reagent, the colonies were coloured red. With the Bacillus typhi abdominalis the above characteristic is awanting. Investigations of the condensation water of agar cultures showed that the alkaline reaction of the colon bacilli was due to the formation of ammonia. This was very distinct in cultures on $1\frac{1}{2}$ per cent. meat infusion agar to which 2 per cent. milk sugar, 1 per cent. urea, and 30 per cent. litmus tincture were added.

BACILLUS EQUI INTESTINALIS.

Found by Dyas and Keith in the intestines of a horse. It is distinguished from the coli communis as follows: It is somewhat thicker, does not grow at a low temperature, and in fermentation tubes produces no gas, but it coagulates milk in one to two days.

Systematic examination of the alimentary contents of both man and animals shows that numerous bacteria are present; but Nuttall and Thierfelder discovered in a course of experiments that animals could live without the presence of bacteria in the alimentary canal. The experiments were conducted with young guinea-pigs, which were born by *Cæsarean section* to prevent bacteria entering their economy, nourished with sterile food, killed in eight days, and examined for bacteria, the results being negative.

INFLUENZA.

Pfeiffer discovered the bacillus and isolated it in pure cultures during the epidemic in 1891–92. Pfeiffer's discovery has been fully confirmed by others.

Microscopical Appearances.—Extraordinarily small bacilli (0·2 to 0·5 μ), and only two to three times as long as broad, with rounded ends. It very seldom forms threads in sputum, but frequently in fresh pure cultures; in three to four-day-old cultures involution forms are already visible. Two particularly small bacilli are frequently observed arranged close together, causing them to be easily mistaken for diplococci. (For Photomicrograph of the bacilli in sputum, see Fig. 65.)

Motility.—Non-motile.

Spore Formation does not appear to exist; it has never been observed in either secretions or cultures, and, moreover, the bacillus exhibits only slight resistance to heat or drying.

Staining Reactions.—The bacillus stains with difficulty. Löffler's methylene blue is a good stain, but a pale red solution of carbol fuchsin in water is better. The preparation must be stained five to ten minutes; if stained for a shorter time or with other stains the middle portion of the rod is often lighter coloured than the end portions. The results with the Gram method are negative.

Biological Characters.—The influenza bacillus is strongly aërobic, and grows only in the presence of hæmoglobin or leucocytes. These latter conditions explain why the cultivation of the influenza bacillus was so long a failure.

Pfeiffer was able sometimes to obtain cultures from lung pus direct on agar, but at other times it was impossible. The cause of the irregularity was that the rods in the first cultures developed when a trace of blood was inoculated with the material, the growth being negative when no blood was present, the same peculiarity applying to all the daughter cultivations. The influenza bacillus can be cultivated regularly and transferred through several generations in nutrient media containing blood. Blood agar is the best medium. To obtain pure cultures Pfeiffer employs the following method:—

The bronchial sputum or exudate from the broncho-pneumonic infiltrated portion of the lung in influenza-pneumonia is thoroughly emulsified with 1 to 2 c.c. of bouillon. Several platinum loops of the bouillon are inoculated, and thoroughly spread over the whole surface of blood agar media, and at the same time control cultures are instituted on ordinary glycerine agar medium.

The dilution of the bouillon has the effect of segregating influenza bacilli, so that they grow in separate colonies on the blood agar medium, while any hæmoglobin present in the original material is so thoroughly diluted, that the influenza bacillus cannot develop on the control agar tubes containing no blood.

The inoculated tubes are placed in the incubator, and in twenty-four hours the influenza colonies appear on the surface of the blood agar medium as closely compressed transparent drops. The control tubes are either sterile or contain colonies of streptococci, diplococci, or other bacteria associated with the influenza bacillus in the original material.

The transparent drops of the influenza colonies are mostly so small that they can only be distinctly seen with a hand lens. They possess a slight tendency to become confluent, and when closely arranged they coalesce into large curved limited drops, the individual arrangement of the colonies being still apparent. When the colonies are widely separated from each other, they sometimes develop as large as a pin-head, and still retain their glassy transparent appearance. The condensation water in the tubes remains usually clear, except when it is mixed with blood that has fallen down the oblique surface of the medium, then delicate white flakes develop in it.

In Bouillon mixed with blood and spread out in a thin layer the growth is somewhat abundant.

Plate Cultures are useful for the isolation of the influenza for diagnostic purposes. A little blood is added to the liquefied agar before it is inoculated, or if Petri-dishes are used the agar is allowed to set and some blood spread on the surface, and several stroke cultures made with the diluted sputum. The developing colonies have the same appearance as those in the agar tubes.

The optimum temperature for the growth of the influenza bacillus is 37° to 38° C., the maximum limit is about 42° C., while the minimum temperature is 26° to 27° C. At room temperature no growth takes place. Oxygen is always necessary for the growth of the influenza bacillus, while in presence of hydrogen and CO_2 with the addition of blood to the media no growth is manifest. Pfeiffer found that when he used blood serum or blood fibrin instead of blood the results were negative, and in further experiments he found that hæmoglobin was the necessary factor in the development of the influenza bacilli, as hæmoglobin agar was just as good a medium as blood agar. Pfeiffer also obtained positive results with the blood of rabbits, guinea-pigs, pigeons, and fish, the growth with the pigeon blood being more luxuriant and quicker than with human blood, owing to pigeon's blood being very rich in hæmoglobin.

Vitality. — Heated to 60° C. the influenza bacilli die in a few minutes. At 43° C. they cease to grow; they are not killed, for when the tubes are again placed at 37° C. they again develop colonies. In non-sterile drinking water the bacilli die in from twenty-four to thirty-six hours. On blood agar and in bouillon they live for fourteen to eighteen days, and in moist sputum they appear to retain their infectivity. They do not resist drying very well. In blood or sputum dried at 37° they are killed in one to two hours, and at room temperature in thirty-six to forty hours.

Pathogenesis.—Pfeiffer experimented with mice, rats, guinea-pigs, rabbits, swine, cats, dogs, and monkeys; and only in monkeys was he able to produce a disease simulating influenza, by inoculating them through the chest wall direct into the lungs, and also by what is a more natural infection in one monkey, viz., by introduction of the influenza culture into the nose. The disease manifested itself with fever and slight coughing for several days; an increase of the inoculated bacilli did not occur. The introduction of large doses kills rabbits somewhat quickly, the temperature falling rapidly before death; it appears in those cases to act as a poison, intoxication symptoms being manifested. Great numbers of bacilli are found in rabbits inoculated intravenously. Dead cultures mixed with chloroform act as a strong poison, a fact that tends

to elucidate the cause of the nervous phenomena frequently observed in cases of influenza in man.

Immunity.—In Pfeiffer's experiments monkeys did not react so strongly to a second injection of influenza bacilli as they did to the first; this fact he considered an indication of immunity. Man can with certainty be attacked several times with influenza, sometimes the same individual during the course of one epidemic. It follows, then, that in man there is no immunity resulting from an attack of influenza; any such condition can only be considered as temporary and only lasting a very short time.

BACILLI OF PSEUDO-INFLUENZA.

In a broncho-pneumonic centre in a diphtheritic child Pfeiffer found a bacillus which in appearance and staining reaction resembled the influenza bacillus, and also grew in a similar manner on blood agar. Similar bacilli have also been isolated by other investigators in *otitis media* and influenza. Pfeiffer considers they are allied to the influenza bacillus, and designates them pseudo-influenza bacilli. They are distinguished from the genuine influenza bacilli by their growth on culture media, being much more pronounced in all dimensions in twenty-four hours, and by their tendency to form long false filaments, a condition rarely occurring in cultures of the genuine bacilli.

The Septicæmia Hæmorrhagica Group of Bacteria.

BACILLUS BOVISEPTICUS.

(*Ger.* Bac der Wild und Rinder-seuche.)

This disease occurs in two forms: one form is characterised by œdema of the skin and subcutaneous tissue—particularly of the head—and swelling of the tongue; the second or pectoral form is characterised by pleuro-pneumonia, swelling of the interstitial tissue of the lungs, pleuritis and pericarditis. Both forms generally run into hæmorrhagic enteritis.

The mortality is about 90 per cent.

The Wild and Rinder-seuche is mentioned as identical with the Schweine-seuche, because experimentally the bacillus of Schweine-seuche was found pathogenic for calves, and the Rinder-seuche likewise pathogenic for swine in several instances.

The bacillus was first observed by Kitt, and further studied by Kitt and Hueppe, and classified as one of the septicæmia hæmorrhagica group of organisms. The Italian buffalo disease (Barbone dei bufali)

is probably identical with the Rinder-seuche, as the Italian investigator found an organism very similar to that of the Schweine-seuche, with which they inoculated the following animals with positive results: a young buffalo, a young pig, a young horse, a young cow, a sheep, also mice, rats, rabbits, guinea-pigs, pigeons, and chickens.

SEPTIC PLEURO-PNEUMONIA OF CALVES.

This disease occurs in certain localities in an enzoötic form, attacking very young calves, the affected animals dying very quickly. The lesions produced are somewhat analogous to those of pleuro-pneumonia contagiosa, but the thickening of the interlobular connective tissue is less marked, and the exudation of lymph not so abundant. There is also a want of uniformity of colour in the individual pulmonary lobules in some cases. Poels found sero-fibrinous exudates and pleuritic adhesions frequent. Vanden also mentions the occasional occurrence of inflammation of the pericardium, liver, kidneys, stomach and intestines.

Microscopical Appearances.—In the lung and muco-pus of the bronchi small ovoid organisms are present, with rounded ends 1 μ to 1·5 μ long, and 0·5 μ thick.

Staining Reactions.—Stain easily with the ordinary aniline stains, but not by Gram's method. When weak solutions of gentian violet are used, they exhibit the bipolar staining characteristic of the septicæmia hæmorrhagica group of bacteria.

Motility.—Very motile.

Biological Characters.—Grows well in bouillon and on solid media.

Pathogenesis.—Rabbits die in twenty-four to forty-eight hours either by ingestion or inoculation. Intrapulmonary injection of 1 drop produces pneumonia. Calves also died by intrapulmonary injection; sheep and dogs are immune. It is a facultative parasite capable of living in the soil, which, according to Poels, explains the presence of the disease on an infected farm.

BACILLUS DYSENTERIÆ VITULORUM.

(Bacillus of White Diarrhœa of Calves—Jensen.)

Described by Jensen as the cause of the so-called white diarrhœa or scour occurring amongst calves.

Microscopical Appearances.—Small bacilli a little larger than the chicken cholera bacillus.

Motility.—Non-motile.

Staining Reactions.—Exhibits polar staining with the ordinary reagents. By the Gram method the reaction is negative.

Biological Characters.—The growth is luxuriant on the usual media, and very similar to the B. coli communis.

On Potatoes it forms a brown-coloured shiny growth; the cultures give off an unpleasant-smelling gas.

Pathogenesis.—When new-born, or very young calves are fed with 5 c.c. of a bouillon culture, a deadly diarrhœa is produced, death occurring in the course of one to two days, the bacilli being found in the intestines and interior of the organs. In sections they were observed in clusters in the small bloodvessels.

BACILLUS CHOLERÆ COLUMBARUM.

This organism was found by Leclainche in an epidemic among wild pigeons, and is probably a variety of the fowl cholera bacillus.

Microscopical Appearances.—Similar to those of the fowl cholera bacillus, but a little larger.

Motility.—Non-motile.

Spore Formation absent.

Staining Reactions. — *On Gelatine* and *Agar Media* growth is similar to fowl cholera bacillus.

Bouillon is not clouded, but a flaky sediment is formed.

On Potatoes at 20° C. it forms a greyish-yellow layer.

Pathogenesis.—Wild pigeons are most susceptible, dying in three to six days by feeding; and in two days, when injected intravenously, the symptoms manifested are drowsiness, diarrhœa, convulsions, and septicæmia. The tame pigeon is not so liable to infection. Fowls are immune, also dogs and cats. Rabbits die in about eight days, and guinea-pigs in about ten days, when subcutaneously inoculated.

BACILLUS OF CHOLERA IN DUCKS.

This organism was found by Cornil and Toupet in an epidemic among the ducks in the Jardin d'Acclimation at Paris. The disease was characterized by diarrhœa, feebleness, and muscular tremors, resulting fatally in two to three days.

Microscopical Appearances.—Morphologically identical with the bacillus of fowl cholera.

Motility.—Non-motile.

Spore Formation absent.

Staining Reactions.—Exhibits the usual bipolar staining with the ordinary aniline stains. Does not stain by the Gram method.

Cultivation.—It is an aërobic, non-liquefying bacillus, growing in the usual culture media at room temperature. The growth in the various media corresponds to that of the bacillus of fowl cholera.

Pathogenesis.—Affects ducks, but not chickens or pigeons, and only kills rabbits when injected in large quantities. Ducks die in one to three days from subcutaneous inoculations, or by ingestion of food containing the bacillus.

BACILLUS OF FOWL CHOLERA.

(*Ger.* Bac der Hühner Cholera ; *Fr.* Cholera des poules).

This disease often occurs in poultry as an epizoötic; it is characterized by diarrhœa and death in one to two days.

Microscopical Appearances.—Very short bacilli of varying size.

Motility.—Non-motile.

Spore Formation absent.

Staining Reactions.—In cover-glass specimens the bacilli stain with the ordinary aniline dyes, more at the pole than in the middle, giving them the appearance of diplococci (see Photomicrograph, Fig. 66), but by intensive staining they appear as genuine bacilli. By the Gram method the bacilli are decolorized.

Vitality.—Exhibit slight resistance to heating and drying, but remain lying a long time in contaminated or mixed cultures.

Biological Characters.—The bacilli grow on the ordinary media at both room and incubator temperature.

On Gelatine Plates, in the deep portions of the medium, they form round, irregular, brownish discs, and on the surface the growth is slow and limited.

In Gelatine Stab Cultures the growth occurs on the surface, as well as along the course of the needle. The surface growth consists of a delicate greyish-white coating.

On Agar and Blood Serum Media a glistening whitish coating is formed.

On Potatoes it does not grow at ordinary temperature, but at higher temperatures a transparent, greyish-white, flat coating is formed.

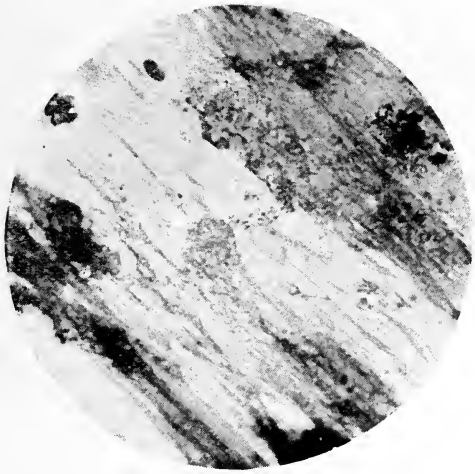


FIG. 65.—Bacillus of Influenza in Sputum. Methylene blue. × 1000.

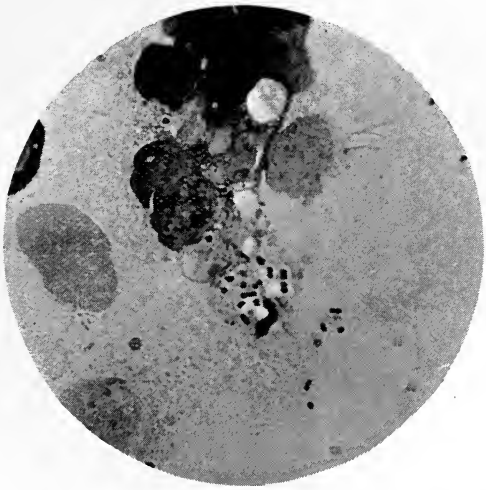


FIG. 66.—Bacillus of Fowl Cholera. Cover-glass specimen from inoculated mouse. Fuchsin. × 1000.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

Bouillon is slightly clouded.
Milk is gradually coagulated by the formation of an acid.
Litmus is reduced. *Indol* and *Phenol* are formed.

Pathogenesis.—The bacilli cause a typical septicæmia in small doses by cutaneous inoculation (and in larger doses by feeding—Günther) in pigeons, fowls, geese, ducks, pheasants, small birds, also birds of prey, rabbits, and mice. Guinea-pigs, sheep, and horses exhibit only a local reaction; suppuration at the point of inoculation. Dogs and cats can consume large quantities of the cadavers of infected animals without becoming sick. Man can also tolerate infected meat.

The bacilli are present in enormous quantities in the bloodvessels of affected animals. In pigeons, and especially in chickens, the point of inoculation is greatly inflamed, tending to necrosis. In the intestines a hæmorrhagic enteritis occurs. Chickens and rabbits exhibit pneumonic lesions. Pericarditis and hæmorrhages on the pericardium are common. The spleen and liver are also enlarged. The bacilli of fowl cholera pass from the mother to the foetus—that is, to the eggs.

Immunity.—Pasteur found that artificial cultures of fowl cholera bacilli, if left exposed to the air for a long time, lost their power of killing chickens. The inoculated birds were only locally affected, and afterwards were immune to infection with virulent cultures.

BACILLUS PHASIANI SEPTICUS.

This organism was discovered by E. Klein in an epizoötic amongst young pheasants. The author isolated a morphologically identical organism in an outbreak amongst some young pheasants, the mortality being very great. The principal lesions were catarrhal inflammation of the bowels and enlargement of the liver, with slight broncho-pneumonia.

Microscopical Appearances.—Small bacilli, very like the Bacillus coli communis, but smaller and shorter (see Photomicrograph, Fig. 67).

Motility.—Strongly motile.

Spore Formation absent.

Staining Reactions.—Easily stained with any of the ordinary aniline dyes, but not by the Gram method.

Biological Characters.—Similar to those of Bacillus coli communis, except that milk is not coagulated.

Pathogenesis.—In the natural course of the disease, death takes place among young pheasants in a few days or within a week. Young pheasants are killed in twenty-four hours with a few drops of a bouillon

culture, with symptoms of drowsiness and stupor. Diarrhœa is an inconstant symptom; septicæmia also occurs. Young chickens, pigeons, rabbits, and guinea-pigs do not die when injected with $\frac{1}{2}$ c.c. of bouillon culture.

It is distinguished from the bacillus of chicken cholera by its motility and its slight pathogenic action on the most of animals, and the fact that the bacillus of chicken cholera coagulates milk.

BACILLUS OF THE GROUSE DISEASE.

This is an infectious disease affecting *red* grouse, due to a bacillus discovered by E. Klein. The affected animals exhibit pneumonic lesions; the mucosa and serosa of the intestines are congested; the liver is also congested and dark coloured. The bacilli are present in the bloodvessels and extravasated blood, and sometimes in the heart's blood.

Microscopical Appearances.—Bacilli 0·4 by 0·6 by 1·6 μ, oval or coccus-like, and sometimes a few are rod-shaped.

Motility.—Motile in recent cultures. When some days old, only a few of the bacilli are motile.

Spore Formation absent.

Staining Reactions.—Stains with the ordinary stains, but not by the Gram method.

Biological Characters.—*On Gelatine Plates* the surface colonies are irregular, and the deep colonies are small and round.

In Stab Cultures.—Nail-like culture with flat head.

On Agar.—A thin greyish coating.

Bouillon becomes clouded.

In Grape Sugar Media gas is formed.

Pathogenesis.—Very virulent for mice, and not so virulent for guinea-pigs, by subcutaneous injection. The bacilli soon lose their virulence, but soon regain it, when cultivated in bouillon to which some small pieces of hard-boiled egg albumen is added. The yellow-hammer and finch are easily affected by subcutaneous injection, while sparrows are not so susceptible. Feeding experiments yield no positive results. Infection probably by means of the air.

BACILLUS OF THE CANARY BIRD SEPTICÆMIA.

Found by Von Rieck.

Microscopical Appearances.—Bacilli somewhat larger than the bacillus of chicken cholera, 1·2 to 2·5 μ long.

Motility.—Motile.

Staining Reactions.—Stains with the ordinary stains, most intensely at the poles. Not by the Gram method.

Biological Characters.—The growth on the various media is more luxuriant than the bacillus of chicken cholera.

On Potatoes it forms a yellow-grey coating.

Vitality.—The cultures are killed when heated for five minutes at 100° C.

Pathogenesis.—Mice die when inoculated cutaneously or subcutaneously with minute quantities, and exhibit a characteristic septicæmia. Feeding experiments yield similar results. Affected canaries exhibit a fuliginous colouring of the skin and multiple necrosis of the liver. Bacilli are present in great quantities in the blood.

Differential Diagnosis.—From chicken cholera it is distinguished by the motility of the organism and its growth on potatoes.

BACILLUS OF PNEUMONIA-PERICARDITIS OF THE TURKEY (M'FADYEAN.)

This organism was described by M'Fadyean in an epizoötic among turkeys characterised by pneumonia-pericardial lesions.

Microscopical Appearances.—In the blood tissues and artificial culture media it occurs as short ovoid bacilli, not distinguishable by shape or size from the bacilli of fowl cholera.

Motility.—Motile.

Spore Formation absent.

Staining Reactions.—Exhibits bipolar staining with the ordinary aniline dyes, but is decolorized by the Gram and Weigert methods.

Cultivation.—*Stab Cultures in Gelatine* kept at 25° C. exhibit a distinctly visible growth in forty-eight hours along the needle track. The gelatine is not liquefied.

In Streak Cultures in Oblique Gelatine it develops in a whitish line, which does not spread far from the needle track.

On oblique surface Agar at the temperature of the body it develops a thin translucent pellicle.

Bouillon becomes turbid in twenty-four hours at 37° C., a ropy sediment finally forming, the upper portions of the bouillon becoming clear.

On Potatoes no appreciable growth takes place.

The bacillus is a facultative anaërobe, growing abundantly in bouillon flasks in an atmosphere of hydrogen.

Pathogenesis.—The results with guinea-pigs and rabbits are similar to those obtained with the bacillus of fowl cholera, very virulent for the rabbit, but not so virulent for the guinea-pig; infection by feeding did not succeed. Fowls and pigeons become slightly affected. Other birds were not affected. A calf and a pony manifested only slight lesions at the point of inoculation. The disease was produced in turkeys inoculated with pure cultures, the symptoms being stiffness, weakness, nasal catarrh, rattling in the throat, and then milk-white evacuations. The autopsy revealed pneumonia and pericarditis. In the lungs and other organs great numbers of the bacilli were present. The bacillus is differentiated from that of fowl cholera by being motile, by its slight action in other birds, and localization in the lungs.

PNEUMO-ENTERITIS OF THE SHEEP.

According to M. Galtier, this disease in sheep is caused by the same organism as swine fever or hog cholera. It sometimes occurs in an epizoötic, causing great ravages in affected flocks. Outbreaks have been observed when recently purchased swine, that had contracted swine fever in the market pens, have been placed in sheep folds. When once established, the disease transmits itself with great rapidity from sheep to sheep, and it is more severe and more frequently fatal in young animals. The general symptoms are lassitude, general loss of vigour, high fever, and loss of rumination; these symtoms are soon followed by tymphany, fœtid and exhaustive diarrhœa, quickened respirations, mucous discharge from the nostrils sometimes tinged with blood, and the special symptoms of broncho- and pleuro-pneumonia. The skin and visible mucous membranes present a more or less vivid red colour, sometimes mixed with hæmorrhagic spots. Abortion is often observed, although the mother does not necessarily succumb to the disease. The intensity of the disease varies; it is sometimes so severe that death occurs in a few hours or days, and again the attack may be so slight as to be hardly perceptible. Convalescence from the severe forms is always prolonged.

M. Galtier further states that pneumo-enteritis (or swine fever), which is generally considered to be peculiar to the pig, extends to all farm animals, especially to the sheep, bovines, and solipeds. The disease being transmitted to the fœtus, calves coming from diseased cows which are or have been subject to coughing, are born with the germ of the disease in them, and die in a few days with the lesions of broncho-pneumonia and enteritis (pneumo-enteritis of calves). Whether this conclusion is correct or justified is largely open to question, as though the morphology of the bacilli of the septicæmic group are very similar, the predilection of the different organisms for different animals is widely diverse.

BACILLUS FELIS SEPTICUS.

This bacillus was isolated regularly by Fiocca from the saliva of cats.

Microscopical Appearances.—Very small short rods, 0·2 to 0·3 μ thick, often occurring as diplococci.

Spore Formation absent.

Staining Reactions. — By ordinary methods, but not by the Gram method.

Biological Characters.—Its growth is similar to the rabbit septicæmia bacillus.

In Bouillon it forms no flakes.
Milk is not coagulated.
On Potatoes a very thin, almost invisible coating.
Sugar Media is not fermented.

Pathogenesis.—Produces septicæmia in mice, rabbits, guinea-pigs, and young rats.

BACILLUS TYPHI MURIUM.

(Mouse Typhoid.)

This bacillus was discovered by Löffler in an epidemic among mice.

Microscopical Appearances.—Small rods, which often form long threads.

Motility.—Strongly motile.

Staining Reactions.—Easily stained with the ordinary stain, but not by the Gram method.

Biological Characters.—*On Gelatine Plates* the deep colonies are small, round, slight, granular, and of yellowish-brown colour; the superficial colonies are flattened, irregularly notched, and possess delicate furrows similar to the colonies of the typhoid bacillus, only more granular and of more luxuriant growth.

In Stab Cultures.—Nail-like growth with a flat top.

On Agar, Blood Serum, and *Bouillon* the growth exhibits no special characteristics.

On Potatoes a whitish, not specially luxuriant coating forms, the surrounding medium being stained a dirty greyish-blue.

In Bouillon containing sugar, gas is formed.
Milk is not coagulated.

Pathogenesis.—Very virulent for white and grey house mice, and for field mice (arvicola arvalis) both by subcutaneous injection and by feeding. The infection is spread by the living mice eating the bodies of those dead of the disease. Löffler, owing to this circumstance, used the cultures in destroying the mice during the plague in Thessaly.

SWINE FEVER.

(Pneumo-enteritis—Klein. *Ger.* Bacillus der Schweine Pest. *America*, Swine Plague—Billings; Hog Cholera—Smith).

The chief veterinary officer, in his report to the Board of Agriculture for 1896, states:—'*It is quite certain that the disease which exists among the swine in America, where it has received the name of hog cholera, is identical with our swine fever, because in the year* 1879 *some cargoes of pigs affected with hog cholera were landed at Liverpool, when an opportunity was afforded of identifying the lesions of that disease with swine fever.*'

The above conclusions are identical with those published by the writer in 1891, when cultures were obtained from an outbreak of swine fever in England, and sent to Billings in America, who wrote that he had made all the tests and control experiments, concluding as follows:—'*It is the same germ as we have here as the cause of hog cholera.*'

Microscopical Appearances.—Short bacilli 1·2 to 1·5 μ long, and 0·6 to 0·7 μ broad.

Motility.—Strongly motile; possessing a multitude of flagella, something like the Bacillus typhi abdominalis peritricha (see Photomicrograph, Plate III., Fig. 13).

Staining Reactions.—Stain easily with any of the usual aniline dyes. The Gram method gives negative results. When freshly obtained from an animal, the condition known as bipolar staining is well-marked (see Photomicrograph, Figs. 68, 69, 70), the clear unstained centres not being so easily differentiated in older cultures, or if the staining process is too long continued. In sections of organs the bacilli are present in the capillaries and small veins.

Spore Formation absent.

Vitality.—It is destroyed by a temperature of 58° C. in from fifteen to twenty minutes. It preserves its vitality in spite of desiccation for nearly two months, and vegetates and multiplies at the ordinary tem-

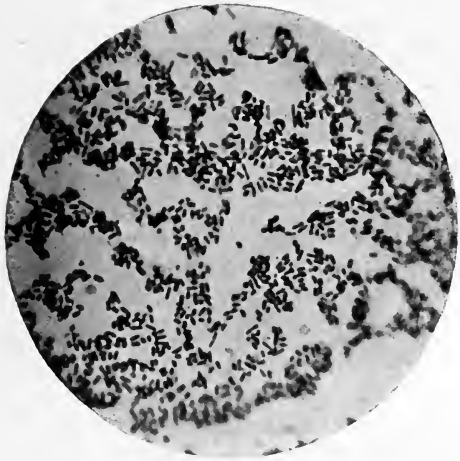


Fig. 67.—B. Phasiani Septicus. Cover-glass specimen. Agar culture. Fuchsin. × 1000.

