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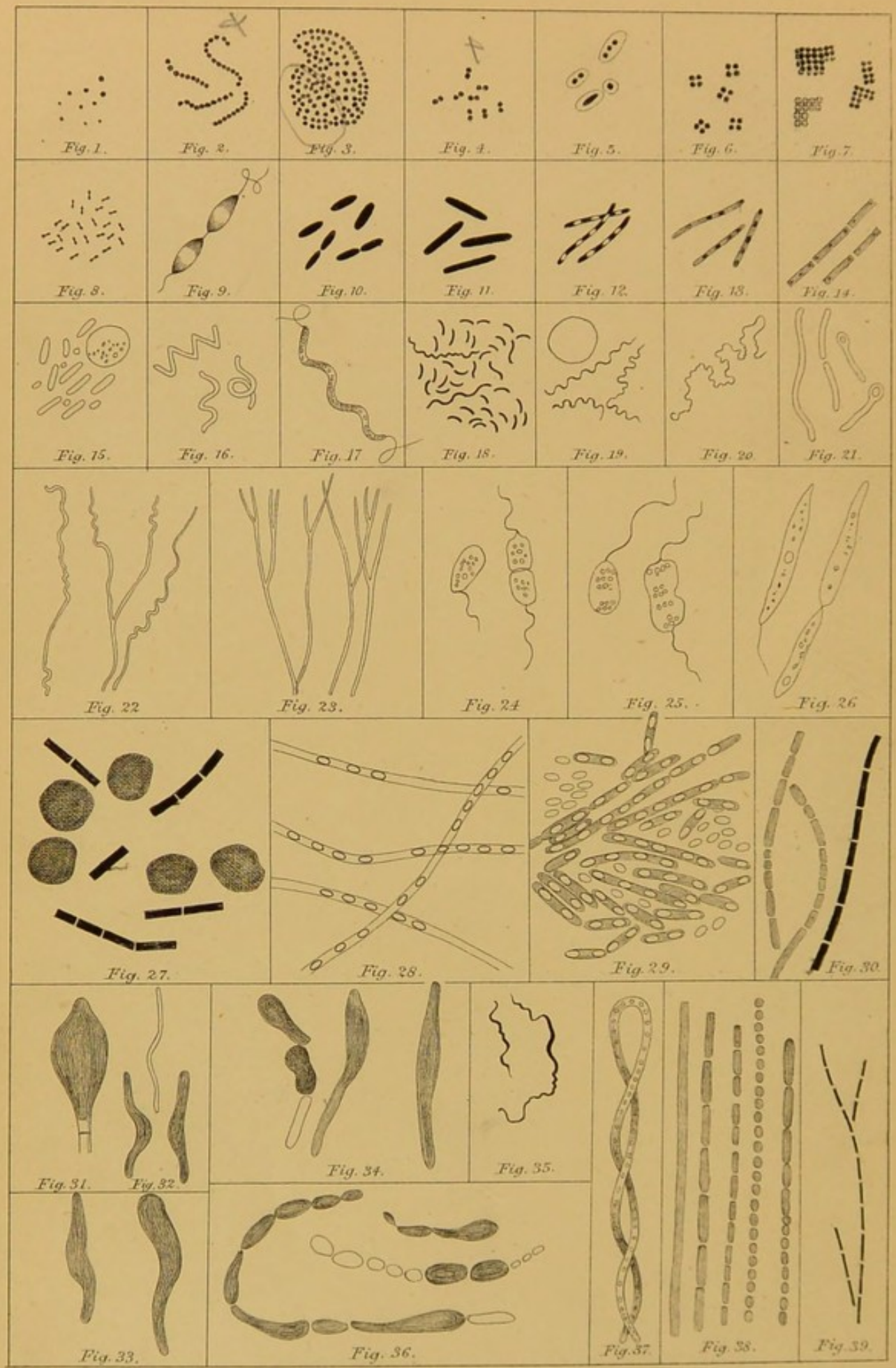
BACTERIOLOGY



BACTERIA, SCHIZOMYCETES OR FISSION-FUNGI.

Edgar Crookshank del. Vincent Brooks, Day & Son, Lith.

AN INTRODUCTION

TO

PRACTICAL BACTERIOLOGY

BASED UPON THE METHODS OF KOCH.

BY

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ILLUSTRATED WITH COLOURED PLATES AND WOOD ENGRAVINGS

LONDON

H. K. LEWIS, 136 GOWER STREET, W.C.

MDCCCLXXXVI.

To

SIR JOSEPH LISTER, BART., M.B., F.R.S.,

WHO HAS CREATED A NEW EPOCH IN

MEDICINE AND SURGERY,

BY APPLYING A KNOWLEDGE OF MICRO-ORGANISMS

TO THE TREATMENT OF DISEASE,

This Work is Dedicated

AS A

TOKEN OF ADMIRATION AND RESPECT

BY THE AUTHOR.

PREFACE.

A BRANCH of study, which has opened fresh paths in pathology and therapeutics, is so important that it may in time become a more essential part of the medical curriculum, and is perhaps worthy of an elementary handbook, which shall include a systematic sketch of the genera and species of micro-organisms, as well as the methods employed in the investigation of their life-histories.

Having myself experienced the want of a practical aid to the study of Bacteriology by the methods introduced by Professor Koch, I thought that it might be useful to those wishing to commence this study by these recent methods, if I embodied my notes made in different laboratories in the form of a Manual for Students. The work is thus intended to help the student beginning the study of a subject, the literature of which, in English, is for the most part diffused in numerous periodicals.

The methods of "pure cultivation" of Bacteria will, after all, be found to be remarkable for their extreme simplicity, and can be easily mastered by

the careful clinical worker, to whom it is hoped this little book may also prove useful.

I take this opportunity of expressing my best thanks to Professor Virchow, who materially furthered my work in the Pathological Institute of Berlin by kind advice and generous assistance.

I am most grateful to Dr. Babes, of Budapest, for his ever-ready co-operation; and to Professor Johne, of Dresden, who also placed his laboratory at my disposal, and to whom I am particularly indebted for much of the material from which the microscopical preparations were made.

I would also wish gratefully to acknowledge the great interest and courteous assistance shown me on the part of Dr. Hauser, of Erlangen; Professor Pettenkofer, Professor Bollinger, and Dr. Büchner, of Munich; and the officials of the new Hygienic Laboratory in Berlin.

The original drawings from which the coloured plates of test-tube- and potato-cultivations are reproduced were made by my wife from cultivations prepared especially for the purpose, and selected as typical. The coloured plates of the microscopic appearances are reproductions of my own drawings, from specimens I had recently prepared. The drawings were made from parts selected as most characteristic from a great number

of preparations, which have been since demonstrated at the meeting of the Royal Microscopical Society, on November 25th.

All the plates have been most faithfully and skilfully reproduced by Messrs. Vincent Brooks, Day, & Son.

In conclusion, I owe much to Professor Gerald F. Yeo, of King's College, London, for many valuable criticisms; and to my colleague, Mr. Herroun, for his kindness in reading the proof-sheets.

EDGAR M. CROOKSHANK.

24, MANCHESTER SQUARE, W.,
December, 1885.

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PART I.

GENERAL METHODS.

ERRATA.

Page 83, line 9, for *should have to be* read *should be.*
„ 93, „ 3, omit *upon.*
„ 159, „ 4, for *It was* read *They were.*
„ 203, „ 26, for BACILLUS VALEI read BACILLUS ALVEI.
„ 231, „ 10, for *methyl-violet* read methyl-violet.

BACTERIOLOGY.

CHAPTER I.

INTRODUCTORY.

THE researches of Pasteur into the *rôle* played by micro-organisms in the processes of fermentation and putrefaction, and in diseases such as anthrax, the silkworm malady, pyæmia, septicæmia, and chicken cholera, have invested the science of Bacteriology with universal interest and vast importance. The further researches of the practical mind of Lister, with the resulting evolution of antiseptic surgery, have demonstrated the necessity for a more intimate acquaintance with the life-history of these micro-organisms; while the more recent investigations which have established the intimate connection between bacteria and infectious diseases, and more especially the discovery by Koch of the tubercle and cholera bacilli, have claimed the attention of the whole thinking world.

The scientific importance of these latter dis-

coveries is evidenced by the fact that in Germany medical men were summoned from all parts of the country to Berlin to attend a course of instruction in the latest methods employed in the investigation of bacteria. In this way the methods of *pure cultivation* devised by Koch, and the means of recognising the cholera bacillus, are being widely disseminated.

To a medical man, those bacteria which are connected with disease, and more especially those which have been proved to be the *causa*, if not the actual *materies morbi*, are of predominant interest and importance. It is, however, impossible by localising one's knowledge to pathogenic species to thoroughly understand the life-history of these particular forms, or to be able to grasp and appreciate the various arguments and questions that arise in comparing their life-history with the progress of disease.

It is not sufficient to know only how to recognise and artificially cultivate a bacterium associated with disease; we must endeavour to establish the exact relationship of the bacterium to the disease in question. To ascertain beyond all doubt whether a micro-organism is actually the *causa causans* of a disease, Koch has laid down the following postulates:—

a. The micro-organism must be found in the blood, lymph, or diseased tissues of man, or animal, suffering from, or dead of, the disease.

b. The micro-organisms must be isolated from the blood, lymph, or tissues, and cultivated in suitable media,—*i.e.*, outside the animal body. These *pure cultivations* must be carried on through successive generations of the organism.

c. A pure-cultivation thus obtained must, when introduced into the body of a healthy animal, produce the disease in question.

d. Lastly, in the inoculated animal the same micro-organism must again be found.

These points would naturally suggest a sequence in the various processes which must be adopted in a practical study of micro-organisms associated with disease. Inasmuch, however, as these processes embrace those which are employed in the isolation, cultivation, etc., of non-pathogenic species, we shall, in studying the bacteria as a whole, adopt the order suggested. After an introduction to the apparatus commonly employed in a bacteriological laboratory, we shall describe the methods of examining liquids, tissues, etc., and the means of recognising micro-organisms. Then will follow the methods of isolating these micro-organisms from such liquids, tissues, etc., and of carrying on pure cultivations in nutrient media. Lastly, we shall refer briefly to experimental researches on the living animal, and the means of isolating micro-organisms from the liquids and tissues of the body after death.

In the second part will be found a classification of bacteria, with a description of each species,

more particularly of those of pathological interest; with a detailed account of the special methods of examination and of staining employed for demonstrating the different species.

In the Appendix a descriptive list of important yeasts and moulds will be given, with any special technique required in their case. Yeasts and moulds are constantly encountered in the special methods of examining bacteria in air, soil, and water, and several are of interest in being, like the pathogenic bacteria, micro-organisms associated with disease.

The special methods just referred to, with description of the apparatus employed, can also be conveniently treated of in the Appendix.

CHAPTER II.

APPARATUS, MATERIAL, AND REAGENTS EMPLOYED IN A BACTERIOLOGICAL LABORATORY.

(A) HISTOLOGICAL APPARATUS.

Microscope.—For the investigation of micro-organisms a good microscope with oil-immersion system and a condenser, such as Abbé's, is essential. Such instruments are supplied by Leitz, Zeiss, and Hartnack in Germany, and Powell and Lealand in England. Zeiss' microscope, with $\frac{1}{12}$ and $\frac{1}{18}$ oil-immersion lenses, or Powell and Lealand's with $\frac{1}{12}$ and $\frac{1}{25}$, is recommended for investigators; while Leitz', with $\frac{1}{12}$, is a serviceable and economical one for students.* In addition to the usual microscopic fittings, Zeiss supplies a micrometer eyepiece, with directions for use. Some such arrangement is essential for the measurement of bacteria. Other accessories to the microscope are—

A large bell-glass for covering the microscope when not in use.

About a foot square of blackened plate-glass.

* Leitz' with $\frac{1}{12}$ costs about £15; Zeiss', with the same, £30 and with $\frac{1}{18}$, £20 more.

A white porcelain slab of the same size.

Glass bottles with ground glass stoppers, for alcoholic solutions of aniline dyes, etc.

Glass bottles with funnels, for aqueous solutions of the dyes, and others provided with pipettes.

A small rod-stoppered bottle of cedar oil. This is recommended by Zeiss in preference to other oils for his immersion lenses.

Set of small glass dishes or capsules, and watch glasses, for section staining, etc.

Stock of best glass slides, in packets of fifty.

Several boxes of round and square thin cover-glasses, in various sizes, of the best quality.

Needle-holders, with a couple of platinum needles, and a packet of ordinary sewing needles.

Glass rods drawn out to a fine point; useful for manipulating sections when acids are employed.

Copper lifters, preferably plated.

One pair of small brass or spring-steel platinum-pointed forceps, for holding cover-glasses.

One pair of brass tongs.

Collapsible tubes for containing Canada-balsam; very serviceable for transport and general use.

Turn-table, used in preparing slides with rings, for mounting preparations of Aspergillus, etc.

Boxes for preparations, book-form.

Tickets and labels, various sizes.

Soft rags or old pocket handkerchiefs, for removing cedar oil after use of immersion lens, cleaning cover-glasses, etc.

Chamois leather for wiping lenses.

Microtome.—Schanze's is much in favour in Germany, but Jung's, of Heidelberg,* though a

* Price lists may be obtained from any of the above-mentioned firms, from which an idea of the instruments can be formed, and a comparison of the prices made.

somewhat cumbrous instrument, is much to be preferred. Smaller accessories, which should be within reach, are—

A small can of sewing machine oil.

A soft rag and chamois leather, for wiping the knives immediately after use.

Stone and leather for setting and sharpening the same.

Two or three camel's hair brushes.

A Freezing Microtome, such as Williams' or Roy's, and a Valentin's Knife, are useful for the examination of tissues in the fresh state, but otherwise are supplanted by the above.

(B) REAGENTS AND MATERIAL EMPLOYED IN THE PROCESSES OF HARDENING, DECALCIFYING, EMBEDDING, FIXING, AND CUTTING OF TISSUES.

Alcohol, absolute.

Bergamot oil.

Celloidin.

Dissolved in equal parts of ether and alcohol.

Cork, or stock of ready-cut corks.

Ebner's solution. A mixture in the following proportions:—

Hydrochloric acid . . .	5·
Alcohol	100
Distilled water	20
Chloride of sodium . . .	5·

Gelatine.

Melted in a small porcelain capsule and set aside ready to be re-melted when required for use.

Glycerine-gelatine (Klebs).

Best well-washed gelatine . .	10
Add distilled water, allow gelatine to swell up, pour off excess of water, melt gelatine with gentle heat, add	
Glycerine	10

Lastly a few drops of phenol for preservation.

Gum.

Kleinenberg's solution.

Saturated watery solution of picric acid	100
Strong sulphuric acid . . .	2

Filter and add

Distilled water	300

Muller's fluid.

Bichromate of potash . . .	2
Sulphate of sodium	1
Distilled water	100

Osmic acid.

Distilled water	100
Osmic acid	·5

Paper trays.

Paraffin.

Spermaceti.

Xylol.

Hardening and decalcifying solutions should be kept in stock in quantities according to requirement. A jacket of brown paper should be pasted round a well stoppered bottle to contain osmic acid to efficiently protect it from light, and it should be kept in a cool place. The celloidin solution may be kept in stock in a wide-mouthed glass bottle, from which small wide-mouthed bottles may be filled according to the number required. To put several pieces of different tissues in the same bottle leads to confusion.

(C) REAGENTS FOR EXAMINING AND STAINING MICROSCOPICAL PREPARATIONS.

1. Acetic acid, strong.
2. Alcohol—absolute.
3. Alcohol—60 per cent.
4. Alcohol—acidulated.

Alcohol	100
Hydrochloric acid	1

5. **Alum-carmine** (Grenacher).

Carmine	1
Five per cent. solution of alum .	100

Boil twenty minutes, filter when cold.

6. **Ammonia, strong.**

7. **Aniline.**

8. **Aniline water.**

Distilled water	100
Aniline	5

Shake well and filter emulsion.

9. **Bismarck brown.**

(*a*) Concentrated solution in equal parts of glycerine and water.

(*b*) Aqueous solution.

Bismarck brown	2
Alcohol	15
Distilled water	85

10. **Borax carmine** (Grenacher).

Distilled water.	100
Borax	4

Add 4 per cent. carmine; heat twice to all but boiling point, add an equal quantity of alcohol at 70°, set aside five hours and then filter.

11. **Cedar oil.**

12. **Eosin.**

(*a*) Saturated alcoholic solution.

(*b*) Aqueous solution.

Distilled water	100
Eosin	5

13. **Ether.**

14. **Fuchsine.**

(*a*) Saturated alcoholic solution.

(*b*) Aqueous solution.

Fuchsin	2
Alcohol	15
Water	85

15. **Gentian violet.**

(*a*) Saturated alcoholic solution.

(*b*) Aqueous solution.

Gentian violet	2·25
Distilled water	100

16. **Gibbes' solution, for double staining.**

Take of

Rosaniline hydrochlorate	2
Methylene blue	1

Triturate in a glass mortar,

Dissolve aniline oil	3
In rectified spirit	15

and add slowly to the above.

Lastly, slowly add distilled water .	15

Keep in stoppered bottle.

17. Glycerine, pure.

18. Hæmatoxylin solution.

Hæmatoxylin	2
Alcohol	100
Distilled water	100
Glycerine	100
Alum	2

19. Iodine solution.

Iodine, pure	1
Iodide of potassium . . .	2
Distilled water	50

20. Iodine solution (Gram).

Iodine	1
Iodide of potassium . . .	2
Distilled water	300

21. Lithium-carmine solution (Orth).

Saturated solution of carbonate of lithium	100
Carmine	2·5

22. **Magenta solution** (Gibbes).

Magenta	2
Aniline oil	3
Alcohol (Sp. Gr. ·830)	20
Distilled water.	20

23. **Methylene blue.**

(*a*) Concentrated alcoholic solution.

(*b*) Aqueous solution.

Methylene blue	2
Alcohol	15
Water	85

(*c*) Koch's solution.

Concentrated alcoholic solution of methylene blue	1
Ten per cent. potash solution	·2
Distilled water	200

(*d*) Löffler's solution.

Concentrated alcoholic solution of methylene blue	30
Solution of potash 1—10,000	100

24. **Methyl violet.**

(*a*) Concentrated alcoholic solution.

(*b*) Aqueous solution.

Methyl violet	2.25
Distilled water	100

(*c*) Koch's solution.

Aniline water	100
Alcoholic solution of methyl violet	11
Absolute alcohol	10

25. **Neelsen's solution.**

Dissolve fuchsine	1
In a 5 per cent. watery solution of carbolic acid	100
Add alcohol	10

26. **Nitric acid, pure.**

27. **Orseille** (Wedl).

Dissolve pure ammonia-free orseille in

Absolute alcohol	20
Acetic acid	5
Distilled water	40

until a dark red liquid results : filter.

28. **Picric acid.**

(*a*) Concentrated alcoholic solution.

(*b*) Saturated aqueous solution.

29. **Picro-carmine** (Ranvier).

Triturate

Carmine	1
Distilled water	10

add

Solution of ammonia	3

30. **Picro-lithium-carmine** (Orth).

To above mentioned Lithium-carmine solution add

Saturated solution of picric acid	2·3

31. **Potash solution.**

(*a*) 1 to 3 per cent.

(*b*) 10 ,, ,,

(*c*) 33 ,, ,,

32. **Safranine.**

(*a*) Concentrated alcoholic solution.

(*b*) Watery solution . 1 per cent.

33. **Sulphuric acid, pure.**

34. **Salt solution** 0·8 per cent.

35. **Turpentine.**

36. **Vesuvin.**

(*a*) Concentrated alcoholic solution.

(*b*) Watery solution.

Water, distilled.

Water, sterilised.

Distilled water can be kept for use in a wash bottle, or far better in a siphon apparatus. Sterilised water is convenient in plugged sterile test-tubes which may be kept close at hand in a beaker, or tumbler, with a pad of cotton wool at the bottom. The *numbered* reagents can be conveniently arranged on shelves within easy reach. Alcoholic solutions of the aniline dyes and other special preparations should be kept in bottles with ground glass stoppers. Aqueous solutions of the dyes may be kept in bottles with funnel filters, and the solution filtered before use. To both aqueous and alcoholic solutions a few drops of phenol, or a crystal of thymol, should be added as a preservative. For the rapid staining of cover-glass preparations, it is convenient also to have the most frequently used stains (fuchsine, methyl-violet) in bottles provided with pipette-stoppers.

(D) REAGENTS FOR MOUNTING AND PRESERVING PREPARATIONS.

Acetate of potash.

Concentrated solution.

Asphalte lac.

Canada balsam.

Dissolved in xylol.

Glycerine gum (Farrant's solution).

Glycerine.
Water.
Saturated solution of arsenious acid.
Equal parts, mix and add solution of gum arabic half a part.

Hollis' glue.

Zinc-white.*

(E) DRAWING AND PHOTOGRAPHIC APPARATUS.

Camera Lucida.—The camera lucida of Zeiss is an excellent instrument, though many prefer the pattern made by Nachet of Paris. Combined with the use of a micro-millimeter objective it affords also a simple method for the measurement of bacteria.

* All instruments and material, microscopes, microtomes, aniline dyes, celloidin, gelatine, etc., etc., for bacteriological work can be obtained in Germany through G. Konig, Berlin, N.W., 35, Dorotheen Strasse. Chemicals, staining reagents, and ready-prepared nutrient gelatine can also be obtained from Dr. Georg Grübler, Leipsig, 17, Dufour Strasse. Solutions of lithium-carmine and picro-lithium-carmine (Orth), picro-carmine (Weigert), alum and borax carmine (Grenacher), etc., ready for use, can be obtained from the latter.

For drawing macroscopical appearances, and for illustrating microscopical specimens with or without the use of a camera lucida, the following materials should be within reach :—

Pencils.
Etching Pens.
Prepared Indian Ink.
Water-colour Paints, and Brushes.
Ordinary and tinted drawing paper and other usual accessories.

Micro-Photographic Apparatus.—The apparatus of Zeiss was recommended to the author by Professor Koch. A large horizontal pattern may also be obtained from Seibert and Krafft, of Wetzlar, but as very little more is required than a flat board, fitted with an ordinary wooden camera, and provided with clamps for fixing the microscope, it is more economical to devise one's own apparatus. The best results are obtained by employing the electric light; and specimens are preferably stained with bismarck brown, vesuvin, or chrysoidin. For mounting the preparations Koch recommends a saturated solution of acetate of potash ; but there is little or no objection to the use of Canada balsam. Dr. Hauser's micro - photographs of specimens stained with bismarck brown and mounted in Canada balsam dissolved in xylol, are the most beautiful results hitherto obtained.*

* Hauser, *Über Fäulniss Bacterien und deren Bezichungen zur Septicämie.* 1885.

The apparatus should be adjustable so that the stage of the microscope may be made horizontal. This will admit of micro-organisms in liquids, or cultivations in nutrient gelatine on plates and in capsules, etc., being photographed. Micro-photography is the most satisfactory method of representing cover-glass, or impression-preparations.

(F) STERILISATION APPARATUS.

Steam-Steriliser.—A cylindrical vessel of tin about half a metre or more in height, jacketed with thick felt, and provided with a conical cap or lid (Fig. 1). The lid is also covered with felt, has handles on either side, and is perforated at the apex to receive a thermometer. Inside the vessel is an iron grating or diaphragm about two-thirds the way down, which divides the interior into two chambers—the upper or "steam chamber," and the lower or "water-chamber." A gauge outside marks the level of the water in the lower chamber; this should be kept about two-thirds full. The apparatus stands upon three legs, and is heated from below with two or three Bunsen, or better, a Fletcher's

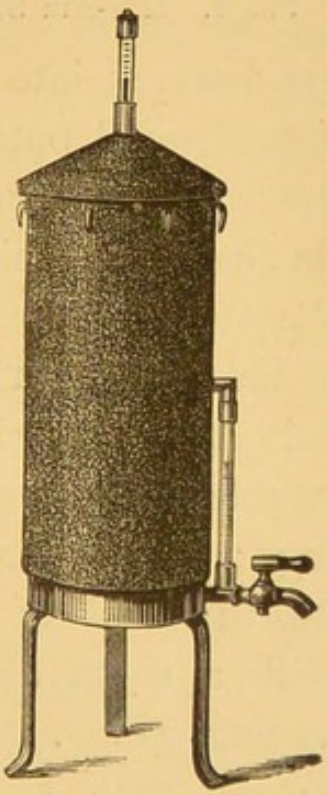


FIG. 1.—KOCH'S STEAM-STERILISER.

burner. It is employed for sterilising nutrient media in tubes or flasks, for cooking potatoes, or hastening the filtration of agar-agar. When the thermometer indicates 100° C. the lid is removed, and test-tubes are lowered in a wire basket by means of a hook and string, and the lid quickly replaced. Potatoes or small flasks are lowered into the cylinder in a tin receiver with a perforated bottom, which rests upon the grating and admits of its contents being exposed to the steam.

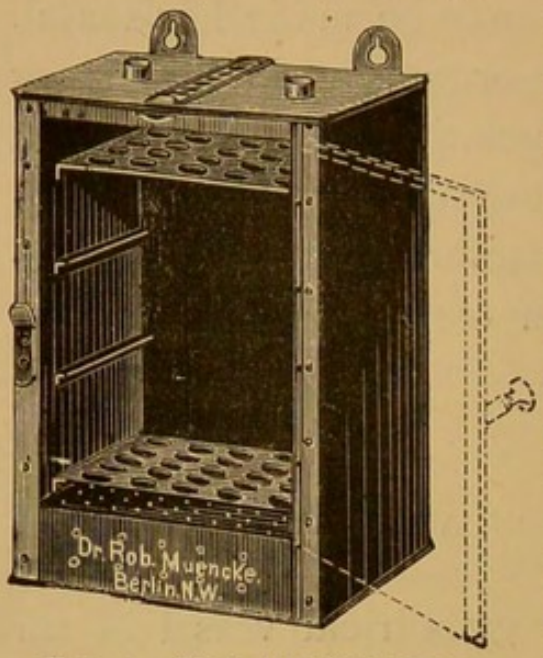


FIG. 2.—HOT-AIR STERILISER.

Hot-air Steriliser.—A cubical chest of sheet iron with double walls, supported on four legs; it may also be suspended on the wall of the laboratory, with a sheet of asbestos intervening (Figs. 2 and 3). It is heated with a rose gas-burner from below, and the temperature of the interior indicated by a thermometer inserted through a hole in the roof; in a second

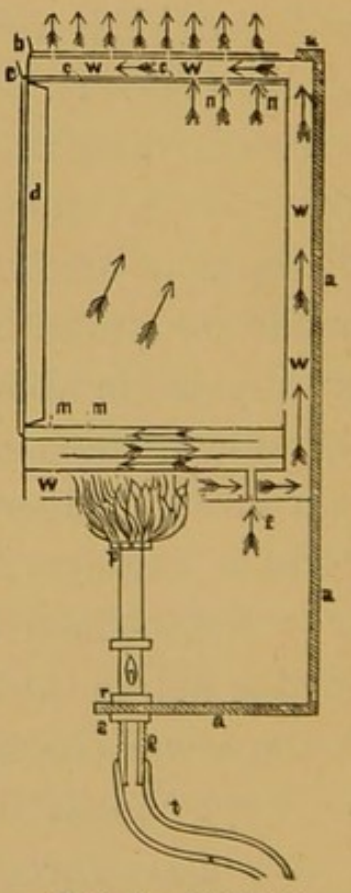


FIG. 3.—SECTION OF HOT-AIR STERILISER.

opening a gas regulator can be fixed. Test-tubes, flasks, funnels, cotton wool, etc., may be sterilised by exposure to a temperature of 150° C. for an hour or more.

(G) APPARATUS AND MATERIAL FOR PREPARING AND STORING GELATINE-, AND AGAR-AGAR-PEPTONE-BROTH.

Water-bath.—A water-bath on tripod stand is required for boiling the ingredients of nutrient jellies and for general purposes. The lid may be conveniently composed of a series of concentric rings, so that the mouth of the vessel may be graduated to any size required.

Test-tube Water-bath. — This consists of a circular rack for test-tubes within a water-bath. It is sometimes employed instead of the steam cylinder for sterilising nutrient jelly in tubes by boiling for an hour, for three successive days.

Hot-water Filter. — A copper funnel with double walls, the interspace between which is filled with hot water. A glass funnel fits inside the copper cone, the stem of the glass funnel passing through and being tightly gripped by a perforated caoutchouc plug, which fits in the opening at the apex of the cone. The water in the cone is heated by applying the flame of a

burner to a tubular prolongation of the water chamber. In a more recent model, as represented in Fig. 4, this prolongation is dispensed with, and the temperature is maintained by means of a circular burner which acts at the same time as a funnel ring.

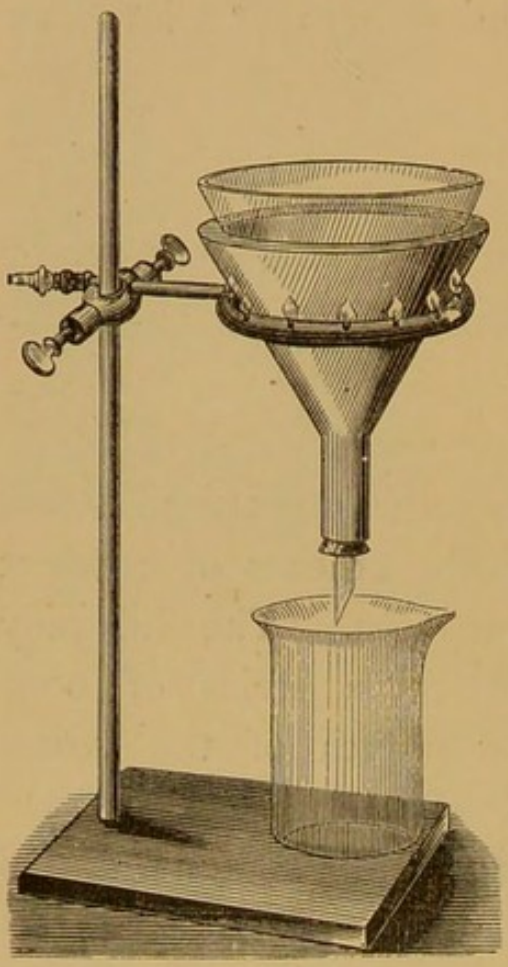


Fig. 4.
Hot-water Filtering Apparatus with Ring Burner.