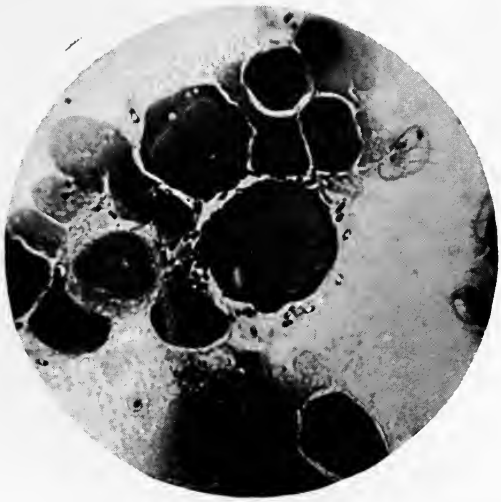


Fig. 68.—Bacillus of Swine Fever. Spleen of pig. Methylene blue. × 1000.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

perature of summer, and retains its vitality for more than fifteen days in sterilized water.

Biological Characters.—*On Gelatine Plates* the surface colonies are spread out flat with round or irregular borders; the deeper colonies are small, almost homogeneous brownish spheres.

Stab Cultures in Gelatine resemble a nail with a flat top (see Photomicrograph, Fig. 73.)

On Agar-Agar Media a greyish-white transparent growth occurs, and in *Grape Sugar Agar* gas is formed.

On Potatoes a yellowish growth occurs.

Bouillon becomes cloudy, and a film forms on the surface.

Milk remains unchanged, and the reaction alkaline.

Neither *Indol* nor *Phenol* are formed.

Pathogenesis.—A small quantity of a culture kills rabbits and mice in seven to twelve days with elevation of temperature. The lesions found are enlarged spleen of a dark red colour, and necrotic patches in the liver. The kidneys are inflamed, and the urine contains albumen. The substance of the heart is flabby, and fatty degeneration is present. The mucosa of the small intestine is swollen, and the contents are shiny, and ecchymoses are often present here and in the duodenum. The characteristic bacilli are present in small masses in all the organs, mostly in the capillaries and small veins. Injection of the virus into the lungs causes pneumonic changes. Feeding with cultures and inhalation also cause infection. Pigeons are somewhat refractory, but can be affected by large doses. Chickens are not affected even with large quantities of the virus. Swine are somewhat refractory to subcutaneous injections, but by intravenous injection of 1 to 2 c.c., and feeding with cultures, a severe diphtheric inflammation of the stomach and large intestines occurs. In other cases the bacilli are less virulent, especially when obtained from chronic cases of swine fever. The characters of the cultures can, moreover, also vary. The necrosis of the liver is not present in the milder forms of the disease, therefore the changes in the bowel are more pronounced. Abscesses sometimes develop under the skin at the point of inoculation. Swine fever sometimes occurs in devastating epizoötics, especially in America, when 90 per cent. of the swine may die. It occurs in an acute or hæmorrhagic septicæmic form, killing the animal in a few days; and in a chronic form, when the disease may last two to four weeks, or even longer. The post-mortem examination of affected animals presents a variety of lesions, which vary according to the severity and length of time the animal has been affected. The following is a summary of lesions observed in cases of swine fever :—

Tumefactions round the head and back, the tongue darkish in colour, and small necrosed patches and ulcers are often present on the lips, gums, and tongue. Along the abdomen numerous dark red-blue

blotches, terminating diffusely in the surrounding tissue. On cutting through the skin, dark red blood escapes from the cut bloodvessels. On section the abdominal cavity usually contains a large quantity of straw-coloured lymph with numerous flocculi floating in it.

The Large Intestines are sometimes agglutinated together with bands of flocculent lymph, the adhesions being very resistant in some parts.

The Mesenteric Lymph Glands are usually enlarged, and on section present a greyish-red striated appearance. The mucosa of the large intestines is usually red and swollen, the ileo-cæcal valve, or valve of Bauhini, swollen and often the seat of extensive ulcerations. The difference between ulceration and the deposition of caseous matter in the follicle of the glands in the neighbourhood of the valve must be carefully noted, otherwise an incorrect diagnosis will result.

The Small Intestines usually present a reddish appearance (typhilitis), the internal surface being studded with numerous dark red spots. The mucosa is swollen, with diffused capillary redness, Peyer's patches enlarged, and contents of intestines fluid. The characteristic ulceration mentioned in connection with the ileo-cæcal valve may also be present, and, although involving the whole thickness of the bowel, perforation is extremely rare.

Liver.—This organ is usually enlarged, edges rounded. On section dark blood exudes, and the acini are considerably enlarged. Occasional centres of necrosis have also been observed.

The Gall Bladder is usually distended, and full of viscid, dark greenish-yellow gall.

The Spleen is sometimes enormously enlarged and the pulp slightly disintegrated. Trabeculæ thickened, the Malpighian corpuscles enlarged, and sometimes a few white spots on the capsules.

The Stomach is sometimes the seat of numerous ulcerations (see Photo., Fig. 71), and in other cases the cardiac portion is healthy, while the pylorus is congested and ulcerated.

The Kidneys are sometimes enormously swollen, and in a state of hæmatogenous nephritis, the pelvis in some instances being almost entirely occluded with blood clots. Sometimes the kidneys are in a state of parenchymatous degeneration, and small petechiæ may also be observed under the capsule, which may or may not be adherent.

The Bladder sometimes contains hæmatogenous urine depending on the condition of the kidneys.

The superficial and deep inguinal glands are usually in the same condition as those of the mesenteric and bronchial regions.

Thoracic Cavity.—It is in this part of the animal that such a wide difference of opinion exists among experts, as to whether pneumonia is or is not a diagnostic symptom of swine fever. The following thoracic

FIG. 69.—Bacillus of Swine Fever. Pure culture from lymph gland of pig. Methylene blue. × 1000.

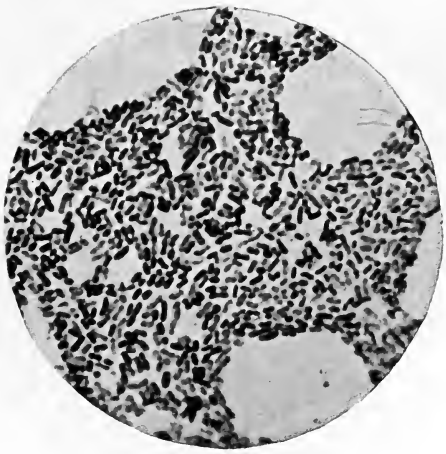


FIG. 70.—Bacillus of Swine Fever. Pure culture from lymph gland of pig. Methylene blue. × 1500.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898

FIG. 71.—Stomach from pig dead of swine fever, showing ulcerations.

FIG. 72.—Broncho-Pneumonia suis, lung of pig dead of swine fever.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

lesions have been observed in genuine cases of swine fever:—Slight effusion into the thorax; attachments or adhesions of the pleuræ may be present or absent. Small ecchymosed spots are sometimes found on the pleura costalis pulmonalis, and also on the pericardium.

The pericardial sac may be distended with an enormous quantity of bright yellow fluid, and the visceral folds studded with numerous bright red ecchymosed spots.

The Bronchial Lymph Glands are usually swollen, juicy, and similar to those of the mesenteric region.

The Lungs are sometimes marked by numerous red or reddish blue-black spots of various sizes, some of which extend beyond the surface of the lung, and often correspond to a single lobulus; large centres of hepatization are also often present (see Photo., Fig. 72). On section some of the lungs reveal a stage of broncho-pneumonia lobularis, the bronchial mucosa being swollen; and a yellowish-red exudate is present in the bronchi.

Immunity.—Billings obtained protection against the disease by inoculating swine with cultures derived from mild cases of the natural disease, but a certain proportion of the animals died from the effects of the inoculation. Smith claims to have obtained better results by injecting small doses of the virus intravenously. Schweinitz succeeded in vaccinating the guinea-pig by means of soluble substances, which he obtained from cultures. The same authority has recently produced immunity by means of an antitoxic serum obtained from the blood of immune animals. A number of guinea-pigs were inoculated with serum from a pig rendered immune to hog cholera, the doses varying from 0·5 to 4·5 c.c. Five of the inoculated guinea-pigs died of blood-poisoning; three withstood the injection, and fifteen days later also resisted the otherwise deadly injection of 1-10 c.c. of a one day old hog cholera culture. In another experiment, three guinea-pigs eight months after being immunized still remained capable of resisting the above-mentioned virulent dose. The immunity did not continue for a longer period, because the serum experimented with was obtained from a pig immunized a long time before. It therefore appears that the antitoxic substance is only present free in the blood, and capable of conferring immunity on other animals, immediately after the animal is immunized; but when already immunized animals receive further inoculations of virus, a new formation of antitoxic substance results. Schweinitz also produced immunity against Schweine-seuche with an antitoxic serum, but it was not possible with the Schweine-seuche serum to produce immunity against hog cholera, or *vice versa*.

BACILLUS SUISEPTICUS.

(Schweine-seuche—Schütz; Swine Plague—Smith. Infectious Pneumonia of the Pig.)

This bacillus was first described by Löffler and Schütz, and is also mentioned by Smith as being associated with his hog cholera germ, as well as occurring alone.

Microscopical Appearances.—Short bacilli morphologically, and in cultures very like the chicken cholera bacillus. (See Photomicrograph, Plate III., Fig. 14.)

Motility.—Non-motile.

Staining Reactions.—Stains easily with the ordinary aniline stains. The condition known as bipolar staining is well-marked in young cultures. The Gram method gives negative results.

Spore Formation absent.

Biological Characters.—*On Gelatine Plates* deep round brownish colonies appear, while the surface growth is limited.

In Gelatine Stab Cultures the growth resembles a nail with a flat top. (See Photograph, Fig. 74.)

On Agar-Agar it forms a thin coating with crenated margins.

On Potatoes it does not grow except they are rendered alkaline, and then it forms a yellowish covering.

Bouillon remains clear, but a thick sediment is formed.

Milk is not coagulated, but a weak acid reaction results. The indol reaction is present.

Pathogenesis.—Rabbits, small birds, and mice are as easily affected as with chicken cholera, and generally die in twenty-four hours from septicæmia. Guinea-pigs are not so easily affected, but young ones die quickly. In all those animals the changes at the point of inoculation are more intense than with the bacillus of chicken cholera (extensive hæmorrhagic œdema); fatty degeneration of the liver is also frequently present. Chickens are affected with large doses. Swine die from subcutaneous injection, with marked œdema at the point of inoculation, and septicæmia. By injection into the thorax they die from a multiple necrotic pleuro-pneumonia, the bacilli being present in the blood; there is also a slight enlargement of the spleen, and catarrh of the mucosa of the stomach. Infection by feeding does not occur. Calves likewise succumb from subcutaneous inoculation with the Bacillus suisepticus. The disease essentially consists of a pleuro-pneumonia, with inflammatory necrosis, and when the processes become chronic caseous deposits occur. The caseous deposits are also found in the large intestines and adjoining mesenteric lymph glands.

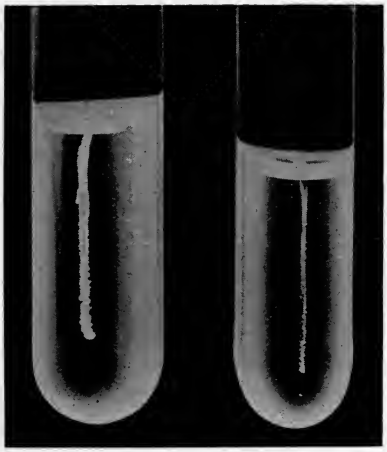


FIG. 74.—Bacillus of Schweine-seuche. Gelatine stab culture.

FIG. 73.—Bacillus of Swine Fever. Gelatine stab culture.

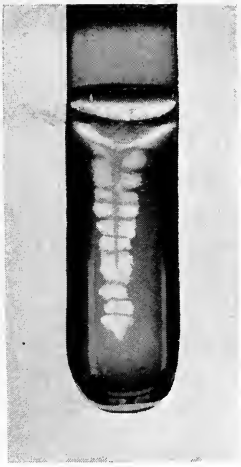


FIG. 75.—Bacillus of Swine Erysipelas. Gelatine stab culture.

FIG. 76.—Bacillus of Mouse Septicæmia. Gelatine stab culture.

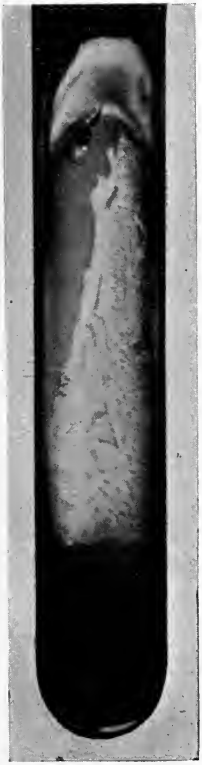


FIG. 77.—Bacillus of Bubonic Plague. Oblique agar culture.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

BACILLUS OF SWINE ERYSIPELAS.

(*Ger.* Bac. der Schweine-rothlaufs; *Fr.* Rouget du porc. Bacillus rhusiopathiæ—Kitt.)

This disease is peculiar to the pig, chiefly affecting adult animals, the improved breeds being more predisposed. The characteristic symptoms are great pyrexia, the appearance of red or purple blotches, at first limited, but afterwards confluent upon the skin, and constipation followed by diarrhœa. In very acute cases the redness of the skin may not be present. The average duration of the disease is about two days, but it may last four or five days, and is sometimes so severe that 60 per cent. of the affected animals die.

Microscopical Appearances.—A very fine cylindrical bacillus, 1 to $1\frac{1}{2}$ μ long, and 0·2 to 0·6 μ broad. Morphologically identical with the bacillus of mouse septicæmia. It is found in the blood, especially in fine capillaries in contact with their internal wall. It is also present in the exudates in all the diseased organs, in the marrow of the bones, in the fæcal matters, and in the urine. Sometimes a blood corpuscle can be observed totally filled with bacilli. (See Photomicrograph of the allied bacillus of mouse septicæmia, Plate III., Fig. 15.)

Motility.—Undecided (Günther).

Staining Reactions.—Stains best with fuchsin, and beautifully by the Gram and Cladius methods, especially in sections of organs.

Spores are not formed, but involution or degenerate forms are frequent. Drying weakens the bacilli quickly, but they can live a long time in filthy fluids, and the bacilli, according to Kitt, are very difficult to kill in large pieces of meat, such as hams, by cooking, pickling, salting, or smoking.

Biological Characters.—*On Gelatine Plates* small transparent, slightly liquefying colonies, which under a low power resemble a mass of threads.

In Gelatine Stab Cultures the growth occurs along the track of the needle, with numerous ramifying out-growths into the surrounding medium, giving the growth the appearance of a test-tube brush. (See Photograph of a culture, Fig. 75.)

On Agar-Agar a fine coating occurs.

On Potatoes no growth.

In Bouillon slight cloudiness, with the formation of a sediment.

Indol is not formed.

Pathogenesis.—Affects white and grey mice, white rats, rabbits, and pigeons. The animals generally die in three to four days, sometimes

later; the day before they die they sit motionless in one place, their eyes being firmly closed with secretion, and their heads retracted as if they were sleeping, and in this position they die. Animals can also be infected by feeding with the virus; but by this method rabbits are less susceptible. Intravenous injection kills rabbits in three to six days. The field and wood mouse, guinea-pig, ox, horse, ass, dog, cat, chicken, goose, and duck are immune. Sheep too seem to be more disposed to infection.

The production of the disease in swine varies according to the breed; well-bred swine die from subcutaneous inoculation by rubbing on the skin, and by feeding with the virus from cases of typical erysipelas.

Post-Mortem Appearances in Swine.—The skin is œdematous, and infiltrated with blood; the flesh is soft, greasy, and of a pale red colour. The lymph-glands, particularly those of the mesentery, are swollen and infiltrated with blood, presenting a red streaky appearance due to engorgement and distension of the blood-vessels of the gland. There is sero-fibrinous exudation of the pleura and pericardium. The peritoneum is congested and covered with ecchymosed spots. The mucous membrane of the bowel is highly congested and swollen, and in many places the epithelium is desquamated, and occasionally partially formed ulcers are present. The bacilli are widely distributed, but not so plentiful in the blood as in the experimental animals. According to Schottelius another bacillus is sometimes found associated with the erysipelas bacillus in the organs. This organism, the *Bacillus coprogenes fœtidus,* is found in the contents of the bowel and neighbouring organs of animals affected with erysipelas. It is non-motile and shorter than the Bacillus subtilis, and possesses spores. The cultures emit a putrid stench. The presence of this organism in animals affected with erysipelas is of no etiological importance. Some of the mild affections of swine Urticaria or nettle rash and skin necrosis, and also endocarditis verucosa bacillosa, are considered by Kitt, Bang, and Jensen to be caused by the bacillus of swine erysipelas. The fæces of affected swine are very virulent. The disease is spread by means of rats and mice.

Immunity.—The repeated passage of the erysipelas bacillus through the pigeon renders it more virulent for swine, while its passage through the rabbit, on the contrary, diminishes its virulence for swine. After a time the virus obtained from the rabbit does not kill the swine, but only makes them sick, rendering them immune to the action of strong virus. The degree of attenuation obtained persists in cultures made in ordinary bouillon, which is used as a vaccine for swine.

Pasteur's method consists in using two vaccines of different degrees of virulence in doses of 0·12 c.c., an interval of ten days intervening between the first and second inoculation. As young pigs are not so

susceptible to the disease as older ones, they are more suitable for immunization. Swine vaccinated by this method remain immune for one year, which is long enough for breeding and fattening purposes.

Lorenz has introduced a method of protective inoculation with the blood serum of swine previously immunized. The serum is obtained in the following manner:—Healthy swine are first inoculated with serum, and then with a virulent culture shortly before the blood is withdrawn. Blood serum of an animal inoculated in this manner confers immunity on other animals. A pig weighing eleven stones, supplies on an average 750 c.c. of serum; out of this quantity about 150 grammes of a stable substance is prepared, containing only $\frac{1}{5}$ volume of serum, 30 per cent. glycerine and 40 per cent. water. It is very difficult to procure a serum of uniform strength in swine erysipelas, because it is not easy, and often impossible, to obtain a uniform grade of virulence in swine erysipelas cultures. The serum is injected subcutaneously behind the ear or between the thighs.

BACILLUS OF MOUSE SEPTICÆMIA.
(Bacillus Murisepticus.)

This is an experimental infectious disease found by Koch. The grey and white mouse, when inoculated subcutaneously with putrid blood or meat infusion, died of septicæmia due to the Bacillus murisepticus.

Microscopical Appearances.—Morphologically identical with the bacillus of swine erysipelas; the bacilli occur mostly in the cells. (See Photomicrograph, Plate III., Fig. 15.)

Motility.—Undecided (Günther).

Staining Reactions.—Are similar to the bacillus of swine erysipelas.

Spore Formation.—Was observed by Koch in one case (Günther).

Biological Characters. — The appearance in gelatine stab cultures is very characteristic, the growth resembling a test-tube brush. (See Photograph, Fig. 76). The growth in the other media is identical with that of the bacillus of swine erysipelas.

Pathogenesis.—Affects grey and white mice, which die in the same characteristic sitting position described with mice inoculated with the bacillus of swine erysipelas. Field-mice, chickens, and guinea-pigs are immune. Schütz considers that the bacillus of mouse septicæmia and swine erysipelas are probably identical.

DIFFERENTIAL DIAGNOSIS TABLE.

	Swine Erysipelas.	Swine Fever.	Infectious Pneumoniæ Suisepticus, or Schweine-seuche.
Size of the organism	1 to 8 μ long; 0·2 to 0·6 broad	0·6 to 1 μ long; 0·4 μ broad	0·6 to 1 μ long; 0·4 μ broad
Motility	Non-motile	Strongly motile peritricha flagella	Non-motile
Staining	With ordinary stains and by the Gram and Cladius methods	Exhibits polar staining with the ordinary aniline stains; negative with the Gram and Cladius methods	Exhibits polar staining with the ordinary aniline stains; negative with the Gram and Cladius methods
Cultivations	The characteristic test-tube brush-like growth in gelatine stab cultures; no growth on potatoes	Grows on agar and in gelatine stab cultures, nail-form like, and on potatoes forms a yellow coating	The growth on agar and gelatine resemble swine fever, but there is no growth on potatoes unless they are alkaline, when a yellowish coating is formed
Indol reaction	None	None	Present
Gas formation	None	Present	None
Inoculation experiments	Mice die in two, three, or four days, rabbits in four to six days. Guinea-pigs and chickens are immune, and swine over three years of age are not affected	Rabbits and mice die in seven to twelve days; pigeons with large doses, and chickens are immune. Inoculation in swine difficult. Feeding experiments yield more positive results	Rabbits, small birds, and mice generally die in twenty-four hours from septicæmia, young guinea-pigs easily, older ones more difficult; chickens with large dose. Feeding experiments negative with swine, but by inoculation positive

THE BACILLUS OF BUBONIC PLAGUE.

The organism causing this disease was discovered by Kitasato and Yersin in the outbreak of bubonic plague at Hong-Kong, China, 1894.

Microscopical Appearances. — Short oval bacilli with rounded ends, usually occurring singly, sometimes in pairs and threes, and very rarely in chains; very often enclosed in a capsule.

Motility.—Non-motile.

Spore Formation.—Absent.

Staining Reactions.—Exhibits polar staining with the ordinary staining methods (see Photomicrograph, Plate IV., Fig. 20); does not stain by the Gram method, although Kitasato says at one time it stains by the Gram method, and at another time it is discolorized; but as mixed infection is often present, the above peculiar reaction towards the Gram stain can perhaps be attributed to other species of bacteria.

Biological Characters.—The bacillus grows best between 36° and 39° C., but also develops very well at ordinary room temperature.

On Gelatine Plates.—Small darkly-defined granular colonies of a brownish colour occur, and the medium is not liquefied.

In Gelatine Stab Cultures.—It develops slowly on the surface, and along the track of the needle.

On Agar Plates.—In twenty-four hours small dewdrop-like colonies appear, which in forty-eight hours resemble grey beads with slightly iridescent borders; sometimes a large colony is observed between the smaller ones. The deep colonies are round and granular.

On oblique surface Agar a viscous shiny coating appears; the above-mentioned colonies like dewdrops being sometimes observed. The water of condensation is clouded, but there is no film formed. (See Photograph, Fig. 77.)

The growth on *blood serum* is similar to that on ordinary agar.

Bouillon becomes diffusely clouded, but if it is inoculated with a cohesive mass of bacteria from an agar culture, the bacilli develop on the bottom of the tube, while the upper portions of the medium remain clear; this mode of growth being somewhat similar to that of streptococci.

Milk is a bad medium, and is not coagulated.

Potatoes.—On the surface at 37° C. a scanty greyish-white growth occurs.

The bacillus of bubonic plague forms no *gas* in sugar-containing media, and no *indol* in either bouillon or peptone water. It grows best on media of neutral reaction (Wlademiroff and Kressling). The addition of glycerine to the media is detrimental.

Vitality of the Bacillus of Bubonic Plague.—It is killed by heating ten minutes at 55° C., and five minutes at 80° C. Corrosive sublimate 1 to 1000 destroys the bacilli immediately; 1 per cent. carbolic acid and 1 per cent. lysol in ten minutes. The mineral acids are very effective. Sulphuric acid 1 to 2000 kills the bacilli in five minutes. Hydrochloric acid 1 to 1000 in thirty minutes. The longest time that infected material on lint, wadding, earth, etc., remained active was eight days. Sputum from patients affected with the pneumonic form kept in a vessel plugged with cotton wool, was no longer virulent in sixteen days. In ordinary drinking water the bacilli die in three days, in sterilized water

in eight days, and in sterilized bilge water in five days; also in direct sunlight in three or four hours.—(Report of German Plague Commission.) The bacilli are killed by drying at ordinary room temperature in four days.

Pathogenesis.—The most susceptible animal is the rat, a minimum quantity of culture being sufficient, when injected subcutaneously, to cause its death. The same results are obtained when the virus is introduced on the mucous membrane of the nose or eye, also by feeding experiments and by gnawing companions dead of the plague. The latter mode of infection is of great importance, as it explains the extraordinary and almost inconceivable rapidity with which this rat-pest spreads, and probably also it is the affected rats that carry the plague from house to house.

Next to the rat the most susceptible animal is the grey monkey. All the ordinary laboratory animals are also susceptible except the pigeon.

Yersin observed the bacillus in the bodies of dead flies by the inoculation of animals.

Ogata found the bacillus in fleas on rats dead of the plague.

Nuttall found that fleas died when fed with plague tissues in seven to eight days at 12° to 14° C., and in about three days at 23° to 31° C. In bed-bugs the bacilli soon died.

In animals subcutaneously inoculated, the point of the inoculation becomes œdematous, and the lymphatics enlarged in a few hours; in twenty-four hours the animal is quiet, hair rumpled with excessive lachrymal discharge from the eyes, finally convulsions set in, ending in death. The post-mortem appearances are as follows:—Bloody œdema at the point of inoculation, reddening and swelling of the lymph-glands; hæmorrhagic extravasation into the abdominal walls; serous effusion into the thoracic and abdominal cavities; intestines congested. Spleen enlarged (see Photomicrograph of section of spleen of mouse, Fig. 78), and greyish points resembling miliary tubercles are sometimes present in the spleen. The supra-renal bodies are also enlarged. The characteristic bacilli can be found in large numbers in the local œdema, lymph-glands, blood, and internal organs.

In cases where the animals do not die quickly, only local evidence of inoculation is present, and the distribution of the organism throughout the body is diminished.

In man, when the virus gains entrance at the foot, the superficial and deep inguinal glands are the first to become affected, and if the infection be through wounds in the hands, then the buboes appear first in the axillary region. A member of the German Commission was infected while performing an autopsy on a victim of plague; two days later a small pustule appeared on the right hand, soon followed by

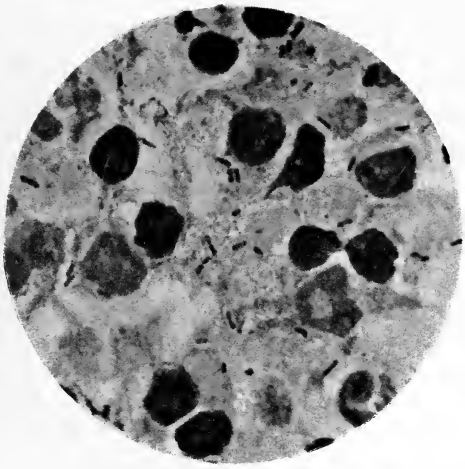


FIG. 78.*—B. of Bubonic Plague. Cover-glass specimen from spleen of inoculated mouse. Methylene blue. × 1000.

FIG. 79.—B. of Broncho-Pneumonia Bovis. Ovoid bacteria. Section of lung of American ox. Weigert's method. × 1000.

* I am indebted to Dr G. W. Nuttall, Berlin, for the specimen from which this photograph was taken.

lymphangitis and enlarged axillary glands. The plague bacilli were found in the secretion from the pustule. Notwithstanding the very alarming initial sickness, recovery took place. In man, the bacilli are most numerous in the enlarged suppurating lymphatics. They are also present in smaller numbers in the blood and the internal organs.

Bacteriological Diagnosis of Plague.—As already mentioned, the characteristic bacilli are very numerous in the pustules or buboes, and the polar staining exhibited with methylene blue enables them to be easily differentiated. The bacillus of chicken cholera resembles it, but is somewhat larger, and has no effect on man. The diagnosis is not so easy in those cases where suppuration has not commenced, and it is in such cases that it is most necessary. The German Commission consider puncture of the bubo somewhat dangerous, on account of opening bloodvessels, but the English doctors make a long incision in the affected gland, which is afterwards dressed with antiseptics. By this method it is easy to obtain material from the gland for cover-glass specimens, plate, and other culture methods. Microscopical examination of the blood is only attended with results in cases of general infection. Culture experiments with the blood yield better results. To separate plague bacilli from the mixture of bacteria in sputum, it is best to make gelatine stroke plate cultures. The suspected material is drawn across the surface of solid gelatine, several strokes being made. The plague bacilli grow very well at the low temperature of 22° to 25° C., while the accompanying bacteria, *i.e.*, diplococcus lanceolatus streptococcus, generally exhibit only a scanty growth. According to Hankin, when the bacilli are grown on agar containing 2·5 to 3·5 of salt at 31° C., in twenty-four to forty-eight hours involution forms, consisting of pear-shaped bodies and spheres, appear, which he considers characteristic enough to form a means of diagnosis. Of further diagnostic importance is the fact that the blood serum of men and animals recovered from plague possesses the power of *agglutination* (German Commission). This power of agglutination appears first in the second week, and in the second and third it is mentioned as being most pronounced. In the cases of mixed infection, particularly those accompanied with suppuration of the buboes, streptococci are found not only in the glands, but also in the blood.

Immunity.—In the Pasteur Institute a plague serum is prepared from a highly immunized horse. The German Commission, experimenting with this serum, found that 3 c.c. was sufficient to protect a brown monkey against an after subcutaneous inoculation. With the susceptible grey monkey 10 c.c. was not sufficient. The serum also exhibited undoubted curative effects on a brown monkey inoculated twelve hours previously. Haffkine prepares his anti-plague serum

from fluid cultures rich in fat. The development is allowed to go on for about a month, the broth being shaken now and then to ensure a series of successive crops. The organisms are then killed by exposure to 70° C. for an hour. This is used as a vaccine, and before use it is shaken and 3 c.c. injected. The efficacy of the serum with plague patients has yet to be determined.

BRONCHO-PNEUMONIA BOVIS.

(Infectious Broncho-pneumonia—Nocard ; Corn Stalk Disease—Billings ; Bacillus Zeæ—Burril.)

The disease occurs among American cattle at certain seasons of the year, and, according to Billings, is caused by an ovoid belted organism which infects cattle eating the leaves and tender top shoots of the corn stalks, and from this circumstance he named it 'The Corn Stalk Disease.' Nocard, on the other hand, found some cattle amongst a lot landed from America affected with a specific lung disease, which at the first glance presented the appearance of a recent lesion of contagious pleuro-pneumonia ; but careful microscopical examination revealed the presence of ovoid belted organisms that could be differentiated in the lymph spaces resembling a state of pure culture. M. Nocard says : 'This single character alone sufficed to affirm that the lesion was not of a pleuro-pneumonic kind.' The author also discovered the presence of morphologically identical organisms in sections of a piece of the lung of an American ox received from Professor Williams (see Photomicrograph, Fig. 79). A portion of this lung, as well as stained sections, were sent to M. Nocard, who replied : 'It is a bacteridian broncho-pneumonia, which in all probability is of the same nature as that I have already described.' In the absence of cultures, M. Nocard, though stating his belief in the identity of the organisms, could not be absolutely definite.

Microscopical Appearances.—Short oval bacteria 1 μ long, and about ·5 μ in width.

Motility.—Strongly motile.

Staining Reactions.—Stains readily with alkaline methylene blue, when the clear spaces in the centre of the organism are well differentiated. By the Gram method the reaction is negative. Sections are best stained with methylene blue, according to Weigert's original method (see Technique, § 37) ; also with fuchsin.

Biological Characters.—According to Billings the growth is more characteristic at ordinary room temperature than in the incubator.

On Gelatine Plates.—Flat, spread out, bluish, transparent, lobulated colonies.

In Gelatine Stab Cultures the growth takes place along the inoculation track and on the surface of the medium as well; there is no liquefaction.

Upon oblique surface Gelatine there is a rapid, pellucid, pearly-white growth, the edges being scalloped.

On Agar-Agar the edges of the growth are deeply scalloped, the separating lines extending into the body of the cultures.

On Potato, greyish-white, somewhat elevated colonies develop.

White of Egg.—Clear yellow colonies with slightly raised edges develop.

Milk is not coagulated.

Pathogenesis.—Mice, rabbits, guinea-pigs, and pigeons inoculated subcutaneously with 2 or 3 drops of the serum or culture died in less than forty-eight hours. Intraperitoneal injection caused death in fifteen to eighteen hours with purulent peritonitis. Sheep and calves inoculated subcutaneously or in the trachea with 1 c.c. of culture serosity or virulent pus did not die, but were seriously affected for several days, when they soon regained their normal condition. An eight-month-old calf and a two-year-old ram, inoculated in the right lung with 5 drops of peritoneal pus from a guinea-pig, died in less than forty-eight hours with fibrinous pleurisy and exudative broncho-pneumonia analogous to that observed at the autopsy on the American cattle. The lesions contained numerous quantities of bacteria.—Nocard.

Pigs, dogs, rats, and chickens are immune. A calf and two sheep previously inoculated subcutaneously, then in the trachea, afterwards resisted the effects of intrapulmonary inoculation with 10 drops of a virulent culture.

PLEURO-PNEUMONIA CONTAGIOSA BOVIS.

Poels and Nolen considered this bovine scourge was caused by a micrococcus, while Arloing isolated a bacillus which he named the Bacillus liquefaciens bovis, but it has been proved that neither of these organisms possess the pathological significance ascribed to them by the above-mentioned investigators. The most recent announcement regarding the cause of this disease was communicated by MM. Nocard and Roux, with the collaboration of MM. Borel, Salimbeni, and Dujardin-Beaumetz, in the *Revue Vétérinaire,* May 1898.

These investigators placed bouillon previously inseminated with a

trace of pleuro-pneumonic serosity in sacs of collodion, which were carefully sealed and inserted into the peritoneal cavity of rabbits. After fifteen to twenty days, the sacs contain an opal, slightly turbid, albuminous fluid, but neither contain cells or bacteria cultivable in ordinary bouillons.

Microscopical Examination of the contents of the sacs with a very high power (about 2000 diameters) revealed infinite, small, refringent, mobile points of such a degree of fineness that it is impossible even after coloration to exactly determine their form. A second sac containing the identical bouillon, minus the pleuro-pneumonic serosity, was inserted into the peritoneal cavity of the same rabbit, in order to ascertain if the modifications found in the fluid of the first sac were not due purely and simply to the osmotic changes which take place in the vicinity of its walls. The liquid contained in this sac was found to have retained its transparency and primitive limpidity. The numerous mobile points that, despite their extreme fineness, had rendered the inoculated liquid opalescent, were in reality living organisms which had exhibited infinite growth, in consequence of the modification undergone by the culture liquid, and thanks to the obstruction by the collodion to the phagocytary action. This is proved when two sacs of inseminated collodion are inserted into the peritoneum of a second rabbit—the first with a trace of the opal liquid thus obtained, the second with several drops of the liquid previously heated, and consequently containing no organisms, comports itself like the sac receiving no serosity, and its contents remain limpid and transparent, whereas the other soon becomes opalescent and contains the innumerable refringent points described above.

Fresh sacs were inseminated with the opal liquid obtained from the fertile sac of the second rabbit, and inserted into the peritoneum of the third rabbit, and so on, successively identical results being always obtained. It is advisable to use several sacs in each passage, as they are frequently ruptured. The rabbits frequently become very thin previous to being destroyed, and sometimes succumb before the time set for the autopsy, when they are found in a profound state of cachexia, being nothing more than skin and bone. No appreciable organic lesions are discovered at the autopsy; the blood and pulp of the parenchymatous organs sown in various media, also in collodion sacs, does not give rise to any cultures, so that it seems in all probability to be an intoxication due to diffusion outwards of the products elaborated by the microbe in the collodion sacs.

It cannot be attributed in every case to digestive or other troubles which might be caused by the sac and foreign body, because several sacs of bouillon containing no serosity can be inserted into rabbits, and conserved there for several months, without the animals showing the

least *malaise,* or losing a gramme of their weight. These accidents appeared more marked, and the cachexia much more profound, when the sacs introduced after inoculation were more numerous, of a greater capacity, or as the cultures concerned were richer. Several attempts were made to obtain cultures in sacs in the guinea-pig, but with negative results. Even after remaining six weeks in the peritoneal cavity of the guinea-pig, strongly inoculated bouillon is found as limpid as at the commencement.

Experimental Inoculation.—A small quantity of the fluid cultivated in the collodion sacs was injected into five Brittany cows, causing the development of the absolutely characteristic pleuro-pneumonic engorgement; one of the cows succumbing with a formidable œdematous infiltration, the other four resisted. Two of them were inoculated in the defended region with a strong dose (1 c.c.) of pulmonary serosity, and did not absolutely manifest any local or general symptoms, whereas a fresh cow inoculated at the same time as they were with 10 drops of the same serosity succumbed twenty-two days after inoculation. The third cow was re-inoculated after four months with 1 c.c. of pulmonary serosity obtained from a subacute lesion, but did not exhibit any local lesion or fever. The fourth cow has not yet been re-inoculated.

FOOT AND MOUTH DISEASE.

(Eczema Epizoötica. *Ger.* Maul und Klauenseuche. *Fr.* Stomatite Aphtheuse.)

In the report of the German Commission, published February 1898, Professors Löffler and Frosch state that the many bacterial bodies heretofore described as the cause of foot and mouth disease do not possess the etiological significance attributed to them. The Commission were able to immunize healthy animals in various ways—calves with lymph heated for twelve hours at 37° C., and also with lymph heated 30 minutes at 60° C., and lastly, with lymph mixed with the blood of animals that had recovered from the disease and acquired immunity to the introduction of the virus into their bodies,—the best results being obtained with the mixture of lymph and blood. The quantities of the blood and lymph mixture used were 1 to 40 c.c. of highly virulent lymph and 10 c.c. of defibrinated blood. Some of the inoculated animals were not affected, while others exhibited slight lesions on the mouth which did not interfere with their general health. These changes appeared ten to fourteen days after the animals were inoculated, as flat, round, or rugged exfoliations of the epithelium, and were localized on the typical spots affected by the characteristic vesicles of the natural disease. These erosions were mostly accompanied by an infiltration of a black or

brownish pigment. The action is the same when the lymph is injected into the vein on one side of the neck, and the immune blood into the vein on the opposite side shortly afterwards. In different outbreaks of the disease the virulence of the lymph is extremely variable, therefore experiments are necessary to obtain, if possible, a lymph of constant action. Further experiments were also conducted with lymph reduced with thirty-nine parts of water and mixed with a considerable quantity of a culture of the Bacillus fluorescens (as a test if any germs passed through the filter), and the whole mixture filtered two or three times through a porcelain filter. Cultivations instituted on various media with the filtrate remained sterile, thus proving that no bacterial elements had passed through the filter. A number of calves were injected intravenously with this filtrate, and at the same time others with lymph. The animals inoculated with the filtrate were affected at the same time as those inoculated with the lymph, presenting all the typical symptoms of the disease, high fever, vesicles in the mouth and feet.

The Commission explain these results by two possibilities. The bateria-free filtered lymph either contains an extraordinary strong working soluble poison, or the not yet discovered causes of the disease were so small that they were able to pass through the pores of the filter, which safely prevents the smallest known bacteria passing through.

In conclusion, the Commission adopt the hypothesis that foot and mouth disease is caused by an organism that is so small that it is able to pass through the porcelain filter. The smallest of all known bacilli is Pfeiffer's bacillus of influenza, which is 0·5 to 1 μ long; and if the supposed cause of foot and mouth disease was only $\frac{1}{10}$ or $\frac{1}{5}$ the size of those, which is not impossible, they would, according to Professor Abbé of Jena, be beyond the limit of the working capabilities of our microscopes, and could not be recognised with the best oil immersion systems. The results obtained by M. Nocard and his colleagues in their researches with pleuro-pneumonia contagiosa (see page 202) tend to strengthen the hypothesis advanced by the Commission. The Commission also consider that other infectious diseases of man and animals, *i.e.* smallpox, cowpox, scarlet fever, measles, spotted typhus, rinderpest, the causes of which are as yet unknown, may possibly belong to this group of extremely small organisms. They also consider that the preparation of a bacteria-free cowpox lymph would remove the agitation against vaccination.

CANINE DISTEMPER.

According to the recent researches of Schantyr, three diseases are included under the name 'Distemper,' which can only be differentiated from each other by bacteriological examination.

1. *Abdominal Typhus of the Dog*, caused by large numbers of short bacilli generally found singly in the blood and internal organs, bearing a great resemblance to the Bacillus typhi abdominalis of man, especially in the growth on potatoes.

2. *Dog Typhoid*, caused by very small thin bacilli occurring in groups, and staining by the Gram method.

3. *Genuine Canine Distemper*, caused by small slightly curved bacilli lying in groups, and not staining by the Gram method, growing very sparingly or not at all on the various culture media.

BACTERIA FOUND IN THE MOUTH.

1. Leptothrix Buccalis Innominata forms manifold, twisted, and intertwined motionless threads, which are stained yellow with a solution of iodine and iodide of potash.

2. Bacillus Buccalis Maximus appears in small tufts running parallel, and with the iodine-iodide of potash solution are stained blue-violet (Granulose reaction).

3. Leptothrix Buccalis Maxima in its form and arrangement presents a great resemblance to the Bacillus buccalis maximus, but is stained yellow with the iodine solution.

4. Iodococcus Vaginatus is found in chains consisting of four to ten cells, which are enclosed in a sheath which is about 0·75 μ thick. The cells are stained a blue-violet with the iodine solution, while the sheaths are stained a pale yellow colour.

5. Spirillum Sputigenum forms comma-shaped strongly-motile rods, which, when two are arranged together, appear like the letter S.

6. Spirochæte Dentium (Sp. denticola).—This organism, like the other above-mentioned, is found under the edges of the gums. It forms long spirals from 8 to 25 μ long, with sharp-pointed ends, which is also common to the spirillum of relapsing fever, whereby both are distinguished from other spirilla.

7. Leptothrix Gigantica.—This organism was found in the coating of the teeth of a dog affected with *Pyorrhœa alveolaris* by Miller, and named gigantica on account of its immense dimensions; like the other mouth bacteria above mentioned, cultivations on artificial media have not yet been obtained.

BACTERIA FOUND IN URINE.

Micrococcus Ureæ (Leube). — Cocci 0·8 to 1·0 μ in diameter, occurring either single, in pairs, tetrads, or in chains. It grows on the surface of gelatine *without causing any liquefaction of the medium.*

Micrococcus Ureæ Liquefaciens (Flugge).—Cocci 1·25 to 2 μ in diameter, occurring either singly or in small chains or irregular groups. It liquefies gelatine media slowly.

Bacillus Ureæ (Leube).—Forms plump rods 1 μ thick with rounded ends, and grows on the surface of gelatine without liquefying the medium.

UROBACILLUS PASTEURI.

Found in putrid urine.

The bacilli are motile and of various lengths, and form threads. Spores are present, situated at one end of the rod. Urea is decomposed by the action of the organism.

BACILLUS GLISCHROGENUS.

Found in slimy urine.

It is motile, and forms in urine, milk, and solutions of starch a slimy substance.

Pathogenesis.—When injected into dogs it causes nephritis; in other animals pyrogenic changes usually result.

The Chief Bacteria occurring in Air, Soil, and Water.

I.—BACILLI.

1. *Nutrient Gelatine is not Liquefied.*

(*a.*) Chromogenic.

Bacillus Auranticus.—Small thick rods, exhibiting slight motility.

On Plate Cultures superficial button-shaped, orange-coloured colonies develop.

In Gelatine Stab Cultures it exhibits a shiny orange-coloured growth.

In Bouillon the growth is very characteristic, the fluid remaining clear, but a membrane with isolated orange-coloured specks forms on the surface, and a somewhat clear layer in the bottom of the tube.

Bacillus Constrictus.—This name is applied owing to the peculiar appearance of the organism when stained by Zimmerman's method. The bacilli exhibit a slight constriction between the individuals, being united in short pointed chains possessing a rod-like appearance. The colonies on plate cultures appear as granular discs with ragged edges, varying in colour from a yellowish-grey to a sulphur-yellow.

Bacillus Fluorescens Non-liquefaciens.—This organism occurs as delicate, short, lively motile rods. The colonies on gelatine possess a glittering appearance like mother-of-pearl, and also exhibit a greenish fluorescence.

On Agar-Agar a greenish-coloured growth occurs. Another form is described, the Bacillus fluorescens non-liquefaciens *immobilis*, which is distinguished by its non-motility and the absence of flagella.

Bacillus Fuscus.—Medium-sized rods, which are often curved, and owes its name to the dark brown-coloured pigment it produces in all the media.

In Gelatine Stab Cultures the growth is nail-like at first, the head finally spreading out.

Bacillus Rubefaciens.—Fine rods, united in two or more joints. The growth in *gelatine* presents a pale rose-red colour.

On Potatoes the substratum is rose coloured, while the colonies themselves vary in colour from a yellowish-grey to a brownish-red.

Bacillus Subflavus.—This organism occurs in rods, often associated in clusters, and are two to four times as long as broad. The cultures form a pale yellow pigment, which in plate cultures shines like mother-of-pearl. The pigment is more apparent in agar-agar cultures.

Bacillus Brunneus.—Small non-motile bacillus. In characteristic cultures the medium surrounding the growth exhibits a diffuse brown colour.

(*b.*) Non-Chromogenic.

Bacilli resembling Typhoid Bacilli (Weichselbaum).—This is a group of motile bacilli which in their morphological and culture characteristics resemble the bacillus of Eberth and Gaffky, and the Bacterium coli commune.

On Plate Cultures the growth is similar to the typhoid and coli commune colonies.

On Potatoes the growth is sometimes brown, sometimes yellow, and sometimes scarcely visible.

Milk is coagulated.

Grape Sugar is fermented by some forms, and by others it is not.

The *Nitroso indol* reaction is sometimes positive, sometimes negative; but the pathogenic effects on experiment animals is awanting. That this group contains a number of different organisms is shown by the fact that it is not possible with any of these species of bacteria to produce immunity against a second species. Also in experiments with the blood serum of animals, rendered immune against one of these species, the power of causing agglutination in the culture of another species was always absent.

2. *Nutrient Gelatine is Liquefied.*

(*a.*) Chromogenic.

Bacillus Arborescens.—Slender bacilli, frequently forming wavy threads; non-motile; distinguished by the branch-like ramifications and iridescence of the colonies in gelatine plate cultures.

On Potatoes it forms a yellow to yellowish-red pigment.

Bacillus Fluorescens Liquefaciens.—A motile bacillus, very like the Bacillus pyocyaneus. It liquefies the gelatine very quickly, with the formation of a greenish-yellow pigment, brightly fluorescent.

On Glycerine Agar the cultures are quite typical, exhibiting an olive-green to olive-brown colour.

Bacillus Rubidus.—Medium-sized, strongly-motile rods, arranged in long threads; a brownish-red pigment is produced in gelatine agar and potatoe cultures; except the formation of pigment it has scarcely any other characteristic.

Bacillus Violaceus.—Small, slender, strongly-motile rods, forming spores in the middle of the rods in agar cultures. Cultures on gelatine exhibit in the liquid portions a bluish violet-coloured bacterial mass. On agar and potatoe media, the formation of the pigment is very abundant, varying from a dark violet to an almost black colour.

Bacillus Viscosus.—A bacillus very like the Bacillus fluorescens liquefaciens, but distinguished by forming a chocolate-coloured coating on the media.

Bacillus Ianthinus.— Medium-sized motile bacilli; the appearance of a colony growing on a gelatine plate culture is compared to a drop of ink. It forms on all media a violet pigment.

Bacillus Helvolus.—Motile rods of varying length, sometimes arranged in shorter threads, producing a yellow to sulphur yellow-coloured pigment. On plate cultures the colonies appear as circular bright yellow discs lying in a funnel-shaped liquefied cavity. On agar media an extensive coating of an intense yellow colour is formed.

Bacillus Prodigiosus.—Very small rods (formerly described as the Micrococcus prodigiosus, or Monas prodigiosa) often arranged in small chains, possessing very slight motility. It is found in the air, less often in water, frequently found in starch-containing media (bread and potatoes), in meat, and in milk.

It grows on all the media, producing a bright red colour, which is most intense on potatoes, exhibiting a blood-red coloured layer.

On Gelatine Plates the deep colonies are like white points, while the surface colonies are round and red in colour, with irregular borders.

The gelatine is very quickly liquefied. On agar-agar a massive dark red substance is formed, while the nutrient medium itself is not coloured. When cultivated in the incubator for several generations, the prodigiosus loses the red pigment. In the cultures on potatoes it also forms, besides the red pigment, *trimethylamin*.

Milk is coagulated. Media containing sugar are fermented. The prodigiosus grows also in the absence of oxygen, but no red pigment is produced. It is somewhat pathogenic; after the introduction of large doses the inoculated animals die with symptoms of poisoning.

Other chromogenic bacteria occur, distinguished only by the colour of the cultures. Bacillus ruber balticus, ruber aquatilis, cæruleus, pavoninus, amethystinus.

(*b.*) Non-Chromogenic.

Bacillus Liquefaciens.—One of the most common and widely distributed water bacilli. Strongly motile rods, often arranged in chains of four or more joints. Gelatine media are liquefied very quickly.

On Plate Cultures, in the form of scales.

In Stab Cultures, in the form of a stocking, with the upper portion distended. The cultures give off an unpleasant stench. It is a facultative anaërobe, and in nutrient media containing nitrates it produces nitric acid.

Bacillus Liquidus.—(This is also a water bacillus.) Short, plump, slightly motile bacilli, liquefying gelatine very quickly; in tubes the grey liquefied gelatine is covered with a thin membrane, which, when the tube is shaken, sinks to the bottom of the medium.

Bacillus Aquatilis.—(Another water bacillus.) Thin motile rods, which liquefy the gelatine slowly (according to some authorities, not at all). In gelatine tube cultures they grow on the surface of the medium as small yellow colonies, and on potatoes with a scanty yellowish coating.

Bacillus Mycoides Wurzel, or Root Bacillus.—Found in the earth and in certain kinds of forage; thick, slightly motile bacilli with rounded poles. Spores are formed in the middle of the rods. Grows only in the presence of oxygen. The greyish-white colonies consist of a net of fine twisted threads. The gelatine is liquefied.

In Stab Cultures the growth resembles a pine tree placed upside down.

On Agar-Agar the growth exhibits a texture like the branching of the roots of a tree. There is another species called the *Bacillus mycoides roseus*, which morphologically resembles the Bacillus anthracis; it grows best at room temperature. On gelatine plates it forms round, scanty, quickly liquefying, red-coloured colonies. On agar-agar a red-coloured growth; the pigment is only formed in the dark; exposed to the light, the growth is white. The pigment is soluble in water.

Bacillus Subtilis (Hay Bacillus).—Large motile rods (for Photomicrograph showing Flagella, see Fig. 81), frequently growing in long straight threads; it is strongly aërobic, and liquefies gelatine media quickly. Optimum temperature, 30°; minimum, 10°; maximum, 45° C.

On Plate Cultures, bright greyish-white colonies appear, surrounded by a radiating margin.

On Agar-Agar, the growth is peculiar, exhibiting a rigid, shrivelled, easily detached coating. Spores form in the middle of the rods, somewhat broader but considerably shorter than the mother cell. The hay bacillus is found in the air, water, dust, fæces, hay, etc. To obtain pure cultures proceed as follows:—Cut some hay into small pieces, place in an Erlenmeyer flask, cover with water, and cook for fifteen minutes; by this means all germs are destroyed, except the *resistant spores* of the hay bacillus. These grow, and a thin membrane of hay bacilli forms in two to three days on the surface of the hay infusion. (See Photomicrograph, Fig. 80.)

Bacillus Mesentericus (Potato Bacillus).—There are three forms of the organism described:—

1. B. mesentericus vulgatus.
2. B. „ fuscus.
3. B. „ ruber.

The last form which invests the potato on which it is growing with a rose colour, possesses extremely resistant durable forms, which can endure boiling for five to six hours. The spores are, in comparison with the bacterial cell, very large. The cultural peculiarities resemble those of the hay bacillus. On potatoes the bacillus forms a strong rugged coating.

Milk is coagulated and peptonised.

Bacillus Spinosus.—Strongly anaërobic motile rods. In gelatine it forms opalescent spherical colonies with bristly outgrowths. The gelatine is liquefied with the formation of gas. The stab culture before liquefaction resembles a hairy caterpillar (Lüderitz). The bacillus grows at room as well as at incubator temperature. The spores are formed in the middle of the rods, which become enlarged like a spindle (clostridium). This bacillus is found in garden soil.

II.—MICROCOCCI.

1. *Nutrient Gelatine is not Liquefied.*

(*a.*) Chromogenic.

Micrococcus Auranticus.—Round to oval cocci, arranged in small clusters. In cultures the colonies are yellow, shiny, club-shaped, and do not extend very far over the media.

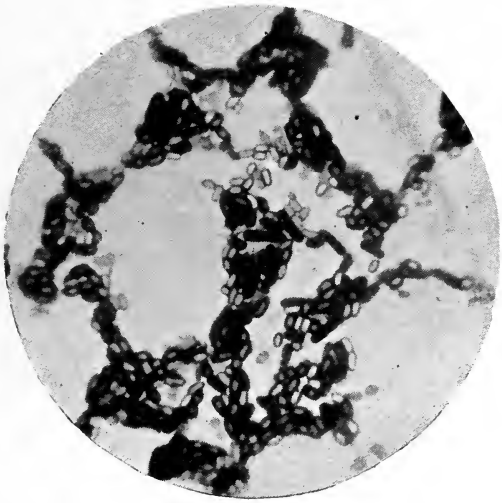


FIG. 80.—B. Subtilis and Spores from culture. Fuchsin. × 1000.

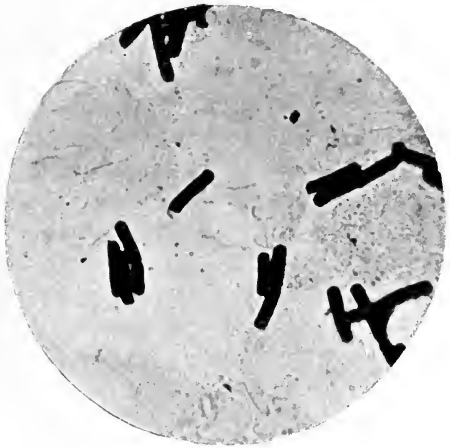


FIG. 81.—B. Subtilis, with Flagella. Agar culture. Stained with Orcein solution. × 1000.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

Micrococcus Versicolor.—Small cocci, arranged in small groups or in the form of diplococci. They are very frequent in the air. The cultures present an irregular form and yellowish-green colour. On gelatine they exhibit a mother-of-pearl like iridescence, and cause fermentation in grape sugar media.

(*b.*) Non-Chromogenic.

Micrococcus Candicans.—Round medium-sized cocci, best known by their growth in gelatine stab cultures, in which they form a nail-like growth with a porcelain white shiny head.

Micrococcus Concentricus.—Small cocci, arranged like bunches of grapes (Staphylococci), characterised by the zonary growth of the colonies on gelatine plates and in stab cultures. The colonies vary in colour from white to a bluish-grey, and are indented on the surface.

Micrococcus Rosettaceus.—Medium-sized cocci. The growth is frequently superficial in rosette-formed masses, with irregular edges of a greyish colour, the central portion varying from dark grey to brown.

Micrococcus Aquatilis.—Round light grey colonies with a mother-of-pearl lustre, the edges appearing indented. Under a low power the colonies resemble a berry in shape.

2. Nutrient Gelatine is Liquefied.

(*a.*) Chromogenic.

Micrococcus Cremoides.—Small cocci, arranged in bunches, and forming a cream-coloured pigment. In the commencement on gelatine yellowish-white to brownish-green granular circular colonies develop; later, the discs appear fixed, and lie in a liquefied depression.

Micrococcus Agilis.—Strongly motile coccus, possessing flagella; grows on the different media, forming a rose-coloured pigment. Gelatine media are liquefied slowly.

Sarcina Lutea (Yellow Sarcina).—Strongly aërobic cocci, arranged in the so-called bale-like forms. On gelatine plates, round, slightly granular yellow colonies develop.

In Stab Cultures a strong surface growth. The cultures form a citron yellow pigment. The gelatine is liquefied very slowly, and the citron yellow growth falls to the bottom of the tube, while the upper portion of the medium remains clear.

Besides the yellow sarcina, there are *white, orange,* and *red* sarcinæ, which are only distinguished from the above-mentioned yellow form by the different colours of the pigment produced. These sarcinæ are all found in the air.

(*b.*) Non-Chromogenic.

Micrococcus Radiatus.—Small cocci with no typical arrangement. On plate cultures the colonies appear surrounded by a radiating border. In stab cultures the growth exhibits horizontal rays, and the gelatine is liquefied slowly.

III.—VIBRIOS.

Vibrio Aquatilis (Günther).—This vibrio is distinguished from the cholera vibrio in the first few days of its development easily, and with certainty by the character of its growth. It forms circular, smooth edged, finely granular colonies. In later stages, when the gelatine commences to liquefy, a faint resemblance to the colonies of the cholera vibrio appears. The vibrio aquatilis is further distinguished from the cholera vibro by the nitroso-indol reaction being negative, and the total absence of any pathogenic properties. The vibro aquatilis at first grew badly in fluid media, but after many generations, cultures were obtained in bouillon and peptone water. Günther failed to observe the formation of spirilla with this vibrio.

Vibrio Berolinensis.—Found by Neisser in Berlin conduit water. On gelatine plates the edges of the colonies are mostly smooth, and exhibit a much more granular appearance than the colonies of the cholera Asiatica vibrio; the gelatine is liquefied slowly, and the nitroso-indol reaction is positive. Guinea-pigs inoculated intraperitoneally, die with the same symptoms as those following the introduction of the genuine comma bacilli of Asiatic cholera. Similar vibrios have been isolated by Weibel, Löffler, Fokker, Kiesling, and also from the river Seine.