Glass Vessels. — A number of glass vessels should be kept in stock according to requirements.

Bohemian hard glass flasks are employed in several sizes, for boiling nutrient media. The conical forms are especially used in the larger sizes for storing nutrient jelly.

Glass funnels large and small are necessary, not only in the processes of preparing nutrient jelly, but for filtering solutions of aniline dyes and for general purposes.

A liberal supply of test-tubes should always be kept in stock, as they are not only employed for the tube-cultivations, but can be conveniently used for storing bouillon, sterilised water, etc.

Cylindrical glasses graduated in cubic centimetres, 10 ccm., 100 ccm., 500 ccm., are required for measuring the liquid ingredients of nutrient jelly, and also in preparing the various staining solutions.

A large wide-mouthed glass jar, with a glass cover, is extremely useful. It must be padded at the bottom with cotton wool for containing a stock of tubes of sterilised nutrient jelly, and should be placed within reach on the working table.

Balance and Weights. — A balance, with large pans and set of gramme weights, is constantly required.

Cotton Wool.—The best or "medicated" cotton wool should be procured.

Gelatine. — The gelatine for bacteriological purposes must be of the very best quality (gold label).

Agar-Agar.—This is also called Japanese Isinglass; it consists of the shrivelled filaments of certain Algæ (Gracilaria lichenoides and Gigartina speciosa).*

Peptonum Siccum (Savory and Moore).

Table Salt.—Prepared table salt can be obtained in tins or packets.

Litmus Papers.—Blue or red litmus paper in cheque books, for testing the gelatine mixture, etc.

* Hueppe, *Die Methoden der Bakterien Forschung.* 1885.

Carbonate of Soda.—A bottle, containing a saturated solution of carbonate of soda, and provided with a pipette-stopper, may be kept, especially for use in the preparation of nutrient jelly.

Lactic Acid.

Filter Paper.—For filtering gelatine stout Swedish filter paper of the best quality is recommended.

Flannel or Frieze.—This is employed as a substitute for, or combined with, filter paper in the preparation of nutrient agar-agar.

(H) APPARATUS FOR EMPLOYMENT OF NUTRIENT JELLY IN TEST-TUBE AND PLATE CULTIVATIONS.

Wire Cages.—These cages or crates are used for containing test-tubes, especially when they are to be sterilised in the hot-air steriliser; or for lowering tubes of nutrient jelly into the steam steriliser, etc. (Fig. 5).

FIG. 5.—WIRE CAGE FOR TEST-TUBES.

Test-tube Stands.—The ordinary wooden pattern, or the metallic folding stands, are called into use for holding cultivations. Pegged racks are also recommended for draining test-tubes after washing.

Caoutchouc Caps.—These are caps for fitting over the cotton wool plugs, and may be used in different sizes for test-tubes and stock-flasks.

Platinum Needles.—A platinum needle for inoculating nutrient media, examining cultivations, etc., consists of two or three inches of platinum wire, fixed to the end of a glass rod. Several of these needles should be made, with platinum wire, of various thicknesses. A piece of glass rod, about seven inches long, is heated at the extreme point in the flame of a Bunsen burner, and a piece

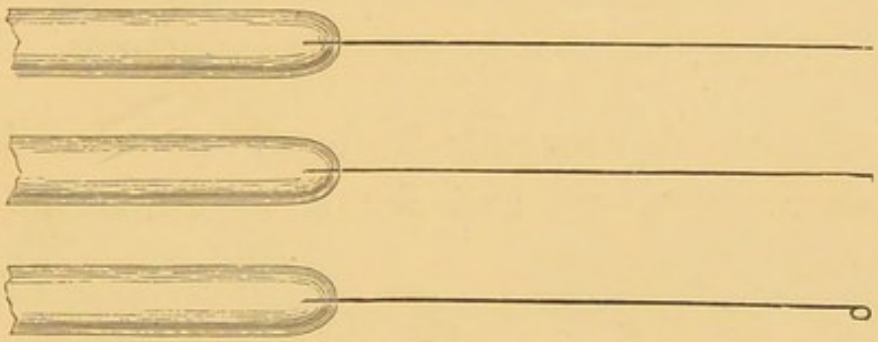


FIG. 6.—PLATINUM NEEDLES.

of platinum wire, held near one extremity with forceps, is then fused into the end of the rod. Some needles should be perfectly straight, and kept especially for inoculating test-tubes of nutrient jelly. For other purposes the needles may also be bent at the extremity into a small hook or a loop* (Fig. 6)

Tripod Levelling-stand.—A triangular wooden frame supported upon three screw-feet, which

* A looped platinum needle is called in Germany an "öse," a term which, on account of its brevity, may be conveniently adopted.

enable it to be raised or lowered to adjust the level.

Large Glass Plate.—A piece of plate-glass, or a pane of ordinary window glass, about a foot square.

Spirit Level.

Glass Bells and Dishes.—Shallow glass bells

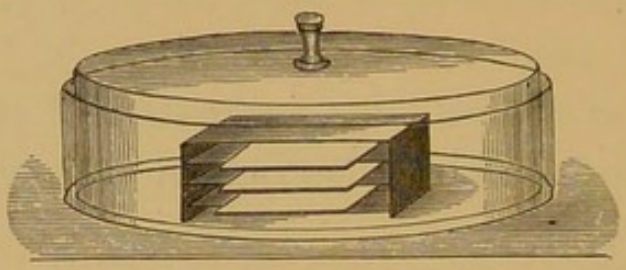


FIG. 7.—DAMP-CHAMBER FOR PLATE CULTIVATIONS.

and dishes, for making a dozen or more damp chambers (Fig. 7), and for completing the ap-

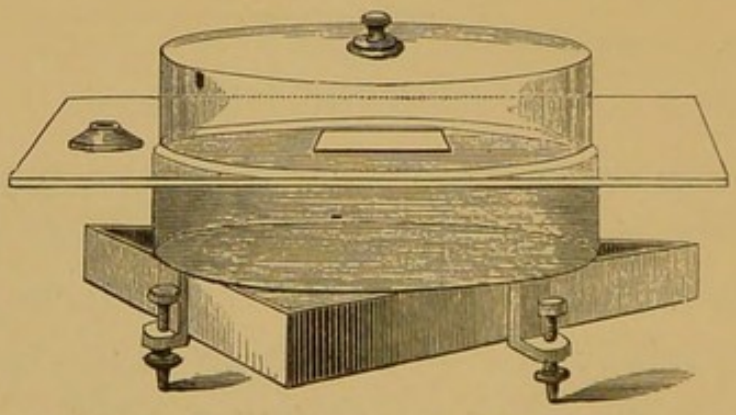


FIG. 8.—APPARATUS EMPLOYED FOR PLATE-CULTIVATIONS.

Tripod stand; Glass dish, filled with cold or iced water; Sheet of Plate-glass; Spirit Level, and Glass Bell.

paratus for pouring out liquefied nutrient jelly on glass plates or slides (Fig. 8).

Iron Box.—A box of sheet-iron (Fig. 9) for

containing glass plates during their sterilisation in the hot-air steriliser, and for storing them until required for use.

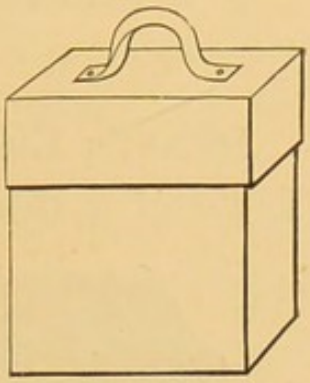
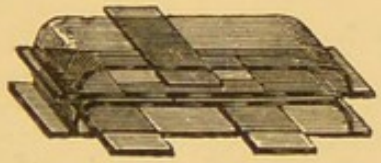


FIG. 9.
BOX FOR GLASS PLATES.

Glass Plates.—Small panes of glass, about six inches by four. Not less than three dozen are required for a dozen damp chambers.

Glass Benches.—These are necessary for arranging the glass plates or slides in tiers in the damp chambers (Fig. 10). Metal shelves may be substituted for them, but the former are to be preferred. They can be easily made, in any number required, by



FIGS. 10, 11.—GLASS BENCHES FOR GLASS PLATES OR SLIDES.

cementing a little piece of plate glass at either end of a glass slip (Fig. 11).

Glass Rods.—One dozen or more glass rods, twelve to eighteen inches in length. They are employed for smoothly spreading out the liquefied nutrient gelatine or agar-agar on the glass plates, etc.

Thermometers. — Two or three centigrade thermometers.

(I) APPARATUS FOR PREPARATION OF POTATO-CULTIVATIONS.

Israel's Case.—Sterilising instruments in the flame of a Bunsen burner is most destructive. It is better, therefore, to have a sheet-iron case (Fig. 12) to contain potato-knives, scalpels, and other instruments, and to sterilise them by placing the case in the hot-air steriliser for an hour at 150° C. The box can be opened at the side, and each instrument withdrawn with a pair of sterilised forceps when required for use.

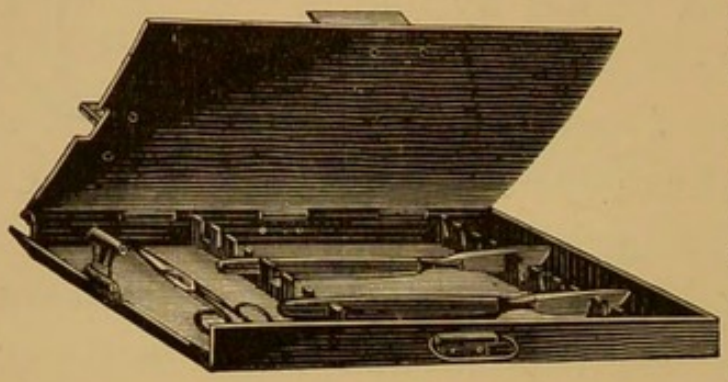


FIG. 12.—ISRAEL'S CASE.

Glass Dishes.—Several shallow glass dishes are required for preparing damp chambers for potato cultivations (Fig. 13). The upper, being the larger, fits over the lower, and having no handle, admits of these damp chambers being placed, if necessary, in the incubator in tiers. The large size may also be used in the same way for plate cultivations.

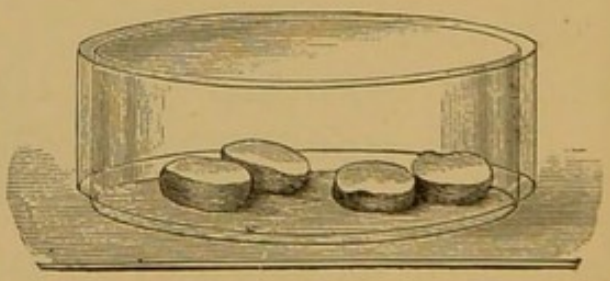


FIG. 13.
DAMP-CHAMBER FOR POTATO-CULTIVATIONS.

Potato Knives.—A common broad smooth-

bladed knife set in a wooden handle is sold for this purpose.

Scalpels. — Half a dozen scalpels, preferably with metal handles, may be kept especially for inoculating sterilised potatoes.

Brush.—A common stout nail-brush, or small scrubbing brush, is essential for cleansing potatoes.

(J) APPARATUS FOR PREPARATION OF SOLIDIFIED STERILE BLOOD SERUM.

Glass Jar.—A tall cylindrical glass jar, on foot, with a broad ground stopper, for receiving blood.

Pipette.—An ordinary or graduated pipette for transferring the serum from the jars to sterile test-tubes or glass capsules.

Serum-Steriliser.—A cylindrical case, with double walls forming an interspace to contain water, closed with a lid, also double walled and provided with a tubular prolongation of the enclosed water chamber (Fig. 14). The water in the cylinder is heated from below, and that in the lid by means of the prolongation.

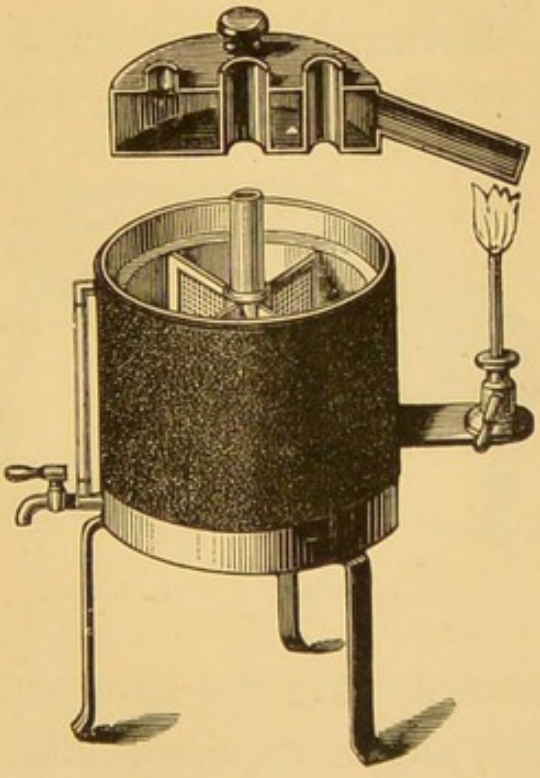


FIG. 14.—SERUM STERILISER.

In the centre of the cylinder is a column which communicates with the water chamber of the cylinder, and from it pass four partitions, which serve to support the test-tubes.

In the lid are three openings, one of which communicates with the water chamber in the lid by which the latter is filled, and into which a thermometer is then fixed. In the centre an opening admits a thermometer, which passes into the central pipe of the cylinder ; through a third opening a thermometer passes to the cavity of the cylinder. The cylinder and cover are jacketed with felt, and the apparatus is supported on iron legs.

Serum Inspissator.—A shallow tin case with glass cover, both case and cover jacketed with felt (Fig. 15). The case is double walled, and the water contained in the interspace is heated from below. It is supported on four legs, and the two front ones move in grooves in the case, so that the latter can be placed obliquely at the angle required, and secured in position by screw-clamps. It is employed for coagulating sterile liquid serum, and for solidifying nutrient agar-agar so as to give them a sloping surface.

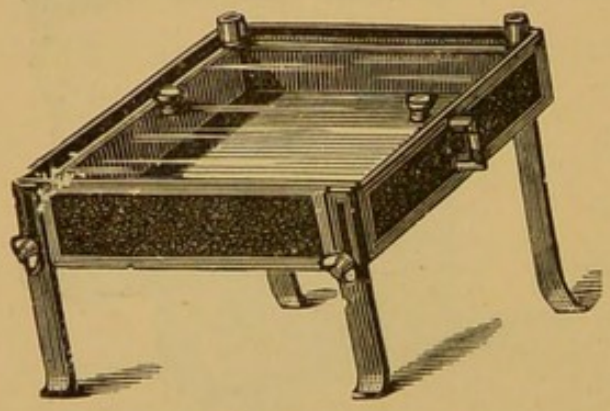


FIG. 15.—SERUM INSPISSATOR.

Glass Capsules.—Small capsules or hollowed-out cubes of crystal glass are employed for cultivations on solid blood serum, on nutrient gelatine, and on agar-agar. They may be procured of white and blackened glass, and are provided with glass slips as covers.

(K) APPARATUS FOR STORING, AND FOR CULTIVATIONS IN, LIQUID MEDIA.

Lister's Flasks.—Professor Lister devised a globe-shaped flask with two necks; a vertical and a lateral one. The lateral one is a bent spout, tapering towards its constricted extremity. When the vessel is restored to the erect position after pouring out some of its contents, a drop of liquid remains behind in the end of the nozzle, and prevents the regurgitation of air through the spout. A cap of cotton wool is tied over the orifice, and the residue in the flask kept for future use. The vertical neck of the flask is plugged with sterilised cotton wool in the ordinary way.

Sternberg's Bulbs.—Professor Sternberg, of America, advocates the use of a glass bulb, provided with a slender neck drawn out to a fine point and hermetically sealed.*

Aitken's Test-tube.—This is an ingenious device for counteracting the danger of entrance of

* Magnin and Sternberg, *Bacteria*. 1884.

atmospheric germs on removal from the ordinary test-tube of the cotton wool plug. Each test-tube is provided with a lateral arm tapering to a fine point, which is hermetically sealed.

Drop-culture Slides.—About a dozen or more thick glass slides with a circular excavation in the centre are required for drop-cultures.

Vaseline.—A small pot of vaseline with a camel's hair brush should be reserved especially for use in the preparation of drop-cultures.

Bulbed Tubes.—Glass vessels such as test-tubes, flasks, and pipettes, which are used in dealing with liquid media, have already been mentioned under other headings, but bulbed tubes, Pasteur's bulbs, and various other forms are also required for special experiments.

(L) APPARATUS FOR INCUBATION.

There are several forms of incubator, each of which has its advocates. They are mostly rectangular chests, with glass walls, front and back, or in front only. A cylindrical model is preferred by some. Two only will be described here, D'Arsonval's and Babes'. The former admits of very exact regulation of temperature, and the latter is a very practical form for general use.

D'Arsonval's Incubator.—The "*Étuve D'Arsonval*" (Fig. 16) is a very efficient apparatus, and is provided with a heat regulator, which

enables the temperature to be maintained with a minimum variation. It consists of a cylindrical copper vessel, with double walls, enclosing a wide interspace for containing a large volume of water. The roof of the water-chamber is oblique, so that the wall rises higher on one side than on the other. This admits of the interspace being completely filled with water. At the highest point is an opening fitted with a perforated caoutchouc stopper, through which a glass tube passes. The mouth of the cylinder itself is horizontal, and is closed by a lid, which is also double-walled to contain water. In the lid are four openings; one serves for filling its water-chamber, and the others for thermometers and for regulating the air supply in the cavity of the cylinder. The cylinder is continued below by a cone, also double-walled, and there is a perforated grating at

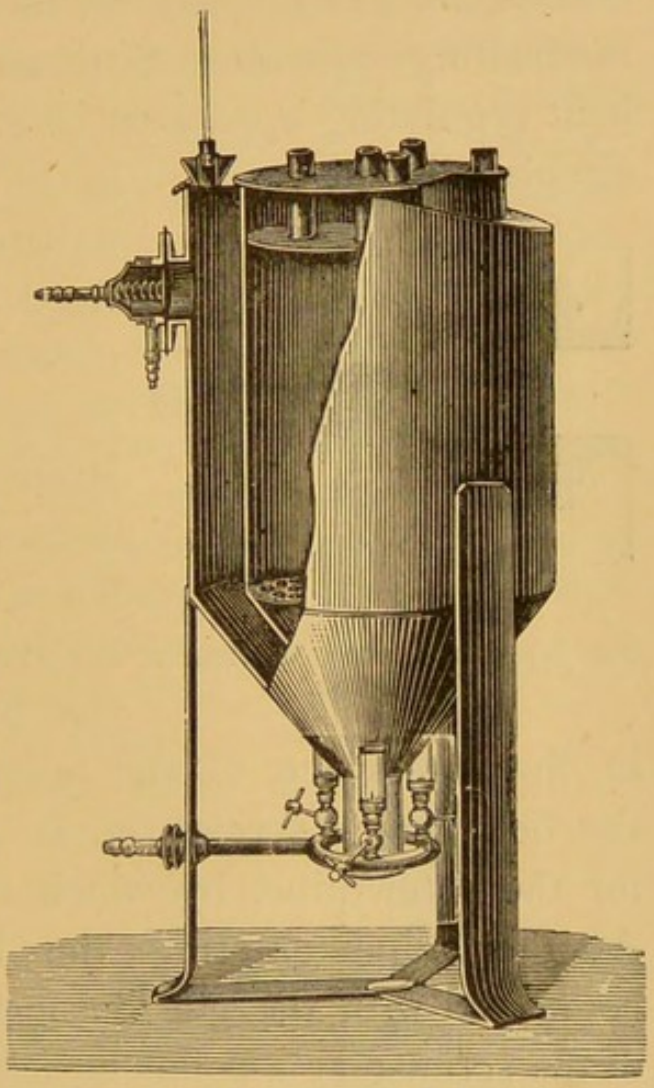


FIG. 16.—D'ARSONVAL'S INCUBATOR.

the line of junction of the cylinder and cone. The cone terminates in a projecting tube provided with an adjustable ventilator. The apparatus is fixed on three supports united to one another below. One of them is utilised for adjusting the height of the heating apparatus. Situated above this leg is the heat regulating apparatus (Fig. 17), attached to a circular, lipped aperture in the outer wall of the incubator. To the lip is fixed with six screws the corresponding lip of a brass box, with a tightly stretched diaphragm of india-rubber intervening. Thus the diaphragm separates the cavity of the box from the water in the interspace of the incubator. The cap of the box, which screws on, is bored in the centre for the screw-pipe, by which the gas is supplied. Another pipe entering the box from below is connected with the gas burners. Around the end of the screw-pipe a collar loosely fits, and is pressed against the diaphragm by means of a spiral wire spring. Close to the mouth of the screw-pipe a small opening exists, so that the gas supply to the burners is not entirely cut off even when the diaphragm completely occludes the mouth of the screw-pipe.

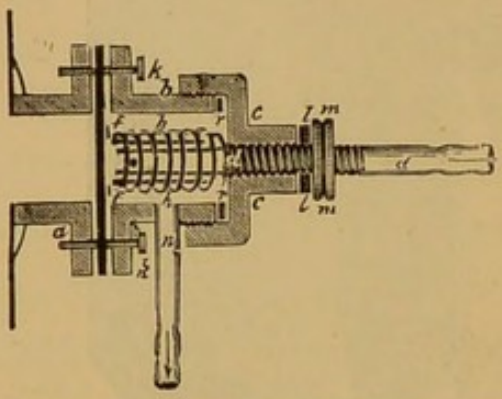


FIG. 17.—SCHLOSING'S MEMBRANE REGULATOR.

To work the apparatus the tube and plug must be

removed, and the water-chamber filled completely with distilled or rain water at the temperature required. The caoutchouc plug is replaced and the tube placed in position. Gas enters through *d* (Fig. 17), and passes through the opening at its extremity into the chamber of the box. Thence it passes through the vertical exit which is connected with the gas burners. As the temperature rises the water rises in the tube, and at the same time exercises a pressure on every part of the walls of the incubator, and hence on the diaphragm. In consequence of this, the diaphragm bulging outwards approaches the end of the tube *d*, and gradually diminishes the gas supply. As a result the temperature falls, the water contracts and sinks in the tube, and the diaphragm receding from *d*, the gas supply is again increased. By adjusting the position of the tube *d* to the diaphragm, any required temperature within the limits of the working of the apparatus can be regulated to the tenth of a degree; *provided*, (1) that the gas supply is rendered independent of fluctuations of pressure, by means of a gas-pressure regulator, (2) that the height of the water in the tube is controlled daily by the withdrawal or addition of a few drops of distilled water, and (3) that the apparatus is kept in a place with as even a temperature as possible, and sheltered from currents of air.

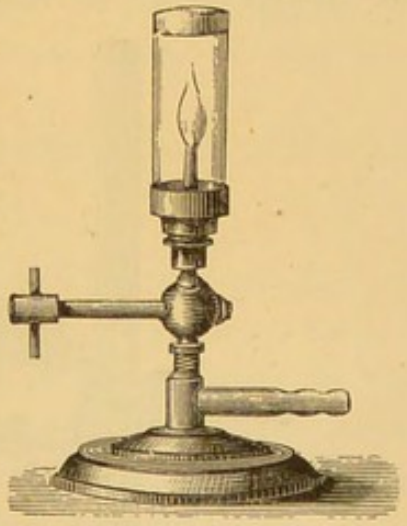


FIG. 18.
GAS BURNER PROTECTED WITH MICA CYLINDER.

The burners in Fig. 16 are protected with mica cylinders similar to the burner represented in Fig. 18. The flames of these burners can be turned down to the smallest length without danger of extinction, and the temperature may be regulated very satisfactorily without using the heat regulator just described, if the gas first

passes through a pressure regulator (Fig. 19). To provide against the danger resulting from accidental extinction of the gas, Professor Koch has devised a self-acting apparatus (Fig. 20), which, simultaneously with the extinction of the flame of the burner, shuts off the supply of gas.

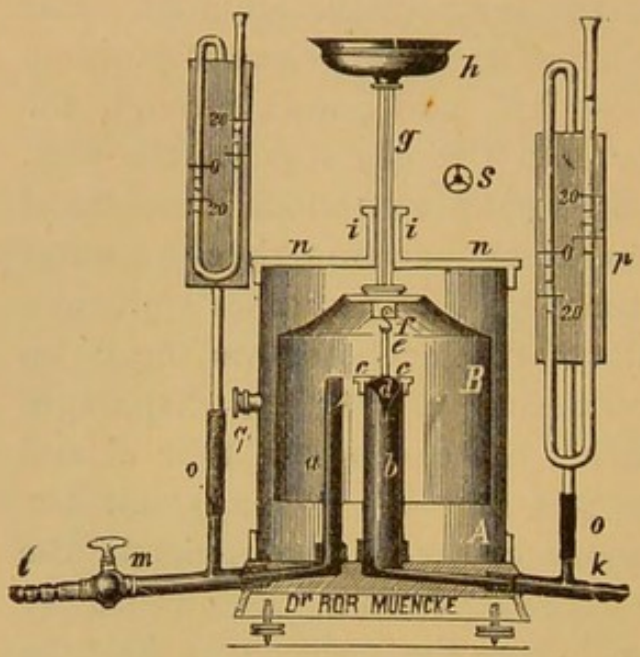


FIG. 19.
MOITESSIER'S GAS-PRESSURE REGULATOR.

Babes' Incubator.—The pattern of Dr. Babes is very simple, and is recommended by the author in preference to all others (Fig. 21).

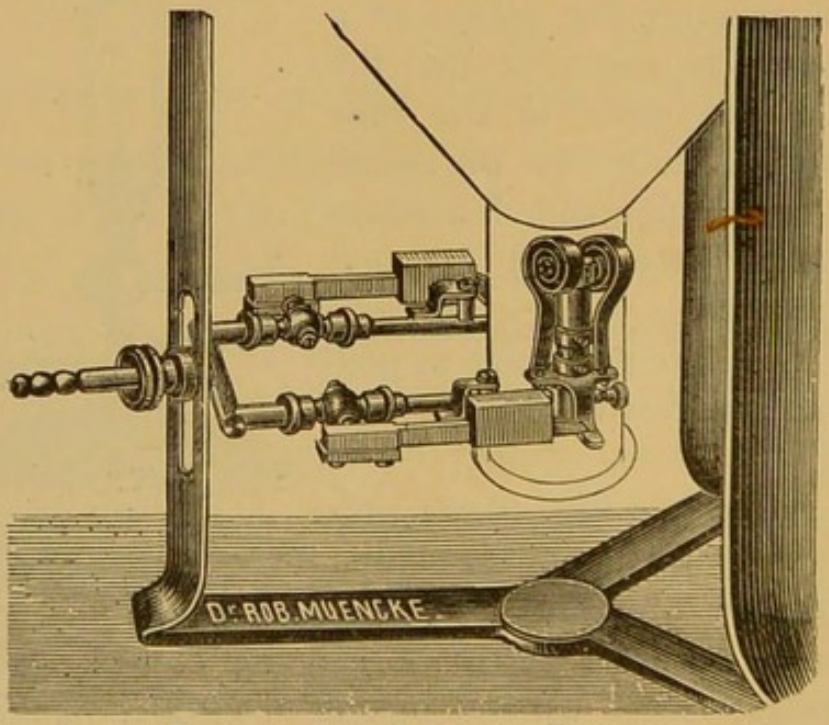


FIG. 20.—KOCH'S SAFETY BURNER.

It consists of a double-walled chest with sides

and roof jacketed with felt. Water fills the interspace between the walls, and on the roof are two apertures, one for a gas regulator, and the other for a thermometer. In front, the chest is closed in by a sheet of felt, a glass door, and a sliding glass panel. The apparatus can be suspended on the wall or supported on legs, and is heated from below by means of protected burners.

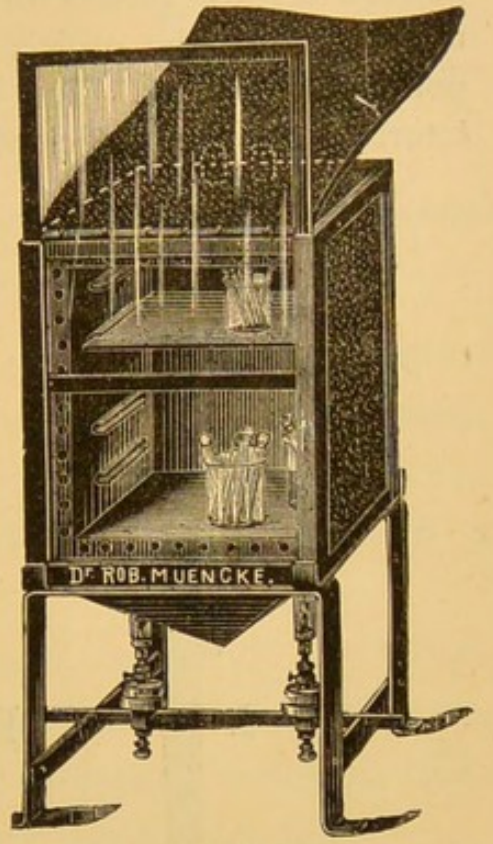


FIG. 21.—BABES' INCUBATOR.