The non-identity of this and the other vibrios with the real cause of Asiatic cholera is determined by the negative results with Pfeiffer's reaction and the agglutination test.

Vibrio Metschnikoff.—This vibrio was first found in an epidemic amongst chickens, then in water from the river Spree. It is somewhat thicker and shorter than the vibrio of Asiatic cholera, often exhibiting a coccoid formation. It is stongly motile. The cultures resemble those of the vibrio of Asiatic cholera, but the liquefaction of gelatine media is more pronounced, and already in twenty-four hours there is a well-marked nitroso-indol reaction present. This vibrio, in contradistinction to Koch's vibrio, is just as pathogenic for pigeons as for guinea-pigs. (See Photo-micrograph from blood of inoculated pigeon, Fig. 82.)

Vibrio Gindha (Pasquale).—Found in well water at Massauah; somewhat long, slightly bent rods, stongly motile, possessing one flagellum. Slightly pathogenic; nitroso-indol reaction negative.

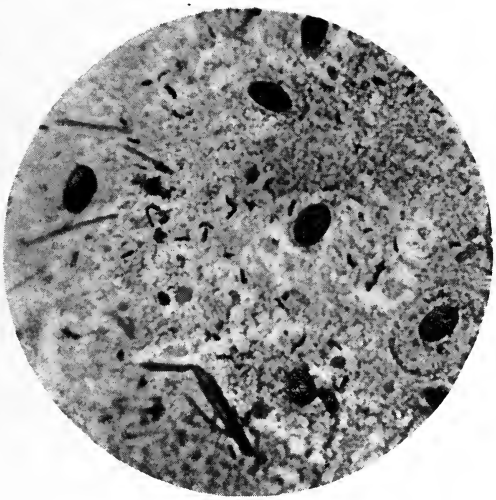


Fig. 82.—V. Metschnikoff, from blood of inoculated pigeon. Fuchsin. × 1000.

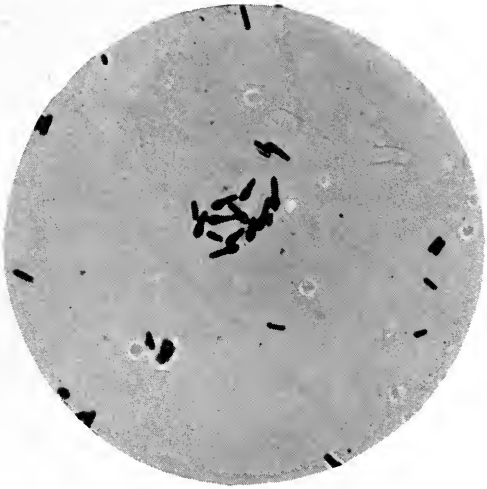


Fig. 82*a*.—Trommelschläger Bacilli. Cover-glass specimen from agar culture. Fuchsin. × 1000.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

Vibrio Lissabon.—Obtained in a widespread cholera epidemic in Lisbon, in which only one death occurred. On gelatine plates it forms circular, sharply limited, slightly liquefying, whitish-yellow colonies. Nitroso-indol reaction negative.

Vibrio Phosphorescens (Dunbar).—Isolated from the river Elbe. Morphologically and in cultures it resembles the vibrio of Asiatic cholera, but is distinguished by being phosphorescent.

Vibrio Massauah.—This vibrio possesses two to four flagella, while the vibrio of Asiatic cholera has only one. The nitroso-indol reaction is positive. It is pathogenic for pigeons, guinea-pigs, and rabbits.

BACTERIA FOUND IN MILK.

Bacteria are always present in milk unless it is drawn from the udder under sterile precautions. Milk can be contaminated in various ways: from the gland direct, *i.e.*, B. tuberculosis, etc.; 'by the hands of the milker,' dirty vessels, hay dust, etc., and by the water added by the thrifty dairyman. Milk is an excellent medium for the development of many forms of bacteria, and under favourable conditions, temperature, etc., it quickly undergoes changes.

Pathogenic bacteria may also contaminate milk. The organism most frequently found being the B. tuberculosis. Pyogenic cocci have also been found. From contamination with diseased matter from affected persons and premises, milk may be the means of carrying and spreading typhoid fever and diphtheria (and according to some authorities, scarlet fever). Such organisms are, however, easily destroyed; the most resistant of all, the B. tuberculosis, being killed by *Pasteurizing* the milk for thirty minutes at 70° C., or by cooking the milk for ten minutes, whereby it is heated between 90° and 95° C. Nothing now remains after the process of sterilization except the resistant spores of some bacteria. (For special methods of examining and staining bacteria in milk, see Technique, § 20.)

A.—COCCI.

MICROCOCCUS ACIDI LACTICI.

Found in fresh milk, occurring either as single large cocci or diplococci.

Biological Characters.—Aërobic.

On Gelatine Plates.—Forms small yellowish non-liquefying colonies.

Milk is first coloured red, then coagulated, finally becoming decolorized.

SPHÆROCOCCUS ACIDI LACTICI.

Found in fresh milk; small cocci, occurring as diplococci or arranged in clusters.

Biological Characters.—Facultative anaërobe.

On Gelatine Plates.—Round, white, non-liquefying colonies.

Milk is coloured red and coagulated with the formation of an acid.

STREPTOCOCCUS ACIDI LACTICI.

Found in curdled milk; small cocci, arranged in chains.

Biological Characters.—

On Gelatine Plates.—Round, white, non-liquefying colonies.

In Gelatine Stab Cultures the growth is entirely confined to the stab.

Milk is coagulated with the formation of an acid.

MICROCOCCUS ACIDI LACTIS LIQUEFACIENS.

Found in butter; oval cocci, occurring in diplococci or tetrads.

Biological Characters.—Facultative anaërobe; optimum temperature 21° C.

On Gelatine Plates.—Small round, white, liquefying colonies.

In Gelatine Stab Cultures.—A liquefying, funnel-shaped growth, with a film on the surface.

Milk is coagulated with the formation of an acid. The casein is not peptonised; in one to two weeks it acquires a musty odour.

STREPTOCOCCUS HOLLANDICUS.

Found in the ropy whey used in making Edam cheese.

Microscopical Appearances.—Occurs in pairs and frequently in long chains.

Actions.—When sterile milk is inoculated with this organism it becomes sour and ropy in twelve to fifteen hours at 25° C.

B.—BACILLI.

BACILLUS ANAËROBIUS (FLUGGE), II., III., IV.

Three different species of rods found several times by Flugge in milk that was cooked one and a half hours. Species III. and IV. form spores.

Biological Characters.—They are all anaërobic and liquefy gelatine media very quickly, and form gas quickly in sugar-containing media.

In Milk, No. II. causes coagulation without putrid gas formation.

No. III. has no effect.

No. IV. coagulates milk with putrid gas formation.

Pathogenesis.—Nos. III. and IV. are poisonous to animals, while No. II. is not.

BACILLUS CYANOGENUS (Bacillus of blue milk).

This organism, the cause of blue-coloured milk, was first cultivated by Hueppe on gelatine plates.

Microscopical Appearances. — Bacilli of various dimensions according to Hueppe and Flugge, 0·3 to 0·5 by 1 to 4 μ.

Motility.—Motile, the flagella being arranged in a bundle at one end (Lophotricha).

Spore Formation.—Absent. The spores described by Hueppe are considered by Heim as nothing but involution changes.

Staining Reactions.—Stains with the ordinary dyes, but not by the Gram method.

Biological Characters.—Grows best at ordinary room temperature; at 37° almost no growth takes place.

On Gelatine Plates.—Greyish-white, granular, non-liquefying colonies with scalloped edges.

In Stab Cultures the deep growth is very limited.

On Agar.—Greyish-blue growth.

On Potatoes.—A yellowish shiny coating. Two pigments are formed, a blue and a fluorescent colour. In gelatine cultures the fluorescence appears first, and later the blue-black pigment. The latter develops more luxuriantly on agar media.

In Milk the blue pigment is only formed in the presence of an acid. In non-sterile milk blue spots appear at first on the cream; finally the whole surface is coloured a sky blue.

In Sterile Milk a grey colour develops, which only turns blue on the addition of an acid. If grape sugar is added to sterile milk it is coloured blue by the bacillus, an acid being formed from the sugar.

Pathogenesis.—Non-pathogenic.

This bacillus was supposed to cause the blue colour in cheese, but such a supposition is untenable in view of the inoculation experiments conducted by Adametz and Beyerinck.

BACILLUS ACIDI LACTICI (HUEPPE).

Found in sour milk. This organism is without doubt identical with the Bacterium lactis discovered by Lister in 1877. Lister also discovered that lactic acid bacteria, although frequently found in dairies, are very seldom found in the open air.

Microscopical Appearances.—Non-motile rods 1·0 to 1·7 μ long, and 0·3 to 0·4 μ broad, mostly in pairs, but sometimes arranged in small chains.

Spore Formation absent.

Biological Characters.—Facultative anaërobe; optimum temperature, 37° C.

On Gelatine Plates.—Flat diffuse superficial colonies with irregular borders, resembling those of Bacterium coli commune.

In Gelatine Stab Cultures.—A nail-shaped growth.

On Agar.—A yellowish-white coating.

On Potatoes.—A yellowish-brown coating.

In Milk, it forms an acid which precipitates the casein, producing alcohol and CO_2.

The above organism is not the only organism that changes milk-sugar into lactic acid, but it is probably the common cause of the spontaneous souring of milk.

BACILLUS LACTIS ACIDI (LEICHMANN).

This bacillus was found by Leichmann in milk, which, when maintained at 50° C., underwent spontaneous lactic acid fermentation. When cultivated in sterile milk it forms lactic acid, which turns polarized light to the left.

BACILLUS LACTICUS (GÜNTHER AND THIERFELDER).

Found in sour milk.

Non-motile bacilli, 0·5 to 0·6 by 1·0 μ, usually arranged in pairs or small chains. It does not produce spores, but stains by the Gram method.

Biological Characters.—Grows best at 28° C., and better on media containing sugar than on the ordinary media.

Gelatine Media are not liquefied. The developing colonies remain very small, but well marked.

On Potatoes the development is very scanty.

In Bouillon.—A turbid growth which does not cause any change in the reaction.

In Grape and *Milk Sugar Bouillon* the growth is luxuriant, the media becoming strongly acid without the production of gas.

To obtain pure culture of the organism from sour milk proceed as follows:—

1. Melt three tubes of gelatine in the water-bath at 30° C.

2. Put some $CaCO_3$ in a clean tube, add a little water, sterilize in the steamer, or boil over the Bunsen flame.

3. Inoculate the first or original tubes with three platinum loops of milk, and reduce in the usual manner, using five platinum loops of milk. Mix the milk and gelatine thoroughly.

4. Put a few drops of the solution of $CaCO_3$ in three sterile Petri-dishes, pour the inoculated gelatine on the $CaCO_3$, and see that they are thoroughly commingled.

After development each acid-forming colony will be found surrounded by a transparent field.

BACILLUS LACTIS (FLUGGE).

Found in bitter milk. Flugge isolated eleven different varieties, which all belong to the group of hay bacilli. They possess the power of peptonising the casein of the milk, whereby it acquires a bitter taste. A few of the varieties produce toxic substances which, when given to young dogs *per os*, caused diarrhœa, muscular weakness, and falling of the temperature. They form very resistant spores, which are not destroyed by several hours' cooking. It is on account of these spores that the sterilization of milk is so difficult. If improperly sterilized milk is placed at a high temperature, the spores germinate, the above-mentioned poisonous substances being formed. Some authors consider that these substances may be the cause of summer diarrhœa in children.

The morphological characters of these eleven species of bacteria are similar to those of the Bacillus subtilis (see page 210). They are all motile rods of various lengths, and all liquefy gelatine media. (See also Bacillus Anaërobius (Flugge), 2, 3, and 4, page 214).

BACILLUS LACTIS INOCUUS.

Found in the fæces of infants and in milk; short, non-motile rods. In the animal body it forms capsules.

Biological Characters.—Aërobic.

On Gelatine Plates white, round, non-liquefying colonies develop.

On Potatoes a brownish coating.

Milk is not changed. In grape sugar agar no gas is formed. Indol is not formed.

Pathogenic only in very large doses.

BACILLUS LACTIS ALBUS (LÖFFLER).

Found in butter-milk; very long motile bacilli, arranged sometimes in threads. Spore formation present.

Biological Characters.—Gelatine media are liquefied.

On Agar a thick whitish coating develops.

On Potatoes dry white colonies.

Milk is coagulated, and the casein peptonised.

BACILLUS LACTIS (BLEISCHII).

Found in butter-milk; large motile bacilli, forming spores.

Biological Characters.—Facultative anaërobe.

Gelatine Media are liquefied.

On Agar and *Potatoes* a light grey coating. The spores are very resistant, and are only killed after being cooked for six hours.

BACILLUS LACTIS ERYTHROGENES (HUEPPE).

Found in red-coloured milk; short, non-motile rods 0·3 to 0·5 by 1 to 1·4 μ.

Spore Formation absent.

Biological Characters.—*On Gelatine Plates,* round, yellow, gradually liquefying colonies; the gelatine surrounding the colonies is coloured red.

In Gelatine Stab Cultures the development is very slow; and kept in a dark place, the medium is coloured red.

On Agar and *Potatoes* a yellowish coating develops, while the surrounding medium is coloured a faint red.

Milk is fermented and peptonised, obnoxious-smelling gases being formed; it is at first a dirty red, then brownish-red, and finally blood-red coloured. The yellow pigment in the colonies is soluble in all extractive materials, while the red pigment is also soluble in water.

Pathogenesis.—The red milk is not pathogenic for man.

BACILLUS LACTIS PITUITOSI (LÖFFLER).

Obtained by Löffler from milk.

Microscopical Appearances.—Somewhat thick, slightly bent rods, that soon break up into coccoid-like segments.

Biological Characters.—In gelatine it forms white colonies which, by transmitted light, appear brown-coloured, usually sharply outlined, but sometimes indented.

On Agar, dirty white-like colonies develop.

On Potatoes a greyish-white, pearl-like, somewhat dry coating.

Milk becomes slightly acid and slimy, giving off a quite specific odour.

Whether this ropy mass is formed from the milk sugar or from the casein has not been determined.

BACILLUS LIMBATUS ACIDI LACTICI.

Found in milk.

Microscopical Appearances. — Short, non-motile rods, mostly arranged in diplococci. Capsules present. Spore formation absent.

Biological Characters.—*On Gelatine Plates* round, white, non-liquefying colonies.

In Gelatine Stab Cultures the growth is mostly on the surface.

Milk is coagulated a red colour, an acid being developed.

BACTERIUM ACIDI LACTICI (GROTENFELD).

Found in sour milk. Small, non-motile bacteria 0·3 to 0·4 by 1 to 1·4 μ.

Biological Characters.—Facultative anaërobe; optimum temperature, 37° C.

On Gelatine Plates round porcelain-white colonies.

In Gelatine Stab Cultures a nail-like growth.

On Potatoes a greyish covering.

Bacteria causing Ropiness in Milk.

I.—BACILLUS GUMOSUS.

Found in slimy milk ; large, slightly motile bacteria.

Spore Formation present.

Biological Characters.—Gelatine medium is liquefied slowly.

On Agar and *Potatoes* a puckered white coating. Cane sugar is fermented, and when alcohol is added to the solution gum is formed.

II.--BACILLUS VISCOSUS LACTIS (ADAMETZ).

Found in water.

Microscopical Appearances.—Non-motile bacilli, 1·1 to 1·3 by 1·2 to 1·7 μ; sometimes occurring in threads with capsules.

Biological Characters.—*In Gelatine Media* the deep colonies are small, while the surface colonies exhibit an extensive development of slimy drops with serrated edges.

On Agar a dirty-white, ropy, slimy coating develops.

Milk at ordinary temperature in five to ten days becomes ropy, and finally transparent, from disintegration of the milk globules.

BACTERIUM ACIDI LACTICI (PETERS).

Found in sour dough. Short motile rods, 0·4 to 1·5 μ.

Biological Characters.—*On Gelatine Plates* round colonies with concentric stratification of a light red colour. In solutions of sugar, to which some yeast is added, this organism produces great quantities of lactic acid.

Bacteria causing Acetic Acid Fermentation.

BACILLUS ACETICUS (HANSEN).

(Mycoderma Aceti.)

The Mycoderma aceti was considered by Pasteur to be the cause of the acetic acid fermentation in wine and beer. (Hansen divides it into two forms, *Bacillus aceticus* and *Pasteurianus.*)

Microscopical Appearance.—Short, non-motile bacilli, forming threads and frequently involution forms.

Spore Formation absent.

Staining Reactions.—Stains by the Gram method.

Biological Characters.—Aërobic; optimum temperature, 30° to 40° C.

On *Solid Media* it grows on the surface, forming porcelain cup-like colonies, especially on *Beer Gelatine;* prepared by adding 5 per cent. gelatine to beer.

In Liquid Media a membrane forms on the surface, the underlying liquid being slightly clouded.

Specific Actions.—It oxidises alcohol, forming acetic acid, and further splitting up the latter into CO_2 and H_2O. It is distinguished from other members of the group by staining yellow when treated with a solution of iodine.

BACILLUS PASTEURIANUS (HANSEN).

(Mycoderma Pasteurianum).

This bacillus is found in beer and wine, but does not occur as frequently as the previous organism, from which it is distinguished by giving a blue reaction with solution of iodine.

BACILLUS ACETICUS PETERSII.

Found in old sour dough; resembles the Bacillus aceticus of Hansen. Forms threads; is strongly aërobic; on gelatine slimy colonies develop.

Bacteria causing Butyric Acid Fermentation.

BACILLUS BUTYRICUS (BOTKIN).

Found in water, milk, and manured earth.

Microscopical Appearances.—Long motile bacilli, forming threads.

Spore Formation present, situated in the middle of the rods, very resistant, and are not killed during the process of sterilizing milk. As they do not germinate under 18° C., milk sterilized for children should be kept at a lower temperature before use.

Staining Reactions.—Stains by the Gram method.

Biological Characters.—Anaërobic.

On Gelatine Plates.—Round or oval liquefying colonies, developing an odourless gas.

In Milk at the bottom of the culture a layer of serum forms, out of which the gas rises upwards; the milk is coagulated. The coagulated albumen rises to the surface and is peptonised, so that eventually only the fat swims on the surface. Butyric and allied fatty acids are formed. In media containing sugar involution forms of the bacilli occur.

BACILLUS BUTYRICUS (PRAZMOSKI).

(Clostridium Butyricum.)

Found in putrid vegetable infusions.

Microscopical Appearances.—Strongly motile bacilli, about 1 μ broad, of various length, forming threads.

Spore Formation present, situated in the middle of the rod, causing a spindle-formed swelling. The spores are 1 μ broad and 2 to 2·5 μ long. When the mature spore germinates the germinating rod escapes at one end, while the spore membrane remains attached at the other parts of the young bacillus like a cap.

Staining Reactions.—With a watery solution of iodine the bacilli, when cultivated on media containing starch, are stained blue, whereby it is called '*The Bacillus Amylobacter.*'

Biological Characters.—Strongly anaërobic. In solutions of starch, dextrin, sugar, and lactic acid salts a great quantity of butyric acid is formed, accompanied by the development of H and CO_2.

BACILLUS BUTYRICUS (HUEPPE).

Microscopical Appearances.—Large rods, frequently occurring in pairs.

Spore Formation present, situated in the middle of the rods.

Biological Characters.—Grows in the presence of oxygen on the ordinary media at both room and incubator temperature.

Gelatine Media are quickly liquefied.

On the surface of Agar Media, a moist yellowish coating.

In Sterile Milk it develops best at incubator temperature, when coagulation occurs similar to that produced by rennet, without the reaction of the milk being changed. The casein is next dissolved and changed into peptone and other products, ammonia being produced. At the same time the milk acquires a bitter taste. This bacillus forms butyric acid out of lactic acid salts.

Bacteria causing Specific Changes in Beer, Wine, and Sugar.

BACILLUS VISCOSUS CEREVISIÆ (VAN LAER).

Found in ropy beer, yeast, in the air, and on slimy bread.

Microscopical Appearances.—Rods 0·8 to 1·6 by 2 to 4 μ, seldom occurring in chains; said to form spores situated in the end of the rods.

Biological Characters.—*In Gelatine Stab Cultures* the growth is uniform along the inoculation track, and on the surface a diffuse white growth develops. The colonies are sharply circumscribed, and when examined under a low power appear brown coloured; old colonies are serrated, and in the middle, white, curly, and slightly thready.

In Beerwort at 27° C. a germine ropiness occurs in twenty-four hours, a large amount of CO_2 being formed; the surface being studded later with yellowish, ropy, slimy islets.

Milk and *Solutions of Peptone and Cane Sugar* also become ropy, gas being formed.

On Potatoes, white, warty, viscous colonies develop, giving off a smell like decayed fish. (Van Laer also describes another similar organism, which is distinguished from the above by only producing slight fermentation and ropiness.) The injurious influence of this organism is only manifest when it obtains access to the wort before the primary

fermentation. When added afterwards no injurious actions were observed. The ropiness in the beer is due to two mucilaginous substances produced by this organism; one contains nitrogen and is insoluble, while the other contains no nitrogen and is soluble in water.

BACILLUS VISCOSUS SACCHARI (KRAMER).

Found in slimy solutions of sugar.

Microscopical Appearances.—Small, non-motile rods, forming threads but no spores.

Biological Characters.—Optimum temperature, 22° C.

Stab Cultures in cane sugar gelatine liquefy quickly, an adherent sediment being deposited.

Sugar solutions containing besides the necessary nitrogenous substances for the growth of the bacteria, are changed into slime.

BACILLUS VISCOSUS VINI (KRUMER).

Found in slimy wine.

Microscopical Appearances. — Non-motile bacteria of various lengths, forming threads.

Biological Characters.— Strongly anaërobic; optimum temperature, 18° C. Develops only on wine and glucose solutions. Wine is fermented in one to two months, a thick slime being formed.

LEUCONOSTOC MESENTEROÏDES (CIENKOWSKI).

(Froschlaich pilz. Pilz der Dextrangarüng. Frog spawn fungus.)

Found on beetroot juice and molasses of sugar factories, where it develops in large gelatinous masses, resembling *frog spawn*. It is also found on raw or cooked carrots and sugar beets.

Microscopical Appearances.—It forms chains of spherical or oval cocci from 1·8 to 2 μ in diameter, enclosed within a thick, tough, membranous envelope. Finally, owing to the anastomoses of numerous chains they appear as large, compact, gelatinous, zoöglœic masses.

Staining Reactions.—The cover-glass specimen is first stained with dahlia violet, which stains the cocci, and then immersed in an aqueous solution of rosolic acid, which stains the gelatinous envelope a rose-red colour.

Biological Characters.—It is a facultative anaërobe; optimum temperature, 30° to 37° C. It is very difficult to obtain in pure cultures,

owing to the gelatinous mass being contaminated with various fungi; to overcome this difficulty heat the cultures continuously for fifteen minutes at 75° C., in order to destroy the fungi. The gelatinous envelopes only develop in cultures on cane or grape sugar media, and in a short time the growth acquires great dimensions. Scheibler considers the gelatinous substance to be dextrin.

On ordinary Gelatine the growth exhibits no special characteristics. *On Gelatine* containing grape sugar it is very diagnostic; consisting in ten to fourteen days of a whitish confluent mass, with slimy, hyaline, gelatinous lumps on the surface. During the first eight days the growth exhibits a dry elastic consistence, but during the next few weeks it becomes softer, moister, finally forming a soft pulp. Individual colonies resemble wart-like balls, sometimes spread out with a puckered film on the surface.

In Grape Sugar Gelatine Stab Cultures proliferations of various dimensions occur along the inoculation track.

As already mentioned, the gelatinous substance is only formed in material containing grape or cane sugar. Other carbohydrates tested by Leisenberg and Zopf were found unsuitable. The organism produces *Invertin*, which splits up the cane sugar. It ferments lactose, maltose, and dextrin, forming lactic acid with a faint evolution of gas. The addition of 3 to 5 per cent. of calcuim chloride to the nutrient medium favours the production of mucus and the fermentation activity of the organism, which is also brisker when oxygen is excluded.

LEUCONOSTOC INDICUM.

This organism is the cause of considerable damage to the Java sugar industry. According to Leisenberg and Zopf the only difference between this organism and the Leuconostoc mesenteroides is a slight difference in the optimum temperature at which it develops.

ASCOCOCCUS BILLROTHII.

Found by Billroth in putrefying meat infusion.

Microscopical Appearances.—Small cocci, arranged in peculiar colonies, which form a creamy layer upon the surface of liquid media, containing numerous small spherical or oval masses. These masses consist of a jelly-like, extremely resistant envelope, from 10 to 15 μ thick; in the interior of the envelope one or more masses of cocci are situated, from 20 to 70 μ or more in diameter. The cocci are closely arranged and united by a firm and scanty intercellular substance.

Biological Characters. — Aërobic. Grows at ordinary room temperature. Produces a strongly alkaline reaction in culture media,

due to the development of ammonia. According to Cohn, it produces a greenish-white slimy mass upon slices of beetroot, and in the juice of sugar beets a slimy fermentation.

THE PHOSPHORESCENT BACTERIA (Photo-Bacteria Beyerinck).

BACTERIUM PHOSPHORESCENS (FISCHER).

Found on dead meat and fish.

Occurs as non-motile, short rods, sometimes in zoöglœa; forms no spores, and stains by the Gram method.

Biological Characters.—Facultative anaërobe. It only grows on media containing chloride of sodium.

In Gelatine Stab Cultures the growth is usually on the surface, and the gelatine is not liquefied.

Ferments all kinds of sugar.

The blue-green phosphorescence is best seen on cultures on dead fish, meat, and sea-water.

BACTERIUM PHOSPHORESCENS PFLUGERI.

Found under similar conditions to the above Bacterium phosphorescens, and also exhibits identical properties. It is, however, longer, more slender, and does not so frequently form zoöglœa. Causes fermentation in all the sugars except maltose.

BACILLUS ARGENTO PHOSPHORESCENS (KATZ).

Found by Katz in Australia in sea-water and on dead sea animals. There are three varieties, which only exhibit a slight differentiation.

They occur in rods 0·6 to 0·8 by 2·5 μ, are motile, and stain by the Gram method.

The culture media are not liquefied. Yellowish colonies develop, exhibiting a silvery-white phosphorescence of a greenish tint, which is however, not so pronounced as in the aforementioned varieties.

BACILLUS PHOSPHORESCENS (GIARDI).

Found on living and dead crustaceous animals. It is pathogenic for the same, especially Talitrus orchestia, which it kills in six to nine days, the whole body of the animal being covered with greenish phosphorescent bacteria. When cultivated on ordinary media they lose

their virulence, but regain it again when cultivated on fish media. Morphologically and in the cultures the appearance is very similar to the Bacterium phosphorescens, only smaller and more coccoid-like.

BACILLUS PHOSPHORESCENS INDICUS (B. FISCHER).

Cultivated by B. Fischer from phosphorescent sea-water in the West Indies.

Microscopical Appearances.—Motile rods, twice as long as broad (0·6 to 0·8 by 2 μ), often arranged in crooked threads.

Staining Reactions.—Do not stain by the Gram method.

Spore Formation absent.

Biological Characters.—Grows under aërobic conditions at medium temperatures.

On Gelatine Plates, round, bluish-green, slowly liquefying colonies, which later become granular and of a brownish colour.

In Gelatine Stab Cultures, funnel-shaped liquefying growth; in the deeper portion of the medium the growth is limited.

On Agar and *on Potatoes* cooked in sea-water, a dirty whitish coating develops. Blood serum is liquefied. The blue phosphorescence is very well marked in cultures on dead sea animals, sea-water, and meat. The organism is non-pathogenic.

BACILLUS PHOSPHORESCENS INDIGENUS (FISCHER).

Found by Fischer in Kiel harbour. It is very similar to the B. phosphorescens indicus, only gelatine medium is liquefied more slowly, while blood serum is not liquefied. It grows at lower temperatures, and causes no phosphorescence on meat.

THERMOPHILIC BACTERIA.

Miquel found a bacillus in the Seine in 1891, which possessed the faculty of growing at a temperature of 69° to 70° C. In 1887, Koch and Globig found bacteria in the surface of the earth, which grew between 50° and 70° C. M'Fadyean and Rabinowitsch have also contributed to our knowledge of this group of bacteria.

The Thermophilic bacteria are mostly bacilli that are facultative anaërobes and non-pathogenic. Most of the forms produce spores which exhibit great resistance. All the various forms grow at a temperature between 56° and 58° C. A few forms grow at 68° C., and at 70° C. Globig observed growth only exceptionally.

THE DRUMSTICK BACTERIA.

(*Ger.* Trommelschlägerbacillen.)

This is a group of saprophytic organisms which form spores at the end of the bacillus, which thus acquires the form of a drumstick. (See Photomicrograph, Fig. 82*a*.) These bacilli are widely distributed organisms.

Microscopical Appearances.—Very small rods, strongly motile.

Gelatine Media are liquefied.

Grether describes a variety that is non-motile, and does not liquefy gelatine.

BACILLUS CAPSULATUS (PFEIFFER).

Pfeiffer isolated the organism from a purulent exudate found in the peritoneal cavity of a dead guinea-pig, also present in the blood.

Microscopical Appearances.—It is a plump bacillus with rounded ends, possessing a well-defined ovoid capsule.

Motility.—Non-motile.

Spore Formation has not been confirmed.

Staining Reactions.—The reaction with the Gram method is negative ; the capsules are easily demonstrated when stained by Johne's method. (See Techniqne, § 22.)

Biological Characters.—Facultative anaërobe growing on the ordinary nutrient media, better at 37° C. than at ordinary room temperature.

In Gelatine Stab Cultures a slimy, white, nail-formed growth develops, and an inodorous gas is formed ; the gelatine is not liquefied.

On the Surface of Agar it forms a thick, moist, white viscid coating.

On Potatoes, a yellowish-white viscid covering.

Pathogenesis.—White and house mice inoculated subcutaneously die in from two to three days. The spleen of the dead animals is found greatly enlarged, and the bacilli are present in the blood and organs, with well-defined capsules. Guinea-pigs, pigeons, and rabbits are also susceptible, guinea-pigs and pigeons only by intraperitoneal infection, and rabbits only when large quantities of the culture are introduced intravenously. The bodies of the dead animals undergo putrefactive changes very quickly. The blood and tissue juices exhibit a stringy consistence.

BACILLUS MEGATERIUM.

(De Barry.)

Found in the earth, air, and on the leaves of cooked cabbages.

Microscopical Appearances.—Very long (sometimes 10 μ and 2·5 μ thick), slightly bent bacilli with round ends. Involution forms often present.

Motility.—Slightly motile; flagella four to eight, arranged on the sides (peritricha).

Spore Formation.—Endogenous, with spores nearly as long as the cells. (For Photomicrograph of rods and spores, see Fig. 83.)

Biological Characters.—Strongly aërobic; optimum temperature, 20° C.

On Gelatine Plates, kidney or sickle-shaped granular colonies, that liquefy the medium slowly.

On Agar, a whitish coating.

On Potatoes, a thick greyish-yellow coating.

On all the media it forms a slimy mass; and according to Günther, should be classified with the capsule bacteria.

BACTERIUM ZOPFII.

Found by Kurth in the intestinal contents of a chicken in 1883. It has also been found in water and fæces. Günther found it in sausages in 1897.

Microscopical Appearances.—Short, plump bacilli, 2 to 5 μ long and 0·75 to 1 μ broad, forming long, short-jointed chains.

Motility.—Motile.

Staining Reactions.—By the ordinary methods and also by the Gram method.

Biological Characters.—Aërobic; at 37° C. the growth is not so luxuriant as at lower temperatures.

On Gelatine Plates.—Examined with a low power the colonies consist of long threads coiled up like tangled balls.

In Gelatine Stab Cultures, the growth, which takes place only on the surface, consists of a coating of finely-arranged radiating threads. Old stab cultures are inodorous.

On Agar, at 37° C., a thin greyish coating develops.

In Bouillon, at 37° C., the growth is hardly visible.

Milk is not altered.

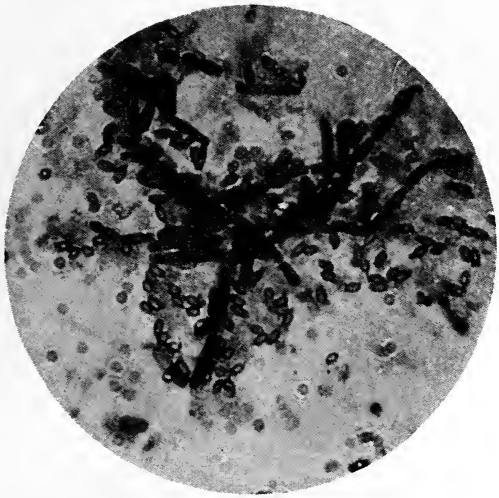


FIG. 83.—B. Megaterium and Spores from a culture. Fuchsin. × 1000.

FIG. 84.—Cladothrix found in water. Cover-glass specimen from gelatine culture. Fuchsin. × 1000.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

The reaction of Grape and Milk Sugar Media is not changed, and no gas is formed.

Indol is not formed.

Non-pathogenic.

THE CLADOTHRICES.

These organisms are found in water, and consist of colourless bacteria, which do not contain sulphur grains; arranged in threads. The principal characteristic of this group is the *false* branching of the threads or pseudo-ramification. Three forms of Cladothrix are well known.

CLADOTHRIX DICHOTOMA (F. COHN).

Found in both standing and running waters, which are more or less rich in organic substances. It is frequently associated with the Beggiatoa. It occurs in stationary tufts, 1 to 3 mm. high, and free in floating flakes. From the point of the Cladothrix threads individual elements (*Rodgonidia*) become detached and float round free for a time, then become stationary, and develop into fresh threads. The individual threads possess a distinct sheath. Artificial cultivation is very difficult. In solutions of extract of meat a thin film forms, which extends over the surface of the media and the walls of the tube. In meat extract gelatine, ramifying colonies, causing very slight liquefaction of the medium, develop. (For Photomicrograph of Cladothrix cultivated from drinking water, see Fig. 84.)

CLADOTHRIX INTRICATA (RUSSELL).

Found in sea slime in the Gulf of Naples. It is always free, and develops no sheath. It forms no tree-like ramifications, but an interlaced mass of threads. In certain threads the pseudo-ramification occurring in C. dichotoma takes place. The fresh threads are homogeneous, and when stained the large bacilli of which they are formed become distinctly visible. When free those bacilli become actively motile, and develop spores which are not thicker than the threads. It is easily cultivated artificially.

On Gelatine Plates, mould-like, quickly liquefying colonies develop, which under a low power appear to consist of a tangled mass of threads.

In Gelatine Stab Cultures radiations are given off from the growth, which become shorter towards the bottom of the stab.

On Agar, a whitish coating develops, from which projections penetrate the medium.

On Potatoes, a whitish coating.
In Bouillon, a gelatinous sediment.

CLADOTHRIX OCHRACEA (WINOGRADSKY).

Found in water containing iron. Very like the C. dichotoma. For its growth the water must contain carbonate protoxide of iron. By oxidation an oxyhydrate of iron is formed, which is deposited in the sheath and not in the threads of the organism. In water and under the cover-glass, cultures can be obtained by the addition of iron salts. In ordinary media the results are negative.

BEGGIATOA.

Found in water containing sulphuretted hydrogen.

Microscopical Appearances.—White threads without any distinct cell membrane, enclosing in their interior dark grains of sulphur, formed by the oxidation of the sulphuretted hydrogen. If a microscopical specimen is prepared and the *sulphur granules* dissolved by adding alcohol or bisulphide of carbon, a distinct system of transverse septa will be visible in the threads.

Biological Characters.—It can be cultivated in water containing sulphuretted hydrogen (also under the cover-glass), where active motility and slow growth is observed. Flagella have, however, not yet been demonstrated. Should the supply of sulphuretted hydrogen be interrupted, then the small grains of sulphur in the threads are gradually changed into sulphuric acid, when the threads appear completely homogeneous. In time degenerative changes occur, vacuoles being formed. The method by which the Beggiatoa increase is not absolutely known.

BACTERIA FOUND IN LEGUMINOUS NODULES.

The earliest description of the leguminous nodules is by Malpighi in his book published in 1687, in which he referred to them as galls, *i.e.* diseased excrescences. In 1853, Treviranus considered the nodules normal growths. In 1866, Woronin discovered that there were numerous entirely closed cells filled with living bacteria within these nodules. In 1879, Frank showed that nodule formation did not occur when the plants were grown in sterilized soil, thus proving that the co-operation of soil bacteria was a necessary factor. In 1888, the true

nature of these nodule bacteria was established without doubt when Beyerinck isolated them from the nodules and cultivated them further on artificial media. These bacteria are now looked upon as the generators of the nodules, by means of which the *Leguminosæ* are enabled to absorb nitrogen from the air and elaborate it into nitrogenous compounds, albumen, etc. The bacteria are situated in the cells of the inner layer of the nodule, which is known as the *bacteroidal* tissue, and under the influence of the surrounding protoplasm are modified into involution forms termed *Bacteroids,* rich in albumen and no longer capable of reproduction.

For further information on this subject, the reader is referred to an interesting article on 'The Bacteria of Soil, with special reference to Soil Inoculation,' by R. Stewart MacDougall, in the *Transactions of the Edinburgh Botanical Society,* July 1897.

BACILLUS RADICICOLA (BEYERINCK).

Found in young root nodules, also in the tissues of plants.

Microscopical Appearances.— In cultures it occurs as large rods 1 to 4 μ long, and in small clusters or *rovers* 0·18 to 0·9 μ. The large rods frequently exhibit knotted, irregular, fork-shaped or three-armed bodies. The bacteria in the nodules exhibit a similar morphology. The *rovers* belong to the smallest of known bacteria, and can escape through the pores of a Chamberland filter.

Motility.—Motile, especially the *rovers,* which sometimes escape from the parent colonies, and found a daughter colony at a distance in the gelatine.

Spore Formation absent.

Staining Reactions.—Sections of the nodules are best stained in a solution of equal parts of fuchsin and methyl-violet in 1 per cent. acetic acid. The plasmal contents and membrane of the nodule cells are coloured *blue,* the bacteria of the infection threads *red,* whilst the membrane of the latter remains uncoloured.

Biological Characters. — These organisms grow under aërobic conditions on ordinary gelatine, but very slowly. The best medium is a decoction of Papilionaceæ leaves or stalks, with 7 per cent. gelatine, $\frac{1}{4}$ per cent. asparagin, and $\frac{1}{2}$ per cent. cane-sugar added. The developing colonies are semi-circular, whitish or slightly clouded. The larger colonies are watery, while the smaller ones are solid and adhere in one piece. According to Beyerinck, the various species of Leguminosæ exhibit a difference in the form of the colonies and the bacteria. Kruse considers that they are probably only varieties of one species.

RHIZOBIUM LEGUMINOSARUM (FRANCK.)

Franck obtained cultures of this organism in liquefied drops of gelatine under microscopic control, and also on plates. According to the same author, the root bacteria occur only in the clusters (0·9 to 1·3 μ in length), oval or rod-shaped, which later form zoöglœa. The gelatine is liquefied slowly. The morphology is the same in all the Leguminosæ.

According to Franck one could at the most accept different varieties or nutrition modifications.

BACILLUS TUBERIGENUS (VON GONNERMANN.)

Found in the root nodules of the lupine bean, etc. Von Gonnermann, with a specially prepared lupine peptone gelatine, isolated ten different species of bacteria, two cocci, the Bacillus fluorescens non-liquefaciens, and seven bacilli which the author named Bacillus tuberigenus, Nos. 1 to 7. When sterilized earth was inoculated with Nos. 3 and 5, the formation of typical root nodules took place.

BACILLUS TUBERIGENUS (No. 3).

Is motile, and 0·3 to 0·6 μ in size.

On Gelatine.—Well defined, yellowish-brown, finely granular colonies develop; the gelatine is liquefied quickly.

On Potatoes, a bright reddish-brown coating.

BACILLUS TUBERIGENUS (No. 5).

Is non-motile and more slender than No. 3 (0·25 to 2 μ).

On Gelatine it forms colonies resembling those of the Bacillus anthracis, but does not give off any offshoots into the surrounding media.

On Potatoes it forms yellow prominent drops.

These bacilli, as well as the Bacillus radicicola, penetrate the root tissue of the Leguminosæ, and form in the cells irregular masses with offshoots and vacuoles, and as such possess the faculty of assimilating the nitrogen of the air. Entire clearness over the importance of these bacteria does not yet exist, nevertheless they appear to play an important rôle in the nourishment of plants, especially the Leguminosæ.

THE NITRIFYING BACTERIA.

According to Winogradsky the many species of nitrifying bacteria can be classified into two sharply divided sub-groups,—*Nitroso-bacteria* and *Nitro-bacteria.* The *Nitroso-bacteria* oxidize ammonia to nitrous acid.

During the oxidation processes the bacteria are protected from injury by the presence of bases which take up the acids with which the ammonia was initially combined, and also neutralize the resulting nitrous or nitric acid. Calcium carbonate performs this function excellently in the soil. Free alkali is unsuitable here for the fixation of the acids, because if present in quantity it would be injurious to the bacteria. In artificial cultures Winogradsky replaces the calcium carbonate by magnesium carbonate (Lafar's *Tech. Mycology*, § 204).

THE NITRO-BACTERIA.

NITROSOMONAS EUROPÆA (WINOGRADSKY).

Found in all samples of European, African, and Japanese earth examined.

Microscopical Appearances.—They occur in short chains of three to four individuals.

Spore Formation absent.

Motility present (Monotricha).

Biological Characters.—For method of isolating this organism see Technique, § 156.

On Silicic Acid Media the colonies are at first compact, with a sharp contour, brownish colour, sometimes resembling a spindle with blunt ends. In ten to fourteen days, round, clear, unstained masses, with irregular offshoots, consisting of motile monas, extend from the growth.

In Fluid Cultures, when quiescent, they collect in zoöglœa, forming a sediment especially around the precipitated carbonate. In seven days or less the fluid becomes cloudy, and in twenty-four to forty-eight hours the motile monas again sink to the bottom. The process of growth and the nitrite formation is now finished.

NITROSOMONAS JAVANIENSIS (WINOGRADSKY).

Found in Java earth, and very similar to the above organism.

NITROSOCOCCUS BRAZILIENSIS.

Found in the soil of Campinas, Brazil. They do not form zoöglœa, and possess no cilia, and attain a diameter of 2 μ. The species grown from Melbourne soil is indistinguishable from that found in Brazil; while that obtained from Quito (Ecuador) is a coccus, 1·5 to 1·7 μ in diameter.

NITRO-BACTERIA (WINOGRADSKY).

This organism was isolated from Quito earth, and forms nitrates out of nitrites.

Microscopical Appearances.—Very small rods, 0·2 to 0·25 by 0·5 μ.

Motility.—Non-motile.

Biological Characters.—*On Silicic Acid Plates* they develop in lenticular or club-shaped colonies.

In Fluid Media it develops in the form of thin films, firmly adherent to the walls and bottoms of the flasks — there is no cloudiness. Burri and Stutzer have with the assistance of silicic acid plates isolated a nitrate builder in European earth, which is distinguished from the above Winogradsky organism by being somewhat larger on solid media, motile, and transferable to ordinary nutrient media (gelatine and bouillon). When grown on the latter medium and again returned to a nitrate solution, have mostly exhibited the remarkable faculty of producing nitrification.

Winogradsky ascertained by comparative investigations that the nitroso-bacteria are the more active of the two.

Both nitroso- and nitro-bacteria are always present in the soil, the latter immediately oxidizing the nitrous acid generated (from the ammonia salts) by the former.

Whether nitrification commences in the dung-heap or in the field is dependent on various circumstances. It takes place whenever a sufficient amount of ammonia salts has been produced by the fermentation of urea, provided there is a free access of air. H. Immendorff showed that in the outer layers of manure heaps (especially horse dung) nitrous acid is produced briskly in a few days. On account of the formation of easily lixiviable nitrates, which may moreover expose the material to wasteful reduction processes, endeavours should be made to minimise the aëration of the manure by battening the heaps well down.

It is well known that the soil has no power of fixing nitrates, as according to P. Dehérain and others a certain portion of the added saltpetre invariably escapes in the drainage water, so that more has to be added to the soil than is recovered in the crop.

In manuring with salts of ammonia no such waste occurs, as they are fixed by the soil and protected from wasteful lixiviation, the nitrifying bacteria then oxidizing the ammonia and supplying the plant with nitrites according to its requirements (Lafar's *Tech. Mycology*, §§ 206, 208).

PART IV.

THE HYPHOMYCETES, OR MOULD FUNGI.

ACHORION SCHÖNLEINII.

This fungus, the cause of favus in man, horses, cattle, dogs, cats, rabbits, and mice, was discovered by Schönlein in 1839. It is found in the so-called favus crusts. According to Unna, there are nine varieties of favus. The disease is most frequently observed on a head possessing hair, although it is also found on skin devoid of hair. It also attacks the nails (Onychomycosis), the parasite being located between the cells of the epidermis and the corium. Kaposi also mentions a case of favus universalis.

Microscopical Appearances.—The mycelium consists of branched radiating hyphæ. Some of the hyphæ swell at their free ends, becoming club-shaped, while others give off lateral buds containing Krals' so-called yellow bodies, which rupture, allowing their contents to escape as free bodies. When this takes place, moss-like offshoots develop in the form of dense twisted threads. Later, the individual threads break up into cell-like oval structures.

Biological Characters.—To obtain the fungus in pure cultures, the favus crusts are mixed with sterile sillic acid, reduced in a sterile mortar, and plate cultures instituted with the mixture. The growth takes place at both room and incubator temperature on all the nutrient media, usually under the surface, because only a few air-hyphæ are formed. In the beginning, from the periphery of the white growth, which later becomes yellow, fine radiating offshoots penetrate the body of the substratum of the medium. (See Photomicrograph, Fig. 86.)

On Gelatine Plates, white, stellate, quickly liquefying colonies, with thick centres.

In Gelatine Stab Cultures a coating forms on the surface, the under-growth being of a yellow colour.

On Agar a puckered whitish coating, the under surface being yellow. (See Photograph, Fig. 85.)

Blood Serum is the only medium on which spores are developed, the most favourable temperature being 30° C.

Pathogenesis. — Artificial infection can only be produced with material containing spores.

TINEA GALLI (SCHÜTZ).

The chicken favus is due to this fungus, which attacks the comb, wattles, and side of the throat. Round spots appear, which usually become confluent, spreading to the neck, breast, and body.

Microscopical Appearances.—The fungus consists of a mycelium formed of pointed and often branched threads of variable dimensions, which often have small wart-like pedunculate projections, while other joints are club-shaped and sometimes found free, and here and there fringed with offshoots. In some cases, fine offshoots can be seen on the sides of the mycelium bearing one or two club-shaped grey-coloured bodies.

Biological Characters.—*On Gelatine* a whitish growth develops; the gelatine is liquefied, acquiring a reddish colour.

It also grows on potatoes and bread paste, the best temperature being about 30° C.

Pathogenesis.—The characteristic symptoms are produced in chickens with pure cultures; while mice, rabbits, and various other experiment animals remain unaffected. According to the conclusions of MM. Constantine and Subrayes, three distinct parasites are the cause of favus in man, the dog, and the fowl. Human favus is nearly related to that of the dog, but distinguished from the latter by its appearance in cultures and by the invariable structure of its mycelium and by its colour.

TRICHOPHYTON TONSURANS.

This fungus is found in the epithelial scales in *herpes tonsurans.*

Microscopical Appearances.—The single mycelial threads are distinctly septate, and from some of them conidia are given off in a similar manner to the Oidium lactis. (For Photomicrograph of this fungus, see Fig. 88.)

Biological Characters.—It grows at room temperature, while the optimum temperature is 30° C.

On Gelatine Plates.—Semi-globular; white, later yellow, liquefying colonies.

In Gelatine Stab Cultures, white coating, which floats on the surface when liquefaction occurs.

FIG. 85.—Achorion Schönleinii. Agar culture.

FIG. 87.—Tricophyton Tonsurans. Agar culture.

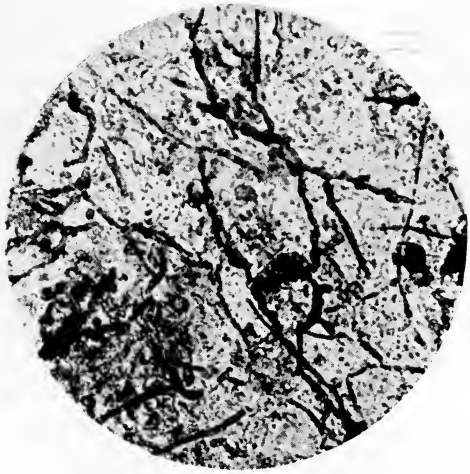


FIG. 86.—Achorion Schönleinii. Section of an agar culture. Fuchsin. × 600.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

On Agar it forms white tufts somewhat puckered (see Photograph, Fig. 87), while the deeper underlying portions are of a yellowish colour.

On Blood Serum, white tufts; the medium is liquefied.

On Potatoes, a slow growth.

In contradistinction to other fungi, the cultures retain their vitality for a long time.

Pathogenesis.—The artificial production of Herpes tonsurans with material containing gonidia has taken place.

Sabouraud distinguishes two groups of trichophyton which he classifies as Botrytis tonsurans, characterised by the grape form arrangement of the fruit. Both groups are distinguished by the size of their spores, and according to Sabouraud, are named *Trichophyton microsporon* and *megalosporon.*

The first form affects only hairy places, and its spores are 3 μ in diameter. It causes the severe affection in children known as the Maladie de Gruby.

The second form has spores from 7 to 8 μ in diameter, and causes in adults a trichophyton affection of the beard as well as of bald parts of the body. According to Sabouraud the trichophyton affecting man and animals exhibit well-marked morphological and clinical differentiation; and furthermore, he states that the trichophyton microsporon when grown on potatoes, in contradistinction to all other varieties which die after three weeks on that media, continues to grow slowly, and after three months further cultures can be instituted in fresh media from the original.

THRUSH.

This disease is caused by a fungus, the *Oidium albicans,* and occurs on all mucous membranes with squamous epithelium, especially in the mouths of infants, where it is manifested by white patches.

Microscopical Appearances.—Sometimes mycelial threads are present, and at other times round or oval conidia like yeast cells.

Biological Characters.—Strongly aërobic; optimum temperature, 37° C.

On Gelatine Plates, white, non-liquefying colonies.

On Gelatine Stab Cultures, yellow-white grains, with processes extending into the medium.

On Agar, a yellowish-white growth.

On Potatoes, a thick white coating, which frequently is observed to consist of small clusters.

On Bread Paste, a thin white coating.

In Media containing Sugar or *Acid Media* it grows more in the form of buds or yeast-like cells.

In Alkaline or *Media deficient in Sugar* it grows more in the form of mycelial threads.

Pathogenesis.—Besides being found in infants' mouths, it has also been observed in adults in the œsophagus, middle-ear, trachea, and in both nasal cavities in a young man after a severe attack of influenza. Pathogenic for rabbits when inoculated intravenously, the fungus becoming localized in the internal organs.

OIDIUM LACTIS.

Found in sour milk, on bread, and decayed fruit. On cream the colonies can be recognised by transmitted light as faintly yellow round spots.

Microscopical Appearances.—Single mycelial threads give off cylindrical joints like yeast-cells (oidien), which towards their extremities become gradually shorter, resembling conidia. The mycelium consists of septate branched threads of various thickness. (For Photomicrograph of this fungus, see Fig. 89).

Staining Reactions.—Stains easily with the ordinary aniline stains; but dried specimens, owing to the heating in their preparation, cause shrinking and alteration in the form of the *oidien*.

Biological Characters.—It grows well on all kinds of nutrient media, especially when the reaction is slightly acid, at both room and incubator temperature. The optimum temperature is 15° to 20° C.

On Gelatine Plates a white, long, hairy, mycelial growth develops, which covers the plate but does not liquefy the medium.

On Agar the growth at first is delicate, but later a viscous yellowish-white coating forms.

On Potatoes, a whitish growth.

On Milk a skin is formed on the surface.

Sugar is fermented and *Albuminoids* decomposed.

Non-pathogenic.

PENICILLIUM GLAUCUM.

This fungus is of universal occurrence. It is found on jam, cheese, decaying fruit, and on barley when it is lying on the malt-house floor. It also occurs on the walls and ceilings of rooms. In the young stage it consists of whitish tufts, which later acquires a green colour, due to the formation of myriads of spores. The free spores are covered externally with a fatty layer which protects them from moisture.

Microscopical Appearances.—A mycelium is formed from which

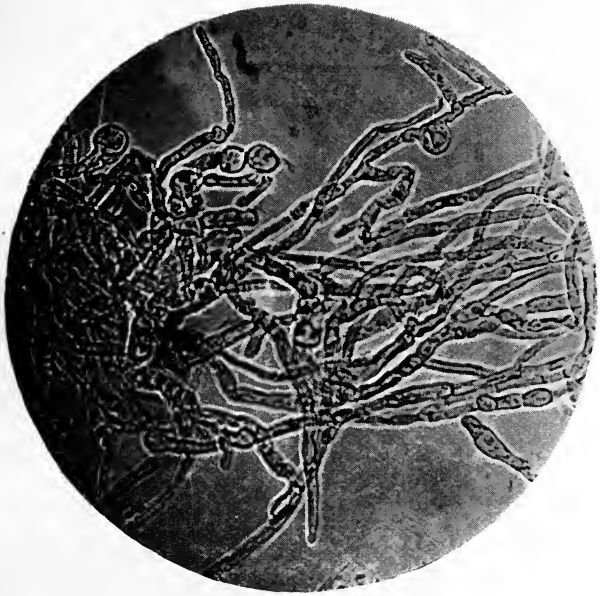


Fig. 88.—Tricophyton Tonsurans, from agar culture. Unstained. × 350.

Fig. 89.—Oidium Lactis, from gelatine culture. × 1000.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

numerous hyphæ are given off. Erect filaments, or *aërial hyphæ*, also extend upwards, shaped like a broom, and bearing at the end of their branches the spores. (See Photomicrograph, Fig. 90.)

Vitality of the Spores.—The spores will germinate at any temperature between 2° and 43° C. 22° to 26° C. is the most favourable, while according to Pasteur dry spores withstand a temperature of 108° C., but are soon killed when immersed in boiling water.

Biological Characters.—It grows best at room temperature on any of the ordinary media. It is also able to propagate itself sexually when placed under certain conditions, especially the absence of oxygen.

It is non-pathogenic.

The green mould growing in the cracks of Roquefort cheese is due to this fungus, which consumes the acid produced by the lactic acid bacteria, thus retarding the development of albumin-degrading organisms.

Experience having shown the favourable action of this fungus, it is grown on bread, which is dried, powdered, and mixed between the separate layers of the sliced curd. In Edam cheese an organism known as the Streptococcus Hollandicus is mixed with the milk before it is made into cheese.

THE ASPERGILLI.

From the mycelium individual threads pass upwards (*air-hyphæ*), and swelling, form clubs without dividing. This swelling of the conidia or fruit-bearers is surrounded by a mass of cylindrical cells, which at their distal ends or extremities produce chains of spherical spores or conidia. Out of each spore a new fungus can develop. When highly nourished, another method of fructification takes place. Some of the terminal branches of the mycelium become twisted like a spiral, and are known as the 'Carpogonium'; from the same thread branches grow towards the carpogonium, one of which becomes fused with the terminal portion of the carpogonium, known as the 'Ascogonium'; while others, the 'Pollinodia,' ramify around the carpogonium like a capsule,—the whole organ being called a perithecium. The ascogonium divides rapidly into a number of oval tubes, inside of which, by endogenous division, small, round, eight-spored asci develop. The aspergilli grows best on bread paste and acid media, beer-wort gelatine, agar, and potatoes.

Asp. Nidulans.—Found on bread; light green tufts. Air-hyphæ present, especially in old cultures which are sometimes coloured light red. Branched sterigmen; optimumt emperature, 40° C. On potatoes and bread it forms a reddish-brown pigment, which penetrates the medium. The pathogenic properties are the same as the A. fumigatus.

Asp. Niger.—Blackish-brown tufts. Branched sterigmen; optimum temperature, 34° C. (See Photomicrograph, Fig. 91).

Asp. Ochraceus.—Yellowish-red to dark-yellow tufts. Branched sterigmen.

Asp. Oryzæ.—Found on rice; at first flesh-coloured, later of an ochre-yellow colour; changes starch and dextrin into sugar. Used in the preparation of the Japanese rice-spirit.

Asp. Repens.—Found on fruits preserved with sugar. Appears at first white, later as greenish tufts, with smooth colourless or greenish spores.

Asp. Subfuscus.—Found on bread. Appears as tufts, yellowish to black in colour. Optimum temperature, 37°. Pathogenic.