The gas should pass first through a pressure-regulator, and then through a thermo-regulator to the burners.

The thermo-regulator of Reichert (Fig. 22) consists of three parts—a hollow T piece, a stem, and a bulb. The T piece fits like a stopper in the upper widened portion of the stem. One arm of the T is open, and connected with the gas supply; the vertical portion terminates in a small orifice, and is also provided with a minute lateral opening. The stem is provided with a lateral arm, and this arm, the stem, and the bulb contain mercury. The regulator is fixed in the roof of the incubator, so that the bulb projects either into the interior of the incubator or into the water chamber. When the incu-

FIG. 22. REICHERT'S THERMO-REGULATOR.

bator reaches the required temperature, the mercury is pushed up by means of the screw in the lateral arm, until it closes the orifice, at the extremity of the vertical portion of the T. The gas which passes through the lateral orifice is sufficient to maintain the apparatus at

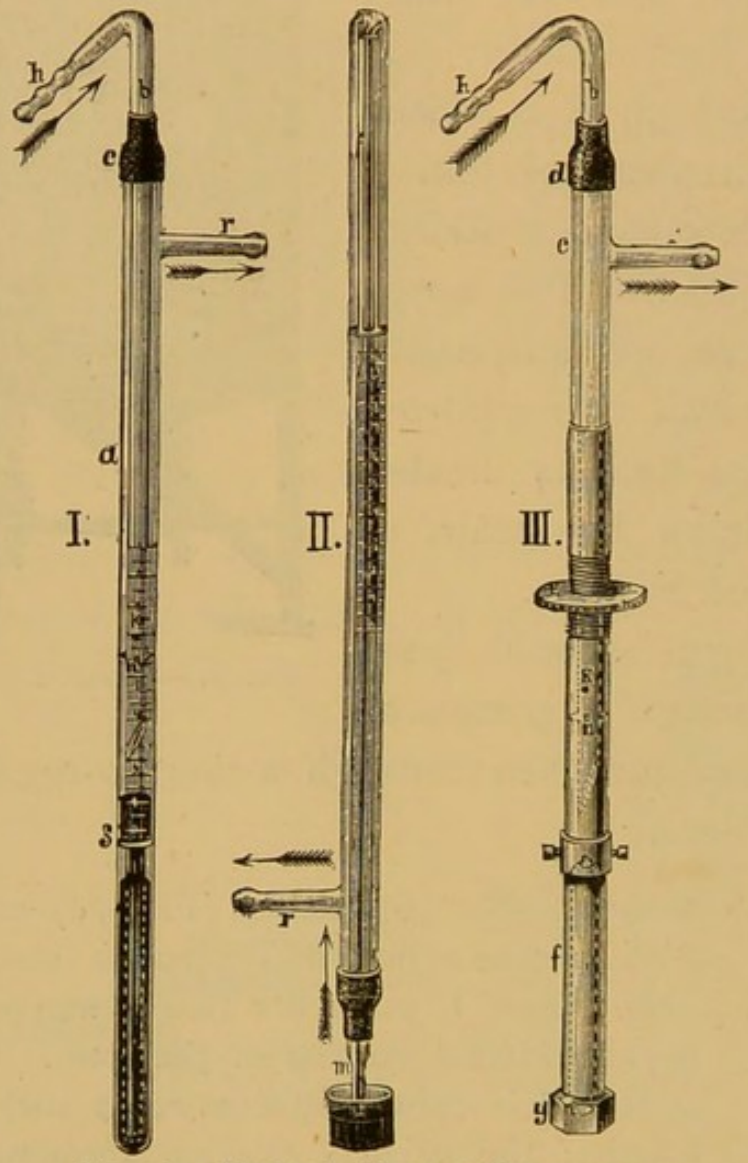


FIG. 23.—MEYER'S THERMO-REGULATOR.

the temperature required. If the temperature of the incubator falls the mercury contracts, and gas passing through the terminal orifice of the T, the flame of the burner increases and the temperature is restored.

Meyer's thermo-regulator is represented in Fig. 23. I. shows the construction of the regulator; its inner tube

terminates in an oblique opening, and is also provided with a minute lateral aperture, which prevents the complete shutting off of the gas supply. II. illustrates the method of introducing the mercury; and in III. the regulator is provided with a metal case for protection.

(M) INOCULATING AND DISSECTING INSTRUMENTS AND APPARATUS IN COMMON USE.

Mouse Cages.—As mice are the animals most frequently employed for experimental purposes, mouse cages have been especially introduced, consisting simply of a cylindrical glass jar with a weighted wire cover.

Dressing-case.—A small surgical dressing-case, with its usual accessories—forceps, knives, small straight and curved scissors, needles, silk, and so forth—will serve for most purposes.

Pravaz' Syringe.—Koch's modification of Pravaz' syringe admits of sterilisation by exposure to 150° C. for a couple of hours.

Special Instruments and Material.—Instruments required for special operations and the materials necessary for strict antiseptic precautions need not be detailed here.*

Dissecting Boards.—Slabs of wood in various sizes, or gutta percha trays, provided with large-headed pins, are employed for ordinary purposes.

Dissecting Case.—A dissecting case fitted with scalpels, scissors, hooks, etc., should be reserved entirely for post-mortem examinations.

* *Vide* Cheyne, *Antiseptic Surgery*. 1882.

(N) GENERAL LABORATORY REQUISITES.

Siphon Apparatus.—Two half-gallon or gallon glass bottles, with siphons connected with long flexible tubes provided with glass nozzles and pinchcocks (Fig. 24), should be employed for the

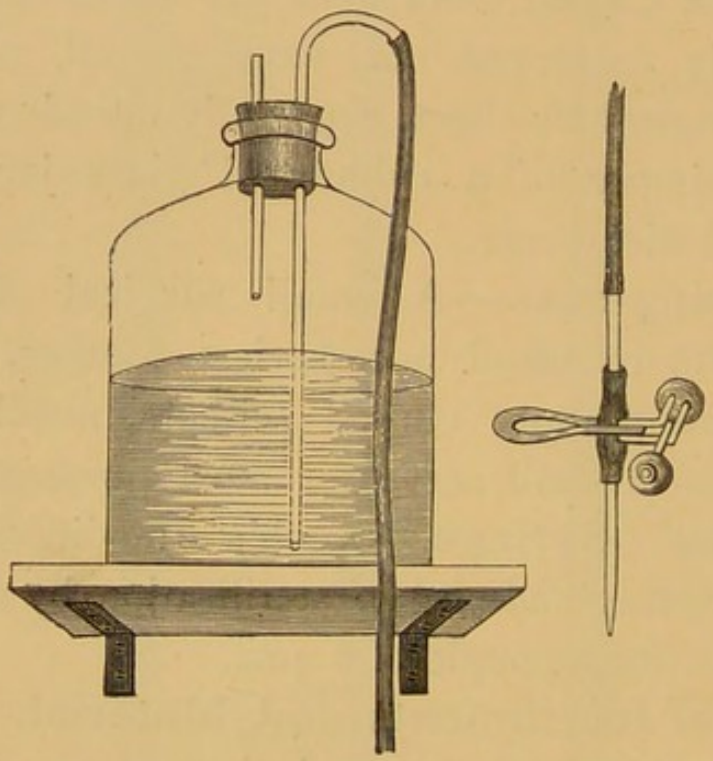


FIG. 24.
SIPHON BOTTLE, WITH FLEXIBLE TUBE, GLASS NOZZLE, AND A MOHR'S PINCHCOCK.

following purposes :—One is used to contain distilled water, with the nozzle hanging down conveniently within reach of the working table; the other is to contain a solution of corrosive sublimate (1 in 1000), and may be placed so that the nozzle hangs close to the lavatory sink or basin. The former replaces the use of the ordinary wash bottle, in washing off

surplus stain from cover glasses, etc., and the latter is conveniently placed for disinfection of vessels and hands after cleansing with water. They should be placed on the top of a cupboard, or on a high shelf.

Desiccator—The Desiccator (Fig. 25) consists of a porcelain pan containing concentrated sulphuric acid, and covered over with a bell-glass receiver.

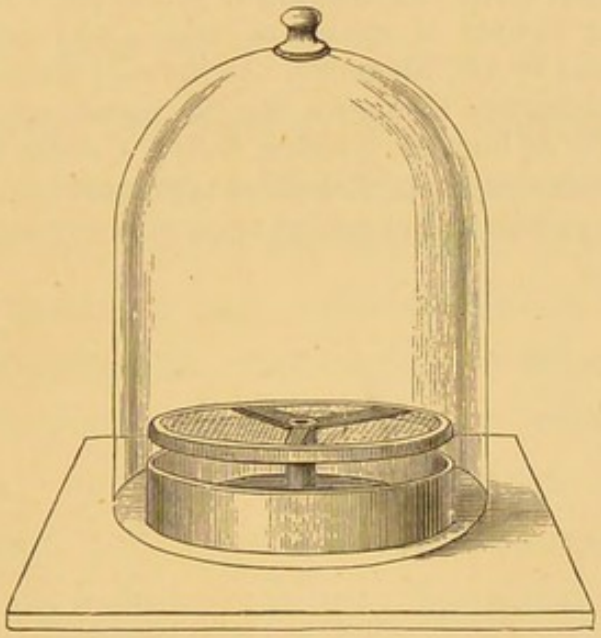


FIG. 25.—DESICCATOR.

The sheet of plate-glass upon which the pan rests is ground upon its upper surface, and the rim of the glass bell is also ground and well greased. In the centre of the pan is a column supporting a circular frame, which is covered with wire gauze. Slices of potatoes, upon which micro-organisms have been cultivated, are rapidly dried by the action of the sulphuric acid in confined air. A cultivation of *micrococcus prodigiosus*, for example,

may be dried in this way, and preserved for subsequent experiments.

Other items commonly in use in a research laboratory cannot be detailed here, and a description of air-pumps, refrigerators, etc., access to which is nevertheless necessary for some special investigations, must be sought for elsewhere.*

* All bacteriological apparatus, as employed by Professor Koch, may be obtained from Dr. Muencke, 58, Louisen Strasse, Berlin. Nearly all the figures of apparatus here given are from blocks, kindly lent to me by Dr. Muencke. As the cost of transport is small, it is more satisfactory to obtain the special apparatus direct from Berlin. Griffin & Son, 22, Garrick Street, Covent Garden, W.C., will make *to order* any bacteriological apparatus required, and from them all glass vessels and chemical apparatus can be obtained.

CHAPTER III.

MICROSCOPICAL EXAMINATION OF BACTERIA IN LIQUIDS, IN CULTIVATIONS ON SOLID MEDIA, AND IN TISSUES.

Preliminary Remarks. — In conducting bacteriological researches, the importance of absolute cleanliness cannot be too strongly insisted upon. All instruments, glass vessels, slides, and cover glasses should be thoroughly cleansed before use. A wide-mouthed glass jar should always be close at hand, containing refuse alcohol for the reception of rejected slide preparations, or dirty cover-glasses. When required again for use, slides can be easily wiped clean with a soft rag. Cover-glasses require further treatment, for unless they are perfectly clean it is difficult to avoid the presence of air bubbles when mounting specimens. They should be left in strong acid (hydrochloric, sulphuric, or nitric) for some hours; they are then washed, first with water and then with alcohol, and carefully wiped with a soft rag. The same principle applies in the preparation and employment of culture media; any laxity in the processes of sterilisation, or insufficient atten-

tion to minute technical details, will surely be followed with disappointing results in the contamination of one's cultures, resulting in the loss of much time. When using platinum needles, either for inoculating fresh tubes in carrying on a series of pure cultures, or in transferring a small portion of a cultivation to a cover-glass for examination under the microscope, the careful sterilisation of the needle by heating the platinum wire till it is white hot in every part, and heating also as much of the glass rod as is made to enter the test-tube, must be carried out with scrupulous care. Indeed it is a good plan to let it become a *force of habit* to sterilise the needle before and after use on every occasion, whatever may be the purposes for which it is employed.

(A) EXAMINATION IN THE FRESH STATE.

Liquids containing micro-organisms such as pus, blood, juices, culture-fluids, can be investigated by transferring a drop with a sterilised öse or a capillary pipette to a slide, covering it with a clean cover-glass, and examining without further treatment. If it is desirable to keep the specimen under prolonged observation, a drop of sterilised water or salt solution must be run in at the margin of the cover-glass to counteract the tendency to dry. *Cultures on solid media* can be examined by transferring a small portion with a

sterilised needle to a drop of sterilised water on a slide, thinning it out, and covering with a cover-glass as already described. A more satisfactory method, by which one can keep micro-organisms under observation and study their movements, spore-formation, etc., will be described under "Drop-cultures." *Tissues in the fresh state* may be teased out with needles in sterilised salt solution, and pressed out into a sufficiently thin layer between the slide and cover-glass. Glycerine may in many cases be substituted for salt solution, especially for the examination of micro-organisms such as Actinomyces, Aspergilli, etc.

There is as a rule no difficulty in recognising the larger micro-organisms such as those just mentioned, but where we have to deal with very small bacilli, bacteria and micrococci, they may possibly be mistaken for granular detritus or fat-crystals, or *vice-versâ*. They are distinguished by the fact that fatty and albuminous granules are altered or dispersed by acetic acid, and changed by solution of potash; alcohol, chloroform, and ether dissolve out fat crystals or fatty particles; on the other hand, micro-organisms remain unaffected by these re-agents. This micro-chemical reaction is made the basis of Baumgarten's method (p. 165).

(B) COVER-GLASS-PREPARATIONS.

The method next to be described is the most commonly employed; in addition to its value as a means of examining liquids, etc., it affords the additional advantage of enabling one to make, if necessary, a large number of preparations which when dried can be preserved, stained or unstained, in ordinary cover-glass boxes; they are then in a convenient form for transport, and can be mounted permanently at leisure.

The method is as follows:—A cover-glass is smeared with the cut surface of an organ, or pathological growth, or with sputum; or a drop of blood, pus, or other fluid to be examined, is conveyed to it with a large öse. By means of another cover-glass, the juice, or fluid, is squeezed out between them into a thin layer, and on sliding them apart each cover-glass bears on one side a thin film of the material to be examined. They are then placed with the prepared side upwards and allowed to dry. After a few minutes, they are held with a pair of flat-bladed or spring forceps, with the prepared side uppermost, and passed rapidly three times through the flame of a spirit lamp or Bunsen burner. To stain them, put two or three drops of an aqueous solution of fuchsine or methyl violet over the film, and after a minute or two wash off the surplus stain with distilled water by means of the siphon apparatus or a wash

bottle. Turn the cover-glass on to a slide, remove excess of water with filter paper, and wipe the exposed surface; examine with Zeiss' DD (about 230 diams.), and if a higher power be required, which is usually the case, place a droplet of cedar oil on the cover glass, and examine with an immersion lens.

If the specimen is to be made permanent, fix the cover-glass at one corner with the thumb, and with a soft rag carefully wipe off the cedar oil; then float off the cover-glass by running in distilled water at its margin, and having made a little ledge with a strip of filter-paper, place the cover-glass up against it upon one of its edges and leave it to dry. When perfectly dry mount in Canada balsam, or put it away in a cover-glass box provided with a label of contents.

A culture from a solid medium may be stained and examined in the same way after spreading it out with a needle into a thin film, with or without the addition of a droplet of sterilised water.

In many cases it is necessary or preferable to apply the stain for a much longer period. This is effected by pouring some of the staining solution into a watch-glass, and allowing the cover-glasses to swim on the surface, with their prepared side, of course, downwards. Throughout all these manipulations it is necessary to bear in mind which is the prepared surface of the cover-glass.

Double coloration of cover-glass prepara-

tions can also be obtained as in Ehrlich's method for staining tubercular sputum, or by staining with eosin after treatment by the method of Gram.

Ehrlich's Method is as follows :—Five parts of aniline oil are shaken up with one hundred parts of distilled water, and the emulsion filtered through moistened filter paper. A saturated alcoholic solution of fuchsine, methyl violet, or gentian violet, is added to the filtrate in a watch-glass drop by drop until precipitation commences. Cover-glass preparations are floated in this mixture for fifteen minutes to half an hour, then washed for a few seconds in diluted nitric acid (one part nitric acid to two of water) and then rinsed in distilled water. The stain is removed from everything except the bacilli, but the ground substance can be after-stained, brown if the bacilli are violet, or blue if they have been stained red (Plate XX., Fig. 1).

Double staining with eosin after the method of Gram is described under tissue staining. The cover-glass preparations are treated by the same processes as employed with sections ; superfluous oil of cloves can be removed by gently pressing the cover-glass between double layers of filter paper.

Babes' Method affords a very rapid means of examining cultivations, etc. A little of the growth, removed by means of a sterilised platinum hook or small öse, is spread out on a cover glass into as thin a film as possible : when almost dry, a drop or two of a weak aqueous solution of methyl violet is

allowed to fall from a pipette upon the film. The cover-glass with the drop of stain is after a minute carefully turned over on to a slide, and the excess of stain gently and gradually removed by pressure with a strip of filter paper. It affords a rapid means of demonstration, for example of such a cultivation as Koch's comma bacilli in nutrient gelatine, enabling the microbes to be seen in some parts of the preparation both stained and in active movement.

His' Method.—The staining of fresh preparations, especially those with no coagulable albumen to fix them, may be also carried out by His' method. A slide is prepared as already described in the examination of micro-organisms in the fresh state. The reagents are then applied by placing them with a pipette drop by drop at one margin of the cover-glass, and causing them to flow through the preparation by means of a strip of filter paper placed at the opposite margin.

(c) COVER-GLASS IMPRESSIONS.

One of the most instructive methods for examining micro-organisms is to make what is called in German a "*Klatsch Präparat.*" It enables us in many cases to study the relative position of individual micro-organisms one to another in their growth on solid cultivating media, and in some cases produces the most exquisite preparations for

the microscope. A perfectly clean, usually small-sized, cover-glass is carefully deposited on a plate or potato culture, and gently and evenly pressed down. One edge is then levered up, carefully, with a needle and the cover-glass lifted off by means of forceps. It is then allowed to dry, passed through the flame three times, and stained as already described. In the case of plate-cultures, especially where no liquefaction has taken place, the growth is bodily transferred to the cover-glass and a vacant area left on the gelatine or agar-agar, corresponding exactly with the form and size of the cover-glass employed (Plate XXV., Figs. 1 and 2).

CHAPTER IV.

PREPARATION AND STAINING OF TISSUE SECTIONS.

(A) METHODS OF HARDENING AND DECALCIFYING PREPARATIONS.

To harden small organs, such as the viscera of a mouse, they must be placed on a piece of filter paper at the bottom of a small, wide-mouthed glass jar, and covered with about twenty times their volume of absolute alcohol. Larger organs, pathological growths, etc., are treated in the same way, but must first be cut into small pieces, or cubes, varying from a quarter of an inch to an inch in size. Muller's fluid may also be employed, and methylated spirit may be substituted for alcohol, from motives of economy. Tissues hardened in absolute alcohol are ready for cutting in two or three days, and those hardened in Muller's fluid in as many weeks.

Teeth, or osseous structures, must first be placed in a decalcifying solution, such as Kleinenberg's. When sufficiently softened they are allowed to soak

in water, to wash out the picric acid, and then transferred through weak spirit to absolute alcohol. Ebner's solution also gives excellent results, especially when the structures to be decalcified are placed in fresh solution from time to time.

(B) METHODS OF EMBEDDING, FIXING, AND CUTTING.

Material to be cut with the freezing microtome, if hardened in spirit, must be well soaked in water before being frozen; if hardened in Muller's fluid, it can be frozen at once.

If Williams' microtome is employed, the hardened tissues must first be well soaked in gum mucilage, then frozen, and cut.

For cutting with Jung's microtome, the tissues are embedded in paraffin, or celloidin, and mounted on cork, or, if firm enough, they may be fixed upon cork without any embedding material at all. Paraffin, dissolved in chloroform, will be found very serviceable as an embedding material, but celloidin is more commonly employed now. The pieces of tissue to be embedded are placed, after the process of hardening is completed, in a mixture of ether and alcohol for an hour or more. They are then transferred to a solution of celloidin in equal parts of ether and alcohol, and left there, usually, for several hours. Meanwhile, corks ready cut for the clamp of the microtome are smeared

over with the solution of celloidin ; this is applied with a glass rod to the surface which is to receive the piece of tissue. The corks are then set aside for the film of celloidin to harden. The pieces of tissue are allowed to remain in the celloidin solution for from one to twenty-four hours, the time varying according to the structure of the specimen. Better results are obtained in the case of lung, or degenerated broken-down tissue, if left for a much longer time than is found to be sufficient for firmer structures. The specimen, when ready, is removed from the celloidin solution with forceps, and placed upon a prepared cork. A little of the solution, which is of syrupy consistence, is allowed to fall on the piece of tissue to cover it completely, and the mounted specimen is finally placed in 60 to 80 per cent. alcohol to harden the celloidin. The specimen will be ready for cutting next day.

The specimen may be more neatly embedded by fixing it with a pin in a small paper tray, pouring the celloidin solution over it, and then placing the tray in alcohol to harden the celloidin. The embedded specimen is then fixed on a cork, which has been cut for the clamp of the microtome. The celloidin in the section disappears in the process of clearing with clove-oil.

Material infiltrated with paraffin must be cut perfectly dry, and the sections prevented from rolling up by gentle manipulation with a camel's-hair brush. They must then be picked off the blade of

the knife with a clean needle, and dropped into a watch-glass containing xylol. This dissolves out the paraffin; the sections are then transferred to alcohol to get rid of the xylol, and then to the staining solution.

In the case of specimens embedded in celloidin, or mounted directly on a cork, the tissue, as well as the blade of the knife, should be kept constantly bathed with alcohol, and the sections transferred from the blade with a camel's-hair brush, and floated in alcohol.

For fixing small organs and pieces of firm tissue directly on cork, such as the kidneys of a mouse, or liver, one employs gelatine, or glycerine-gelatine, liquefied over a Bunsen burner in a porcelain capsule. The cork with specimen affixed is placed in alcohol, and is ready for cutting sections next day.

The advantage of glycerine-gelatine consists in that it may be used for fixing irregular pieces of tissue, as it does not become of a consistency that would injure the edge of the knife.

(c) General principles of staining bacteria in tissue sections: methods of Weigert, Gram, and Weigert-Ehrlich.

Sections of fresh tissues made with the freezing microtome are to be floated and well spread out in ·8 per cent. salt solution, and then carefully transferred, well spread out on the copper lifter, to a watch-glass containing absolute alcohol. Simi-

larly sections selected from those cut with Jung's microtome, may be transferred from the spirit to absolute alcohol. The sections may be then stained by any of the methods to be described.

It is often advisable to employ some method which will enable one to study the structure of the tissue itself. In the same way with sections however prepared, one should always examine with a low power (Zeiss' AA) first; this enables one to recognise the tissue under examination in most cases, and even to examine in many cases the topographical distribution of masses of bacteria. With Zeiss' DD., Oc. 2, a power of about 250 diams., very many bacteria can be distinguished, and with the oil immersion lenses the minutest bacilli and micrococci can be recognised, and the exact form of individual bacteria accurately determined. As Zeiss' microscopes are, like most good modern instruments, provided with a triple nosepiece, there is no loss of time in examining a preparation successively with these different powers.

Weigert's Method.—A very useful method for staining both the tissue and the bacteria is as follows:—Place the sections for from six to eighteen hours in a one per cent. watery solution of any of the basic-aniline dyes (methyl violet, gentian violet, fuchsine, bismarck brown). To hasten the process place the capsule containing the solution in the incubator, or heat it to 45° C. A stronger solution may also be employed, in which case the sections

are far more rapidly stained, and are easily over-stained. In the latter case they must be treated with a half-saturated solution of carbonate of potash. In either case the sections are next washed with distilled water, and passed through 60 per cent. alcohol into absolute alcohol. When almost decolorised spread out the section carefully on a copper lifter and transfer it to clove-oil, or stain with picro-carmine solution (Weigert's) for half-an-hour, wash in water, alcohol, and then treat with clove-oil. After the final treatment with clove-oil, transfer with the copper lifter to a clean glass slide. Dry the preparation by pressure with a piece of filter paper folded four times, and preserve in Canada balsam dissolved in xylol.

Gram's Method.—In the method of Gram the sections are stained for three minutes in aniline-gentian-violet solution. This is prepared by shaking up one ccm. of pure aniline with twenty-four parts of water, and filtering the emulsion. Half a gramme of the best finely powdered gentian-violet is dissolved in the clear filtrate, and the solution filtered before use. The sections are then transferred to a solution of iodine in iodide of potassium till they become dark brown in colour, and then decolorised in absolute alcohol. The time required for complete decolorisation in alcohol varies from a few minutes to twenty-four hours. They are then treated with clove-oil and mounted in Canada balsam. It is much better, however, to employ

the aniline-gentian-violet solution quite freshly prepared, and the following useful method is invariably used by the author:—Place four or five drops of pure aniline in a test-tube, fill it three-quarters full with distilled water, close the mouth of the tube with the thumb and shake it up thoroughly. Filter the emulsion twice, and pour the filtrate into a watch-glass or glass capsule. To the perfectly clear aniline water thus obtained add drop by drop a concentrated alcoholic solution of gentian-violet till precipitation commences. Stain sections in this solution from ten minutes to half an hour, then transfer to iodine-potassic-iodide solution, and decolorise in alcohol. The process of decolorisation may be hastened by placing the section in clove-oil and returning it to alcohol, and again to clove-oil. If examined, after it has been finally treated with clove-oil and mounted in Canada balsam, the tissue appears colourless or tinged faintly yellow, while micro-organisms, *e.g.*, bacilli and micrococci, are stained blue or blue-black. Double staining is obtained by transferring the sections after decolorisation to a solution of eosin, bismarck brown, or vesuvin, again rinsing in alcohol, clearing in clove-oil, and mounting in balsam. Another instructive method is to place the decolorised sections in picro-carminate of ammonia for three or four minutes, and then treat with alcohol, oil of cloves, and balsam. In this way the nuclei are well stained. A somewhat similar

result is obtained by placing the sections for a few minutes in Orth's solution (picro-lithium-carmine); transferring to acidulated alcohol, then to an alcoholic solution of picric acid, and treating with clove-oil and balsam (Plate XVII., Fig. 1).

Weigert-Ehrlich Method.—This is a method in which nitric acid is employed as a decolorising agent. It is as follows:—Filtered, saturated, watery solution of aniline one hundred parts; saturated alcoholic solution of a basic aniline dye (methyl-violet, gentian-violet, fuchsine), eleven parts; are mixed and filtered. Rapid staining is obtained by warming the solution. Decolorise with nitric acid (1 in 2), and stain with a contrast colour. As delicate sections are apt to be injured by immersion in the nitric acid, they may be transferred from the fuchsine solution to distilled water, then rinsed a few minutes with alcohol, and finally placed in the following contrast stain for one to two hours :—

Distilled water . . .	100 cc.
Saturated alcoholic solution of methylene blue. . .	20 cc.
Formic acid	10 mm.

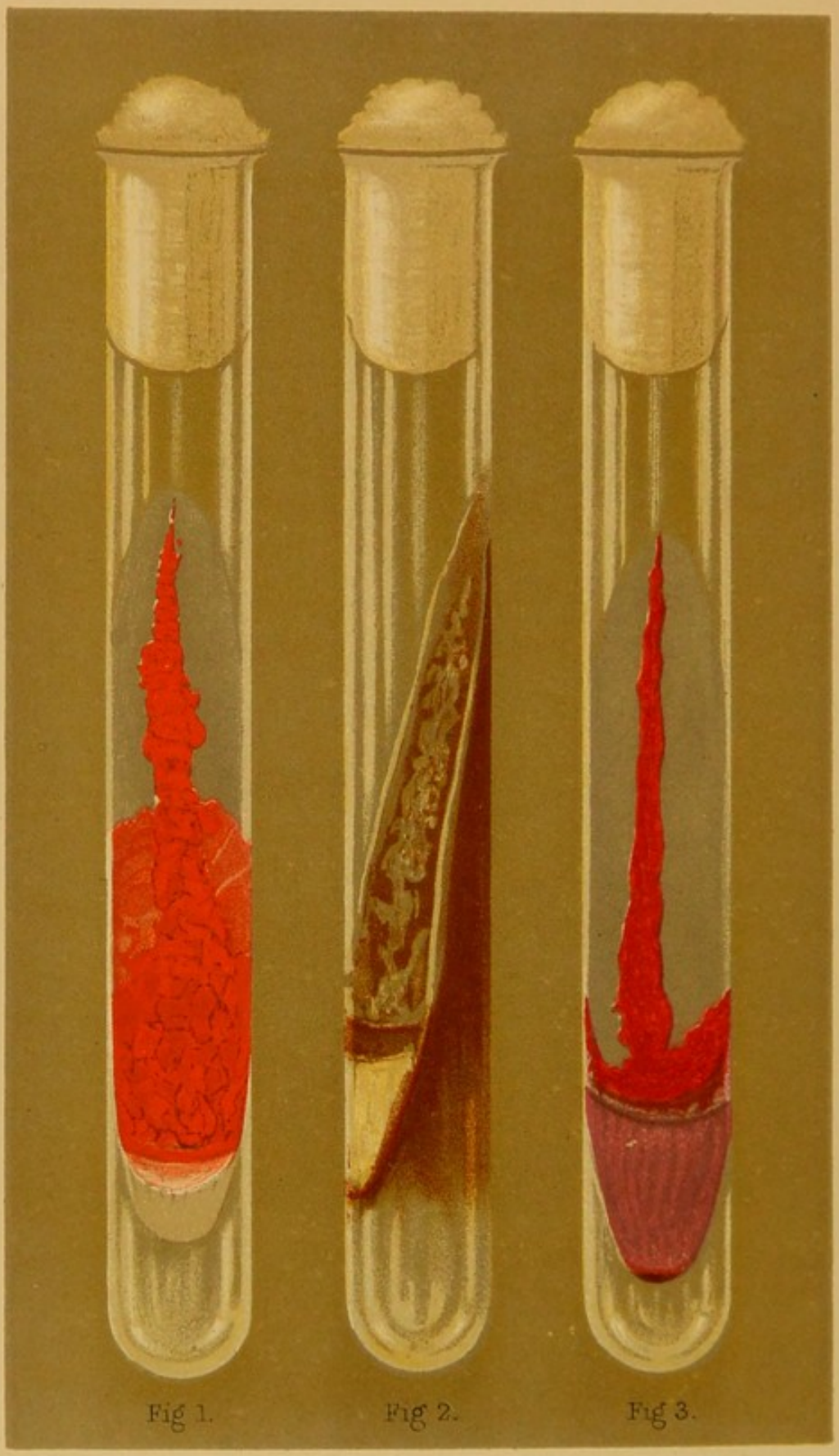
The method of Weigert-Ehrlich is employed for staining both leprosy and tubercle bacilli. The more especial methods which have been employed by Koch, Klein, Gibbes, Friedländer, Schütz, Lustgarten, and others will be given with the description of those species of micro-organisms to which they apply.