Asp. Albus.—Whitish tufts and branched sterigmen.

Asp. Clavatus.—Greenish tufts and club-shaped fruit bearers and small conidia.

Asp. Flavescens, or Flavus.—Found in bread, greenish-brown tufts, and yellowish-brown spores, with rugged surface, small black sclerotien. The optimum temperature for its development is 28° C. The pathogenic properties are the same as the Asp. fumigatus.

Asp. Fumigatus.—Found in the trachea and bronchi of birds, and also on bread; bluish-green tufts, which later acquire a bluer colour; very small smooth spores; optimum temperature, 37° to 40° C. (For Photomicrograph of this fungus, see Fig. 92.)

Pathogenesis.—When rabbits and dogs are injected intravenously with the spores, death occurs in about twenty-four hours. In all the organs, especially in the substance of the heart and kidneys, the fungi can be detected in small clusters. In man an aspergillimycosis also occurs in the lungs, auditory canal, and on the cornea.

Asp. Glaucus.—Found in fruit, cabbage, and damp wooden walls. Greenish-coloured tufts and round spores with rugged surfaces; optimum temperature, 10° to 15° C.; killed at 25° C.; non-pathogenic.

MICROSPORON FURFUR.

Found in the scales cast off in pityriasis versicolor. When these scales are treated with a 5 per cent. solution of caustic potash, and examined microscopically, short, slightly branched mycelial threads are seen, with very large conidia lying together in clusters.

Cultivations have not yet been obtained.

MICROSPORON MINUTISSIMUM.

Found in the scales cast off in erythrasma, and similar to the above, except that the mycelia and conidia are here excessively fine.

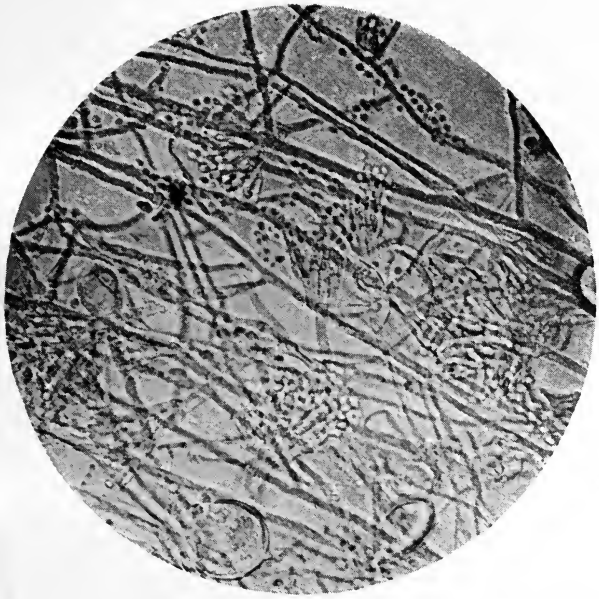


FIG. 90.—Penicillium Glaucum, from gelatine culture. Unstained. × 300.

FIG. 91.—Aspergillus Niger, from agar culture.
Unstained. × 350.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

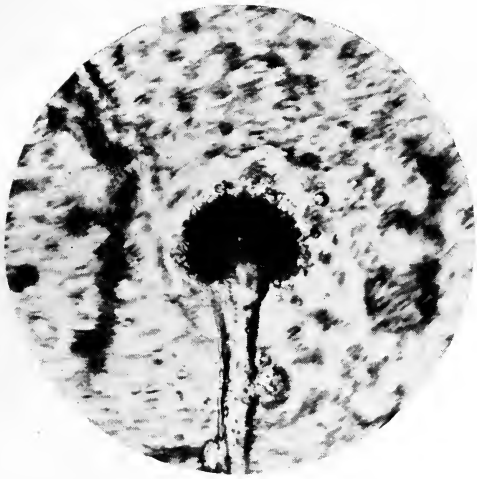


Fig. 92.—Aspergillus Fumigatus, from agar culture.
Unstained. × 350.

Fig. 93.—Mucor Corymbifer, from potato culture.
Unstained. × 350.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

THE MUCORS.

Specially characterised by aseptate hyphæ springing from the mycelium. In these hyphæ a large sporangium develops apically and by fission. Inside it spores are produced, which later are liberated by the bursting of the sporangium. Under some conditions proliferation occurs by conjugation of two bodies forming what is called the zygospores.

Mucor Corymbifer.—Occurs as greyish tufts. The hyphæ come off from the mycelium at an angle, and are branched with colourless sporangia. The spores are small, 3 μ to 2 μ. Optimum temperature, 37° C.

Pathogenesis.—When injected into the veins of rabbits, death follows in from two to three days. The clumps of fungus are mostly found in the kidneys and lymph-follicles of the intestinal mucosa. Dogs are immune. (For Photomicrograph of this fungus, see Fig. 93.)

Mucor Mucedo.—Found on horse manure as a whitish growth, like thistledown. The hyphæ are 1 to 13 cms. in length, and may be single or branched; crystals of oxalate of lime occur on the outside of the sporangia, which later are coloured brown or black. (See Photomicrograph, Fig. 94.) Optimum temperature, 37° C.; non-pathogenic.

Mucor Pusillus.—Found in moist bread, and occurs in tufts, which are at first white and get grey. The mycelium is unusually fine. The sporangia are covered with a spinous membrane; optimum temperature, 45° C.

Pathogenesis.—Same as Mucor corymbifer.

Mucor Ramosus.—Occurs on moist bread as tufts, at first white, and later brownish. The mycelium is very much branched, and has long branched air hyphæ and large spores. Optimum temperature, 40° C.

Pathogenesis.—Same as Mucor corymbifer.

Mucor Racemosus.—Found on sugar and starchy substances. Has many delicate fruit hyphæ not exceeding 1 to 5 cms. in length. Sporangia are yellow or yellowish-brown, with round spores.

Mucor Stolonifer.—The mycelium has branches which at first ascend and then descend, and are covered with fine root hairs.

The sporangia are deep black and tuberculated with brown globose spores, 10 to 20 μ in diameter. The zygospores are blackish-brown.

Mucor Rhizopodiformis.—The mycelium is at first quite white, and later turns greyish. The hyphæ are twisted, at first ascend, and then grow down again into the medium, where they get covered with root hairs. Spores colourless; diameter 5 to 6 μ.

Pathogenesis.—Same as Mucor corymbifer, but is distinguished from it by the pleasant fruity smell of the cultures.

The mucors are the lowest members of the vegetable kingdom exhibiting a method of reproduction which is universal in the higher forms of plant life. Two cells are concerned in this process, known as the male and female elements, and the process, which is called sexual reproduction, only takes place by this method when growing on a solid substratum. When there is a plentiful supply of nourishment at hand reproduction takes place asexually, that is, by spore formation.

FUSISPORIUM MOSCHATUM (KITASATO).

This fungus is described by Kitasato. It was found as an accidental growth in a vegetable infusion. The principal characteristics are the formation of sickle-shaped spores (see Photomicrograph, Fig. 95), and an odour of musk given off by the cultures.

Biological Characters.—It grows only at room temperature on all the ordinary media, on rice paste, and infusion of peas.

Gelatine Media are liquefied slowly; the cultures at first are greyish-white, eventually becoming rose or brick-red coloured.

In preparing and mounting microscopic specimens of the various fungi, illustrated by the accompanying photomicrographs, the author adopted the following method:—

1. Hammer out a piece of platinum wire quite flat at the point, in the shape of a shovel.

2. Select an isolated growth, heat the shovel, and cut through the medium a short distance from the growth.

3. Push the platinum spade under the growth and transfer the mass *en bloc* to a clean cover-glass.

4. Place the cover-glass on a clean slide, heat gently over the flame (just enough to melt the medium), and press the cover-glass gently to remove superfluous medium.

By this process the fungus is mounted on the medium it is growing in, and the character of the growth is readily observed. In making permanent mounts by this method, a drop of a solution of formalin is added before the slide is heated.

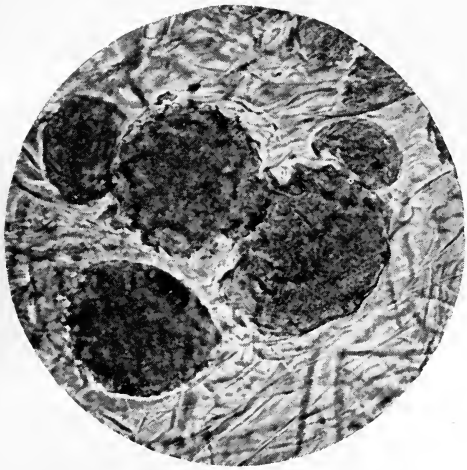


Fig. 94.—Mucor Mucedo, from gelatine culture, *showing Zygotes.* Unstained. × 350.

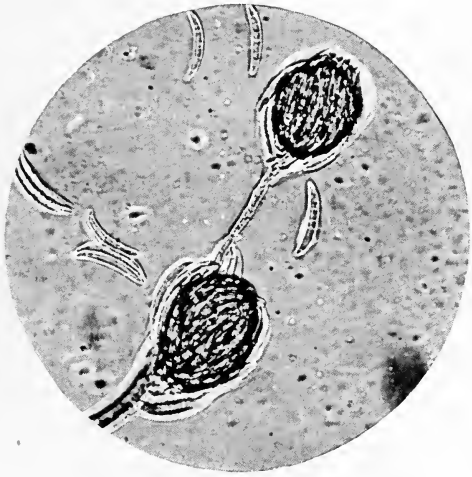


Fig. 95.—Fusisporium Moschatum, from potato culture, *showing sickle-shaped Spores.* Unstained. × 350.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh,* 1898.

PART V.

THE BLASTOMYCETES, OR YEAST FUNGI

The yeast fungi are divided into two groups:—

(*a*) Saccharomycetes or true yeasts, in which true spore formation occurs.

(*b*) Torulæ, in which no spore formation has been observed.

The Blastomycetes reproduce themselves by gemmation or budding, which distinguishes them from the schizomycetes or bacteria, which reproduce themselves by fission or simple division. From the hyphomycetes or mould fungi the blastomycetes are distinguished by being unicellular and by asexual reproduction.

The yeasts employed for commercial purposes consist of a mixture of different species. We are indebted to the researches and experiments of Hansen for our present knowledge of the various changes produced by the different species during fermentation, as well as for the method of isolating them by means of plate cultures instituted with acid or beer-wort gelatine. Observation of the following conditions enables the various species to be differentiated during development.

1. The temperature at which ascospores develop.

2. The characters of the film or zoöglœa—mass of cells forming on the surface of the fluid during fermentation.

3. The changes produced in the various kinds of sugar.

4. Whether the variety under observation causes a top or bottom fermentation.

A surface or top fermentation yeast grows and forms spores readily at 14° to 18° C.; while a bottom fermentation yeast grows at 4° to 10° C. in the bottom of the fluid, and forms spores only with difficulty.

METHOD OF OBTAINING PURE CULTURES ON A SMALL SCALE.

1. A Pasteur flask containing the wort to be experimented with is started and carried on as vigorously as possible.

2. To this growth a quantity of sterilized water is added, and the yeast cells in a given drop counted under the microscope.

3. Supposing 10 cells to be present, a similar-sized drop is now transferred to a flask containing 20 c.c. of water, which is equivalent to 1 yeast cell for each 2 c.c. of water.

4. The flask containing the 20 c.c. of water with the 10 yeast cells is thoroughly shaken, and this liquid divided equally, 1 c.c. being placed in each of twenty flasks containing sterilized wort.

5. If the separation has been complete, 10 out of 20 flasks should contain one organism each, but this of course cannot be absolutely depended on.

6. At this stage Hansen shakes the flasks very vigorously to separate the cells as much as possible, and places the flasks in the incubator, allowing them to remain perfectly still, in order that the cells may sink to the bottom or become attached to the walls of the flasks.

7. At the end of several days the flask is carefully lifted and examined, and it is noted whether one or more white specks have been formed on the walls of the glass; if only one such speck is found, *it is a pure culture.*

This method is especially useful when the yeast plants are at all weakly. In mixed and vigorous species, wort gelatine plate cultures should be instituted. For the methods of obtaining pure cultures on a large scale, see the works of Hansen and Jörgensen.

SACCHAROMYCES CEREVISIÆ I.

This is known as the Old English top-fermentation yeast, and is used by brewers and bakers.

Microscopical Appearances.—Large round or oval cells, 11 to 4 μ, most frequently 8 to 6 μ in diameter. These cells give off small cells by budding. In the earlier stages of film formation delicate mycelial-like threads are formed, which, as the film becomes older, grow longer and more regular. Nuclei can be demonstrated in the cells, especially in old cultures, when stained with hæmotoxilin, hæmatine, alum solution, or osmic acid. The cells are sometimes very granular.

Spore Formation.—Ascospores develop after twenty-four hours at 10° to 37° C., but most rapidly at 30° C., most slowly (after ten days) at 11° to 12° C., and below this temperature the formation ceases. For the development of the ascospores, Hansen employs plaster-of-Paris blocks, which are first thoroughly sterilized by heat. A small portion of yeast is laid on the upper surface, and the lower surface set in a small vessel containing water, within a sterile air chamber, the whole apparatus being placed in an incubator, or left at room temperature. Ascospores can also be grown

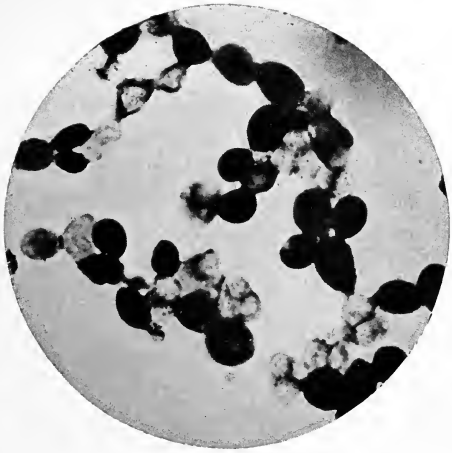


FIG. 96.—Saccharomyces Cerevisiæ I. and Ascospores. Cladius stain. × 1000.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

on potatoes prepared according to Globig's method. (See Technique, § 78.)

The author has found the following simple arrangement yield most satisfactory results. An oblique plaster-of-Paris block is prepared so that it will rest in the bottom of an ordinary test-tube, about 1 in. in diameter, containing a little water in the bottom, which is plugged and sterilized. Some yeast is placed on the upper portion of the oblique surface of the plaster block, when the tube can be placed in the incubator or left at room temperature.

Staining Reactions.—Dried specimens can be stained with fuchsin and methylene blue. This stain is also used to differentiate living and dead cells in hanging-drop cultures, the latter alone staining. The author has found that ascospores can be beautifully demonstrated by the Cladius method of staining, § 12, the cells being stained blue, and the spores and background remaining yellow with the picric acid (see Photomicrograph, Fig. 96). The ascospores can also be stained by the ordinary method for staining spores (see § 26).

Film Formation.—This takes place most rapidly (seven to ten days) at a temperature of from 20° to 22° C., most slowly (two to three months) at 6° to 7° C., and ceases altogether above 38° C. and below 5° C.

Biological Characters.—*On Gelatine Plates* it forms small white colonies; under a low power the individual yeast cells forming the surface colonies can be observed.

It secretes a peculiar substance which, acting on saccharose or crude cane sugar, inverts it to invert sugar. This latter substance is fermented, a similar change taking place in dextrose and maltose, alcohol and carbonic acid gas being formed, accompanied with an evolution of heat and great multiplication of the yeast cells. On lactose or milk sugar it does not seem to cause any change.

SACCHAROMYCES ELLIPSOIDEUS.

(Divided by Hansen into I. and II.)

SACCHAROMYCES ELLIPSOIDEUS I.

This is a 'wild' species of wine ferment. It is found on the surface of fruit, chiefly on wine grapes.

Microscopical Appearances.—Round or oval cells, which sometimes assume a sausage form.

Spore Formation.—The spores are from 2 to 4 μ in diameter, two to four being found in a single ascus. They are developed between 7½°

and 31½° C., most rapidly (in twenty-one hours) at 25° C.; above 32·5° C. and under 4° C. the development ceases.

Film Formation.—The surface membrane is formed most rapidly (eight to twelve days) at 33° to 34° C., most slowly (sixty to ninety days) at 6° to 7° C. It is always a delicate membrane, and above 38° C. and under 5° C. development ceases. The growth is most characteristic between 13° to 15° C., when it consists of a complicated branching mass of elongated cells or threads, arranged in rows with lateral processes coming off at the point of junction. Secondary branches are formed at the constrictions of the primary branches.

On Wort Gelatine the colonies present a net-like appearance. It causes as powerful and rapid fermentation as the Saccharomyces Cerevisiæ on the various carbohydrates on which that ferment acts.

SACCHAROMYCES ELLIPSOIDEUS II.

A 'wild' or wine fermentation yeast, which gives rise to the muddiness of beer.

Microscopical Appearances.—In young cultures at 15° C. the cells are usually somewhat rounded or egg-shaped, while older cultures exhibit longer mycelial rods with forked transverse shoots given off at the joints.

Spore Formation.—The spores are from 2 to 5 μ in diameter, two to four being found in a single ascus, and may be egg-shaped, slightly irregular, or elongated. They are developed most rapidly at 29° C., most slowly at 8° C.; above 34° C. and below 4° C. the development ceases.

Film Formation.—It is essentially a low yeast, and the film that forms is very delicate. At 33° to 34° C. it appears in three to four days, but not for five to six months at 3° to 5° C. At 2° and at 40° C. no film is developed.

SACCHAROMYCES PASTORIANUS.

Hansen considers this an impure species, and divides it into three.

SACCHAROMYCES PASTORIANUS I. (HANSEN).

A 'wild' yeast, the spores frequently occurring in the atmosphere of breweries. It gives an unpleasant bitter taste and bad smell to beer.

Microscopical Appearances.—It occurs as elongated ellipsoidal or pear-shaped cells, from which small apical or lateral branches are sometimes given off.

Spore Formation.—The asci are usually elongated or rounded, and may contain two spores or multiples of two up to eight or even more, which vary in size from 1·5 up to 5 μ. They are developed most rapidly (seven to ten days) at 27·5° C., most slowly (fourteen days) at 3° to 4° C. The development ceases at ·5° C. and at 31° C.

Film Formation.—The films, which are usually very delicate, are developed most readily (seven to ten days) at from 26° to 28° C., most slowly (five to six months) at from 3° to 5° C.; development ceases at 34° and 2° C. Mycelial-like threads develop freely in the film at from 3° to 15° C., and most irregular forms appear. In the older films numerous irregular club, skittle-shaped, and other forms occur. In the younger films the cells are usually smaller and the irregular forms less frequent.

SACCHAROMYCES PASTORIANUS II. (HANSEN).

This was also separated from the air of the brewery. It is a feeble top fermentation yeast when growing in beer-wort. It gives rise to neither cloudiness nor to any unpleasant bitter taste.

Microscopical Appearances.—The sedimentary cells are mostly elongated, but may be slightly rounded, varying considerably in size. The cells found in the film are rounded, egg-shaped, or somewhat elongated.

Spore Formation.—The asci are usually elongated, the spores occurring in multiples of two from 2 to 5 μ in diameter. They are developed most rapidly (twenty-seven hours) at 23° C., most slowly (seventeen days) at 3° to 4° C.; formation ceasing at 29° C. and at ·5° C. This yeast secretes an invertase and causes fermentation of all the carbohydrates that are fermented by the other yeasts of this group. In old cultures of the films the cells are small, thread-like, and very irregular in shape.

SACCHAROMYCES PASTORIANUS III. (HANSEN).

According to Hansen this yeast is one of the causes of turbidity in beer.

Microscopical Appearances.—The cells are very similar to those of the sedimentary yeast, but at a temperature of from 15° down to 3° C. elongated mycelial-like threads develop, which in old cultures become still more characteristic. In the Saccharomyces Pastorianus I. the mycelial threads are most characteristic at 13° to 15° C., while at 15° to 3° C. the cells in Saccharomyces Pastorianus II. are oval and rounded.

Spore Formation.—Similar to the Saccharomyces Pastorianus II.

It takes place most rapidly (twenty-eight hours) at 25° C., most slowly (nine days) at 8·5° C., and ceases at 29° C. and at 4° C.

Film Formation.—This appears in the form of small flakes, most rapidly (seven to ten days) at 26° to 28° C., most slowly (five to six months) at 3° to 5° C., and ceases altogether at 34° and 2° C. The elongated or sausage form cells predominate, but large and small round and ovoid cells are also found in the sediment in the films at from 20° to 28° C.

Biological Characters.—In cultures grown on yeast-water gelatine, at the end of sixteen days the colonies exhibit peculiarly fringed edges. Grown in wort, it gives rise to a top fermentation, causing considerable turbidity, and producing alcohol and carbonic acid gas.

SACCHAROMYCES APICULATUS.

Found in fermented wine and spontaneously fermented beer, and in hot seasons on sweet succulent fruits—cherries, plums, grapes, etc. In winter it is found in the soil beneath the trees that bear these summer fruits.

Microscopical Appearances.—In cultivation fluids the cells are lemon-shaped, the buds are lemon-shaped, and in older cultures oval.

Spore Formation absent. Hence it cannot be classified as a true yeast.

Biological Characters.—When dried in a thin layer it is killed, which accounts for it not developing on unripe fruit. It is a bottom fermentation yeast, causing feeble alcoholic fermentation. It does not invert cane sugar, but acts on dextrose in yeast water, the fermentation being incomplete. Mixed with Saccharomyces it retards the action of the latter.

SACCHAROMYCES ANOMALUS.

Found in brewery yeast.

Microscopical Appearances.—Small oval cells.

Spore Formation present, in the form of hemispheres with projecting rims, whereby they are shaped like a hat; the optimum temperature for their development is 25° C.

SACCHAROMYCES MARXIANUS.

First found in wine.

Microscopical Appearances.—Small ellipsoidal and egg-shaped cells, with sausage-shaped cells here and there; often arranged in colonies.

Spore Formation. — Spores are not freely developed; on solid media they are more frequent, being usually oval or kidney-shaped.

Film Formation.—The film develops very slowly, and consists of oval and short sausage-shaped cells.

Biological Characters.—In beer-wort it is not very active. It does not ferment maltose, but acts strongly on saccharose, which it inverts and then ferments with great rapidity. It also acts upon dextrose.

SACCHAROMYCES MEMBRANÆFACIENS.

Forms a bright yellow, tough scum on beer-wort, composed of long and sausage-like cells, occurring either singly or closely packed together.

Spore Formation.—Spores are formed rapidly.

Biological Characters.—Nutrient gelatine is liquefied. It does not cause any fermentation of ordinary carbohydrates and does not invert cane sugar.

SACCHAROMYCES EXIGUUS.

Found in German yeast by Hansen. It forms no mycelial threads on beer-wort or on solid media. It forms spores but sparsely. The *film* is very delicate, and consists of short rod-shaped or ovoid cells. It causes the same changes in sugars as the Saccharomyces marxianus.

SACCHAROMYCES ACIDI LACTICI.

Occurs as ellipsoidal cells.

On Gelatine Plates and *Agar Surface Cultures* it forms white, glistening colonies.

In Gelatine Stab Cultures, club-shaped outgrowths are given off from the inoculation track.

On Potatoes it forms a brownish coating.

Milk is coagulated, an acid being formed. In solutions of milk sugar it forms alcohol.

MYCODERMA CEREVISIÆ ET VINI.

Like all other forms of mycoderma it is distinguished from the saccharomyces by forming no spores (ascospores).

Microscopical Appearances.—Long cells, which do not transmit light so strongly as the saccharomycetes.

Biological Characters.—On wort gelatine, small dim light grey spots, which either spread over the surface or cause a shell-shaped cavity in the medium.

On Wort a greyish-white, thickly matted film develops, the optimum temperature being 15° C.

Actions.—It causes only a weak alcoholic fermentation, no acetic acid fermentation, but exerts a deleterious action on fermented fluids by producing abnormal chemical changes.

THE TORULÆ.

These yeasts are widely distributed in nature, and occur in round or elongated forms and develop no spores. Reproduction takes place by budding, and often at the same time a mycelium is formed. Gelatine media are not liquefied. The various forms are distinguished by the colour of the pigment produced.

1. White, Hefe.
2. Rose, „
3. Black, „

They cause only a very weak alcoholic fermentation. There are, however, some species that grow with distinct fermentation properties.

PATHOGENIC BLASTOMYCETES.

SACCHAROMYCES HOMINIS.

Found in an infectious disease, which began with a subperiosteal inflammation of the tibia, terminating in a chronic pyæmia.

Microscopical Appearances.—Round or oval cells, double contoured, and possessing capsules.

Biological Characters.—*On Gelatine Plates,* prominent, round, non-liquefying colonies.

On Agar, a white coating.

On Potatoes, a greyish-brown coating.

On Blood Serum, a dim drop-like coating.

Bouillon is clouded, a film growing on the surface.

In Grape Sugar Bouillon fermentation takes place, alcohol and CO_2 being formed.

Pathogenesis.—In rabbits a local abscess forms. Mice die from septic changes.

SACCHAROMYCES LITOGENES.

Found in the lymph-glands of an ox affected with carcinoma of the liver.

Microscopical Appearances.—Large and small round cells with a membrane.

Biological Characters. — The growth in the various media is similar to the Saccharomyces neoformans, except on *potatoes*, when an intense brown coating develops.

Pathogenesis—In guinea-pigs a tumour forms at the point of inoculation and nodules in the organs. The yeast cells in the centre of the nodules very frequently degenerate.

SACCHAROMYCES NEOFORMANS.

Found in the juice of fruits.

Microscopical Appearances.—Round or elliptical cells with refractile granules and a double contour, which increase by budding.

Biological Characters. — *On Gelatine Plates*, round, cup-shaped, non-liquefying colonies.

In Gelatine Stab Cultures, a granular growth along the inoculation track.

On Agar, a dry film develops.

On Potatoes, a white elevated growth.

Milk is not coagulated.

In Sugar Bouillon a sediment forms, and often a film is developed.

Pathogenesis.—In guinea-pigs a tumour forms at the point of inoculation, and nodules develop in the internal organs.

SACCHAROMYCES SUBCUTANEUS TUMEFACIENS.

Found in a myxomatous tumour of the upper part of the thigh.

Microscopical Appearances.—Oval or round cells, frequently possessing large transparent capsules. The cells increase by budding.

Biological Characters. — *In Gelatine Stab Cultures* the development occurs in small colonies ; it causes no liquefaction of the medium.

On Agar, a thick creamy growth.

On Potatoes, an extensive white coating, which later becomes brown.

On Acid Beer-wort Agar, a brown coating.

In Alkaline Bouillon, a slight sediment.

In Beer-wort, a thick sediment without film formation on the surface.

It causes a slight fermentation of saccharose. It forms ethylic alcohol and acetic acid.

Pathogenesis.—White mice and rats are susceptible, extensive local vegetations being produced. Microscopically examined, the tumours exhibit no definite structure, but appear to consist of an extensive parasitic infiltration.

HYDROPHOBIA.

Memmo, of the Hygienic Institute, University of Rome, has recently stated in a contribution regarding the etiology of hydrophobia, that in some cases he found a blastomycete possessing pathogenic properties which he considers the cause of the disease. The fungus was found in the cerebro-spinal fluid, the substance of the brain aqueous humour, stroma of the parotid gland, and in the saliva, but never in the heart's blood or other organs. Liquid culture media were used, especially bouillon containing tartaric acid, and glucose of weak acid reaction. The cultures did not begin to develop for ten days. In material from ten healthy dogs and twenty rabbits, no similar cultures were produced. Culture fluids exposed to the air, and mixed with dust found in the laboratory, yielded no growth of the above fungus.

With cultures of three or four generations from four different outbreaks of hydrophobia, the purity of some being tested by plate cultures, rabbits, guinea-pigs, and dogs were inoculated, mostly subcutaneously, but also by intraperitoneal and intradural methods. In eleven to twenty days some of the guinea-pigs and rabbits were affected with paralysis of the hind extremities, followed shortly by death.

In thirty to sixty days the dogs began to emaciate, became rabid, vomited, and finally paralysis of the extremities preceded death. The brain substance of the infected animals was infectious for other animals. The fungus has also been found in sections of the spinal cord of a boy dead of hydrophobia, stained by Sanfelice's method.

PART VI.

THE PROTOZOA, OR ANIMAL PARASITES.

AMŒBA COLI.

Found in the human intestines, in the stools, in dysentery, and in the diarrhœa in abscess of the liver.

Microscopical Appearances.—During the resting stage they appear as almost homogeneous cells, while during motility a slightly refractive ectoplasm and strongly refractive entoplasm with a granular appearance can be distinguished. Vacuoles are also frequently present in the entoplasm, as well as foreign bodies, red blood-corpuscles, and bacteria. The large nucleus, with a nucleolus, is plainly visible in dead forms, stained specimens, or by the addition of acetic acid.

Motility.—This is due to the presence of pseudopodia, in which rounded blunt continuations of the ectoplasm extend into the entoplasm.

The Amœba coli multiply by dividing in two. All attempts at cultivation have up to the present failed. The Amœba coli found in the normal intestinal contents cannot be distinguished morphologically from that found in dysentery, but the latter, when introduced into the rectum of cats, causes an ulcerative hæmorrhagic inflammation of the large intestines. Furthermore, the dysentery amœba are genuine tissue parasites, penetrating deep into the submucous, sometimes as far as the serous coating of the intestines.

Method of Examining Stools for Bacteria.—The stool for examination should be quite recent, and should, if possible, be received in a vessel previously warmed to body-heat. In a watery stool a drop is taken, preferably from the red jelly-like part showing bloody mucus, and examined on a cover-glass. If more solid but unformed, the stool is diluted with warm normal saline solution, and treated as above.

In solid formed stools some of the mucous slime attached is examined. In making a permanent preparation some of the material is smeared very thinly on the cover-glass, and before there is time for drying it is passed into absolute alcohol to fix. Their staining reactions are not as pronounced as those of the bacteria, and methylene blue gives the best

results, and we get the nucleus and the protoplasm both stained, but the former more deeply. Sections stain with eosine and hæmatoxylin as well as methylene blue.

PARAMÆCIUM COLI.

Found in the intestines of man and swine.

Microscopical Appearances.—Elliptical infusoria, their whole surface being covered with cilia. In man they are 60 to 70 μ long, and in swine 70 to 100 μ long. The mouth is funnel-shaped, the nucleus bean-shaped, and they possess two contractile vacuoles. They multiply by dividing into four and by conjugation, also sometimes becoming encysted.

COCCIDIA.

The best known coccidia are those found in the rabbit and the fowl, and, according to Rieck, there are two different varieties—a liver and a bowel species, the former causing peculiar white or abscess-like cavities in the liver (due to local dilatation of the bile-ducts), the other inhabiting the intestines and causing a most acute and fatal inflammation. M'Fadyean records an outbreak amongst pheasants of the intestinal variety, where the coccidia penetrated the glands of Lieberkühn and invaded their epithelium. The same author also mentions an outbreak amongst lambs.

Zschokke, Hess, and Guillebeau mention a form of dysentery amongst cattle in Switzerland, produced by coccidia, and known as 'red dysentery' and 'dysenteria hæmorrhagica coccidiosa.' It has also been produced experimentally in cattle with sporulating coccidia.

The coccidia are unicellular animal parasites belonging to the class *Sporozoa*. In the adult state they are ovoid in shape and enclosed in a double-contoured shell or cyst, flattened at one of its poles, 30 to 50 μ long and 14 to 28 μ broad. The protoplasmic contents of the encysted forms separate from the wall and contract into a ball-shaped mass.

COCCIDIUM OVIFORME.

This parasite is found in the rabbit's liver, and sometimes in the intestinal canal. It enters the gall-ducts by way of the ductu, choleduchus, thence into the epithelial cells, and, increasing in size, becomes encysted. It is surrounded by a slender external membrane and a shining double inner membrane. The permanent cysts have granular contents and a nucleus. The development does not proceed

FIG. 97.—Coccidium Oviforme. Cover-glass specimen from liver of rabbit. Stained by Ehrlich's method. × 750.

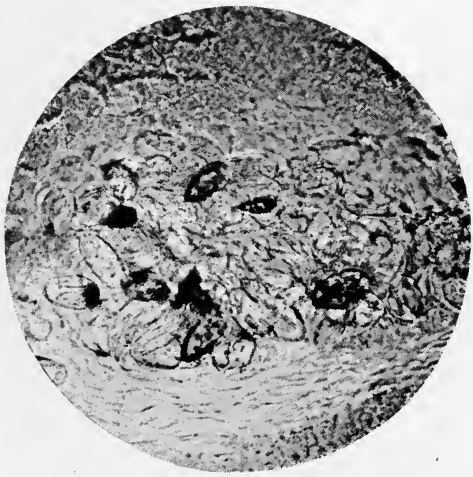


FIG. 98.—Section of a rabbit's liver, showing Coccidia. Unstained. × 300.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

further in the rabbit's liver, but outside the animal's body, and in contact with moisture, the plasma of the cell divides into four oval mother spores, each of which again divides into two sickle-formed daughter spores. When these sickle-formed spores gain entrance to an animal's stomach, the membrane of the mother spores is dissolved by the gastric juice, the free spores entering the intestines and ultimately the gall-ducts. All the parasites in an affected liver do not complete the above cycle of development, but sporulate during their growth in the epithelial cells, exhibiting 4 to 50 sickle-formed nucleus-containing bodies, which finally cause a general disease of the liver. White particles are sometimes found floating in the bile, consisting of masses of coccidia, and, according to Rivolta, the parasite sometimes develops in the epithelium of the gall-bladder.

Staining Reactions.—The parasite stains well with hæmatoxylin and eosin in sections and cover-glass specimens. The author obtained some specimens from a Jack-rabbit's liver in California in 1895, which exhibited the same reactions towards Ehrlich's aniline water fuchsin as the bacillus of tuberculosis. (See Photomicrograph of same, Fig. 97; for Photomicrograph of section of the same liver, showing the Coccidia unstained, see Fig. 98.)

According to Leuckart, the intestinal species is known as Coccidium perforans, but that it is a distinct species is not exactly proven, as both forms frequently coexist in the same host, the difference probably arising from the different positions of the parasites in the affected animals.

In man, cases of coccidia are recorded in the liver, kidneys, and pleural exudate.

Rivolta also mentions the presence, along with the Coccidium oviforme in the rabbit's liver, of other coccidia belonging to the species Elimeria falciformis.

PLASMODIUM MALARIÆ.

This parasite was discovered in the blood of malarial patients by Laveran in 1882.

Microscopical Appearances.—The young forms of the parasite are about 1 to 2 μ in size, colourless and motile; in stained specimens small nuclei are visible. The parasites either cling to the outside of the blood-corpuscles, or enter the interior of the same. The parasites developing in the interior of the red blood-corpuscles, form at the expense of the hæmoglobin, or brown or black granular pigment. At the height of its growth the parasite may fill the whole blood-cell; and in this condition, when it sporulates, the spores rupture the remains of the blood discs, thus liberating themselves.

Various Types of Malarial Parasites.

(1.) *The Quartan Parasite.*—The cause of quartan malaria has an incubation period of seventy-two hours; they are not strongly motile. At first the parasite occupies $\frac{1}{4}$ or $\frac{1}{2}$ of the blood-cell, and large quantities of granular pigment are present. Losing its motility it increases in size, until it appears to fill the whole blood-cell. It then forms spores, arranged like the ray-flowers in a daisy-marguerite form, having eight to twelve round spores, the whole period of development occupying about three or four hours. The segmentation takes place both before and during the febrile stage; and about three hours before the outbreak of the rigors, the first mature spores are visible in the blood.

The red blood-corpuscles affected with the quartan parasite do not alter their size. The parasites sometimes sporulate before completely filling the blood-cell, when they only form four to five spores. Cilia are only observed in young forms. If many generations of the quartan parasite are present in the blood, then it causes quartan duplex or triplex, or an irregular type of fever.

(2.) *The Tertian Parasite.*—The cause of the tertiana form of malaria develops in forty-eight hours; the young form resembles the quartan parasite. It is strongly motile, possessing pseudopodia, contains pigment, enlarges the red blood-corpuscles containing them, and sporulates in the form of a rosette, or like a sunflower containing fifteen to twenty spores which are smaller than those of the quartan parasite, the nucleus only being observed with difficulty. The free spores infect fresh blood-corpuscles in a short time, completing the same above-mentioned cycle of development. All the tertian parasites do not sporulate, a large number remaining sterile. These sterile parasites are as large or larger than the sporulating forms, and the pigment remains motile. (Laveran considers this a degenerative process.) They can be observed in the blood hours after the attack, and also during the fever-free days. The process of sporulation is coincident with the fever paroxysms in the tertiana form. Golgi found that three hours before the rigors commenced, the temperature had already begun to rise, and that the first spores were already apparent in the blood, but they were most plentiful during the rigors. The full-grown tertian parasite often possesses flagella. (Kruse considers this condition likewise a degeneration.) The tertian parasites cause typical *tertiana;* two generations of the same can produce a false *quotidiana. Tertiana duplex* generates several generations separated about twenty-four hours from each other, causing irregular fever.

(3.) *The Quotidian Parasite.*—The commencement of the cycle of development of this parasite, which requires twenty-four hours for its completion, is similar to that of the previous forms. The young form

consists of plasma and a nucleus, is devoid of pigment, and infects the red blood-corpuscles. The parasite is strongly motile, which assists in its identification, as on account of its fine contour and colour, which is only a little paler than the blood-corpuscle, it is scarcely distinguishable from the latter. The parasite loses its motility in blood preparations quickly, one hour at the latest. A whitish ring is now formed, and owing to an outgrowth at one part of the periphery, it frequently resembles a signet ring.

Mannaberg considers these forms do not live within the blood cell, but adhere firmly to the outside. The red spot in the centre, according to the same authority, is due to thinning of the plasma, and the shining through of the underlying red blood-corpuscle. The ring-form can again return to an amœboid condition. The amœboid parasite does not grow very large, occupying at the most about one-third of the red blood-corpuscle, forming a very fine pigment on the periphery, and exhibits slight motility. In twenty hours the pigment concentrates in the middle or edges of the cell in dark dormant lumps, and the parasite divides inside the red blood-corpuscle into small spores (five to ten).

According to Marchiafava and Celli, the sporulation takes places only in the internal organs of the body, almost not at all in the peripheral blood. The spores are plentiful in blood taken from the spleen, while in blood from the finger they are either absent, or only present in very scanty numbers. Red blood-corpuscles affected with the quotidian parasites shrink, and acquire a yellow copper-colour, and when the parasite is several days in the blood, the Laveran half-moon or disc-shaped bodies, and additional forms (spindle or cigar-shaped, and spherical) appear. The quotidian parasite causes *quotidiana*, and when several generations are in evidence, *a continual or irregular fever*. The fever caused by the tertian and quartan parasites is distinguished from that of the quotidian parasite by the malignant clinical symptoms of the latter—obstinate recurrence, great anæmia, and other pernicious symptoms (diarrhœa, cachexia, coma, etc.), the relapse appearing about fourteen to fifteen hours after the first cycle. The half-moon forms are considered answerable for the recurrent form. They are found in the blood during the fever-free stages, and by segmentation or genuine spore formation can give rise to the formation of new amœboid forms. Then the new paroxysms do not exhibit a genuine recurrence, but, according to Golgi, the manifestation of a long-interval type. According to other authorities this is disputed, and the half-moon variety is considered a degenerative form incapable of forming new individuals.

(4.) *The Malignant Tertian Parasite* is considered by Marchiafava and Begnami as a distinct species closely resembling the quotidian parasite, but distinguished from the latter by completing its cycle of development

in forty-eight hours, being larger, and at the time of sporulation occupies one-half to two-thirds of the blood-corpuscle; no pigment is formed for over twenty-four hours, and when it is formed the motility of the parasites is not impaired. Circular forms, and later on half-moon forms, also develop. It has usually 8 to 15 spores. The infected blood-corpuscles become copper-coloured, shrivel easily, but never hypertrophy. These peculiarities distinguish this parasite from Golgi's ordinary tertian parasite, which, moreover, in all described stages is larger, richer in pigment, and forms more spores, 15 to 20. The malignant tertian parasite generates a severe tertian form of fever, the chart showing peculiar curves, with very short fever-free intervals, often only of a few hours' duration, and with regular pseudo-crises ending in continued or irregular fever. The quotidian parasite also exhibits malignant characters.

Mixed Infection.—In many cases of intermittent fever various forms of the above-mentioned parasites are present at the same time in the blood. Quotidian parasites frequently occur beside non-pigmented, and the quartan sometimes beside the tertian parasite. Golgi found in one case three generations of the quartan parasite and two of the tertian parasite. In the fever curve of the chart we may find the different parasites showing their effects simultaneously, or an irregular type of fever may be manifested. It also happens that during the course of the fever only one form of parasite causes the changes, the others apparently having no influence.

According to Lekowicz's investigations of malignant and ordinary (tertiana and quartana) malarial fever, the parasites of both groups develop in a similar manner, but are differentiated as follows:—(*a*) Length of the period of incubation; (*b*) the size and form of the parasite found in the same stage of development; (*c*) the presence of melanin; (*d*) the presence of flagella and the number of spores present. However, the greatest differentiation is that the parasites of ordinary malaria develop within the cell (endoglobular), while those of the malignant forms are developed without the cell (extraglobular). The former grows within the red blood-corpuscles, the latter rolling the red corpuscles around themselves like a caterpillar does a leaf, whereby a part of their surface remains free, enabling them to attach themselves to the wall of the blood-vessels. When the parasite is separated from the blood-corpuscles by pressure of the cover-glass in cases of ordinary malaria forms, the wall of the corpuscle is ruptured, and the pigment escapes outwards, the remainder of the corpuscle being immediately decolorized. In cases of malignant malaria the wall and the pigment of the blood-corpuscle remain intact, and the parasite is not in the interior of the cell, but lies in a small cavity formed by the rolling process. The growth of the parasites in both instances is identical. At the end of the febrile stage

small protoplasmic bodies appear, possessing amœboid movement, which at rest assume a *ring form*, finally either penetrating the red blood-corpuscles, or rolling the corpuscles around themselves and absorbing the hæmoglobin. The duration of the vegetative period of the parasite causing the malignant types is disproportionately longer than by the parasites of tertiana and quartana. The fully-developed parasite is about the size of a red blood-corpuscle, and in ordinary malarial fever is spherical, and in malignant malaria half-moon or disc-shaped ; from these forms further oval and spherical forms also originate. At the height of this stage the parasites appear (probably owing to the lower temperature) to possess flagella, the tendency thereto varying with each group. The protozoa of the malignant forms develop a distinct double-contoured cuticle, while those of ordinary malarial fever possess an outer fine membrane ; nevertheless, spore formation, excapsulation, and dissemination of the young spores is analogous in both forms. In the sporulating bodies of the half-moon variety, Lekowicz has counted as many as thirty spores. He also distinguishes, as characteristic of the group, an extraglobular incubative period of more than three days' duration ; and of the half-moon or disc-shape of the fully developed individuals, four forms are determined, namely—

1. Hæmosporidium undecimanæ. (Period of incubation, ten days.)
2. Hæmosporidium sedecimanæ (fifteen days).
3. Hæmosporidium vigesimo-tertianæ (twenty-two days).
4. Hæmosporidium (?) (incubative period unknown).

The fever paroxysm coincides with the sporulation of a generation of these parasites. In the malignant forms a quotidian and tertian type is frequently observed, because many generations of parasites, differing in age from twenty-four to twenty-eight hours, are present. The presence of the tertian type in these cases is explained by a severe attack interfering with the formation of the active living spores of the immediately following twenty-four hours' younger generation, without influencing the forty-eight hours' younger generation, in which encapsulation has not yet taken place. The half-moon forms are by no means inaccessible to the action of quinine as heretofore accepted. Their resistance is only so altered that the quinine impairs the vitality of, or destroys, the complete half-moon forms capable of forming spores of several generations of simultaneous existing parasites, but it does not prevent previously existing young and half-grown sporidia in the internal organs developing into half-moon forms. After treatment with quinine the half-moon forms are still present one to two weeks later in the blood.

Method of Preparing Dry Specimens.

1. The cover-glass with the drop of blood in its centre is drawn quickly across another cover-glass, and both are *air*-dried, protected from dust.

2. Fix five to thirty minutes in a mixture of equal parts of absolute alcohol and ether.

3. Dry between two pieces of filter paper.

4. Stain in a watery solution of methylene blue; wash with water.

5. Contrast stain in a 2 per cent. solution of eosin in 60 per cent. alcohol.

Plehn's Method.

Stain.

Methylene blue concentrated watery solution, . .	60
½ per cent. eosin solution in 75 per cent. alcohol, .	20
Aqua distillata, ...	40

1. Place the specimen in the stain five to ten minutes.

2. Wash in water and dry.

3. Mount in xylol balsam.

Results.—The cells containing hæmoglobin are stained red, the plasma and body of the parasite a more or less intense blue.

Many other staining and fixing methods are used, but examination of the fresh specimens and staining by the above-mentioned methods will be found sufficient for diagnostic purposes. To observe the nucleus substance, the ordinary methylene blue method is sometimes sufficient, or the specimen is fixed in a mixture of acetic and picric acids and stained with hæmotoxylin, or examined in fresh blood to which some methylene blue or fuchsin is added.

Mode of Infection.—The cultivation of the parasite of malaria has not yet been accomplished, and infection of animals has also not succeeded. The parasite is only known as a parasite of the blood of man, and of where and in what manner they exist outside the body nothing is known, therefore the knowledge of the mode of infection is very limited. Malaria can be communicated from man to man by means of the blood. Gerhardt did it subcutaneously, and later it was accomplished both by subcutaneous and intravenous injections of the blood of malarial patients. Nevertheless, malaria is not a contagious disease; it is not communicable from man to man under natural conditions. The parasite is not found in the secretions, but it appears to be present in the herpetic vesicles of malarial patients, because the contents of the vesicles are capable of transmitting the disease by inoculation. The parasite undoubtedly exists in certain swampy districts, endemic, and at certain times epidemic, in a saprophytic condition in either the earth, water, or air. The transmission by means of the bite of insects is theoretically possible, and by some authorities it is advanced, but it has not yet been satisfactorily demonstrated. The incubative period in the majority of cases is from eight to fourteen days; but cases are known

where the disease did not break out for months, and others where it occurred in one to two days, or occupied only a few hours. These differences are probably due to the number of the parasites in the system, and the reaction of the affected individuals to the poison, or to varieties at present unknown in the stages of the parasite outside the body.

SOUTHERN OR TEXAS CATTLE FEVER.

(Ixodic Anæmia in Jamaica, Williams. Australian Tick Fever.)

This disease is due to the ravages of a blood parasite, the 'Pyrosoma bigeminum,' originally discovered by Smith in the red blood-corpuscles of cattle affected with southern fever. Williams found it in Jamaica, and Koch has also recently discovered it in Africa, in cattle diseases associated with the presence of ticks (Ixodes or Boöphilus bovis).

The disease affecting cattle, known as Texas or Southern fever, commences with a high fever, which, unless death occurs sooner, lasts about a week, and is associated with severe anæmia and hæmaturia; a chronic condition often follows when the symptoms are not so exaggerated.

Microscopical Appearances of the Parasite.—When the blood of an affected animal is examined in the fresh state at ordinary temperature, small bodies are seen inside the red blood-corpuscles. These may be pale rounded masses with amœboid movement and distinct contour, or pear or spindle-shaped bodies, distinctly outlined, with a granular body or vacuole at the thick end. There may be two of the pear-shaped bodies with narrow ends opposed in a single corpuscle. Very rarely three or four ovoid forms occur in the same corpuscle (see Photomicrograph, Fig. 99). The bodies measure from $\frac{1}{2}$ to 2 μ in diameter, and are usually situated in the disc of the red corpuscles.

The Motility when present may persist for an hour. In the blood circulation the infected cells rarely exceed 1 to 2 per cent., and in some cases in a later stage the parasite may be found free.

If an animal dies or is killed in the acute febrile stage a great number of infected corpuscles are found in the capillaries of the peripheral circulation. The parasites are most plentiful in the vessels of the kidney, next in the liver (see Photomicrographs, Figs. 99 and 100), spleen, and heart substance.

Staining Reactions.—Cover-glass specimens heated after Ehrlich's method, and stained with alkaline methylene blue one-half to two minutes, washed with water, and placed in a 1 per cent. solution of acetic

acid for a few seconds and then washed in water; examined in water, or dried and mounted in xylol balsam.

Anatomical Changes.—The principal post-mortem lesions observed by the author during an extensive outbreak in California in 1888, where one 'rancher' lost 700 out of a herd of 1200 in from three to four weeks, were as follows. The blood was a bright brick-red colour, very thin, seeming to coagulate more rapidly than normal blood. The flesh in the majority of cases was of a bright red colour, the fat between the muscles, as well as that of the alveolar tissue, of a deep brownish colour. The spleen was enormously enlarged, of a dark purple colour, the capsule sometimes being studded with petechiæ. On section the splenic pulp consisted of a disintegrated mass, having lost all consistency, pressure causing light red blood to escape. The largest measurement obtained was 24 in. long and 16 in. in circumference. The stomach, when examined *in situ*, shows the following appearances :—The *reticulum* is sometimes the seat of red imbibitions. The *abomasum* is always the seat of distinct and pathognomonic changes. The mucosa often presents a pink or dark-red colour, with minute ecchymoses studding its surface, and erosions of the epithelium are also frequently present. The *duodenum* is sometimes of a deep red colour, the mucosa deeply tinged with bile, especially close to the pylorus. The *jejunum* is frequently reddened, and circumscribed hæmorrhagic centres are often seen. The *cæcum* and *colon* are generally the seat of more or less ecchymoses. The *rectum* is generally of a red colour, and when diarrhœa is one of the symptoms, the mucosa is the seat of extensive hæmorrhages. The *liver* is the most affected organ of any in the body, and the condition of it may be said to be diagnostic of Southern fever. It is always enlarged, and in most cases enormously so, records showing it to weigh 20 lbs., and in one case 27½ lbs. In colour it is light brick-red, inclining to a dark yellow, almost resembling powdered cinnamon; on section it was generally fatty and light red coloured; blood escaped as well as an excess of bile. Under the capsule yellow streaks could be seen, some as large as straws, due to engorgements of the bile-ducts. Microscopical examination of a section of such reveals a condition like artificial injection of the gall capillaries with bile. The *gall-bladder* was in all cases more or less distended, and in some as large as a urinary bladder; the record shows it to have weighed 4 lbs. Its walls were hypertrophied and full of gall, sometimes of a dark yellow, and again of a dark green colour, almost black, sometimes slightly inspissated, and again so much so that it resembled boiled starch in consistency. The *kidneys* were usually of a dark brown colour, from the intense congestion; in some cases they were enlarged, in others not; there was always more or less parenchymatous degeneration present, and diffuse capillary engorgement. The cortex was softer than usual, and numerous petechiæ could be seen throughout its substance. In some cases pus was present in the pelvis, and in others a thin yellow exudate; the fat

of the kidney had the same peculiar yellow colour already described. The *bladder* was usually distended with bloody urine, and in such cases the mucosa was more or less congested. The *lungs* in some cases were emphysematous, in others a broncho-pneumonia was present. Again, some, in fact all, presented a more or less hypostatic pneumonia, due to weakness of the heart and kidney complications. Pleurisy was also present in some cases. The *pericardium* was studded with petechiæ; the fat around the heart exhibited the same peculiar colour already mentioned. *Serous infiltrations* and hæmorrhages were sometimes found beneath the skin of the lower jaw and neck.

Mode of Infection.—According to Smith and Kilborne the natural infection is caused by the blood-sucking tick (Ixodes or Boophilus bovis) which lives on the skin of the cattle, in an enzoötic territory becoming charged with the parasite.

The mature female tick on the animal's body has an oblong, oval-shaped body, and somewhat resembles a castor-oil bean; it presents a dull leaden colour, is rarely more than $\frac{1}{2}$ an inch long, and $\frac{5}{16}$ of an inch in breadth, possessing four pairs of legs, situated on the antero-lateral portion of the body. It attaches itself to its host by what is known as the rostrum, in the centre of which is a barbed dart furnished on either side with several rows of teeth arranged obliquely, which enable the tick to adhere more securely to the skin. The male tick is usually found attached to the skin immediately underneath the anterior portion of the female, the body being of a dark brown colour and triangular in shape. When fully grown it is very much smaller than the fully developed female, and never being in an engorged condition, is much more active and stronger in comparison with the female. As the female tick engorges herself she is fecundated by the male, and at maturity withdraws the barbed mouth organs or rostrum, falls to the ground, lays a great number of eggs, shrivels up gradually and perishes. The parasite is carried over in the eggs, and in from two to six weeks the young ticks are hatched, creep upon the cattle, and infect them with the disease. By this means fresh cattle arriving in infected districts become affected, while the native cattle exhibit no diseased symptoms. It is possible that they were infected as calves, and since then continued to harbour the blood parasites; in fact, inoculation of the blood of such animals produces the infection in other cattle in regions where the disease does not exist. Cattle travelling from infected regions can infect healthy pastures, not by direct infection, but in a round-about manner. The ticks that the cattle bring with them fall from the animals and deposit their eggs on the ground, and the young ticks infect the previously healthy cattle, the disease breaking out in forty-five to sixty days after the appearance of the infected cattle, because so much time must elapse before the young brood of ticks is capable of

producing the disease. The infection can also be conveyed to healthy districts by the artificial dissemination of mature forms of the ticks, which does not take place when the ticks are removed from the infected cattle before they are driven on new tracts of land. The Pyrosoma bigeminum, in certain respects, is somewhat similar to the malaria organism, but is distinguished by dividing into two, and producing no pigment. Although many particulars in their development are not sufficiently cleared up, their place with the amœba is certain.

Immunity.—Koch recently stated in his address to the Colonial Society, Berlin, that he was able to infect healthy cattle with ticks taken from diseased ones, and had succeeded in conferring immunity against Texas fever as follows:—Healthy animals were inoculated with the blood of the animals infected by young ticks, and Texas fever produced, and the process continued for several generations, until the disease was produced in a number of animals which recovered. These recovered animals were infected a second time with the blood of sick animals, being immune to the mild form of the disease produced under the conditions of the experiment. The immune animals were next taken to the coast and turned out with an affected herd, but did not become affected. They were finally inoculated with the blood of an animal affected with a severe form of the disease, when only one of the immunized animals became affected.

BABESIA BOVIS (STARCOVICI).

This parasite was first found by Babes in the red blood-corpuscles of the blood of cattle affected with hæmoglobinuria in Roumania.

Microscopical Appearances.—It is found throughout the vascular system, either as round or lanceolate-shaped bodies, mostly lying in pairs or undergoing a process of division, and staining like bacteria. In the fresh state they are non-motile, and measure about 1 μ, and in stained specimens about 0·6 μ in diameter. In the commencement of the attack large pear-shaped, irregular, staining bodies were also observed.

The symptoms of the disease in the affected cattle are similar to those of Southern fever.

Hæmaturia is frequent during the life of the animal, the attack lasting about five days.

The chronic stage sometimes occurring in Texas fever was not observed. According to Babes, the ticks play an important part in the production of the Roumanian disease, the blood parasites increasing in the bodies of the ticks.

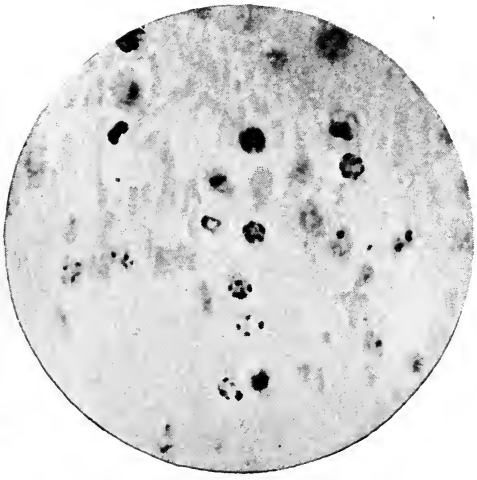


Fig. 99.—Pyrosoma Bigeminum in bloodvessel. Section of liver of affected ox. Methylene blue. × 600.

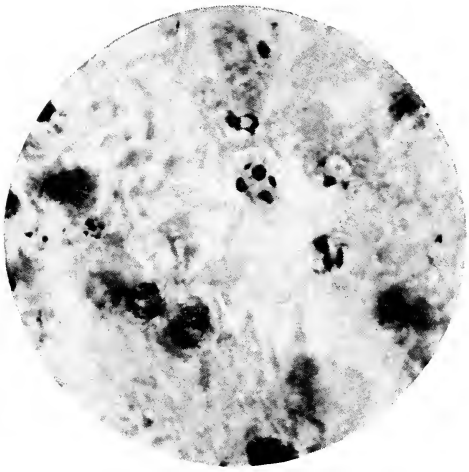


Fig. 100.—Pyrosoma Bigeminum in bloodvessel. Section of liver of affected ox. Methylene blue. × 1000.

[*T. Bowhill, F.R.C.V.S., Photo., Edinburgh*, 1898.