CHAPTER V.

PREPARATION OF NUTRIENT MEDIA AND METHODS OF CULTIVATION.

To cultivate micro-organisms artificially, and, in the case of the pathogenic bacteria, to fulfil the second of Koch's postulates, they must be supplied with nutrient material free from pre-existing micro-organisms. Hitherto various kinds of nutrient liquids have been employed, and in many cases they still continue to be used with advantage, but as a general rule they have been in a great measure supplanted by the methods of cultivation on sterile solid media about to be described. The advantages of the latter method are obvious. In the first place, in the case of liquid media, in spite of elaborate precautions and the expenditure of much labour and time, it was almost impossible or extremely difficult to obtain a pure culture. If a drop of liquid containing several kinds of bacteria be introduced into a liquid medium, we have a mixed cultivation from the very first. If in the struggle for existence some bacteria were unable to develop in the presence of others, or a change of temperature and soil allowed

one form to predominate over another, then one might be led to the conclusion that many bacteria were but developmental forms of one and the same micro-organism; while possibly the contamination of such cultures might lead to the belief in the transformation of a harmless into a pathogenic bacterium. In the case of solid cultivating media, on the other hand, the chance of contamination by gravitation of germs from the air is avoided by the fact that test-tubes, flasks, etc., can be inverted and inoculated from below. The secret of the success of Koch's method, however, depends upon the possibility, in the case of starting with a mixture of micro-organisms, of being able to isolate them completely one from another, and to obtain an absolutely pure cultivation of each distinct form. When sterile nutrient gelatine has been liquefied in a tube and inoculated with a mixture of bacteria in such a way that the individual micro-organisms are distributed throughout it, and the liquid is poured out on a plate of glass and allowed to solidify, the individual bacteria, instead of moving about freely as in a liquid medium, are fixed in one spot, where they develop individuals of their own species. In this way colonies are formed each possessing its own characteristic biological and morphological appearances. If an adventitious germ from the air falls upon the culture, it also grows exactly upon the spot upon which it fell, and can be easily recognised as a stranger. To maintain the individuals isolated from one another



CULTIVATIONS ON NUTRIENT AGAR-AGAR

Fig 1. Micrococcus indicus.
Fig 2. Bacillus cyanogenus.
Fig 3. Micrococcus prodigiosus.

H. Crookshank Pinxt.

Vincent Brooks, Day & Son, Lith.

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during their growth, and free from contamination, it is only necessary to thin out the cultivation, and to protect the plates from the air. The slower growth of the micro-organisms in solid media, and the greater facility afforded thereby for examining them at various intervals and stages of development, is an additional point in favour of these methods; and the characteristic macroscopical appearances so frequently assumed are, more especially in the case of morphological resemblance or identity, of the greatest importance. The colonies on nutrient gelatine (examined with a low power) of the bacillus anthracis and proteus mirabilis; the naked eye appearances in test-tubes of nutrient gelatine of the bacillus of mouse-septicæmia (Figs. 38, 39), and of anthrax (Fig. 37), and the brilliant and curious growth of micrococcus indicus upon nutrient agar-agar (Plate II., Fig. 1), may be quoted as examples in which the appearances in solid cultivations are pathognomonic.

Solid Media.

(A) Preparation of sterile gelatine-, and agar-agar-peptone-broth.

Sterile Gelatine-Peptone-broth, or Nutrient Gelatine, is prepared as follows:—Take half a kilogramme of beef (one pound), as free as possible from fat. Chop it up finely, transfer it to a flask or cylindrical vessel, and shake it up well with a

litre of distilled water. Place the vessel in an ice-pail, ice-cupboard, or in winter in a cold cellar, and leave for the night. Next morning commence with the preparation of all requisite apparatus. Thoroughly wash, rinse with alcohol, and allow to dry, about 100 test-tubes. Plug the mouth of the test-tubes with cotton wool, taking care that the plugs fit firmly, but not too tightly. Place them in their wire cages in the hot-air steriliser to be heated for an hour at a temperature of 150° C. In the same manner cleanse and sterilise several flasks and a small glass funnel. In the meantime the meat infusion must be again well shaken, and the liquid portion separated by filtering and squeezing through a linen cloth. The red juice thus obtained must be brought up to a litre by again transferring it to a large measuring glass and adding distilled water. It is then poured into a sufficiently large and strong beaker; and set aside after the addition of

10 grammes of peptonum siccum.
5 grammes of common salt.
100 grammes of best gelatine.

In about half-an-hour the gelatine is sufficiently softened, and subsequent gently heating in a water-bath causes it to be completely dissolved. The danger of breaking the beaker may be avoided by placing a cloth several times folded at the bottom of the water-bath.

The next process requires the greatest care and attention. Some micro-organisms grow best in a slightly acid, others in a neutral or slightly alkaline medium. For example, for the growth and characteristic appearances of the comma bacillus of Asiatic cholera a faintly alkaline soil is absolutely essential. This slightly alkaline medium will be found to answer best for most micro-organisms, and may be obtained as follows :—

With a clean glass rod dipped into the mixture, the re-action upon litmus paper may be ascertained, and a concentrated solution of carbonate of soda must be added drop by drop, until red litmus paper becomes faintly blue. If it has been made too alkaline it can be neutralised by the addition of lactic acid.

Finally, the mixture is heated for an hour in the water-bath and filtered while hot. For the filtration the hot-water apparatus can be used with advantage, furnished with a filter of Swedish paper made in the following way.

About eighteen inches square of the best and stoutest filtering paper is first folded in the middle, and then, as in Fig. 26, creased into sixteen folds. The filter is made to fit the glass funnel by gathering up the folds like a fan, and cutting off the superfluous part. The creasing of each fold should be made firmly to within half an inch of the apex of the filter, which part is to be gently inserted into the tube of the funnel. To avoid

bursting the filter at the point, the broth when poured out from the flask should be directed against the side of the filter with a glass rod. During filtration the funnel should be covered over with a circular plate of glass, and the process of filtration must be repeated, if necessary, until a pale, straw-coloured, perfectly transparent filtrate results.

The sterilised test-tubes are filled for about a third of their depth by pouring in the gelatine

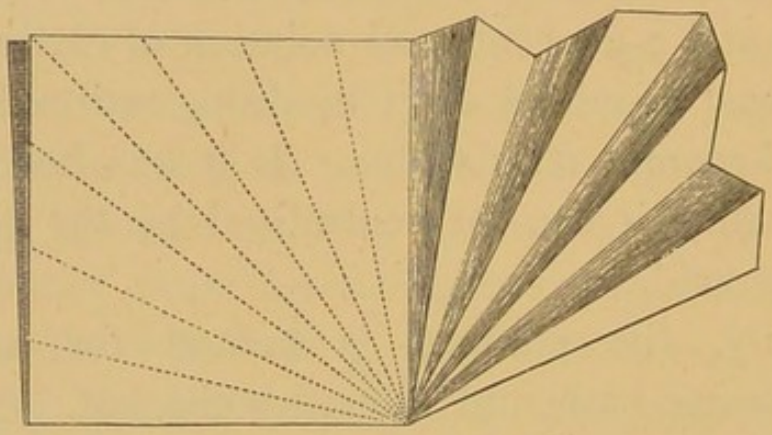

Fig. 26.—Method of Making a Folded Filter.

carefully and steadily, or by employing a small sterilised glass funnel. The object of this care is to prevent the mixture touching the part of the tube with which the plug comes into contact; otherwise, when the gelatine sets, the cotton wool adheres to the tube, and becomes a source of embarrassment in subsequent procedures. As the tubes are filled they are placed in the test-tube basket, and must then be sterilised. They are either lowered into the steam steriliser, when the

thermometer indicates 100° C., for twelve minutes for four or five successive days; or they may be transferred to the test-tube water-bath and heated for an hour a day for three successive days.

If the gelatine shows any turbidity after these processes, it must be poured back from the test-tubes into a flask and boiled up for ten minutes, after the addition of the white and shell of an egg beaten up together. It is once more filtered, and the processes of sterilisation just described must be repeated.

Sterile Agar Agar Peptone broth, or Nutrient Agar-Agar.—Agar-Agar has the advantage of remaining solid up to a temperature of about 45°. The preparation of a sterile nutrient jelly is conducted on much the same principles as those already described. Instead, however, of 100 grammes of gelatine, only about 20 grammes of agar-agar are employed (1·5—2 per cent.), and to facilitate its solution it must be allowed to soak in salt-water overnight. For the filtration, flannel is substituted for filter paper, or may be used in combination with the latter. The hot-water apparatus is invariably employed, unless, to accelerate the process, the glass funnel and receiver are bodily transferred to the steam steriliser. If the conical cap cannot be replaced, cloths laid over the mouth of the steriliser must be employed instead. It may be necessary to repeat the process of filtration, but it must not be

expected that such a brilliant transparency can be obtained as with gelatine. The final result, when solid, should be colourless and clear, but if only slightly milky it may still be employed.

After the final treatment in the steam steriliser some of the tubes may be placed in the blood-serum apparatus, and left to gelatinise with an oblique surface. A little liquid gradually collects at the bottom of the surface, being expressed by the contraction of the nutrient jelly.

(B) METHODS OF EMPLOYING NUTRIENT JELLY IN TEST-TUBE- AND PLATE-CULTIVATIONS.

Test-Tube-Cultivations.—To inoculate test-tubes containing nutrient jelly, the cotton wool plug must be *twisted* out, by which means any adhesions that may exist are broken down. A sterilised needle charged with the blood, pus, etc., containing the micro-organisms, or with a colony from a plate-culture, is steadily thrust once, and once only, into the nutrient jelly. The tube should be held with its mouth downwards, to avoid, as far as possible, accidental contamination from the gravitation of germs in the air, and the plug, which has been removed with the thumb and index finger of the right hand and held between the fourth and fifth fingers of the left, is replaced as rapidly as possible (Fig. 27).

Where, however, one tube is inoculated from

To face p. 66.

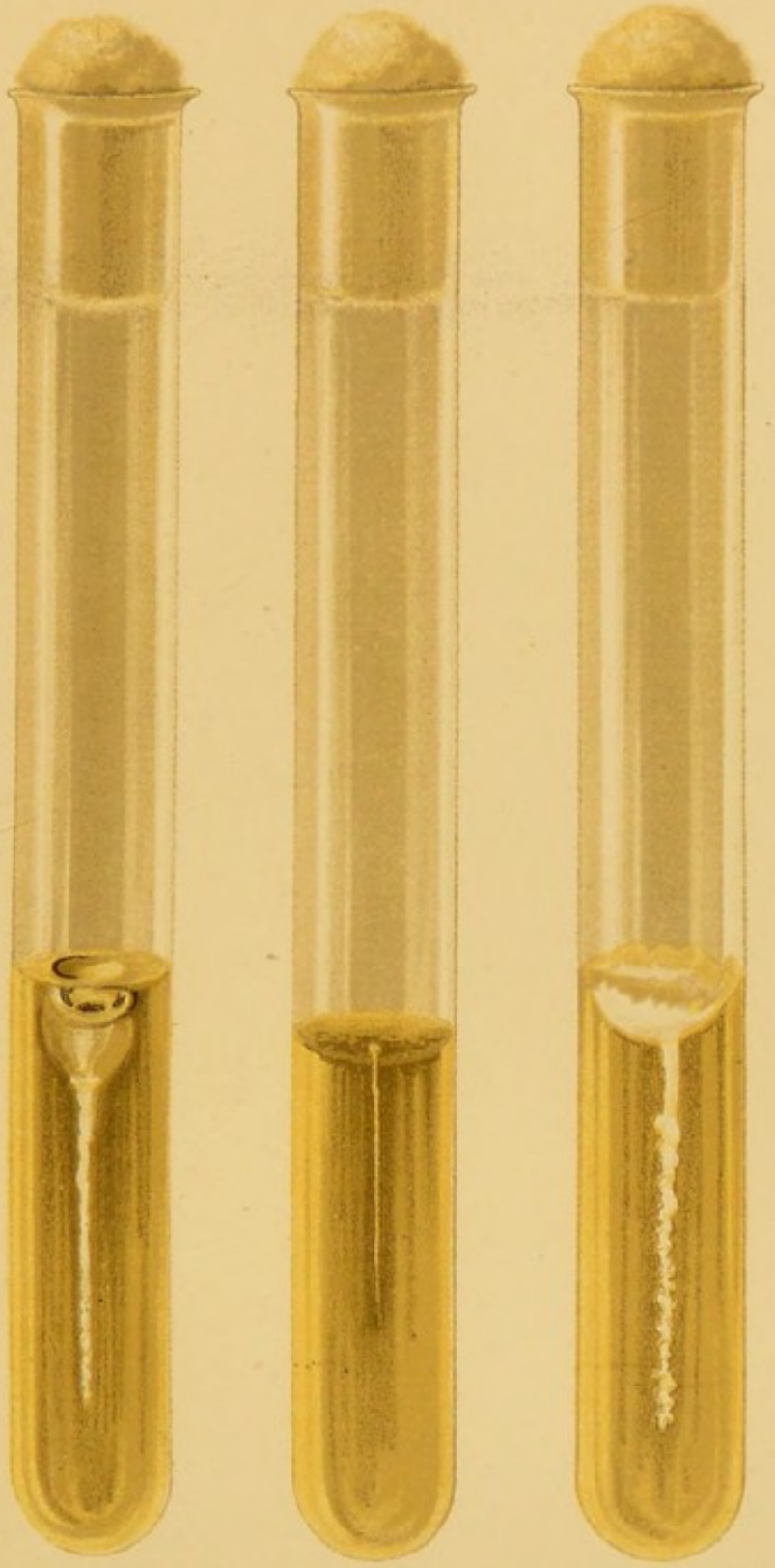

Fig 1. Fig 2. Fig 3.

CULTIVATIONS IN NUTRIENT GELATINE

Fig 1. Spirillum cholerae asiaticae
Fig 2. Micrococcus cholerae gallinarum.
Fig 3. Staphylococcus cereus albus.

H. Cookshank Pinx.

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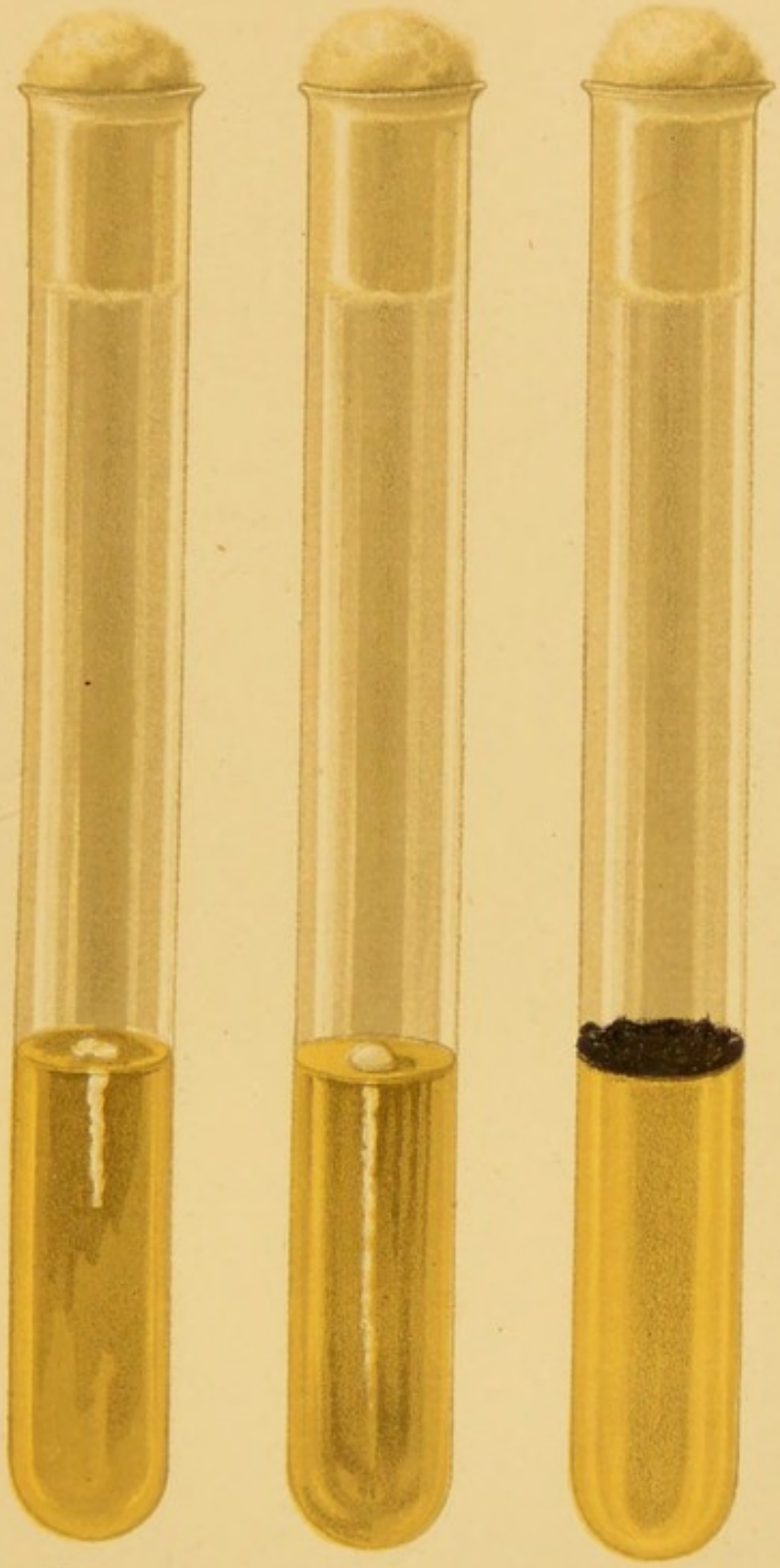

Fig 1. Fig 2. Fig 3.

CULTIVATIONS IN NUTRIENT GELATINE.

Fig 1. Micrococcus tetragonus.
Fig 2. Bacterium pneumoniæ crouposæ.
Fig 3. Saccharomyces niger.

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To follow Plate 4.

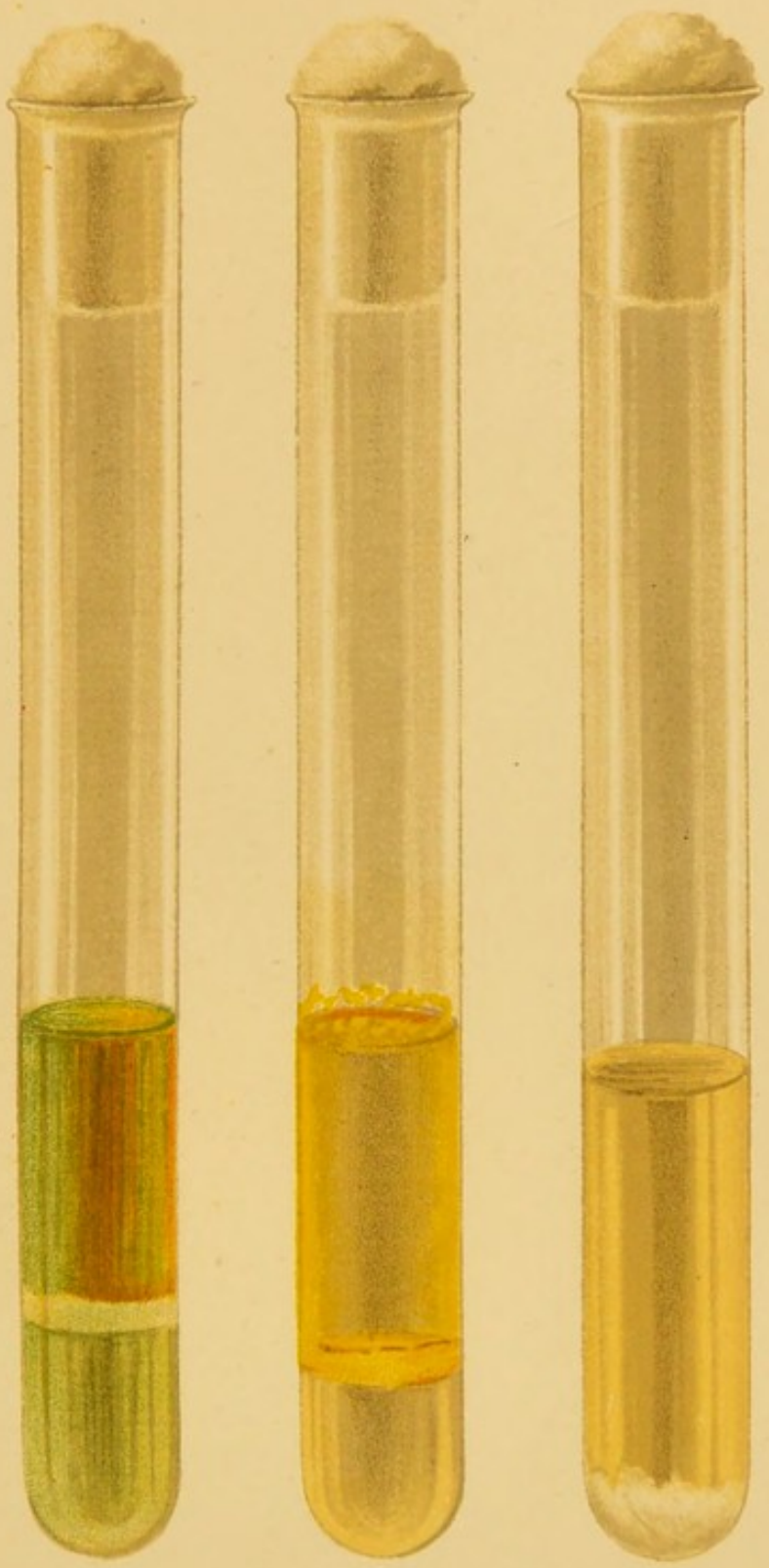

Fig 1. Fig 2. Fig 3.

CULTIVATIONS IN NUTRIENT GELATINE.

Fig 1. Bacterium æruginosum.
Fig 2. Sarcina lutea.
Fig 3. Bacillus anthracis.

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another in the liquid state, as in the process of preparing plate-cultures, or where a culture is made from a tube in which the growth has liquefied the gelatine, it is obvious that the tubes cannot be inverted, and they must then be held and inoculated as in Fig. 28. To inoculate those tubes of nutrient agar-agar which have been gelatinised obliquely, the sterilised needle with the material to be cultivated is streaked over the surface from below upwards.

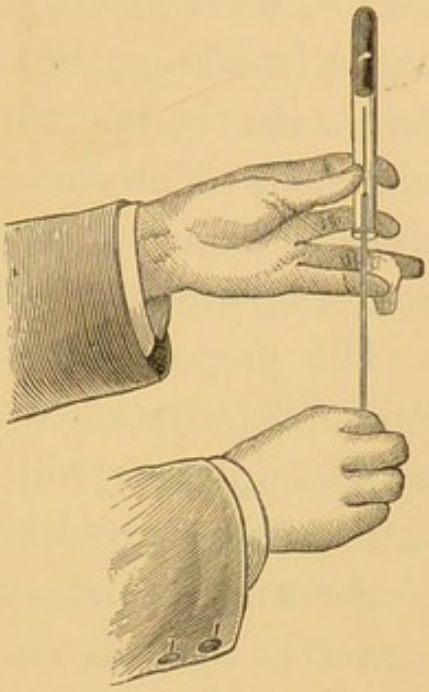

FIG. 27.
METHOD OF INOCULATING A TEST-TUBE CONTAINING STERILE NUTRIENT JELLY.

PLATE-CULTIVATIONS.

The key to the success of Koch's method of cultivation on solid media consists in the employment of plate-cultivations. By this means, as has already been mentioned (p. 60), a mixture of bacteria, whether it be in fluids, excreta, or in artificial cultivations, can be so treated that the different species are isolated one from the other, and perfectly pure cultivations established in various nutrient media of each of the constituent bacteria in the original mixture. We are enabled

the tubes. Take the sterilised öse in the right hand and hold it like a pen. Remove the plug from the culture-tube by using the fourth and fifth fingers of the right hand as forceps, and place it between the fourth and fifth fingers of the left. Remove the plug of the "original" in the same way, placing it between the third and fourth fingers of the left hand (Fig. 28). With the öse take up a droplet

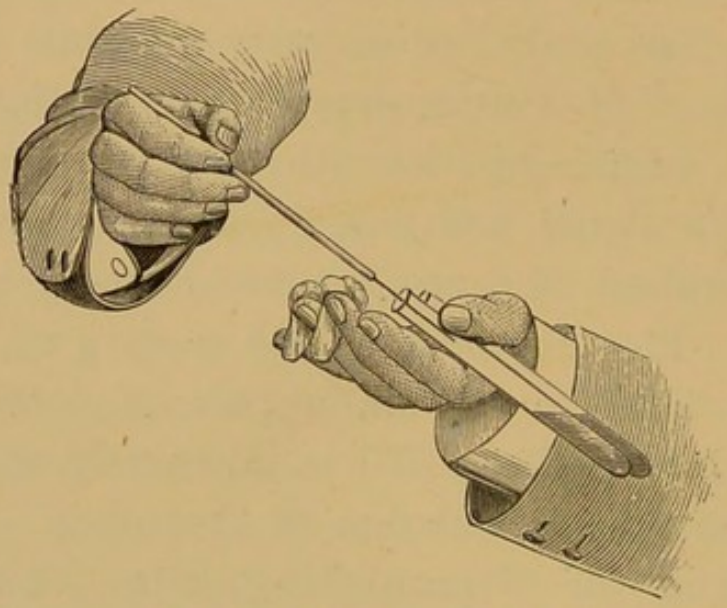

FIG. 28.—METHOD OF INOCULATING TEST-TUBES IN THE PREPARATION OF PLATE-CULTIVATIONS.

of the cultivation and inoculate the "original," twisting the öse several times in the liquid gelatine. Replace the plugs and set aside the cultivation. Hold the freshly inoculated tube between the fore-finger and thumb of either hand, almost horizontally, then raise it to the vertical, so that the liquid gelatine gently flows back. By repeating this motion and rolling the tube between the fingers and thumbs the micro-organisms which have been introduced are

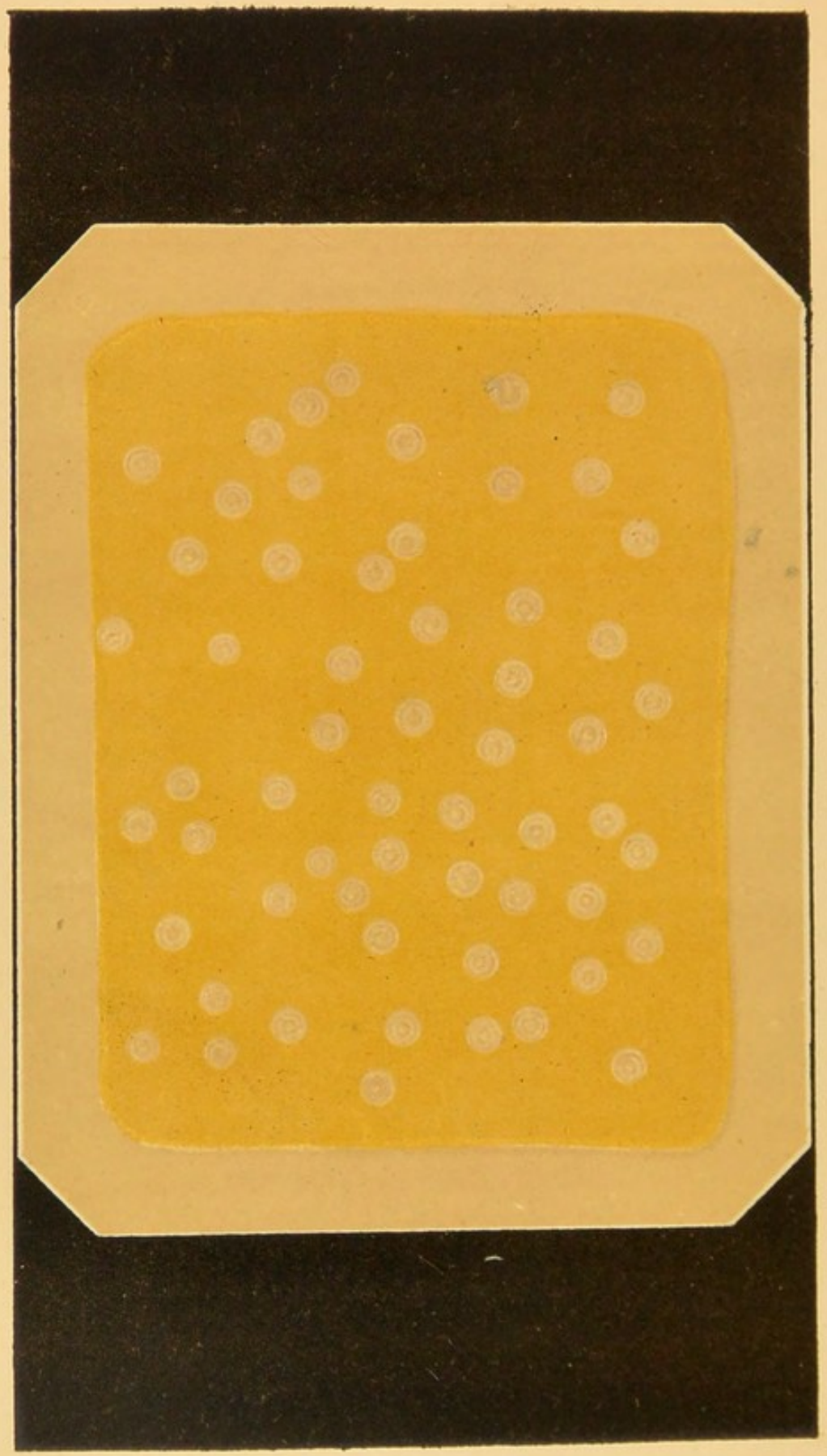

PLATE - CULTIVATION.