FIG. 101.—Klossia. Section of liver of snail (Aquolimax.)
× 1000.

T. Bowhill, F.R.C.V.S., Photo., Edinburgh, 1898.

BABESIA OVIS (STARCOVICI).

This parasite is the cause of ictero-hæmoglobinuria of Roumanian sheep, and was also found by Bonome in an outbreak in Italy. The transmission of affected blood to healthy sheep in large quantities yielded, according to Babes and Bonome, no genuine reproduction of the disease.

BOVINE MALARIA.

Celli and Santori describe a disease amongst cattle in the Roman Campagna which they call *cattle-malaria*. The disease is characterised by acute anæmia, enlargement of the spleen, emaciation, and fever. Native cattle do not appear to be affected with the disease, but Swiss, Lombardy, and Dutch cows are affected, and sometimes death results. In the blood of the affected animals parasites were found inside the red blood corpuscles, which sometimes exhibited Brownian and at other times amœboid movement. The endoglobular parasites were sometimes pear-shaped and united in pairs, and identical with Smith's *Pyrosoma bigeminum* of Texas fever.

Celli and Santori consider this malady is probably identical with diseases described by other observers, while, on the other hand, they find many resemblances between this disease and human malaria. Quinine administered to the affected cattle produced favourable results.

KLOSSIA SOROR (A. SCHNEIDER).

This parasite is frequently found in the kidneys of land and water snails (Helix, Succinea, Neritina, etc.), and probably also in other species.

Microscopical Appearances.—Cysts containing a large number of permanent spores. Each of these mother spores divides into four to six sickle-shaped embryo (or daughter spores). The sickle-shaped spores are 1 to 7 μ in size, and exhibit a serpentine motility for a short time. The daughter spores often increase in the epithelial cells of the kidney, causing hypertrophy of the same. Sickle forms have also been observed in the secretion of the kidneys. (For Photomicrograph of Klossia in the liver of a snail, see Fig. 106.)

PHOTOMICROGRAPHY.

The four plates of photomicrographs produced at the end of this work were made with the large Zeiss Photomicrographic Apparatus (see

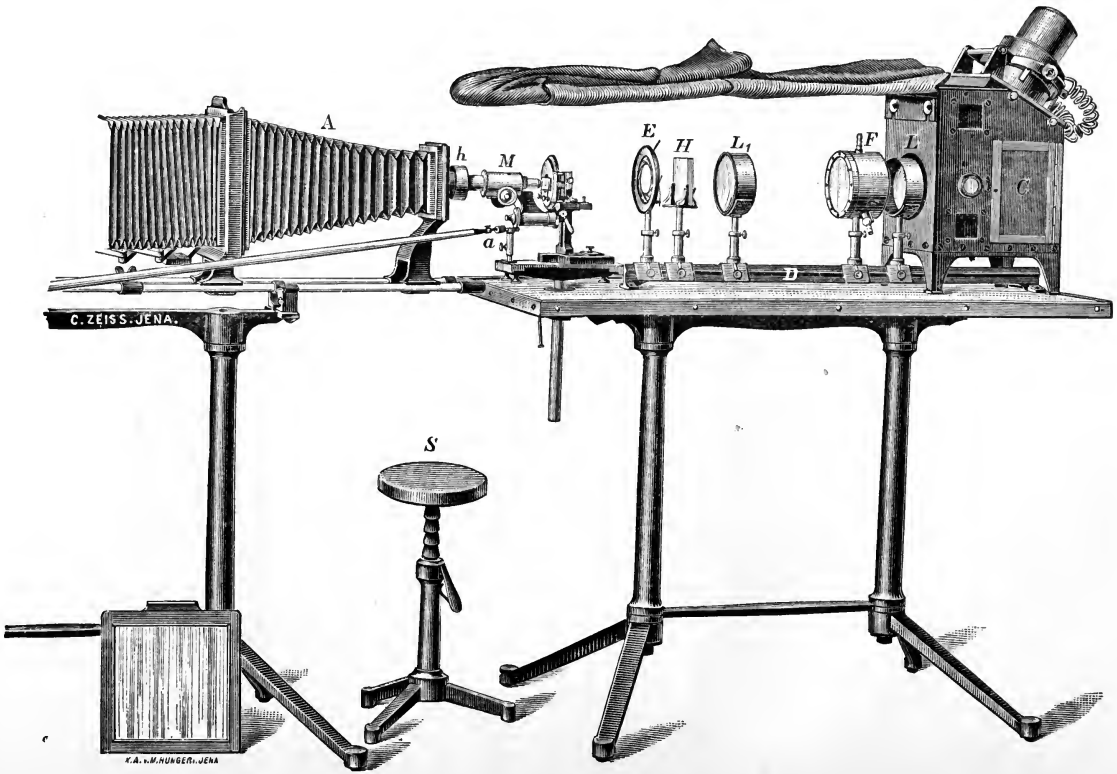


FIG. 102.—Large Zeiss Photomicrographic Apparatus.

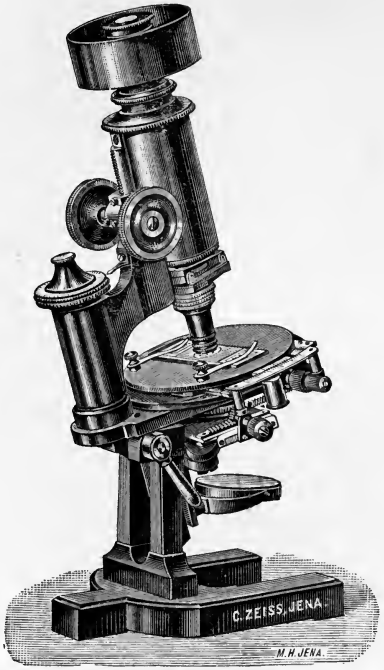


FIG. 103.— Zeiss Special Microscope and Stand for Photomicrography.

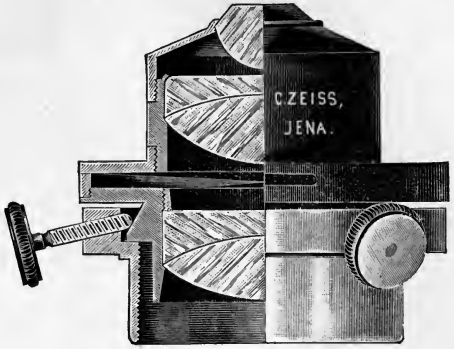


FIG. 104.—Special Centering Achromatic Condenser.

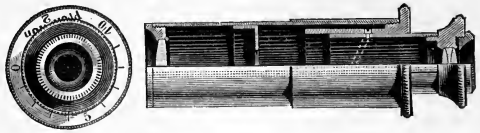


FIG. 105.—Projection Eye-piece.

Fig. 102) with a Schuckert & Co. electric lamp, enabling the work to be done at any time.

Between the electric lamp and the microscope the following articles are placed in a direct line on a sliding bar :—

1. A *Collecting Lens,* diameter $4\frac{1}{4}$ inches, consisting of an anterior and posterior lens. The concave surfaces of these lenses are turned towards the light, and adjusted so that the distance between the luminous centre and the edge of the lens mount is about $4\frac{3}{4}$ inches, rendering the rays nearly parallel, without introducing excessive aberrations. The *anterior* lens is placed close to the lamp, and the posterior lens, which again collects the parallel rays, on a point situated about 16 inches from the anterior lens (the collecting lenses can be rotated).

2. Between the anterior and posterior lenses an *absorbing cell* is placed for the absorption of the heat rays generated by the electric lamp. The walls of the cell consist of plate-glass discs about $4\frac{3}{4}$ inches in diameter, while the distance between the glass discs is about $2\frac{1}{2}$ inches ; an inlet tap is fitted on the bottom and an outlet tap on the top of the body of the cell. (This cell can also be used with sunlight.) The cell is filled with water, previously boiled, from the lower tap, because if filled from above the almost inevitable result is cracking of the glass discs.

3. An *Iris diaphragm* is placed between the *microscope* and the *posterior collecting lens,* with a total aperture of $2\frac{5}{8}$ inches, fitted with spring clips for holding objects, coloured screens, and ground glass for indirect illumination.

The iris diaphragm is particularly adapted for centering the entire apparatus, for shutting off false light, and when closed the illuminating rays are focused in the small opening of the closed diaphragm (about $\frac{1}{2}$ an inch) by means of the posterior collecting lens.

4. The microscope used with the large Zeiss apparatus is their special stand for photomicrography (see Fig. 103), which is placed on an adjustable sole plate with levelling screws, and instead of the ordinary Abbé condenser, a special centering achromatic condenser is used (see Fig. 104), with an aperture of 1·0, and is focused with respect to the plane of the object by means of the rack and pinion movement of the illuminating apparatus. The aperture of the illuminating pencil is regulated by an iris diaphragm placed between the lenses of the condenser. The best lenses for photomicrographic work, especially for bacteria and other high-power photography, are the apochromatic lenses of Zeiss. The photomicrographs in this work, reproduced by the colotype process, were made with the apochromatic homogeneous immersion lens 2 mm., numerical aperture 1·40, while those reproduced by half-tone process were made with a 1·5 mm. lens with a numerical aperture of 1·30, and illuminated by means of a special heliostat of my

own construction. Projection eye-pieces, Nos. 2 and 4 (see Fig. 105) were used in conjunction with these lenses.

The microscope, electric lamp, and other fittings connected therewith, just described, are placed on a slide on a table as shown in Fig. 102, while the camera is placed on a separate stand (see Fig. 102).

For sunlight a light filter is used, while with the electric light spectroscopic-tested glass plates are used—for red stained specimens, green; for blue stained specimens, yellow; for violet, yellow, and green, and for unstained specimens, blue.

For focusing the image on the ground glass of the camera a special improved aplanatic focusing lens is used with an exceptionally large field and a magnification of 6—a higher power is a disadvantage. The fine adjustment during focusing is manipulated by means of a Hook's key and rod connection.

Magnification.—This depends upon the distance of the specimen from the ground glass slide, and can be accurately measured as follows:—Take a stage micromillimeter (which is one millimeter divided into hundredths); place it under the lens and project the picture on the ground glass slide; adjust the focus, and measure the size with an ordinary glass 100 millimeter measure. When 1 millimeter of the projected stage micrometer is equal to 10 millimeters on the glass measure, then the magnification = 1000 diameter. Other magnifications are calculated in a similar manner.

APPENDIX

KISCHENSKY'S QUICK METHOD OF STAINING BACTERIA IN COVER-GLASS AND SLIDE PREPARATIONS.

1. A drop of a weak solution of carbol fuchsin (10 drops to 10 c.c. of water) is placed on a cover-glass and mixed with a minimum quantity of the pure culture under investigation, and spread out in a thin layer.

2. The preparation is now heated gently over the flame.

3. In examining blood, pus, etc., the above stain is mixed with an alcoholic solution of methylene blue.

The *results* are: the quickness of the method, beautiful staining, by which means the flagella can also be demonstrated, while the field remains unstained.

SEMENOWICZ AND MARZINOWSKY'S SPECIAL METHOD OF STAINING BACTERIA IN COVER-GLASS SPECIMENS AND SECTIONS.

1. The cover-glass specimens and sections are stained two (three to four) minutes in a solution of carbol fuchsin (one part of a concentrated solution and two parts of water).

2. The specimens are now washed with water.

3. Stain three to four (four to five) minutes with Löffler's methylene blue.

4. Wash in alcohol.

5. Clarify in oil and xylol.

Results.—Löffler's blue displaces the carbol fuchsin in the bacteria and appears to work on the principle of a mordant. The nuclei and the bacteria are stained blue, the interstitial tissue and the protoplasm of the cells red, while degenerated bacteria also appear red.

GASTROMYCOSIS OVIS (NIELSEN).

This organism was isolated in a very rapidly fatal endemic disease amongst sheep in Iceland and Norway, characterised by a hæmorrhagic

inflammation of the abomasum and the other compartments of the stomach, portions of the intestines, as well as the whole body. The bacilli were found in the walls of the stomach and frequently in the internal organs three hours after death. This disease, from the description given by Nielsen, is probably identical with a disease affecting sheep in the country known as 'Braxy.'

Microscopical Appearances.—Bacilli from 1 to 2 by 6 μ, often occurring in pairs, seldom arranged in chains, frequently containing refractile bodies.

Staining Reactions.—Stains by the Gram method.

The bacillus has not been cultivated on artificial media. Experiments conducted with pieces of tissue containing the bacilli, introduced into rabbits and lambs, as well as feeding experiments, yielded negative results.

SPECIAL BACILLUS ISOLATED FROM MILK IN PIORKOWSKI'S LABORATORY, BY CAMPBELL M'CLURE

(*Deutsche Med. Wochenschrift*, 1898, No. 26).

Microscopical Appearances.—It occurs as short rods a little shorter and thicker than the diphtheria bacillus. Club-shapes are often observed, especially in old cultures.

Motility.—Non-motile.

Spore Formation absent.

Staining Reactions.—Stains with any of the ordinary dyes, but not by the Gram method.

Biological Characters.—*On Gelatine Plates.*—In two to three days a luxuriant growth develops on the surface of the medium of small white, slightly transparent, granular points, with well-defined borders, becoming later (in forty-eight hours) yellowish-brown in colour, dark in the middle, and clear at the periphery.

In Gelatine Stab Cultures opaque white points develop along the inoculation track, tending to become confluent in the centre.

On Agar Plates at 37° C., light brown granular colonies develop.

On Glycerine Agar, a similar growth.

Bouillon at 37° C. becomes cloudy, a flaky sediment forming on the bottom and sides of the tube.

On Potatoes, at 37° C. a copious, greyish-white, moist coating, with dentated edges, which become yellow in older cultures.

On Grape Sugar Agar Media the growth exhibits no special characteristics.

Milk inoculated and kept at 37° C. for forty-eight hours has an acid

reaction, is coagulated, and emits an odour of acetic acid (this feature is not constant).

Pathogenesis.—A mouse inoculated subcutaneously died in fourteen days. Investigation of the heart's blood, spleen, liver, and lungs yielded negative results.

BACELLI'S METHOD OF TREATING TETANUS IN MAN.

Bacelli injects subcutaneously 3 to 4 centigrammes of a 2 to 3 per cent. solution of carbolic acid until about 35 centigrammes have been injected in the twenty-four hours.

The carbolic solution acts as curative serum on the tetanus poison circulating in the blood, besides on the nerve elements already affected by the poison. The fact that so large a quantity of carbolic solution can be taken into the system without appearances of intoxication, the author declares to be due to a special resistance of the tetanic poison against the remedy—(*Munich Med. Woch., Dtsch. Med. Zg.*)

List of some of the Principal Works consulted in the Compilation of the Manual.

BOOKS.

C. Flugge—'Die Micro-organism.'
C. Günther.—'Einfuhrung in das Studium der Bacteriologie.'
Schurmayer.—'Bacteriologische Technik.'
Fraenkel.—'Bacteriologie.'
Kalden's 'Technik.'
Lehmann and Neumann.—'Bacteriologie.'
Bohm and Oppel.—'Mikroskopischen Technik.'
Levy and Klemperer.—'Bacteriologie.'
Kitt.—'Atlas der Thierkrankheiten.'
Friedlander and Eberth's 'Mikroskopische Technik.'
Robert Behla.—'Die Amöben.'
'Atlas der Klinischen Mikroskopie des Blutes.'
Mosselman and Liénaux.—'Veterinary Microbiology.'
Cornel and Babes.—'Les Bacteries.'
Woodhead.—'Bacteria and their Products.'
Abbot.—'Principles of Bacteriology.'
Kanthack and Drysdale.—'Practical Bacteriology.'
Sternberg.—'Manual of Bacteriology.'
Klein.—'Micro-organisms and Disease.'
Simon.—'Clinical Diagnosis.'
Lafar.—'Technical Mycology.'

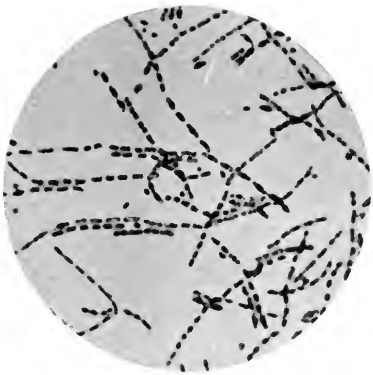
Sykes.—'Principles and Practice of Brewing.'
Neuman.—'Parasites and Parasitic Diseases of Domestic Animals.'
Billings.—'Investigations in Nebraska.'

PERIODICALS.

'Hygienische Rundschau.'
'Centralblatt für Bacteriologie.'
'Archiv für Wissenschaftliche und Praktische Thierheilkunden.'
'Berliner Thierartz Wochenschrift.'
'Recueil de Médecine Vétérinaire.'
'The Lancet.'
'British Medical Journal.'
'Science Progress.'
'The Veterinary Record.'
'The Veterinarian.'
'The Veterinary Journal.'
'Journal of Comparative Pathology and Therapeutics'—Reports of the U.S. Bureau of Animal Industry.

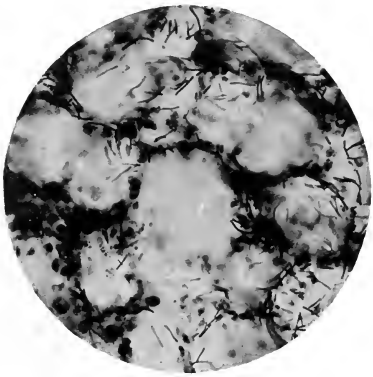
I.

Bacillus Anthracis with capsules in Mouse's blood stained by Johne's Method × 1.000.



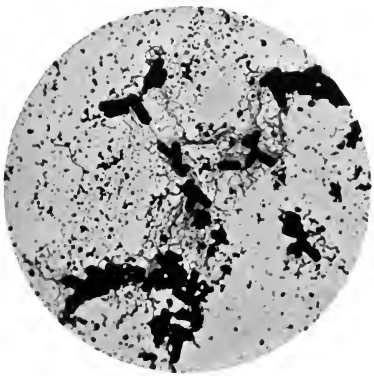
II.

Bacillus Anthracis and Spores Agar Culture Fuchsin × 1.000.



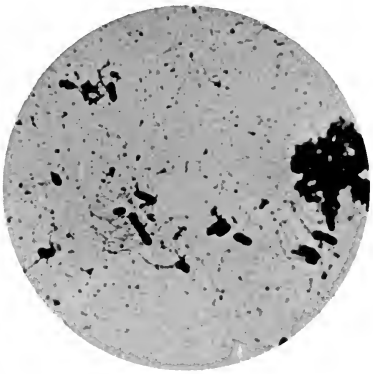
III.

Bacillus Anthracis section of Mouse's lung. Methylene-blue × 350.



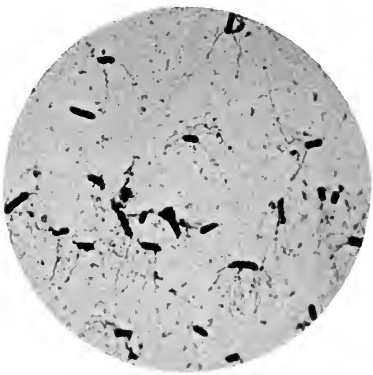
IV.

Bacillus of Symptomatic Anthrax with flagella. Grape-sugar-agar culture stained with Orcëin Solution × 1.000.



V.

Bacillus of Malignant Oedema with flagella. Grape-sugar-agar culture stained with Orcëin Solution × 1.000.

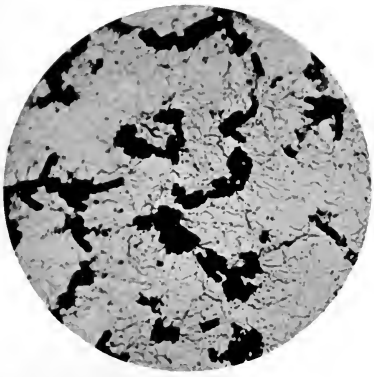


VI.

Bacillus of Tetanus with flagella. Grape-sugar-agar culture stained with Orcëin Solution × 1.000.

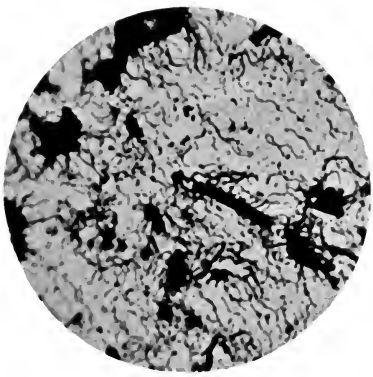
Bowhill, photo. Berlin 1898.

Collotype by C. G. Röder, Leipzig.



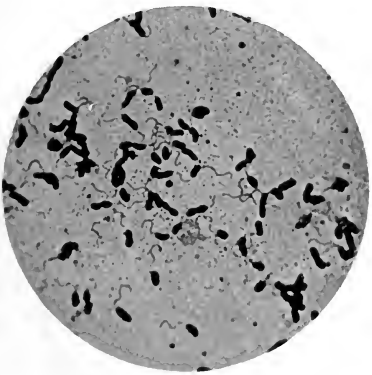
VII.

Bacillus of Typhoid Fever with flagella. Agar culture stained with Orcëin Solution × 1.000.



VIII.

Bacillus of Typhoid Fever with flagella. Agar culture stained with Orcëin Solution × 1.500.

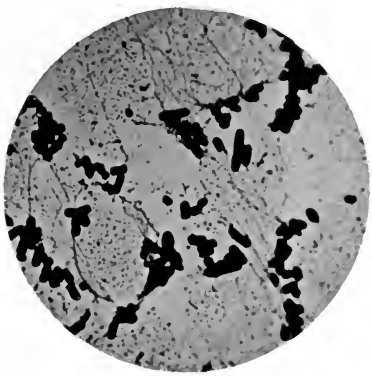


IX.

Finkler and Prior's Spirillum with flagella. Agar culture stained with Orcëin Solution × 1.000.

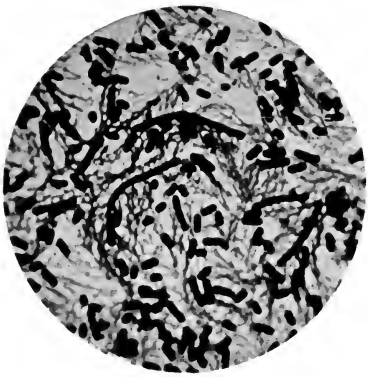
X.

Cholera Spirilla with flagella. Agar culture stained with Orcëin Solution × 1.000.



XI

Bacillus Coli communis with flagella. Agar culture stained with Orcëin Solution × 1.000.

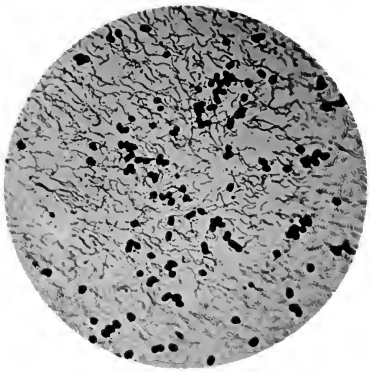


XII.

Proteus Vulgaris with flagella. Agar culture stained with Orcëin Solution × 1.000.

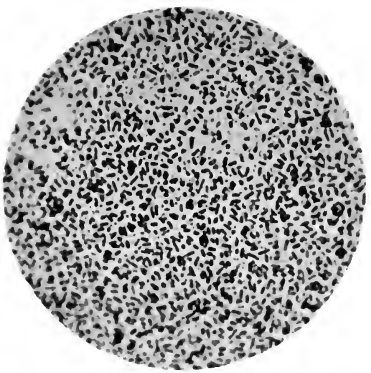
Bowhill, photo. Berlin 1898.

Collotype by C. G. Röder, Leipzig.



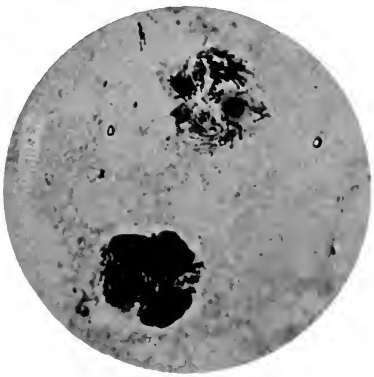
XIII.

Bacillus of Swine Fever with flagella. Agar culture stained with Orcëin Solution × 1.000.



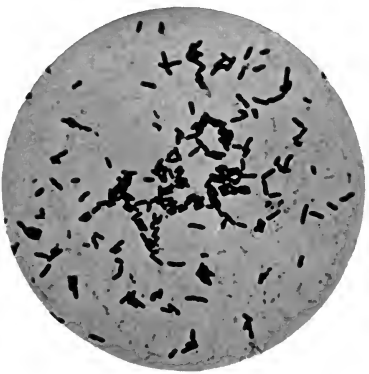
XIV.

Bacillus of Schweine-Seuche "German". Agar culture stained with Orcëin Solution × 1.000.



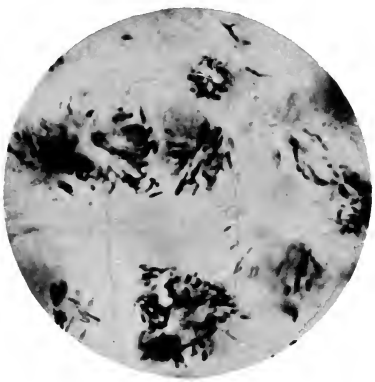
XV.

Bacillus of Mouse septicaemia in Mouse's blood. "Cladius stain". × 1.000.



XVI.

Bacillus of Glanders. Glycerine Agar culture Fuchsin × 1.000.



XVII.

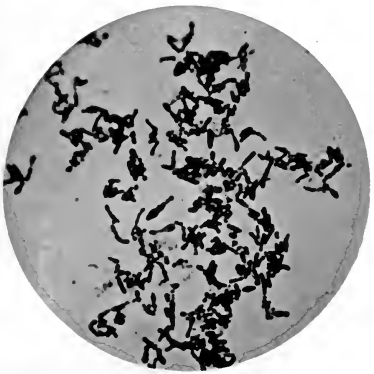
Bacillus of Leprosy section of skin-nodule "Cladius stain" × 1.000.

XVIII.

*) Bacillus of Tuberculosis × 1.000. Cover glass preparation from a Guinea pig inoculated with a culture from a Guinea pig inoculated with butter.

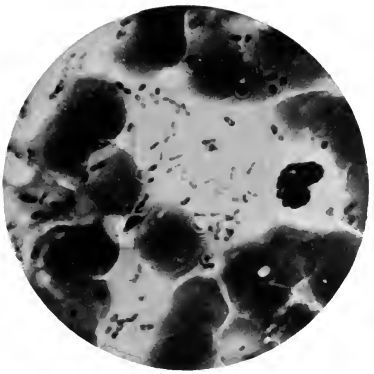
*) For the specimen from which this photograph was obtained I am indebted to Drs. Morgenroth and Hormann, Hygienic Institute, Berlin.

Bowhill, photo. Berlin 1898. Collotype by C. G. Röder, Leipzig.



XIX.

Bacillus of Diphtheria Blood-Serum. Agar culture "Cladius stain" × 1.000.

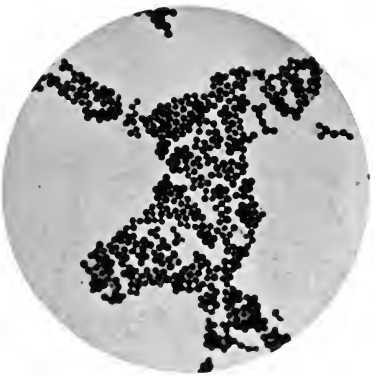


XX.

Bacillus of Bubonic Plague in human blood × 1.000.

XXI.

Fraenkels Diplococcus of pneumonia. Agar culture Fuchsin × 1.000.

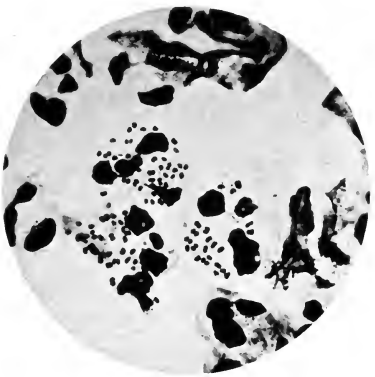


XXII.

Staphylococcus pyogenes aureus. Agar culture "Cladius stain" × 1.000.

XXIII.

Schütz's Streptococcus of Strangles in pus from abscess Cladius stain × 1.000.



XXIV.

Gonococci, in urethral discharge stained by Knacks Method × 1.000.

Bowhill, photo. Berlin 1898.

Collotype by C. G. Röder, Leipzig.

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