First attenuation of the Spirillum Finkleri, after twenty-four hours.

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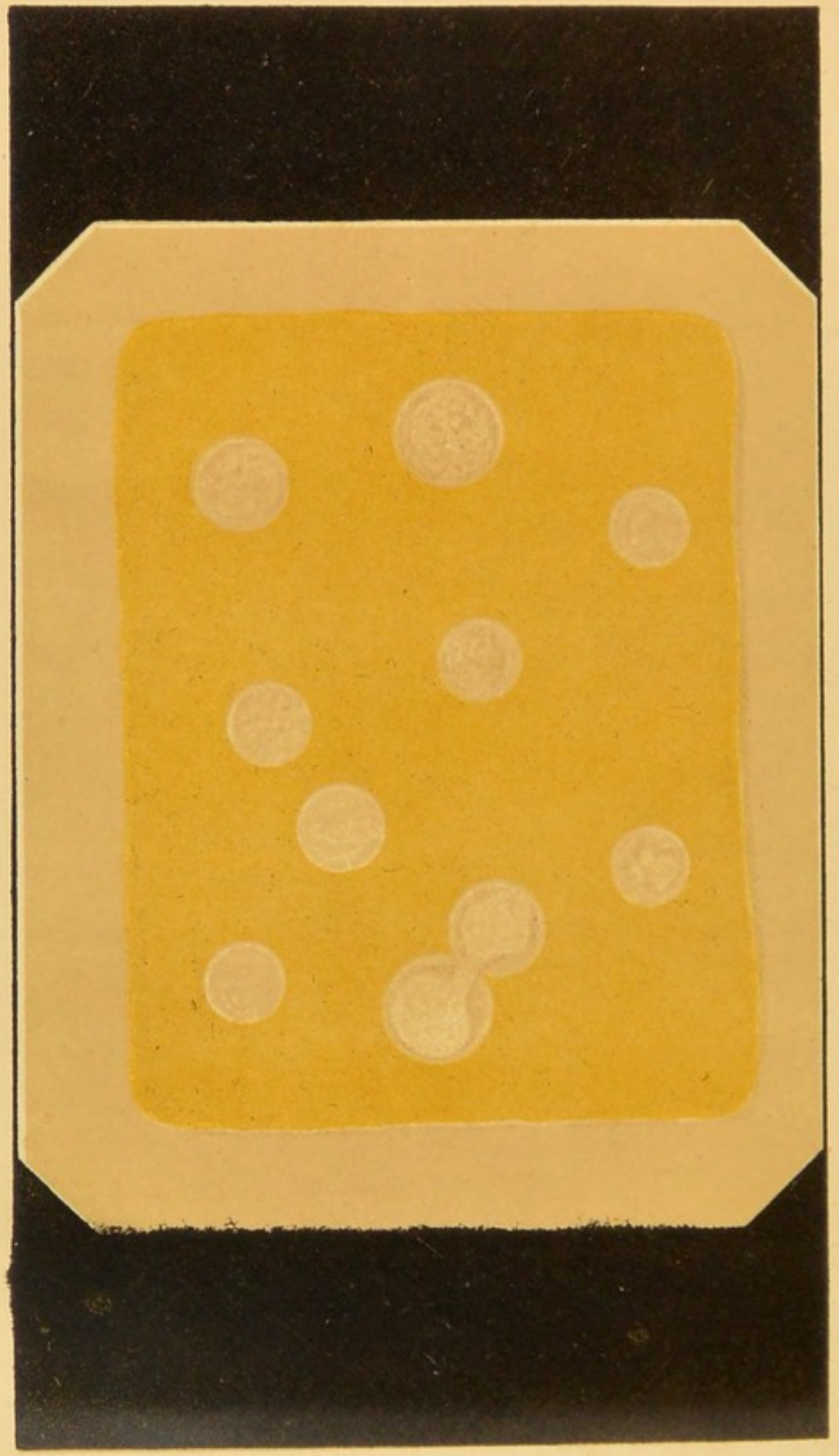

PLATE CULTIVATION.

Second attenuation of the Spirillum Finklerii, after thirty-six hours.

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distributed throughout the gelatine. Any violent shaking, and consequent formation of bubbles, must be carefully avoided. From the so-called "original" inoculate in the same manner a fresh tube of liquefied gelatine, introducing into it three droplets with a sterilised öse. This tube is then called the "first attenuation," or No. 1. After treating No. 1 as has been already described in the case of the original, the same process is repeated with a third tube, which is inoculated in the same way from No. 1. This is the "second attenuation" or No. 2, and in some cases a "third attenuation" is carried out from No. 2. The last tube must be inoculated in different ways, according to experience for different micro-organisms. Sometimes a sufficient separation of the micro-organisms is attained by inoculating the last tube with a fine straight needle dipped from one into the other from three to five times.

The next process consists in pouring out the gelatine on a glass plate and allowing it to solidify.

Preparation of the Gelatine-plates.—The directions to be observed in pouring out the gelatine are as follows:—

Place the box containing sterilised plates horizontally, and so that the cover projects beyond the edge of the table; remove the cover, and withdraw a plate with sterilised forceps; hold it between the finger and thumb by opposite margins, rapidly transfer it to the filter paper under the bell-glass, and

quickly replace the cover of the box. On removing the plug from "the original," an assistant raises the bell-glass, and the contents of the tube are poured on to the plate; with a glass rod the gelatine must be then rapidly spread out in an even layer within about half an inch of the margin of the plate. The assistant replaces the bell-glass, and the gelatine is left to set. Meanwhile a glass bench or metallic shelf is placed in the damp chamber, ready for the reception of the plate-cultivation, and when the gelatine is quite solid the plate is quickly transferred from under the bell-glass to the damp chamber; precisely the same process is repeated with tubes 1 and 2, and the damp chamber, labelled with the details of the experiment, is set aside for the colonies to develop. Not only plate-cultures should be carefully labelled with date and description, but the same remark applies equally to all preparations, tube-cultures, potato-cultures, drop-cultures, etc. In plate-cultivations write the source of the material, the date, and the number of inoculations; for example, thus :—

Finkler's comma-bacilli.

From tube-cultivation on "agar-agar," 5th February, 1885.

Lower plate (Orig.)	.	1 öse from cultivation.
Middle plate, No. 1	.	3 ösen from Orig.
Upper plate, No. 2 .	.	3 ösen from No. 1.

Corresponding with the fractional cultivation of the micro-organisms obtained in this manner, the colonies will be found to develop in the course of a day or two, varying with the temperature of the room. The lower plate will contain a countless number of colonies which, if the micro-organism liquefies gelatine, speedily commingle, and produce, in a very short time, a complete liquefaction of the whole of the gelatine. On the middle plate, with the first attenuation, the colonies will also be very numerous, but retain their isolated position for a longer time; while on the uppermost plate, the second attenuation, the colonies are completely isolated from one another, with an appreciable surface of gelatine intervening.

Examination of Plate-cultivations.—The macroscopical appearances of the colonies are best studied by placing the plate on the slab of blackened glass, or on the porcelain slab if the colonies are coloured.

To examine the microscopical appearances a selected plate is placed upon the stage of the microscope; it is better to have a larger stage than usual for this purpose. The smallest diaphragm is employed, and the appearances studied principally with a low power. These appearances should be carefully noted, and a rapid sketch of the colony made. The morphological characteristics of the micro-organisms of which the colony is formed can then be examined in the following way. A small

öse, or a platinum needle bent at the extremity into a miniature hook, is held like a pen, and the hand steadied by resting the little finger on the stage of the microscope. The extremity of the needle is steadily directed to the space between the lens and the gelatine without touching the latter, until,

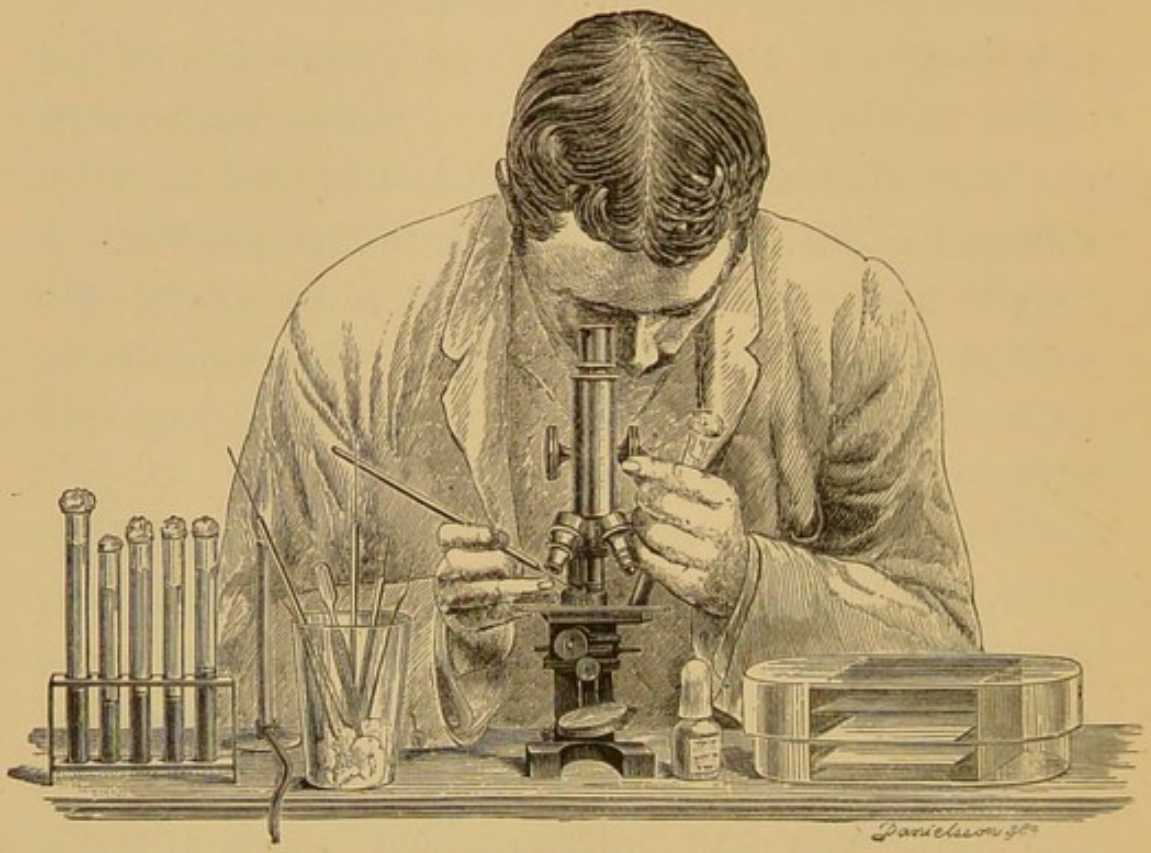

FIG. 29.—MICROSCOPICAL EXAMINATION OF COLONIES ON PLATE-CULTIVATIONS, AND METHOD OF ISOLATING COLONIES BY INOCULATION OF TEST-TUBES.

on looking through the microscope, it can be seen in the field, above or by the side of the colony under examination (Fig. 29). The needle is then dipped into the colony, steadily raised, and withdrawn. Without removing the eye from the microscope this manipulation can be seen to be successful by the colony being disorganised or completely re-

moved from the gelatine. It is, however, not easy to be successful at first, but with practice this can be accomplished with rapidity and precision. A cover-glass-preparation is then made in the manner already described, viz., by rubbing the extremity of the needle on a perfectly clean cover-glass and examining by Babes' rapid method, or by thinning out the micro-organisms in a droplet of sterilised water previously placed on the cover glass, drying, passing three times through the flame, and staining with a drop of fuchsine.

Inoculations should be made in test-tubes of nutrient gelatine and agar-agar, from the micro-organisms transferred to the cover-glass before it is dried and stained, from any remnants of the colony which was examined, or from other colonies bearing exactly similar appearances. In this way pure cultivations are established, and the macroscopical appearances of the growth in test-tubes can be studied. The plates should be replaced in the damp chambers as soon as possible; drying of the gelatine, or contamination with micro-organisms gravitating from the air during their exposure, may spoil them for subsequent examination. Nutrient agar-agar can also be employed for the preparation of plate-cultivations, but it is much more difficult to obtain satisfactory results. The test-tubes of nutrient agar-agar must be placed in a beaker with water and heated until the agar-agar is completely liquefied. The gas is then

turned down and the temperature of the water allowed to fall until the thermometer stands just above 50° C. The water must be maintained at this temperature, and the test-tubes must be in turn rapidly inoculated and poured out upon the glass plates, as already described. Glass plates may also be employed in a much simpler method. The nutrient jelly is liquefied, poured out, and allowed to set. A needle charged with the material to be inoculated is then streaked in lines over the surface of the jelly. This method is of especial value in inoculating different organisms side by side, and watching the effect of one upon the other, or a micro-organism in this way may be sown upon gelatine which has been already altered by the growth of another micro-organism; the change produced in the gelatine, as in the case of the fluorescing bacillus, extending far beyond the limits of the growth itself (Plate VIII., Fig. 3).

Nutrient jelly may also be spread out on sterilised microscopic slides and inoculated as just described, or cultivations may be made in shallow glass dishes, glass capsules, etc., which must be sterilised on the principles already laid down, and after inoculation placed in damp-chambers for the growths to develop.

To face p. 76.

CULTIVATIONS ON NUTRIENT AGAR-AGAR.

Fig 1. Sarcina lutea.
Fig 2. Micrococcus pyogenes aureus.
Fig 3. Bacillus fluorescens.

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(c) PREPARATION AND EMPLOYMENT OF STERILISED POTATOES, POTATO-PASTE, BREAD-PASTE, VEGETABLES, FRUIT, AND WHITE OF EGG.

Potato Cultivations.—Sterilised potatoes form an excellent medium for the cultivation of many micro-organisms, more especially the chromogenous species. Potato-cultivations also give in some cases very characteristic appearances, which are of value in distinguishing bacteria which possess morphological resemblances.

Preparation of Sterilised Potatoes.—Potatoes, preferably smooth skinned, which are free from "eyes" and rotten spots, should be selected. If they cannot be obtained without eyes and spots, these must be carefully picked out with the point of a knife with as little destruction of the surface as possible. The potatoes are well scrubbed with a stiff brush and allowed to soak in sublimate solution for half an hour. They are then transferred to the potato-receiver and steamed in the steam-steriliser for twenty minutes to half an hour, varying according to the size of the potatoes. When cooked, the potato-receiver is withdrawn and left to cool, the potatoes being retained in it until required for use.

Damp chambers are prepared ready for their reception, the vessels being cleansed and washed with sublimate as described for plate-cultivations. Small glass dishes of the same pattern as the

large ones (Fig. 13) may be employed for single halves of potatoes. Potato knives and several scalpels which have been sterilised in an Israel's case by heating them in the hot-air steriliser to 150° for one hour, must be ready to hand. The potato knives may also be sterilised by heating them in the flame of a Bunsen burner and placing them on their backs with their blades projecting over the edge of the table. Scalpels may be sterilised in the same way and laid upon a sterilised glass plate and covered with a bell-glass. It must not be forgotten, however, that heating the blades in the flame destroys the temper of the steel, and therefore knives and other instruments should preferably be sterilised in the hot-air steriliser, enclosed in an Israel's case, or simply enveloped in cotton wool.

Inoculation of Potatoes.—The coat sleeves should be turned back, and the hands, after a thorough washing with good lathering soap, be dipped in sublimate solution. An assistant opens the potato receiver, and a potato is selected, and held between the thumb and index finger of the left hand (Fig. 30). With the knife held in the right hand, the potato is almost completely divided in the direction which will give the largest surface. The assistant raises the cover of the damp chamber, and the potato is introduced, and while withdrawing the knife, allowed to fall apart. The cover is quickly replaced, and another

potato treated in the same way is placed in the same damp chamber. The four halves are then quite ready for inoculation. As an extra precaution the left hand is again dipped in sublimate, and one half of a potato is taken up between the tips of the thumb and index fingers, care being taken to avoid touching the cut surface. Holding

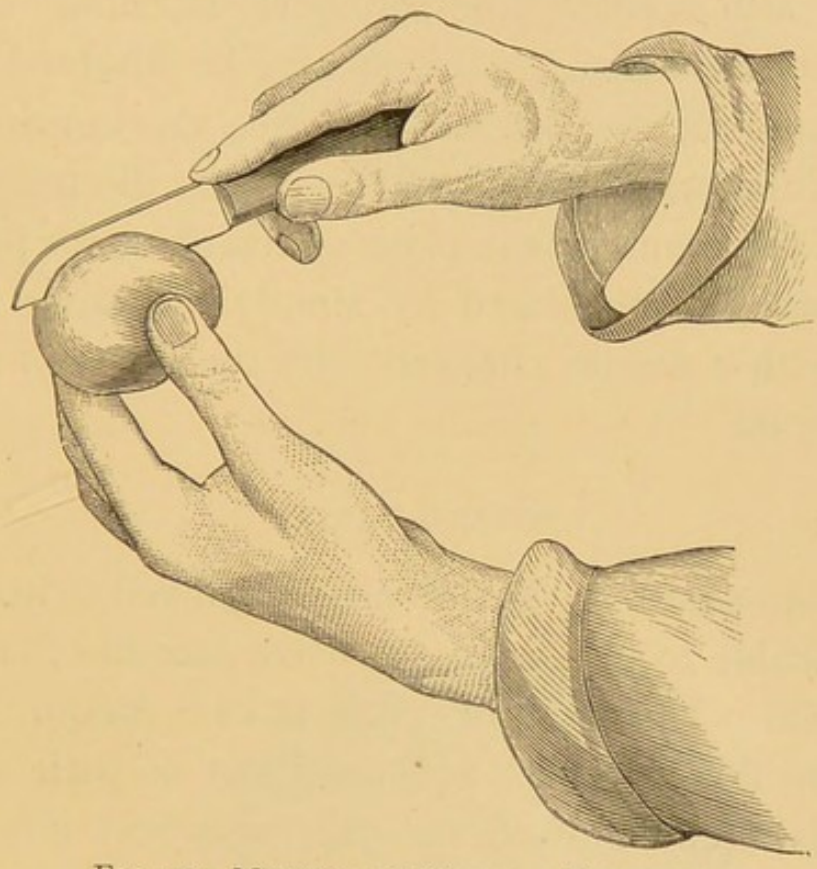

FIG. 30.—METHOD OF DIVIDING POTATOES.

it with its cut surface vertical, a small portion of the substance to be inoculated is placed on the centre with a sterilised needle or öse. With a sterilised scalpel the inoculated substance is rapidly spread over the surface of the potato with the flat of the blade to within a quarter of an inch of the margin, and the potato is then as

quickly as possible replaced in the moist chamber. With another sterilised scalpel a small portion of the potato from the inoculated surface of the first half is in the same way spread over the surface of the second half, this forming as in plate-cultivations a "first attenuation." Exactly the same is repeated with a third potato, and even a fourth, so that a still further attenuation or fractional cultivation of the micro-organisms may be obtained. In some cases it is necessary to place the cultures in the incubator, others grow very well at the temperature of the room. As in plate-cultivations, the potato may also be inoculated by simply streaking it in lines with a needle charged with the material to be inoculated.

POTATO-PASTE.

Potato-paste is sometimes employed where it is desirable to obtain an extensive growth of certain bacteria. The potatoes are boiled for an hour, and the floury centre squeezed out of their skins. This is then mashed up with sufficient sterilised water to produce a thick paste, and is heated in the steam steriliser for half an hour for three successive days.

BREAD-PASTE.

Some micro-organisms, more especially mould fungi, grow very well on bread-paste. This is prepared by removing the crust from a stale loaf

Fig 1. Micrococcus prodigiosus.
Second attenuation after three days

Fig 2. Penicillium glaucum.
after three weeks growth.

POTATO CULTIVATIONS.

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To follow plate 9.

Fig 1. Sarcina lutea

Growth five days after inoculation.

Fig 2. Saccharomyces rosaceus.

Growth two days after inoculation

POTATO CULTIVATIONS.

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of bread and allowing it to dry in the oven. It is then broken up, and reduced to a fine powder with a pestle and mortar. Small, carefully cleansed, conical, or globe-shaped flasks are plugged with cotton wool and sterilised in the oven. When cool a small quantity of the powder is placed in them, and sterilised water added in the proportion of one part for every four of the powder. The paste is sterilised by steaming in the steriliser at 100° C. for half an hour for three successive days. The flasks can be reversed, and are inoculated in the usual way with a platinum needle.

CULTIVATIONS ON VEGETABLES, FRUIT, WHITE OF EGG, ETC.

Boiled carrots and other vegetables, and various kinds of stewed fruit, are also occasionally employed for the cultivation of bacteria. The sterilisation of these media must be carried out on the principles already explained. White of egg may be poured out on sterilised glass plates, or in shallow glass dishes, boiled in the steam-steriliser and after inoculation, placed in a damp chamber.

(D) PREPARATION AND EMPLOYMENT OF STERILE BLOOD SERUM.

The tubercle-bacillus, the bacillus of glanders, and a few other micro-organisms, thrive best when culti-

vated on solid blood serum. This medium has the additional advantage of remaining solid at all temperatures. The technique required for its preparation and sterilisation is as follows:—Several cylindrical vessels, about 20 cm. high, are thoroughly washed with sublimate solution (1-1000), and then with alcohol, and finally rinsed out with ether. The ether is allowed to evaporate, and the vessels are then ready for use. The skin of the animal selected—calf, sheep, or horse—is washed with sublimate at the seat of operation, and the bleeding is performed with a sterilised knife. The first jet of blood from the vein is rejected, and that which follows is allowed to flow into the vessels until they are almost full. The ground-glass stoppers, greased with vaseline, are replaced, and the vessels set aside in ice, as quickly as possible, for from twenty-four to thirty hours. By that time the separation of the clot is completed, and the clear serum can then be transferred to plugged sterile test-tubes. These should be filled with a sterilised pipette for about a third of their length, and are then placed in Koch's slow steriliser with the temperature maintained for an hour at 58° C. The same process is repeated for six successive days, the temperature on the last day being gradually raised to 60°. This completes the sterilisation, but to solidify the serum it is necessary to arrange the tubes in the inspissator at the angle required. The temperature of this apparatus is kept between 65°

Fig. 1.

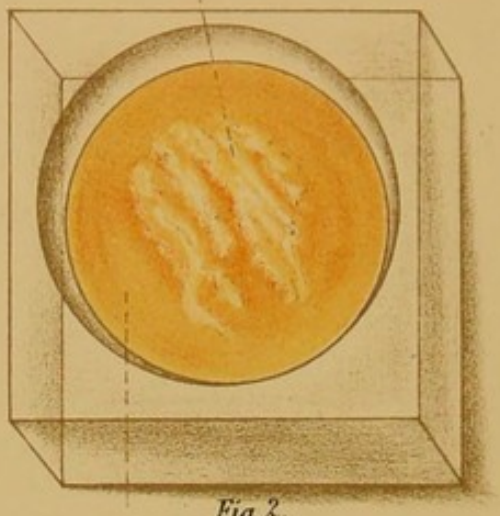

Fig. 2.

Fig. 3.

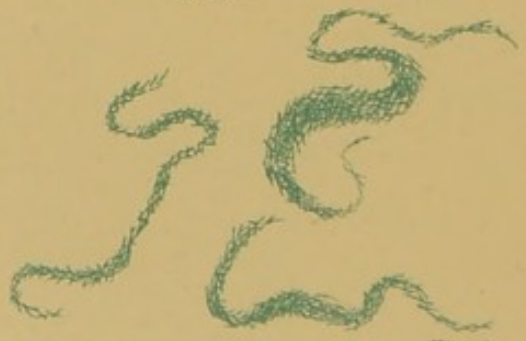

Fig. 4.

BACILLUS TUBERCULOSIS.

Fig. 1. Pure-cultivation on solid blood serum in a test-tube.
Fig. 2. Pure-cultivation on solid blood serum in a glass-capsule.
Fig. 3. The same preparation as Fig. 2 (x 80).
Fig. 4. Cover-glass impression-preparation of colonies x 700 (Ehrlich's method).

After Koch Mittheilungen a. d. Kaiserl Gesundheitsamte.

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and 68° C. Directly solidification takes place the tubes must be removed, and they should then present the character of being hard, solid, of a pale straw colour, and transparent. A little liquid collects at the lowest point, and the serum is sometimes milky in appearance at its thickest part. The serum may not only be employed in test-tubes, but also in small flasks, glass capsules, or other vessels, all of which should have to be cleansed and sterilised in the usual way. Hydrocele fluid and other serous effusions may be prepared in the same manner, or gelatine may be added to the serum in the proportion of 5 per cent.

Inoculation of the Tubes.—A small portion of the material to be inoculated is taken up with a sterilised needle or öse, and drawn in lines over the sloping surface of the serum; or a minute piece of tissue, tubercle, etc., may be introduced into the tube and deposited on the surface of the nutrient medium. The precautions that are to be observed in isolating the material to be inoculated will be referred to later (p. 97).

Liquid Media.

(E) Preparation of sterilised bouillon, liquid blood serum, urine, milk, vegetable infusions, and artificial nourishing liquids.

Nutrient liquids are still largely employed, and by some observers even in preference to the solid

media advocated by Koch. It must not be supposed, however, that the methods of cultivation in liquids are discarded entirely by the German school, for there is no more instructive method than the employment of so-called drop-cultures. For inoculation experiments where the presence of gelatine is undesirable, for studying the physiology and chemistry of bacteria and where for any object a rapid growth of micro-organisms is necessary, the employment of liquid media is not only advisable, but is absolutely necessary. Liquid media comprise two distinct groups—*natural* and *artificial*. The natural group includes meat broths, blood, urine, milk, and vegetable infusions; the artificial are solutions built up from a chemical formula representing the essential food constituents.

Natural Media.

Bouillon.—A broth or bouillon of beef, pork, or chicken may be made in the same manner as described for the preparation of gelatine-peptone-broth, with simply omission of the gelatine. After the neutralisation with carbonate of soda solution drop by drop, the flask of broth is placed in the steam steriliser for half an hour at 100° C. A clear liquid results on filtration, which is transferred to plugged sterilised flasks or test-tubes, and sterilisation effected by exposing them in the steam steriliser

for half an hour at 100° C. for two or three successive days.

Liquid Blood Serum.—The preparation of sterile blood serum has already been described. It may be used for cultivation, especially in the form of drop-cultivations, before the final treatment by which it is solidified. Hydrocele fluid, peritonitic and pleuritic effusions, can also be employed after sterilisation in the steam steriliser. The fluid should be withdrawn with a sterilised trocar and canula, and received into plugged sterilised flasks.

Urine.—In order to obtain urine free from micro-organisms the following precautions must be observed:—The orifice of the urethra must be thoroughly cleansed with sublimate solution. The first jet of urine should be rejected, and the rest received into sterilised vessels, which must be quickly closed with sterile plugs. If these precautions be not attended to the urine must be rendered sterile by the means described for the sterilisation of bouillon.

Milk.—If milk has been drawn into sterile flasks after thoroughly cleansing and disinfecting the teats and hands, it may be kept without change. If procured without these precautions it must be steamed in the steriliser for half an hour for five successive days.

Vegetable Infusions. — Infusions of hay, cucumber, and turnip are used for special purposes, and more rarely decoctions of plums, raisins, malt, and horse-dung. They are mostly prepared

by boiling with distilled water, after maceration for several hours. The filtrate is received into sterile flasks and sterilised in the usual way in the steam steriliser.

Artificial Fluids.

Pasteur's Fluid.—This solution is prepared by mixing the ingredients in the following proportions :—

Distilled water	100
Pure cane sugar	10
Ammonium tartrate . . .	1
Ash of yeast	·075

Cohn-Mayer Fluid.—Mayer's modification of the nourishing fluid employed by Cohn is as follows :—

Distilled water	20
Phosphate of potassium . . .	·1
Sulphate of magnesium . . .	·1
Tribasic calcium phosphate . .	·01
Ammonium tartrate . . .	·2

(f) Methods of storing and employing liquid media, Lister's flasks, Aitken's test-tubes, Sternberg's bulbs, Pasteur's apparatus, Miquel's bulbs.

Cultivations in liquid media can be carried on in test-tubes, but it is more satisfactory to employ

special forms of flasks, bulbs, U tubes, etc. As test-tubes and flasks containing liquid media cannot be inverted, inoculation with a sterilised needle must be carried out as rapidly as possible, with the additional precaution of closed windows and doors.

Lister's Flasks.—These flasks (p. 31) were especially introduced by Sir Joseph Lister as a means of storing liquid nutrient media. They are so constructed that after removal of a portion of the contents, or restoring the vessel to the vertical position, a drop of liquid always remains in the extremity of the nozzle, which prevents the regurgitation of unfiltered air.

Sternberg's Bulbs.—The method of introducing liquid into the bulbs employed by Professor Sternberg, and of sterilising and inoculating it, is as follows:—The bulb is heated slightly over the flame, and the extremity of the neck, after breaking off the sealed point, is plunged beneath the surface of the liquid. As the air cools the liquid is drawn into the bulb, usually filling it to about one-third of its capacity. The neck of the flask is again sealed up, and the liquid which has been introduced is sterilised by repeatedly boiling the flasks in the water-bath. They should then be placed in the incubator for two or three days, and if the contents remain transparent and free from film, they may be set aside as stock-bulbs, to be used when required.

To inoculate the liquid in the bulb the end of the neck is heated to sterilise the exterior, the bulb

is gently warmed, and the extremity of the neck nipped off with a pair of sterilised forceps. The open extremity is plunged into the liquid containing the micro-organism, a minute quantity enters the tube and mingles with the fluid in the bulb, without fear of contamination by atmospheric germs. The extremity of the neck is once more sealed up in the flame of a Bunsen burner.

Aitken's Tube.—These tubes are plugged and sterilised, and the nutrient medium introduced as into ordinary test-tubes. Instead of withdrawing the cotton wool plug they are inoculated by means of the lateral arm. The sealed extremity of the arm is nipped off with sterilised forceps, and the inoculating needle is carefully introduced through the opening thus made. It is directed along the arm until it touches the opposite side of the test-tube, where it deposits the material with which it was charged. The needle is withdrawn, and the end of the lateral arm again sealed up in the flame; the test-tube is then tilted until the liquid touches the deposited material; on restoring the tube to the vertical the material is washed down into the body of the nutrient liquid.

Pasteur's Apparatus. — Special forms of tubes, bulbs, and pipettes are employed by the school of Pasteur. The tubes are provided with lateral or with curved arms drawn out to a fine point, and with slender necks plugged with cotton wool. A double form shaped like a tuning fork,

each limb with a bent arm, is a convenient form for storing sterilised bouillon. The sealed end of an arm is nipped off with sterilised forceps, the sterile bouillon aspirated into each limb, and the arm again sealed in the flame; a series of such tubes can be arranged upon a rack on the working table.*

Bulbs with a vertical neck drawn out to a fine point; others with a neck bent at an obtuse angle plugged with cotton wool, and a lateral curved arm drawn out to a fine point, are also employed. For a description of these various vessels and their special advantages the works of Pasteur and Duclaux must be consulted.

Miquel's Bulbs.—The *tube à boule* of Miquel† is also a very useful form. It consists of a bulb of 50 cc. capacity blown in the middle of a glass tube. The part of the tube above the bulb is contracted about half way between the bulb and its extremity, and can either be left quite straight or can be made to curve slightly. On either side of the contraction the tube is plugged with asbestos. The portion of the tube below the bulb is S shaped, and drawn out at its extremity into a fine point. The bulbs are charged with nutrient liquid and inoculated by aspiration, and the point of the S tube sealed in the flame of a Bunsen burner.

Drop-Cultures.—This method of cultivation

* Duclaux, *Ferments et Maladies*. 1882.

† Miquel, *Les Organismes Vivants de l'Atmosphère*. 1883.

has already been referred to as a particularly instructive one. It enables us to study many of the changes which take place during the life history of micro-organisms. This is illustrated, for example, by the anthrax bacillus, where we can watch the gradual growth of a single bacillus into a long filament, and the subsequent development of bright oval spores. It is necessary carefully to observe the minutest details to maintain the cultivation pure. An excavated slide is thoroughly cleaned, and then sterilised by being held with the cupped side downwards in the flame of the Bunsen burner. A ring of vaseline is painted round the excavation, and the slide is then placed under a glass bell. Meanwhile a carefully cleansed cover-glass is also sterilised by passing it through the flame, and should be deposited on the plate of blackened glass. With a sterilised öse a drop of sterile bouillon is transferred to the cover glass, and this is inoculated by touching it with a sterilised needle charged with the material without disturbing the form of the drop. It is quite sufficient just to touch the drop instead of transferring a visible quantity of blood, juice, or growth, as the case may be. The slide is then inverted and placed over the cover-glass, so that the drop will come exactly in the centre of the excavation, and is gently pressed down. On turning the slide over again the cover-glass adheres, and an additional layer of vaseline is painted round the edges of the cover-glass itself. The slide must be

labelled, and, if necessary, placed in the incubator, and the results watched from time to time. Instead of bouillon liquid blood serum may also be employed in this form of cultivation. If it is required to preserve the drop cultivation as a microscopic preparation, the cover-glass is gently lifted off and allowed to dry. Any vaseline adhering to the cover-glass should be wiped off, and the cover-glass can then be passed through the flame and stained in the usual manner.

CHAPTER VI.

EXPERIMENTS UPON THE LIVING ANIMAL.

To carry out the last of Koch's postulates, and so complete the chain of evidence in favour of the causal relation of micro-organisms to disease, and to study the mode of action of a pathogenic bacterium, it is necessary to introduce into a living animal a pure cultivation of the micro-organism in question. For this purpose various animals are employed—such as mice, guinea-pigs, rabbits, pigeons, and fowls.

Inhalation of Micro-organisms. — The animals may be made to inhale an atmosphere impregnated with micro-organisms by means of a spray. In this way Friedländer succeeded in administering the micrococci of pneumonia to mice, and the production of tuberculosis by experimental inhalation has thrown light upon the clinical records of cases reported as instances of the infectiousness of phthisis.

Administration with Food.—A sheep fed upon potatoes which have been the medium for the cultivation of the anthrax bacillus dies in a

few days. Similarly, animals fed upon the nodules of bovine tuberculosis become tubercular, and even upon the flesh and milk of tuberculous animals will occasionally set up tuberculosis.

Cutaneous and Subcutaneous Inoculation.—Cutaneous inoculation may be carried out by making a superficial wound, and inoculating it with a sterilised platinum needle, charged with the micro-organisms to be inoculated. Another simple method is to take a sterilised knife, infect the point with the material to be inoculated, and then make a minute wound or incision. In either case a situation should be selected, such as the root of the ear, which cannot be licked by the animal after the operation.

Subcutaneous inoculation is very simple and effectual, and consequently the method most frequently employed. The animal selected—for example, a guinea-pig—is held by an assistant, who covers it with a towel, leaving only the hinder extremities exposed. By so doing, and gently laying it upon its back, with its head low, a guinea-pig passes apparently into a state of hypnotism, and the trivial operation can be performed with little or no movement on the part of the animal. From a spot on the inner side of the thigh the hair is cut close with a small pair of scissors curved on the flat, and the skin must be thoroughly purified with sublimate solution. A small fold of skin is then pinched up

with a pair of sterilised forceps, and with a pair of sharp sterilised scissors, or with a tenotomy knife, a minute incision is made. A sterilised platinum öse is charged with the material to be inoculated, and the loop is gently inserted under the skin, forming a small pocket in the subcutaneous tissue. The needle is then withdrawn, and the sides of the wound gently pressed into apposition. In a mouse the same process is adopted, with the exception that the root of the tail is the usual site of the operation. In a method suggested by Koch an assistant can be dispensed with: a glass bell reversed is placed as a cover to a wide-mouthed glass jar, in which a mouse is held by the tail with a pair of forceps, while the cover is so placed over the mouth of the jar as to leave a small interval near the rim uncovered. The mouse rests with its head downwards and with its feet against the inner wall of the jar, and in the interval between the cover and the rim the root of the tail is exposed, and must be cleansed and treated as already described.

Special Operations. — In many cases it is absolutely necessary to perform an operation of greater severity. After the administration of an anæsthetic, infective material may be introduced into the peritoneal cavity by the performance of abdominal section, or injected into the duodenum in the manner employed in the case of Koch's

comma bacilli by Nicati and Rietsch. In such cases antiseptic precautions must be rigidly followed, and use made of iodoform and other antiseptic dressings. The disinfection of the skin of the animal, of the instruments employed, and of the hands of the operator, are details essential to secure success. To inoculate tubercular matter, sputum may be rubbed up with distilled water, filtered, and the filtrate injected into a tracheal fistula, or the first steps of the operation of iridectomy may be performed, and tubercular material inserted in the anterior chamber of the eye. The advantage of the latter method consists in that it enables the results and changes to be observed from day to day. A cultivation of micro-organisms may also be mixed with sterilised water, and then injected with a syringe directly into the circulation. In rabbits this may be performed without difficulty by injecting the large vein at the base of the ear with a Pravaz' syringe. Before every inoculation the instruments must be sterilised, as already explained, by employing an Israel's case, and after each operation all instruments should be placed in sublimate solution, wiped dry, and sterilised in the hot-air steriliser, before they are put away. If these precautions are not observed, instances of accidental infection are sure to occur.

CHAPTER VII.

EXAMINATION OF ANIMALS EXPERIMENTED UPON AND THE METHODS OF ISOLATING MICRO-ORGANISMS FROM THE LIVING AND DEAD SUBJECT.

METHOD OF DISSECTION AND EXAMINATION.

ALL animals that die after an experimental inoculation should be examined immediately after death. Every precaution must be taken, in conducting the dissection, to exclude extraneous micro-organisms, and all instruments employed must have been sterilised in the hot-air steriliser, or heated in the Bunsen burner. If a mouse, for example, has died after an inoculation, it should be at once pinned out by its feet on a slab of wood or in a gutta-percha tray, and bathed with sublimate solution. In the same way, before examining a dead rabbit, a stream of sublimate should be directed over it to lay the fur, which otherwise interferes with the dissection. The hair should be cut away with sterilised scissors from the seat of inoculation, which is the first part to be examined, and any suppuration, hæmorrhage, œdema, or other pathological

change should carefully be noted. From any pus or exudation that may be present, material for inoculations should at once be taken, and cover-glass-preparations made for microscopical examination.

To examine the internal organs and to make inoculations from the blood of the heart or spleen, the skin is cut through from below upwards in the median line of the abdominal and thoracic regions. The abdominal cavity is then opened, and the walls pinned back on either side of the animal. Any abnormal appearances should be noted, and especially the state of the spleen should be examined, by turning the intestines aside. After noting its appearances it should be removed with a sterilised forceps and scissors, and deposited upon a sterilised glass slide. After washing it with sublimate solution by means of a camel's hair brush or strip of filter paper, it should be incised with sterilised scissors; the pulp may be squeezed out from the cut surface, and test-tubes of nutrient gelatine and agar-agar can be inoculated from it, and, if necessary, potato and drop-cultivations also established. Precisely the same care must be taken in examining lymphatic glands, tubercles, or pathological nodules; any chance putrefactive micro-organisms on the surface are destroyed by the sublimate solution, and a section is then made, and a minute fragment snipped out of the centre of the nodule, to be examined or transferred to the nutrient medium. The examination of the thorax is made by cutting

through the ribs on either side of the sternum with sterilised scissors, and turning the sternum up where it will be out of the way. The pericardium is then opened, and the right auricle or ventricle pierced with the point of a sterilised scalpel, and inoculations and cover-glass-preparations are made from the blood which escapes.

The lungs also require to be especially studied. They should be incised with a sterilised scalpel, and inoculations and cover-glass-preparations made from the cut surface. It may be necessary to imbed a piece of lung or fragment of spleen, so that it shall be free from air. This may be done by isolating a fragment with the precautions just described and depositing it upon the surface of a test-tube of nutrient agar-agar. The contents of another tube, which have been liquefied, and allowed to cool almost to the point of gelatinisation, must then be poured over it. From a potato a little cube must be cut, the tissue deposited in the trough thus formed, and the cube replaced. Blood may also be taken directly from a vein by laying it bare by dissection, making a small section with sterilised scissors, and inserting an öse, the needle of a Pravaz' syringe, a capillary tube, or the extremity of the capillary neck of a Sternberg's bulb. If the cultivation is contaminated by the presence of putrefactive or other micro-organisms they must be isolated subsequently by carrying out a series of plate-cultivations.

Having completed the dissection, the organs of such a small animal as a mouse may be removed *en masse* and transferred to absolute alcohol for subsequent examination. In other cases it may be only necessary to reserve portions of each organ. In any case it should be remembered that with a virulent micro-organism, *e.g.*, anthrax, any remaining part of the animal should be cremated, and the hands and all instruments should be thoroughly disinfected.

Isolation of Micro-organisms from the Living Subject.—Micro-organisms in the living subject may be isolated from pus of abscesses, or other discharges, and from the blood and tissues. Abscesses should be opened, and other operations performed, when practicable, with Listerian precautions, and a drop of the discharge taken up with an öse or capillary pipette as already explained.

To make a cultivation from the blood of a living person, the tip of a finger must be well washed with soap and water and bathed with strong sublimate, or 1 in 20 carbolic, solution. Venous congestion is produced by applying an elastic band or ligature to the finger, which is pricked with a sterilised sewing needle. From the drop of blood which exudes the necessary inoculations and examinations can be made.

PART II.

SYSTEMATIC AND DESCRIPTIVE, WITH SPECIAL MICROSCOPICAL METHODS.

CHAPTER VIII.

HISTORY OF OUR KNOWLEDGE OF BACTERIA.

LEEUWENHOECK,* two hundred years ago, recognised, and described, microscopic organisms in putrid water and saliva, which probably correspond with organisms, such as vibrios and leptothrix of modern times. During two centuries these minute beings have afforded histologists a subject for controversy and dispute. Existing as they do upon the very borderland of the vegetable and animal kingdoms, not only have they been transferred from one to the other, but even the question has been raised whether the smaller forms should be considered as living beings at all.

In reviewing the history of the various classifications which have from time to time been proposed, we shall see that the gradual improvements in the means of studying such minute objects, the methods of cultivating them artificially, and of studying their chemistry and physiology, and the ever-increasing revelations of the microscope, have resulted in

* Leeuwenhoeck, *Op. Omnia* (Lugd. Batav., 1722).

establishing these microscopic objects as members of the vegetable kingdom, ranking among the lowest forms of fungi. While enabling us to settle their position as a whole, these improved methods have further given us so great an insight into the life-history of individual forms, that, with regard to the division into genera and species, we are up to the present time still in a position of doubt and uncertainty.

Müller, in 1773, was the first to suggest a classification. He established two genera, *Monas* and *Vibrio*, and grouped them with the *Infusoria*. In 1824 Bory de Saint Vincent also attempted a classification; but it was not until Ehrenberg in 1838, and Dujardin in 1841, worked at the subject, that a scientific distinction of species was attempted.

Ehrenberg described four genera:—

I. Bacterium .	. filaments straight, rigid.
II. Vibrio .	. filaments snake-like, flexible.
III. Spirillum .	. filaments spiral, rigid.
IV. Spirochæte .	. filaments spiral, flexible.

Dujardin united *Spirillum* and *Spirochæte*, and classed them thus:—

I. Bacterium .	filaments rigid, vacillating.
II. Vibrio .	. filaments flexible, undulatory.
III. Spirillum .	. filaments spiral, rotatory.

Up to that time bacteria were still considered as

Infusoria; but the year 1853 marked the commencement of a new era in their history, for Robin then pointed out the affinity of the *Bacteria* and *Vibrios* to *Leptothrix*. Davaine, in 1859, still more definitely insisted that the *Vibrios* were vegetables, and that they were in fact allied to the *Algæ*.

Since that time a flood of light has poured in upon the subject through the writings of Hoffmann, Pasteur, Cohn, Rabenhorst, Hallier, Billroth, Warming, Nägeli, Magnin, Marchand, Sternberg, Van Tieghem, Lister, Klein, Koch, Flügge, De Bary, Zopf, Cornil, Babes, and many other workers in the recent widespread revival of bacteriological research.

Of all these writers we are most indebted to Cohn,* not only on account of his researches, which extended over very many years, but also for his system of classification, which has since been almost universally adopted.

In his first classification, published in 1872, Cohn considered the Bacteria as a distinct group belonging to the *Algæ*, and divisible into four tribes, including six genera:—

I. Sphærobacteria	globules (Micrococcus).
II. Microbacteria .	short rods (Bacterium).
III. Desmobacteria .	long rods (Bacillus and Vibrio).
IV. Spirobacteria .	spirals (Spirochæte and Spirillum).

* Cohn, *Beiträge zur Biologie der Pflanzen*, 1872, *et seq.*

Cohn noted, in spite of placing them with the *Algæ*, that the absence of chlorophyll connected the *Bacteria* to *Fungi*, and we find Nägeli subsequently adopting this view, and employing the term *Schizomycetes*.

Billroth, in 1874, disputed the division into species, and considered that all the forms described by Cohn were but developmental forms of one micro-organism, *Coccobacteria septica*. In the following year Cohn answered the criticism of Billroth, and produced a second classification, in which he still maintained that distinct genera and species existed. The genera Cohn considered to be distinguished by definite differences in shape, which were adhered to throughout life, while some special feature, as a difference in size or physiological action, or some minute difference in form, determined the various species. Cohn illustrated, by his well-known comparison of a sweet and a bitter almond the appearances of which are similar but the properties very different, that a distinction into species might depend upon a difference in physiological action only. Others strongly support Cohn's views. By cultivating various micro-organisms through several generations, many conclude that a micrococcus cannot be transformed into a bacterium, or a bacterium into a bacillus or spirillum. Koch does not believe—and in this he is supported by Klein—that a bacillus can change its nature, and be converted

from a harmless into a pathogenic form, as asserted by Büchner.*

The second classification of Cohn (1875) only differed from the first in that, instead of keeping the bacteria as a separate group, he placed them, from their close relationship with the *Phycochromaceæ*, under a new group, the *Schizophytes*, and added the genera *Leptothrix*, *Beggiatoa*, *Crenothrix*, *Sarcina*, *Ascococcus*, *Streptococcus*, *Myconostoc*, and *Streptothrix*.

Nägeli maintained that *Bacteria* were allied to *Yeasts*, and should be included in the class of *Fungi*. In fact, he divided the *fungi producing decomposition* into :—

Mucorini	moulds
Saccharomycetes	yeasts
Schizomycetes	fission-fungi

(This last class comprising bacteria.)

Flügge,† following Rabenhorst, maintains the term *Schizomycetes*, and divides them as follows :—

* Büchner, *Ueber d. experim. Erzeugung d. Milzbrandcontagiums aus d. Heupilzen.*

† Flügge, *Fermente und Mikroparasiten.* 1883.

SCHIZOMYCETES (FLÜGGE).

Cells round or ovoid.	Isolated, or in chains, or united in amorphous gelatinous families		*Micrococcus.*
	Forming gelatinous families of definite form.	Colonies solid, filled with cells . .	In large numbers, in irregular colonies . . . *Ascococcus.*
			In small but definite numbers, in regular groups . . . *Sarcina.*
		Colonies, with simple layer of cells at the periphery.	*Clathrocystis.*

- Cells cylindrical.
  - Short, isolated, or in small heaps loosely united, or in irregular gelatinous families . . . . . *Bacterium.*
  - Long, cylindrical, forming filaments.
    - Filaments isolated, interlaced, or in bundles.
      - Without ramifications.
        - Straight filaments
          - Short, distinctly jointed . . *Bacillus.*
          - Long, indistinctly jointed
            - Very thin *Leptothrix.*
            - Thicker *Beggiatoa.*
        - Wavy, or in spirals
          - Short rigid . . *Spirillum (vibrio).*
          - Long flexile . *Spirochæte.*
      - Pseudo-ramifications . . . . *Streptothrix.* / *Clathrothrix.*
    - Cells shut in roundish gelatinous masses . . . *Myconostoc.*

The belief is nevertheless rapidly gaining ground that the lowest forms of vegetable life cannot be divided by a hard and fast line into a series with chlorophyll (*Algæ*), and a series without it (*Fungi*), and the tendency now is to solve the difference of opinion between Cohn and Nägeli by following the example of Sachs, and amalgamating the two series into one group, the *Thallophytes*.

Researches by competent observers have quite recently clearly demonstrated that several micro-organisms in their life cycle exhibit successively the shapes characteristic of the orders of Cohn. This had as early as 1873 been observed by Lister in a bacterium in milk. Lister detected forms of cocci, bacteria, bacilli, and streptothrix genetically connected. Among the recent observers Cienkowski and Neelsen have worked out the different forms assumed by the *bacillus of blue milk;* Zopf has in a similar manner investigated *Cladothrix*, *Beggiatoa*, and *Crenothrix*, and traced out various forms; Van Tieghem has investigated *Bacillus amylobacter* with a similar result; Klein and others have met with an involution-form of the filamentous growth of *Bacillus anthracis;* Hauser has quite recently described bacillar, spirillar, and spirulinar, and various other forms in the *Proteus mirabilis* and *Proteus vulgaris*. These facts obviously shake the very foundation of Cohn's classification, and we are left without possessing a sound basis for classification into

genera or species. The mode of reproduction is not sufficiently known to afford a better means for distinction than the other morphological appearancestaken alone; nor can we depend upon physiological action, which is held by many to vary with the change of form, according to altered surroundings.

Zopf, who has warmly supported the *pleomorphism* of bacteria, has suggested as a result of his investigations a division of the *Schizomycetes*, *Spaltpilze*, or *Fission-fungi*, into the following four groups :— *

1. **Coccaceæ.**—Possessing (so far as our knowledge at present reaches) only cocci, and thread forms resulting from the juxtaposition of cocci. The fission occurs in one or more directions.

Genera :—Streptococcus, Micrococcus, Merismopedia, Sarcina.

2. **Bacteriaceæ.**—Possessing mostly cocci, rods (straight or bent), and thread-forms (straight or spiral) The first may be absent, and the last possess no distinction between base and apex.

Division (as far as is known) occurs only in one direction.

Genera :—Bacterium, Spirillum, Vibrio, Leuconostoc, Bacillus, Clostridium.

3. **Leptotricheæ.**—Possessing cocci, rods, and thread-forms (which show a distinction between base and apex). The last straight or spiral.

* Zopf, *Die Spaltpilze*, 1885.

Genera :—Leptothrix, Beggiatoa, Crenothrix, Phragmidiothrix.

4. **Cladothricheæ.**—Possessing cocci, rods, threads, and spirals. Thread-forms provided with false branchings.
Genus :—Cladothrix.

Zopf, however, does not assert that all the fission-fungi exhibit this pleomorphism, nor does he pretend that his classification will include all the micro-organisms described. Cohn, on the other hand, was ready to admit that all the forms described by him were not truly independent species. These facts indicate that a need exists for a thorough investigation of the whole subject, and any classification at present can only be considered a provisional arrangement. In determining the distinctions into species we must take into account not only microscopical appearances of the micro-organisms themselves and their physiological action, but the character of their colonies in plate-cultivations under a low power of the microscope, and the macroscopical appearances displayed in the various nutrient media. In this way, by considering each individual characteristic, Koch showed that the comma bacillus of Finkler was a different organism from the bacillus which was present in Asiatic cholera.

Meanwhile in these pages we shall adopt the classification of Zopf. It not only serves in a practical handbook as a convenient form for

arranging the micro-organisms for reference, but it may lead the investigator to work upon the same lines as Zopf, and by tracing out the life-history of individual forms *in pure cultivations*, either to extend his work in establishing Protean species, or to restrict the doctrine of pleomorphism to a few forms.

CHAPTER IX.

SYSTEMATIC AND DESCRIPTIVE.

THE *Schizomycetes*, *Spaltpilze* or *Fission-fungi* have already been described as divisible, according to Zopf, into four groups; *Coccaceæ*, *Bacteriaceæ*, *Leptotricheæ*, and *Cladotricheæ*. They comprise the following genera and species:—

GROUP I.—COCCACEÆ.

Genus I. *Streptococcus* (Chain-cocci).—Division in one direction only. Individual cocci remain united together to form chains.

Genus II. *Merismopedia* (Plate-cocci).—Division in two directions, forming lamellæ or plates.

Genus III. *Sarcina* (Packet-cocci).—Division in three directions, forming colonies in cubes or packets.

Genus IV. *Micrococcus* (Mass-cocci).—Division in one direction, cocci after division remain aggregated in irregular heaps or botryoidal masses.

Genus V. *Ascococcus* (Pellicle-cocci).—Like micrococcus, but the cocci grow in gelatinous pellicles.

Genus I. Streptococcus (Zopf).

Streptococcus pyogenes, Rosenbach (*Chain micrococci in pus: Coccus of Pyæmia*).—Cocci, singly

and in chains; occur in acute abscesses. Inoculation of guinea-pigs or mice with pus containing these cocci, or with a pure cultivation of the same, causes suppuration at the site of injection, and death with symptoms of blood-poisoning. In the blood, in the tissue around the abscess, and in the pus the cocci are found singly or in zoogloœa or chains. Their appearances in cultivation-media have been very minutely described.* Cultivated in a streak on the surface of nutrient gelatine on a glass plate, they form at first whitish, somewhat transparent, rounded spots, of the size of small grains of sand. They develop but slightly on this medium, even at the highest temperature attainable without liquefying the gelatine. On nutrient agar-agar they grow most energetically at a temperature of 35° — 37° C. On this medium also, they show a tendency to form little spots, which finally become about the size of a pin's head. If a streak is made with a needle well charged with a fresh cultivation, growth in a continuous line is obtained, but still showing an inclination to form centres. In its further development the middle of the cultivation is heaped up, and presents a pale brownish coloration, while the periphery is flattened, except at the extreme margin, which is again raised up, and often with a spotted appearance. Still later, the periphery develops successive

* Rosenbach, *Mikro-organismen bei den Wund-Infections-Krankheiten des Menschen.* 1884.

layers or terraces. The growth is so slow that in two to three weeks the maximum width of the culture-streak is about 2 to 3 mm. On solidified blood serum the cocci grow as on agar-agar. They do not liquefy any nutrient medium. In a vacuum they rapidly cause the decomposition of white of egg or beef which are energetically peptonised.

Streptococcus erysipelatis.—Minute cocci ·4 μ to ·3 μ occur in chains in human erysipelatous skin, and in the fluid of erysipelatous bullæ. They occupy the lymphatic channels of the skin, and spread along them as the disease progresses.*

They can be cultivated artificially in nutrient gelatine or agar-agar, and produce typical erysipelas when re-inoculated in man or animals.† The characteristic erysipelatous blush is produced by inoculating these micro-organisms in the ear of a rabbit. In the human subject the disease was produced in fifteen to sixty hours after inoculation. A beneficial result was obtained in cases of lupus, cancer, and sarcoma, this being the object for which the latter inoculations were undertaken.‡

The appearances of cultivations very strongly resemble those already described in *streptococcus pyogenes*. There is less tendency, however, to the formation of terraces, the edge of the growth is

* Lukomsky, *Virch. Archiv*, vol. lx.
† Orth, *Archiv für exp. Pathol. u. Pharmacol.*, Bd. i., 1873.
‡ Fehleisen, *Aetiologie des Erysipels.* 1883.

thicker and more irregular, and the appearance of the streak is more opaque and whiter.*

Streptococcus diphtheriticus.—Oval cocci, ·35 to 1·1 μ in diameter, have been described as characteristic of diphtheria.† The cells lie singly, in pairs or in rosaries, and in spherical or cylindrical masses in diphtheritic membranes and the surrounding connective and muscular tissues. From the point of infection they can be traced along the lymphatics, and are found in the blood, heart, liver, kidneys, and other organs. In severe cases the blood capillaries and uriniferous tubules are blocked up.

The attempt to get pure cultivations in nutrient media and to make inoculation experiments has not yet succeeded; a bacillus and a bacterium have also been described as the specific micro-organisms of diphtheria (p. 136).

Streptococcus of Progressive Tissue Necrosis in Mice.‡—Cocci ·5 μ in diam., in chains or rosaries, and zoogloœa; their invasion causes tissue necrosis with destruction even of cartilage cells, and spreading from the point of inoculation, causes death in about three days. The cocci are absent from the blood and internal organs. These observations were made after the injection of putrid fluids in the ear of mice, and

* Rosenbach, *ibid.*

† Oertel, *Deutches Archiv f. Klin. Med.* 1871.

‡ Koch, *Untersuch. über die Aetiologie d. Wundinfections krankheiten.* 1878.

a pure infection was obtained by the inoculation of field-mice, which have an immunity from bacillary septicæmia.

Streptococcus bombycis, Béchamp, (*Microzyma bombycis*).—Oval cocci ·5 μ in diam., singly, in pairs, or chains. They occur in the contents of the alimentary canal, and in the gastric juice of silkworms suffering from "*flacherie*" ("*Maladie de morts blancs*," "*flaccidezza*," "*schlaffsucht*").

Streptococcus vaccinæ. — Cocci, ·5 μ in diam., singly, in pairs, and in long or short chains, and colonies. They are found in the fresh lymph* of human and cow-pox, and in the pustules of true small-pox. They are regarded as the active principle of vaccine lymph, since filtration deprives the latter of its infectious element.† The lymphatics of the skin in the region of the pustule of both human and sheep-pox are filled with cocci. Successful vaccination has been stated to result from artificial cultivations.‡

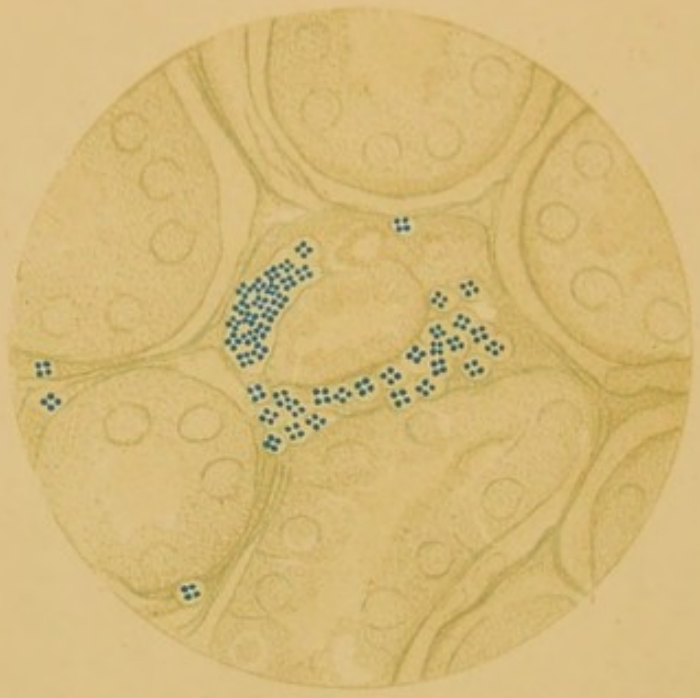
Streptococcus perniciosus (*Parrot disease*). —Cocci, singly, in chains, and in zoogloœa have been described in connection with a disease of the grey parrot (Psittacus erithacus).§ This disease is fatal to about 80 per cent. of these parrots imported to Europe. They suffer from diarrhœa

* Cohn, *Virchow's Archiv.* 1872.

† Chauveau, *Comptes Rendus*, 1868, and Burdon Sanderson, *Reports on the Intimate Pathology of Contagion.*

‡ Quist, *St. Petersburgh Med. Wochenschr.* 1883.

§ Wolff, *Virchow's Archiv.* 1883.



MICROCOCCUS TETRAGONUS.

Fig. 1. From a section of Kidney of a mouse. Gram's method. Zeiss' $\frac{1}{18}$ o. i. Oc. 4.

MICROCOCCUS PYOGENES AUREUS.

Fig. 2. From a section of Liver of a rabbit. Gram's method. Zeiss' $\frac{1}{18}$. o. i. Oc. 4.

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and general weakness; their feathers are ruffled, their wings hang loosely, and their eyelids close; convulsions set in, and death follows. At the autopsy greyish nodules are found in the lungs, liver, spleen, and kidney; in and around the capillaries of these nodules, and in the blood of the heart, the cocci are found in great numbers in zoogloea, and more rarely in chains. Inflammatory change in the surrounding tissue is absent.

Genus II. Merismopedia.

Merismopedia Gonorrhoeæ (*Coccus of Gonorrhœa*).—Cocci 0·83 μ in diam., singly, in pairs, or tetrads, or zoogloea groups. They are found in gonorrhœal pus adhering to the pus corpuscles and epithelial scales. Artificial cultivations have been carried out,* and the pathogenic character of the cocci established by inoculation.

Micrococcus tetragonus.—Cocci about 1 μ in diam., in groups of four (tetrads), surrounded by a hyaline capsule. They are found in the sputa of phthisical patients and in the walls of tubercular cavities. In a test-tube of nutrient gelatine they form an irregular white growth, more especially in the upper part of the needle track (Plate IV., Fig. 1). On the sloping surface of nutrient agar-agar thick, whitish, heaped-up masses develop. Guinea-pigs and mice inoculated with a

* Bockhart, *Sitzungsberichte der Phys. Med. Gesell. Würzburg.* 1882.

minute quantity of a pure cultivation die in two to ten days, and the groups of the characteristic tetrads may be found in the capillaries throughout the body, especially in the spleen, lung, and kidney (Plate XII., Fig. 1).

Double infection can be produced by inoculating a mouse with a pure cultivation of bacillus anthracis two or three days after inoculation with micrococcus tetragonus. On examination after death the capillaries of the lungs, liver, and kidney are filled with both anthrax bacilli and masses of tetrads* (Plate XVI., Fig. 2).

Genus III. Sarcina.

Sarcina ventriculi, Goodsir.†—Cocci reaching 4 μ in diam., united in groups of four, or multiples of four, producing cubes or packets with rounded-off corners. Contents of the cells are greenish or yellowish-red. They occur in the stomach of man and animals in health and disease, and were first detected in vomit.

Sarcina intestinalis, Zopf.‡—Cocci in groups of four or eight. Very regular in form; never in the large packets which occur with Sarcina ventriculi. They are found in the intestinal canal, especially the cæcum, of poultry, particularly fowls and turkeys.

Sarcina lutea.—Cocci singly, in pairs, in

* Crookshank, *Notes from a Bacteriolog. Laboratory. Lancet*, 1885.

† *Edinburgh Med. and Surg. Journal.* 1842.

‡ Zopf, *Die Spaltpilze.* 1885.

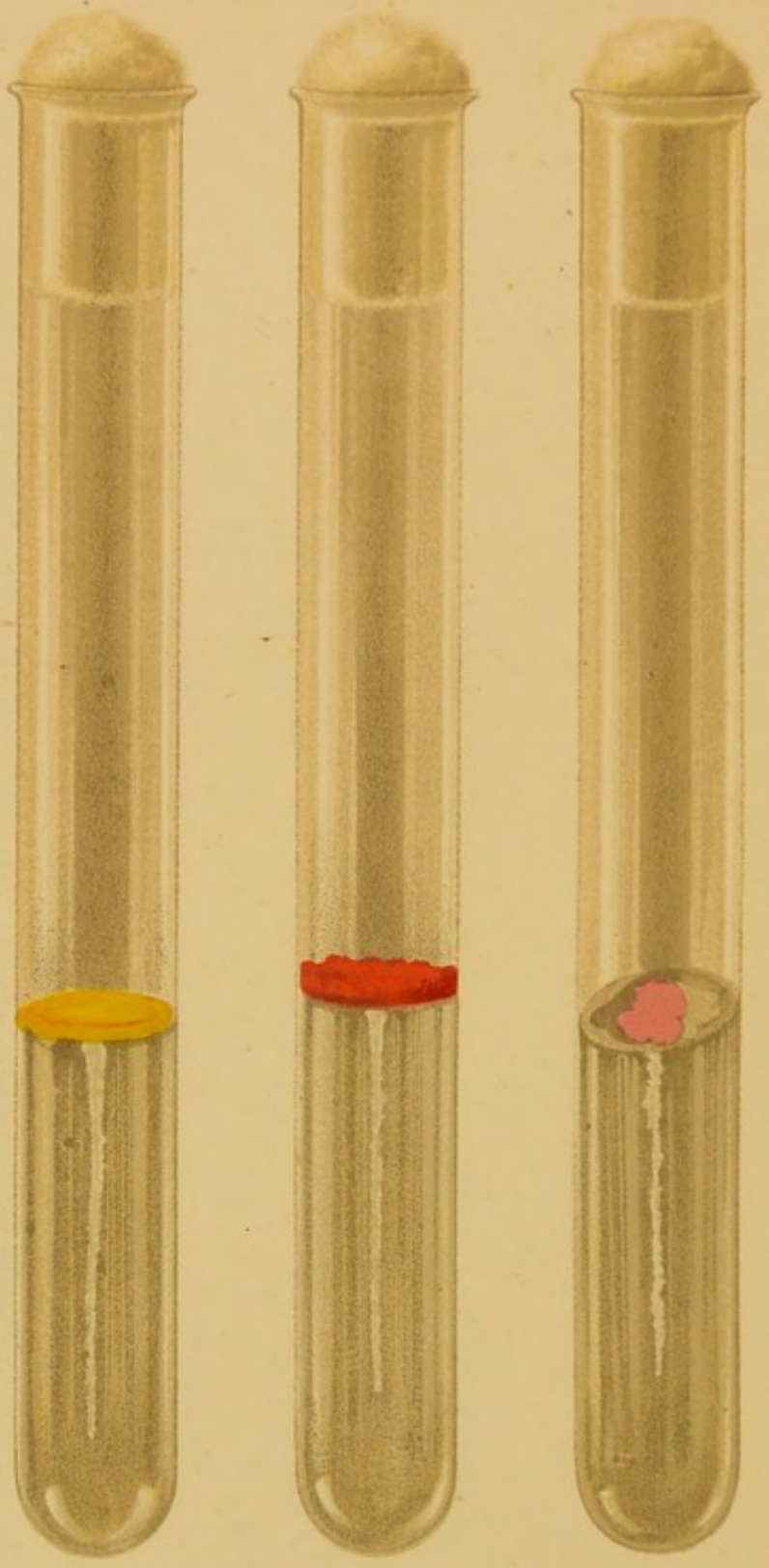

Fig 1. Fig 2. Fig 3.

CULTIVATIONS IN NUTRIENT AGAR-AGAR

Fig 1. Sarcina lutea.
Fig 2. Micrococcus indicus.
Fig 3. Saccharomyces rosaceus.

H. Cruickshank Pinx.

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tetrads, and in packets. A single individual in a tetrad may be divided into two, or into four, so that a tetrad within a tetrad results. Cultivated in nutrient agar-agar in a test-tube, they form a colourless growth along the track of the needle, and a bright, canary-yellow layer upon the surface, where they have access to the air (Plate XIII., Fig. 1, Plate VIII., Fig. 1). In plate-cultivations the colonies are round, slightly granular in appearance, and yellow. Cultivated in a test-tube containing nutrient gelatine, they grow rapidly, the gelatine becoming liquid, the yellow growth forms a wad about the middle of the tube (Plate V., Fig. 2), or, liquefying the whole of the gelatine, subsides to the bottom of the test-tube. Cultivated on sterilised potatoes they form a yellow layer (Plate X., Fig. 1). In drop-cultures in bouillon the subdivision into tetrads within tetrads and formation of groups of 8, 16, and 24 can be studied (Plate I., Fig. 7). Inoculation of mice produces negative results. The cocci are occasionally present in the air.

Sarcina urinæ, Welcker.—Very small cocci; 1·2 μ in diam., united in families of 8 to 64. Observed in the bladder.

Sarcina litoralis, Oersted.—Cocci 1·2—2 μ in diam., bound together in 4 to 8 families, which, in their turn, may unite and include as many as 64 tetrads. Plasma colourless; in each cell 1—4 sulphur granules. Discovered in sea-water containing putrefying matter.

Sarcina Reitenbachii, Caspary.—Cocci about 1·5 to 2·5 μ in diam., at the time of division lengthened to 4 μ. Mostly united together from 4 to 8 in number; occasionally 16 or more. Colourless cell-wall, lined with rose-red layer of plasma. Found on rotting water plants.

Sarcina hyalina, Kützing.—Cocci round, 2·5 μ in diam., almost colourless. United in families of 4 to 24 cells, reaching 15 μ in diam. In marshes.

Genus IV. Micrococcus.

Micrococcus pyogenes aureus (*Staphylococcus pyogenes aureus* Rosenbach. *Yellow coccus in pus. Coccus of acute infectious osteomyelitis*).—Cocci singly, or aggregated in masses. Cultivated on nutrient agar-agar an orange-yellow culture develops, looking like a streak made with oil paint* (Plate VIII., Fig. 2). Cultivated in a test-tube of nutrient gelatine, the gelatine is rapidly liquefied, and the growth subsides as an orange-yellow sediment. On potatoes and blood serum a similar orange-yellow culture grows luxuriantly.

The micro-organisms injected into the pleura or knee of a rabbit produce, as a rule, a fatal result on the following day, but if it survives longer, it eventually dies of severe phlegmon. If injected into the knee of a dog, suppuration occurs, followed by disintegration of the joint. The cocci do not cause any septic odour in pus, nor does

* Rosenbach, *ibid.*

any gas develop. Albumen is converted by their action into peptones.

They occur in the pus of boils and in the abscesses of pyæmia, puerperal fever, and acute osteomyelitis. Injected into the peritoneal cavity of animals, they set up peritonitis, and introduced into the jugular vein they produce septicæmia and death. When a small quantity of a cultivation was introduced into the jugular vein after previous fracture or contusion of the bones of the leg, the animal died in about ten days, and abscesses were found in and around the bones, and in some cases in the lungs and kidneys. Similar cocci were found in the blood and pus of the animals.*

Micrococcus pyocyaneus, Gessard. — Cocci ellipsoidal. Cultivated in sterilised nourishing solution, they colour it blue, especially on the surface. The deeper part of the medium becomes decolorised again by the action of the micro-organism, but when shaken with air develops the colour. The pigment formed by the cocci is a definite principle, pyocyanin.† It can be extracted with chloroform from the pus and washings of bandages; it is soluble in acidulated water, which it colours red. In neutral solution it becomes blue. It crystallises in chloroform in long needles, and forms sometimes lamellæ and prisms.

* Becker, *Deutsche Med. Wochenschr.*, Nov. 1883.

† Gessard, *De la pyocyanine et de son microbe. Paris thèse.* 1882.

The cocci are present in the pus of those cases in which the wounds and pus-stained bandages exhibit a green-blue or blue colouring.

Micrococcus coleræ gallinarum (*Bacterium of Fowl-cholera. Microbe du choléra des poules*).—Cocci 2—3 μ in diam., sometimes united in pairs, and then presenting a figure of 8 appearance. In the tissues they appear as rods 2 to 3 μ in length and 5 μ in diam., with their extremities stained more deeply than their middle* (*vide* p. 130). When cultivated by introducing a drop of the infected blood into sterile chicken-broth, a number of round bodies, undergoing rapid movement and as a rule united as diplococci or elongated and contracted in the middle, appear in the broth, which is at first slightly milky, but becomes limpid, and the microbes at the same time pass into a finely granular state. From this, however, fresh cultures can still be started. Cultivated in a test-tube of nutrient gelatine, after from three days to a week there develops along the needle track a fine, almost imperceptible, greyish thread without liquefaction of the gelatine (Plate III., Fig. 2). The growth is exceedingly scanty, even after several weeks.

Fowls suffering from the disease usually die very rapidly. In the less acute cases they are somnolent, weak in their legs, and their wings trail. They suffer from diarrhœa, and pass into a state of sopor and die. The micro-organisms are found in large

* Cornil and Babes, *Les Bacteries*. 1885.

numbers in the blood and organs after death, and in the intestinal discharges.

A drop of the broth injected into the connective tissue in the region of the pectoral muscles causes the death of the fowl the following day, with characteristic pathological changes.* If a culture be kept for some time, and a fowl be then inoculated with it, instead of death only local changes are produced, and the fowl is protected against the action of a virulent culture; thus affording an example of so-called *mitigation of the virus*.† The microbe is ærobic, and its toxic effect is said to be due to the abstraction of oxygen from the blood producing asphyxia.‡

Micrococcus prodigiosus (*"Blood-rain," "Bleeding host," Pilz der rothen Milch*).—Cocci slightly oval, ·5—1 μ in diam., forming at first rose-red, and then blood-red zoogloea. They grow luxuriantly when cultivated on sterilised potatoes (Plate IX., Fig. 1), and on the sloping surface of nutrient agar-agar (Plate II., Fig. 3). They appear occasionally on bread, boiled rice, and starch-paste, and more rarely on boiled white of egg and meat. Milk sometimes becomes coloured blood-red by the growth of this fungus, an appearance formerly attributed to a disease of the cow.

* Cornil, *Observ. hist. sur les lésions des muscles déterminées par l'injection du microbe du choléra des poules* (*Archives de Physiologie*, 1882), and Cornil and Babes, *Les Bacteries*. 1885.

† Pasteur, *Sur le choléra des poules, Compt. Rend.* 1880.

‡ Cornil et Babes, *Les Bacteries*. 1885.

In Paris, in 1843, the fungus was peculiarly prevalent, attacking especially the bread produced in the military bakehouses.

The cocci themselves are colourless. The colouring matter resembles fuchsine; it is insoluble in water, but soluble in alcohol. The addition of acids changes it to a carmine red, and of alkalies to a yellow colour.

Micrococcus of septicæmia in rabbits, Koch.*—Ellipsoidal cocci ·8—1 μ in largest diam. The disease was produced by the injection of putrid meat infusion. After death slight œdema was noted at the site of injection, slight extravasation of blood, and great enlargement of the spleen. No emboli or peritonitis resulted. Masses of cocci were found in the capillaries of different organs, especially in the glomeruli of the kidneys. Rabbits and mice inoculated with blood from the heart proved susceptible to the disease.

Micrococcus of pyæmia in rabbits, Koch.†—Round cocci and diplococci ·25 μ in diam. The disease was produced by the subcutaneous injection in a rabbit of distilled water, in which the skin of a mouse had been macerated. At the autopsy there were found great infiltration around the site of injection, peritonitis, and accumulations in the liver and lungs; in short, the appearances of pyæmia. In the capillaries of the

* Koch, *Wundinfect. Krankheit,* 1878.
† Koch, *ibid.*

organs examined, masses of cocci were observed enclosing blood corpuscles. Fresh inoculations in rabbits with exudation-fluid, or blood from the heart, reproduced the same disease.

Micrococcus of progressive suppuration in rabbits, Koch.*—Cocci only about ·15 μ in diam., principally in thick zooglœa. The disease was induced by the injection into rabbits of decomposing blood. At the place of injection a spreading abscess formed, which was fatal to the animal in about twelve days. No bacteria were observed in the blood, but in the walls of the abscess thick masses of cocci were found. The pus is infectious, causing the same disease in healthy rabbits.

Micrococcus aurantiacus, Schröter.—Cocci, oval, 1·5 μ in diam., singly or in pairs, or in zooglœa. They occur as orange-yellow spots which coalesce into patches. A golden-yellow pellicle develops when they are cultivated in nutrient liquids. The colouring matter is soluble in water. They were observed on boiled potatoes and white of egg.

Micrococcus chlorinus, Cohn.—Cocci occur in the form of a finely granular zooglœa, causing a yellowish green or sap-green layer on boiled eggs and nourishing solutions. The colouring matter is soluble in water and is decolorised by acids.

Micrococcus violaceus, Schröter.—Cocci elliptical, larger than *M. prodigiosus*, uniting into

* Koch, *Wundinfect. Krankheit*, 1878.

violet blue gelatinous spots, which again unite to form larger patches. They occasionally grow on boiled potatoes exposed to the air.

Micrococcus luteus, Schröter.—Cocci somewhat larger than *M. prodigiosus*, elliptical, with highly refractive cell contents. They form yellow drops of 1—3 mm. diam. on boiled potato, and a thick, wrinkled, yellow skin on nutrient liquids. The colouring matter is insoluble in water, and unchanged by sulphuric acid or alkalies.

Micrococcus fulvus, Cohn. — Cocci round 1·5 μ in diam., frequently in pairs. They form rusty-red conical drops of a firm consistency, and about ·5 mm. diam., on horse dung.

Micrococcus hæmatodes, Zopf. — Cocci, which cultivated on boiled white of egg in a damp chamber in the incubator, form a red layer. The reaction of the colouring matter is similar to that produced by *M. prodigiosus*. They have been observed in human sweat, especially from the axilla, colouring the surrounding parts and the linen an intense brick or blood-red colour.*

Genus V. Ascococcus.

Ascococcus Billrothii.—Small globular cocci, united into characteristic colonies. They form on the surface of nourishing fluids a cream-like skin, divisible into an enormous number of globular or oval families. Each family is surrounded by a

* Babes, *Vom rothen Schweiss. Biol. Centralbl.*, Bd. 2, 1882.

thick capsule of cartilaginous consistency. In a solution containing acid tartrate of ammonia the fungi generate butyric acid, and change the originally acid fluid into an alkaline one. They were first observed on putrid broth, and later on ordinary nourishing solutions; they also readily develop upon damp slices of boiled roots, carrots, beetroots, etc.

Methods of Staining Cocci.

Cocci stain well with watery solutions of gentian-violet, methyl-violet, fuchsine, methylene blue, and bismarck brown. For examining cocci in liquids such as pus or blood, or in cultivations in solid media, a little of the material should be spread out on a cover-glass [page 46], and stained with a drop or two of a watery solution of fuchsine or methyl-violet. The former is especially recommended for staining *Merismopedia gonorrhœæ*.

For a zooglœa, or pellicle of micrococci Klein recommends transference bodily to a watch-glass containing the dye, leaving it there till deeply tinted, then taking it out with a needle, washing in water, and then in alcohol till excess of colour is removed. It must then be transferred to a glass-slide, spread well out, and a drop of clove-oil placed on it; after a minute or two the clove-oil is drained off, a drop of Canada balsam added, and covered with a cover-glass.*

Cocci in the tissues may be stained by immersing the sections in an aqueous solution of gentian violet, or in aniline-gentian-violet solution, then rinsing in water, decolorising in alcohol, treating with clove-oil, and preserving in balsam (p. 55); or, after washing with alcohol they

* Klein, *Micro-organisms and Disease*, 1885.

may be rinsed with water, and stained for half an hour with Weigert's picrocarmine. From this they are again removed to water, then to alcohol, clove-oil, and Canada balsam.

The method of Gram is much more satisfactory (p. 56, Plate XII., Fig. 2). Sections should be examined with and without a contrast stain. The *after-stain* most commonly employed is eosin. The sections after the process of decolorisation should be placed in a weak alcoholic solution of eosin (two or three drops of a concentrated alcoholic solution added to a watch-glass full of alcohol), till stained a delicate pink. They are then rinsed in fresh alcohol, treated with clove-oil, and preserved in Canada balsam.

Sections containing cocci of osteomyelitis may be *after-stained* with weak solution of vesuvin. Safranine and picro-lithium-carmine may also be used as contrast stains (p. 58).

Nuclear stains, such as carmine, hæmatoxylin, may also be employed. Sections may be left one minute in Grenacher's solution, then washed out in weakly acidulated alcohol (2—1000); and finally treated in the usual way, with alcohol, oil of cloves, and balsam.

Sections containing *micrococcus tetragonus* are best stained with Gram's method and eosin (Plate XII., Fig. 1), but they may also be treated by the method of Friedländer, to demonstrate their capsules (p. 135).

To stain the cocci of *rabbit-septicæmia* and of *chicken-cholera* in the tissues, place the sections twenty-four hours in Löffler's solution, wash in water faintly acidulated with acetic acid, then treat with alcohol, oil of cloves, and balsam. Babes recommends staining the microbe of chicken cholera by immersing the sections for twenty-four hours in a concentrated solution of methyl-violet B, or of safranine.*

* Cornil and Babes, *Les Bacteries*, 1885, p. 208.

Group II.—Bacteriaceæ.

Genus I. *Bacterium.*—Cocci and rods, or only rods, which are joined together to form threads. Spore-formation absent or unknown.

Genus II. *Spirillum.*—Threads screw-form, made up of rods (long or short) only, or of rods and cocci. Spore-formation absent or unknown.

Genus III. *Leuconostoc.*—Cocci and rods. Spore-formation present in cocci.

Genus IV. *Bacillus.*—Cocci and rods, or rods only, forming straight or twisted threads. Spore-formation present either in rods or cocci.

Genus V. *Vibrio.*—Threads screw-form in long or short links. Spore-formation present.

Genus VI. *Clostridium.*—Same as bacillus, but spore-formation takes place in charactreistically enlarged rods.

Genus I. Bacterium.

Bacterium ureæ (*Micrococcus ureæ*, Cohn).—Cocci 1·25—2 μ in diam., singly or in chains, and rods. The rods split up by division into chains of cocci, and the latter are finally set free. The cocci increase further by subdivision, and a jelly-like membrane develops around them. Masses of cocci exist in the form of irregular or roundish lumps. Cultivations, after twenty-four hours, consist exclusively of rods, after forty-eight hours, of cocci-chains, and, in fourteen days, of zoogloœa; the cocci transplanted into fresh nourishing solution again grow

into rods. These observations point to the existence of a pleomorphic species, *Bacterium ureæ*, and the former nomenclature *micrococcus ureæ* must be regarded as untenable. They are ærobic; occurring in urine they set up ammoniacal fermentation, converting urea into carbonate of ammonia.*

Bacterium aceti.—Cocci, short rods, long rods, leptothrix-forms, and zooglœa. Cocci and short rods may occur in the same thread. The long rods and threads may develop irregular swellings, so-called involution-forms, which have a thickened membrane and a grey colour. The effect of the action of this microbe is to oxidise alcohol in wine and other fruit juices into vinegar. The masses of zooglœa united together form a membranous layer which must not be mistaken for the pellicle formed by *Saccharomyces mycoderma*. The latter prepares the medium for the action of the bacterium aceti.

Bacterium Pasteurianum, Hansen.—Morphologically similar to *Bacterium aceti*, but the cells contain a starch-like substance, which is turned blue by iodine. They occur in beer-wort.

Bacterium Zopfii, Kurth.—Cocci 1—1·25 μ in diameter, rods and threads. Cultivated in a streak on nutrient gelatine spread out on a glass slide, a peculiar development takes place. In twenty-four hours after inoculation threads have developed, in forty-eight hours, windings of the threads are

* Zopf, *Die Spaltpilze*, p. 62, 1885.

observed, and in six days the threads have broken up into cocci. They were observed in the intestine of fowls, especially in the contents of the vermiform appendix. Inoculation of rabbits is followed by negative results.

Bacterium merismopedioides, Zopf.—Forms threads 1—1·5 μ in thickness; these subdivide into long rods, short rods, and finally into cocci. The cocci divide first in one and subsequently in two directions, forming characteristic groups, which appear like merismopedia. These groups may eventually consist of 64 × 64 cells or more, and ultimately form zooglœa. The cocci develop again into rods and threads. They were observed in water containing putrefying substances (River Panke, Berlin).*

Bacterium pneumoniæ crouposæ (Plate I., Fig. 5).—Cocci ellipsoidal and round, singly, in pairs (diplococci), rods and thread forms. The cell membrane thickens, and develops into a gelatinous capsule, which is round if the coccus is single, and ellipsoidal if the cocci occur in pairs or in rod forms. Cultivated in a test-tube of nutrient gelatine it grows along the needle track in the form of a round-headed nail (Plate IV., Fig. 2) without liquefaction of the gelatine. The cocci when artificially cultivated have no capsule, but it again appears after their injection into animals. The coccus can also be cultivated on blood serum and on boiled potatoes. They occur in pneumonic exuda-

* Zopf, *Die Spaltpilze*, p. 66, 1885.

tion.* Inoculation of dogs with a cultivation of the cocci occasionally gave positive results; but in rabbits no results followed. Guinea-pigs proved to be susceptible in some cases, but thirty-two mice, after injection of a cultivation diffused in sterilised water into the lungs, died without exception. The lungs were red and solid, and contained the cocci, which were also present in the blood, and in enormous numbers in the pleural exudation. Inhalation experiments by spraying the cocci diffused in water into mouse cages succeeded in producing pneumonia and pleurisy in three out of ten mice. The nail-shaped cultivation is not always produced, nor are these conclusions accepted by all investigators.†

METHODS OF STAINING THE BACTERIA OF PNEUMONIA.

(*Pneumonie-Coccen, Friedländer.*)

Cover-glass-preparations (p. 46) of pneumonic sputum or exudation may be treated as follows :—

(*a*) Stain by the method of Gram, and after-stain with eosin (p. 48).

(*b*) Treat with acetic acid, then stain with gentian-violet or bismarck-brown. Examine in distilled water, or dry and preserve in Canada balsam.

(*c*) Float them on weak solutions of the aniline dyes twenty-four hours; differentiation between coccus and capsule is thus obtained.

(*d*) Stain with osmic acid; the contour of the capsules is brought out.

* Friedländer, *Fortschr. d. Med.*, 1883.

† Klein, *Micro-organisms and Disease*, 1885.

Sections of pneumonic lung should be stained by

(*a*) Method of Gram.

(*b*) *Method of Friedländer.* This method is employed to demonstrate the capsules in tissue sections. It consists in placing the sections twenty-four hours in the following solution :—

Fuchsine	1
Distilled water	100
Alcohol	5
Glacial acetic acid . . .	2

They are then rinsed with alcohol, transferred for a couple of minutes to a 2 per cent. solution of acetic acid, and in the usual way treated with alcohol and oil of cloves, and preserved in Canada balsam.

Bacterium Pflügeri, Ludwig.—Large, round cocci, mostly in zooglœa, and thread-forms composed of rods. They can be cultivated on boiled white of egg and potatoes. They were observed to produce phosphorescence in putrid fish and meat.

Bacterium ianthinum, Zopf.—Long and short rods, which aggregate in swarms, and finally break up into cocci. Observed on pieces of pig's bladder floated on the surface of water, rich in bacteria, from the river Panke. They formed spots of an intense violet colour, measuring from 1—10 mm. They occurred only on the surface of the bladder exposed to the air, and never on the part under water. The colouring matter is soluble in alcohol.

Bacterium synxanthum, Ehrenberg (*xanthinum. Bacterium of yellow milk*).—Cocci ·7—1 μ long, and rod-forms.* They are actively motile,

* Zopf, *Die Spaltpilze.*

and differ very little from *Bacterium termo.** They produce a yellow colour in boiled milk, which at first becomes acid, and then strongly alkaline. They also occur on boiled potatoes, carrots, etc., where they form small lemon-yellow masses. The colouring matter soluble in water, insoluble in ether and alcohol, unchanged by alkalies, decolorised by acids. It is similar to yellow aniline colours both spectroscopically and in ordinary reactions.

Bacterium of Diphtheria in Man and Pigeons.—Elongated cocci, or short, stout rods, mostly twice as long as broad. Inoculation with cultivations from a human source on nutrient gelatine and potatoes produced the disease in pigeons, mice, and rabbits. A bacillus has also been isolated from diphtheritic membranes; *vide* also *Streptococcus diphtheriticus* (p. 117).

Panhistophyton ovatum, Lebert (*Nosema bombycis*, *Micrococcus ovatus*, *Corpuscles du ver à soie*).—Shining oval cocci, 2—3 μ long, 2 μ wide, singly and in pairs, or masses;† or rods, 2·5 μ thick and twice as long.‡ They multiply by subdivision. They were experimentally proved to be the cause of *pébrine*, *gattine*, *maladie des corpuscles*, or *flecksucht;* and were discovered in the organs of diseased silkworms, as well as in the pupæ, moths, and eggs.

* Flügge, *Fermente und Mikro Parasiten.* 1883.
† *Ibid.*
‡ Zopf, *Die Spaltpilze.*

Genus II. Spirillum.

Spirillum choleræ Asiaticæ, (*Comma-bacillus*) Koch.—Curved rods, spirilla, and threads (Plate I., Fig. 18). The curved rods or commas are about half the length of a tubercle-bacillus. They occur isolated, or attached to each other forming S-shaped organisms or longer screw-forms; the latter resembling the spirilla of relapsing fever. Finally they may develop into spirilliform threads. In old cultivations threads are found with bulgings or irregularities, which may be involution-forms (Plate I., Fig. 35).* The commas are actively motile; their movements and development into spirilla may be studied in drop-cultivations. In plate-cultivations, at a temperature of from 16°—20° C., the colonies develop as little specks, which commence to be visible after about twenty-four hours. Examined with a low-power, and a small diaphragm, these colonies have the following characteristics. They appear as little masses, granular, and of a very faintly yellowish red tinge. They liquefy the gelatine, and sink down in a little excavation, at the bottom of which one recognises the punctiform colony.

In test-tubes of slightly alkaline nutrient gelatine (10 p. c.), the appearance is strikingly distinctive. The cultivation commences to be visible in about

* Compare also Van Ermengem, *Recherches sur le Microbe du Chol. Asiat.* 1885

twenty-four hours. Liquefaction sets in very slowly, commencing at the top of the needle track around an enclosed bubble of air, and forms a funnel continuous with the lower part of the growth (Plate III., Fig. 1); the latter preserves for several days its resemblance to a white thread (Figs. 33 and 34).* In about eight days, however, this too is liquefied, with the exception of the deepest part of the needle track, which disappears only at the moment when all the rest of the gelatine is liquefied.

On a sloping surface of agar-agar the cultivation develops in the form of a white, semi-transparent *plaque*, with well-defined margin, and the liquid which is found at the bottom of the oblique surface becomes milky. In potato-cultivations the microbe will only grow at the temperature of the blood (37° C.), forming a slightly brown, transparent layer. Inoculation of a cultivation of the bacillus in the duodenum of guinea-pigs, with † and without ‡ ligation of the bile duct, has given positive results. More recently these results have been confirmed by the following method. A 5 per cent. solution of potash was injected into the stomach of guinea-pigs, and twenty minutes after, a cultivation of comma-bacilli diffused in broth was similarly in-

* Reprinted from *Remarks on the Comma-Bacillus of Koch. Lancet*, 1885.

† Nicati et Rietsch, *Communication à l'Academie de Médecine.* 1884.

‡ Van Ermengem, *Le Microbe du Choléra Asiatique.* 1885.

troduced. Simultaneously with the latter, an injection of tincture of opium was made into the abdominal cavity, in the proportion of 1 ccm. for every 300 grammes' weight of the animal. Those who have had success with inoculation experi-

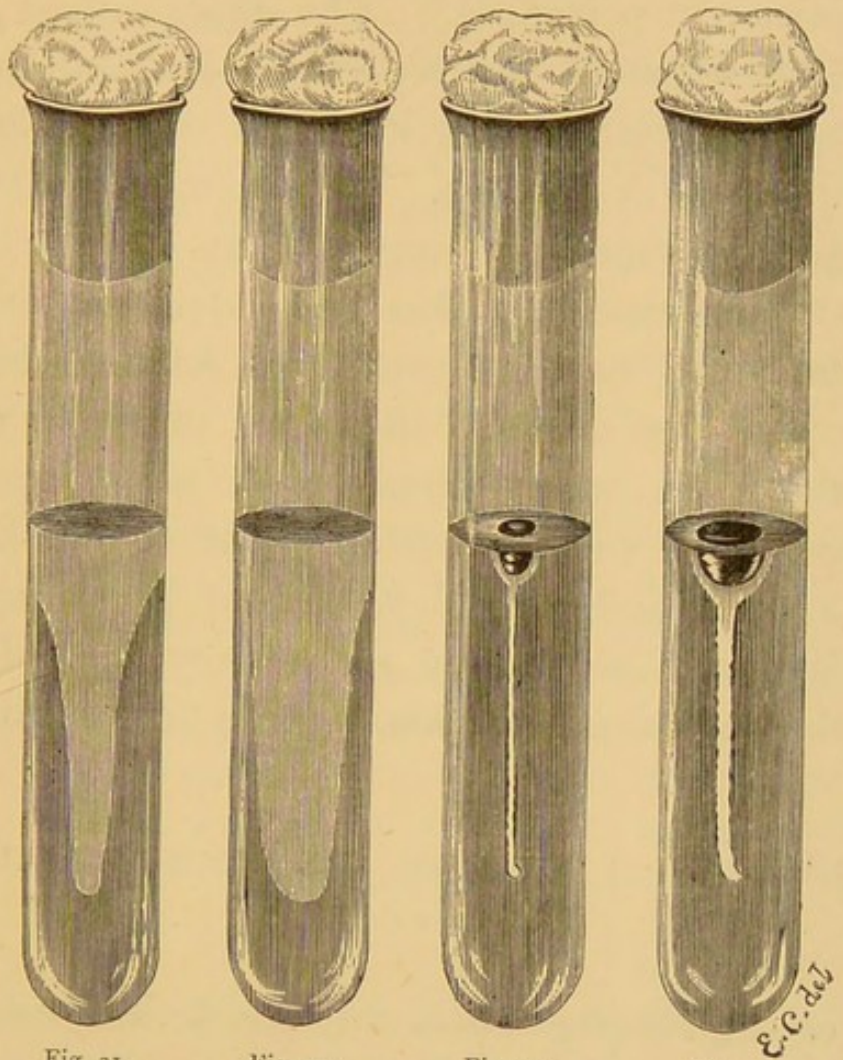

Fig. 31. Fig. 32. Fig. 33. Fig. 34.

PURE CULTIVATIONS IN GELATINE-PEPTONE BROTH.

Fig. 31. Finkler's bacillus, twenty-four hours old.
Fig. 32. ,, ,, two days old.
Fig. 33. Koch's cholera bacillus, twenty-four hours old.
Fig. 34. ,, ,, two days old.

ments maintain that choleraic symptoms were produced without any trace of peritonitis or putrid

infection, and that the comma-bacilli of Koch were again found in the intestinal contents, and fresh cultivations established.

On the other hand, these researches have been discredited, and the results of inoculation attributed to septicæmic poisoning.* The whole subject must be re-investigated, and reports of results arrived at by recent investigations in Spain will be looked forward to with interest. The comma-bacilli are found in the superficial necrosed layer of the intestine, in the mucous flakes and liquid contents of the intestinal canal of cases of Asiatic cholera. They were also detected in a tank in India, which contained the water supply to a neighbourhood where cholera cases occurred. The comma-bacilli are ærobic, and their development is arrested by deprivation of air. They are destroyed by drying and the presence of various antiseptic substances.

METHODS OF STAINING THE COMMA-BACILLI OF KOCH.

In cover-glass preparations they may be well stained in the ordinary way with an aqueous solution of methyl violet or fuchsine, or in the rapid method, without passing through the flame (p. 48, Babes' method).

In sections of the intestine their presence may be demonstrated by

* Klein, *Brit. Med. Journal*, 1885; Lankester, *Nineteenth Century*, July, 1885.

(*a*) *Koch's method.**

Sections of the intestine, which must be well hardened in absolute alcohol, are left for twenty-four hours in a strong watery solution of methylene-blue, or for a shorter time if the colour solution is warmed. Then treated in the usual way.

(*b*) *Babes' method.*†

Sections, preferably from a recent case of cholera, and made as soon as possible after death, are left for twenty-four hours in a watery solution of fuchsine (fabrique de Bâle), then washed in distilled water faintly acidulated with acetic acid, or in sublimate solution (1—1000), passed rapidly through alcohol and oil of cloves, dried with filter paper, and preserved in Canada balsam.

(*c*) *Nicati and Rietsch's method.*‡

A small quantity of the stools or of the scraping of the intestinal mucous membrane is spread out on a glass slide and dried, then steeped during some seconds in sublimate solution or in osmic acid (1 — 100). It is then stained by immersion in fuchsine-aniline solution (1 or 2 grammes of Bâle fuchsine dissolved in a saturated aqueous solution of aniline), washed, dried, and mounted in Canada balsam.

Spirillum Finklerii (*Comma-bacillus of Cholera nostras*).—Curved rods thicker than the comma-bacillus of Koch, and spirilla. The colonies on plate cultivations (Plates VI. and VII.) are very much larger than those of the comma-bacillus

* *Berliner Klinische Woch.*, No. 31.

† Cornil and Babes, *Les Bacteries*, p. 458, 1885.

‡ *Brit. Med. Journal*, Sept. 1885.

of Koch of the same age. They have the faintest yellowish-brown tinge, a well-defined border, and a distinctly granular appearance. They liquefy nutrient gelatine very rapidly, so that the first plate of a series is, as a rule, completely liquefied on the day following inoculation, and the second plate in two or three days more. In a test-tube-cultivation in nutrient gelatine the appearances are especially characteristic; the gelatine is very rapidly liquefied along the whole track of the needle, so that the cultivation resembles a conical sack, or the finger of a glove turned inside out (Figs. 35 and 36). On a sloping surface of nutrient agar-agar a white moist *plaque* forms very quickly. On potatoes they grow at the ordinary temperature of the air, producing a brownish layer, and corrosion of the surface of the potato. They were discovered in the evacuations of cases

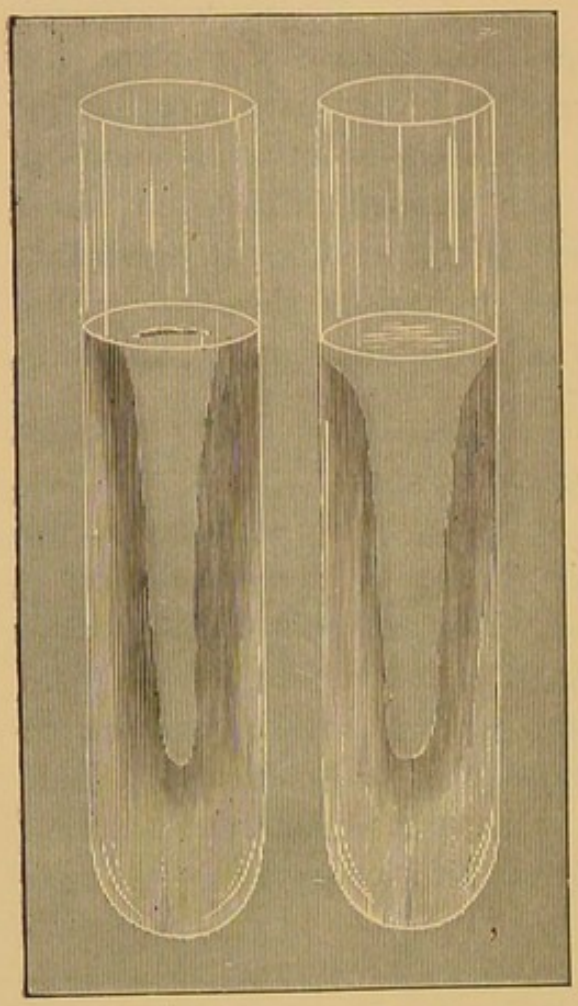

Fig. 35. Fig. 36.

PURE CULTIVATIONS OF THE SPIRILLUM FINKLERII IN GELATINE-PEPTONE-BROTH.

Fig. 35. In twenty-four hours.
Fig. 36. In thirty-six hours.

of cholera nostras, and claimed at first to be identical with the comma-bacillus of Koch. They are now regarded as quite distinct, though, like the *Spirillum of Asiatic Cholera*, they quite recently have been shown to be also pathogenic.*

Spirillum plicatile, Ehrenberg (*Marsh-Spirochæte*).—Thin threads, 2·25 μ in breadth, with numerous narrow windings, 110—125 μ long, occurring also in spirulinar forms. The threads have primary and secondary windings; the former are in each example of equal size, but the latter are often irregular; their ends are cut off bluntly, and they exhibit rapid movement. They occur abundantly in marsh-water in summer, and can be obtained by allowing algæ to decompose in water. On cultivation the threads break up into long rods, short rods, and finally cocci. This change is rendered visible by making cover-glass-preparations, and staining with aniline dyes.

Spirillum Obermeieri (*Spirochæte Obermeieri* Cohn: *Spirillum of Relapsing Fever*).—Threads similar to the *Spirillum plicatile*. In length mostly 16—40 μ, with screw-curves regular (Plate I., Fig. 19). They move very rapidly, and exhibit peculiar wave-like undulations. They have been observed in the blood of patients suffering from relapsing fever,† but never in the secretions. They only occur

* Finkler and Prior, *Ergänzungshefte zum Centralblatt für allgemeine Gesundheitspflege, Erster Band*, 1885.

† Obermeier, *Med. Centralbl.*, 1873.

during the relapses, and are absent during the non-febrile intervals. Their number is variable, but usually is strikingly great. Outside the body, in blood serum and 50 per cent. salt solution, the threads preserve their movements. From analogy to the *Spirillum plicatile* it is presumed that these threads are composed of articulated rods and cocci. Monkeys have been inoculated with success from man,* but inoculations of mice, rabbits, sheep, and pigs, gave negative results.

The spirilla were found in the blood of the inoculated monkeys in great numbers, and also in the brain, lung, liver, kidney, spleen, and skin ; and are believed to be the cause of the disease.

METHODS OF STAINING THE SPIRILLUM OBERMEIERI.

In cover-glass preparations of blood the spirilla stain strongly with fuchsine, methyl-violet, gentian-violet, or bismarck-brown.

In sections, brown aniline stains must be employed.

Genus III. Leuconostoc.

Leuconostoc mesenteroides, Cienkowski (*Gomme de sucrerie, Froschlaichpilz, Frogspawn fungus*).—Cells singly, in chains, or in zooglœa, surrounded by a thick gelatinous envelope. The life-history has been very thoroughly investigated.† The

* Carter, *Lancet*, 1879 and 1880 ; Koch, *Cohn's Beiträge.*

† Cienkowski, *Die Gallertbildungen d. Zuckerrübensaftes*, 1878 ; and Van Tieghem, *Sur la gomme du sucrerie, Ann. Sc. Nat.*, 1879.

spores, 1 8—2 μ in diameter, are of a round or ellipsoidal form, with thick membrane and shining contents. The outer membrane-layer bursts, and a middle lamella oozes out, and forms a thick gelatinous envelope, while the inner layer remains adherent to the plasma. Thus the spore-germination leads to the formation of a coccus with a gelatinous envelope. The coccus then elongates into a short rod-form, and the gelatinous envelope becomes ellipsoidal. The rod divides into two cocci, and each of these lengthens into a rod and divides. By repetition of this process a chain of cocci results, encased in a cylindrical or ellipsoidal envelope. The chains increase in length, become twisted up, and eventually fall apart into pieces of various lengths. In nourishing liquids a great number of little masses are formed, which adhere together, and produce pseudo-parenchymatous structures. These latter may join together, forming still larger agglomerations. The masses of zoogloœa are of almost a cartilaginous consistency, and admit of sections being made with a razor. After a long time the envelope liquefies, and the cocci are set free; the latter introduced into fresh nourishing media develop new colonies. In the chains here and there some of the cells become enlarged without changing their form. In these cells spores originate, which, when the gelatinous envelope liquefies, are set free.

This micro-organism occurs occasionally in beet-root juice and the molasses of sugar-makers,

forming large gelatinous masses resembling frog-spawn. The vegetation is so rapid that forty-nine hectolitres of molasses, containing 10 per cent. of sugar, were converted within twelve hours into a gelatinous mass; consequently, it is a formidable enemy of the sugar manufacturers.

Genus IV. Bacillus.

Bacillus subtilis (*Hay bacillus*).—Cylindrical rods as much as 6 μ in length, and about three times as long as broad. Single forms grow to double their length, and then undergo division. They also form threads which may be composed of long rods, short rods, and cocci. They are motile, and provided with a flagellum at each end. If the nourishing medium is impoverished, the multiplication of the rods by division gradually ceases, and spore formation commences. The rods become motionless, and a dark spot is visible, either in the middle or towards one end. This gradually develops into a shining spore with a dark contour. The rods swell slightly during this process, their contour becomes undefined, and soon disappears entirely, so that the spores are set free in about twenty-four hours. The spores are 1·2 μ long, and ·6 μ broad. They develop into rods in the following way. On one side of the spore a swelling appears, at the summit of which an opening in the spore-membrane results, and the germ escapes. This lengthens into a rod,

and remains for a time attached to the empty spore-membranes. These spores are widely distributed, and occur in the air, soil, dust, etc. On the excrement of herbivorous animals the bacilli form a white efflorescence, and on infusion of horse-dung a thick crumpled skin. They flourish equally in liquids and upon damp, solid nourishing media. On potatoes they grow as a yellowish-white skin; on ordinary nutrient liquids they develop a thin, and subsequently a thick, dense, crumpled pellicle, with copious spore-formation. They are ærobic; deprivation of carbonic acid causes the growth of the bacilli to cease, and the rods degenerate. They may be cultivated in various other nourishing media, such as blood-serum, nutrient gelatine, and nutrient agar-agar (Plate XIV., Fig. 2).

The simplest way to obtain a culture of the bacillus is to make a decoction of hay. The hay is chopped into small pieces, and boiled with distilled water in a flask for a quarter of an hour; it is then filtered into a beaker, which must be covered with a glass plate, and set aside in a warm place. In two or three days the liquid swarms with the bacilli, the spores of which exist in great numbers in ordinary hay. A more sure method for obtaining a pure cultivation is as follows:—

(*a*) Add only a small quantity of water to some finely chopped hay, and set aside for four hours at 36° C.

(*b*) Pour off the extract, and dilute it to the Sp. Gr. 1·004.

(*c*) Boil gently for one hour in a bulb plugged with cotton wool.

(*d*) Set aside 500 ccm. of the extract at 36° C.

In about twenty-four hours, as a rule, a pellicle has commenced to develop upon the surface of the liquid. If the reaction is definitely acid, carbonate of soda solution must be added to the decoction.

METHODS OF STAINING HAY BACILLUS.

To demonstrate the flagella of the bacilli, they must be stained with hæmatoxylin solution (Koch).

The linking together of cocci, long rods, and short rods in the threads, is shown by treating with alcoholic solution of fuchsine, or with iodine solution (Zopf).

To stain the spores the cover-glass preparations must be heated to a very high temperature (210° C.), in the hot-air steriliser for half an hour, or they may be exposed for a few seconds to the action of concentrated sulphuric acid (Büchner).

Bacillus anthracis (*Bactéridie du charbon, Bacillus of splenic fever, woolsorters' disease*, or *malignant pustule*).—Rods, 5—20 μ long and 1—1·25 μ broad, and threads, made up of rods and cocci. As a thorough knowledge of the life-history of this bacillus is of the greatest importance, inasmuch as it is without any doubt the actual cause of wide-spread disease, the various steps to be followed

in a practical study of it will be successively treated in detail. Its morphological and biological characteristics have been very completely worked out, and it serves as an excellent subject for gaining an acquaintance with the various methods that should be employed in studying micro-organisms. It is found that a mouse inoculated with the bacillus or its spores will die in from twenty-four to forty-eight hours, or more rarely in from forty-eight to about sixty hours.

Examination after death.—The details to be observed in the autopsy have already been described (p. 96). The spleen is found to be considerably enlarged, and may be removed (p. 97), and examined by making cover-glass preparations, inoculations, and subsequently sections.

Cover-glass-preparations.—In cover-glass-preparations of the blood of the spleen the bacilli are found in enormous numbers. Preparations should be made similarly with blood from the heart and exudations from the lungs, etc. In the last-mentioned the bacilli are present in very small numbers, or altogether absent They should be examined both unstained and stained (p. 46). The rods are straight, or sometimes curved, rigid, and motionless, and vary in size in different animals. They stain intensely with aniline dyes, and are then seen to be composed of segments with their extremities truncated at right angles; between the segments a clear linear space exists, which is typically characteristic (Plate I.,

Fig. 27). By double staining (p. 47), the rods are seen to consist of a membrane or hyaline sheath with protoplasmic contents.

Drop-cultures.—A little of the blood from the spleen or heart is employed to inoculate the liquid medium, bouillon or blood serum. Several of these cultures should be prepared, and some of them placed in the incubator. Examined from time to time it will then be observed that the rods grow into long homogeneous filaments, which are twisted up in strands, and then untwisted in long and graceful curves. In a few hours they begin to swell, become faintly granular, and finally, bright, oval spores develop (Plate I., Fig. 28). The cultures in the incubator develop rapidly, a temperature of 25°—40° C. being most favourable for the growth of the bacillus. The spores are eventually set free, and by making a fresh cultivation, or by injecting them into a mouse or guinea-pig, they germinate again into the characteristic bacilli, which in their turn grow into filaments and spores. When the spore germinates it swells, the outer layer becomes jelly-like, and giving way at one or other pole, the contents escape and grow into a rod. With the precautions previously described (p. 97) cultivations should be established in nutrient gelatine, nutrient agar-agar, and on sterilised potatoes.

Test-tube-cultivations in nutrient gelatine.—Typically characteristic appearances are obtained by inocu-

CULTIVATIONS ON NUTRIENT AGAR-AGAR

Fig 1. Bacillus anthracis.
Fig 2. Bacillus subtilis.
Fig 3. Staphylococcus cereus albus.

H. Cookshank Pinxt. *Vincent Brooks, Day & Son, Lith.*

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lating a 5 to 8 per cent. nutrient gelatine. A whitish line develops in the track of the inoculating needle, and from it fine filaments spread out in the gelatine (Fig. 37). Occasionally a little isolated spot develops, from which rays extend in all directions, like the silky filaments of the thistle-down. The filaments are more easily observed with a magnifying glass. In a more solid nutrient-gelatine the growth appears only as a thick white thread. As liquefaction of the gelatine progresses, these appearances rapidly disappear, and the growth subsides as a white flocculent mass (Plate V., Fig. 3). In exhausted culture-media, and sometimes in the blood, filaments are seen in a state of degeneration. This has also been observed in sections of the kidney, etc., of a rabbit inoculated with the anthrax bacillus, which had died of septicæmia the following morning.

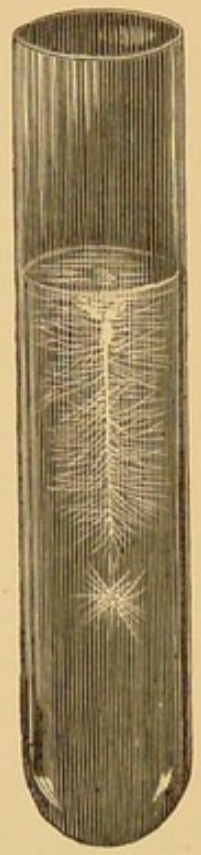

FIG. 37.* PURE CULTIVATION OF THE BACILLUS ANTHRACIS IN GELATINE-PEPTONE-BROTH.

Test-tube-cultivations in nutrient agar-agar.—Cultivated upon a sloping surface of nutrient agar-agar a viscous snow-white *plaque* is developed (Plate XIV., Fig. 1). Without access of air no cultivation can be obtained, the bacilli being ærobic. This can be demonstrated by embedding a piece of lung or

* Crookshank. Reprinted from *Lancet*, 1885.

spleen pulp containing bacilli in nutrient agar-agar (p. 98). No growth of the bacilli takes place.

Potato-cultivations.—A very characteristic growth results from the inoculation of sterilised potatoes. The damp-chamber containing the potatoes is placed in the incubator, and in about thirty-six to forty-eight hours a creamy, very faintly yellowish layer forms over the inoculated surface, with usually a peculiar translucent edge (Plate XV., Fig. 1). On removing the cover of the damp-chamber a strong, penetrating odour of sour milk is encountered.

Plate-cultivations.—From the spleen or blood of the heart, cultivations must be established in nutrient gelatine on plates. The colonies develop in about two days, according to the temperature of the room. They appear to the naked eye as little white spots or specks, which, on examination with a low power of the microscope and small diaphragm, exhibit two distinct forms. One form, on careful focussing, has the appearance of a little compact ball of twisted thread; in the other, liquefaction of the gelatine has commenced, and the thread bundles are spreading out like locks or plaits of hair in the neighbouring gelatine. These appearances are perfectly characteristic.

Cover-glass-impressions. — The plate-cultivations should be also examined as soon as the colonies appear, by making cover-glass-impressions (p. 49),

Fig 1. Bacillus anthracis.
Growth at 37° C three days after inoculation.

Fig 2. Micrococcus indicus.
Growth three days after inoculation.

POTATO CULTIVATIONS.

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and staining them with aniline dyes. The filaments, examined with a high power, will then be seen to consist of a number of rods or segments (Plate I., Fig. 30). On the other hand, filaments from a tube cultivation in a solid medium will be found to be composed, not only of rods, but here and there of torula-like involution-forms (Plate I., Fig. 30). In a cover-glass-impression from a potato-culture (Plate I., Fig. 29) the individual segments have a great tendency to be isolated one from the other, and there is copious spore-formation.

Preservation of spores.—Spores may be preserved simply by allowing anthracic blood to dry and sealing it in a tube. The spores from a potato cultivation are treated as follows:—The inoculated surface containing the creamy cultivation is sliced off in a thin layer, and is mashed up with distilled water in a glass capsule. Sterilised silk-thread is cut up into lengths of about a quarter of an inch, and allowed to soak in the paste for some hours, under a bell-glass. The threads are then picked out with a pair of forceps, and laid upon a sterilised glass plate, covered with a bell-glass, and allowed to dry. From the plate, when perfectly dry, they are transferred to a small test-tube, which can be plugged with cotton-wool, or sealed in the Bunsen burner.

Examination in the tissues.—The organs must be hardened in absolute alcohol, cut and stained, (pp. 51, 157). The method of Gram is the most

instructive, and eosin a very satisfactory contrast stain. The capillaries all over the body, lungs, liver, kidney, spleen, skin, mucous membrane, etc., will be found to contain bacilli. In some cases the bacilli are so numerous, (*e.g.*, in the capillaries of the kidney, Plate XVI., Fig. 2), that examination with a low power gives the appearance of an injected specimen.

Inoculation of animals.—A thread containing spores, a drop of blood from an infected animal, or a minute portion of a cultivation, introduced under the skin of a mouse or guinea-pig, causes its death, as a rule, in from twenty-four to forty-eight hours. Sheep fed upon potatoes which have been the medium for cultivating the bacillus, die in a few days. Goats, hedgehogs, sparrows, cows, horses, are all susceptible. Rats are infected with difficulty. Pigs, dogs, cats, white rats, and Algerian sheep have an immunity from the disease. Frogs and fish have been rendered susceptible by raising the temperature of the water in which they lived.

Dissemination of the disease and mode of infection.—It has been stated that when carcases of animals which have died of anthrax are buried under the soil, the development of the bacilli into spores can take place. The spores were supposed to be taken up by earth worms, carried to the surface, and deposited in their castings; animals then grazing or sojourning on the soil are thus liable to be infected.* This has not

* Pasteur, *Bulletin de l'Académie de Médecine.* 1880.

To face page 154.

Fig. 1. From a section of mucous membrane of the stomach of a mouse. Gram's method and Eosin. Zeiss' ⅛. o. i. Oc. 2.

BACILLUS ANTHRACIS.

Fig. 2. From a section of Kidney of a mouse. Gram's method and Eosin. Zeiss' ⅛. o. i. Oc. 2.

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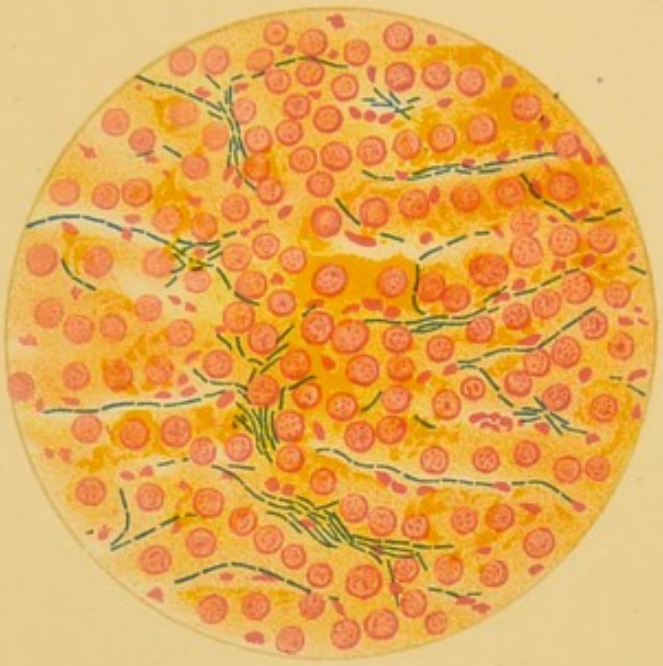
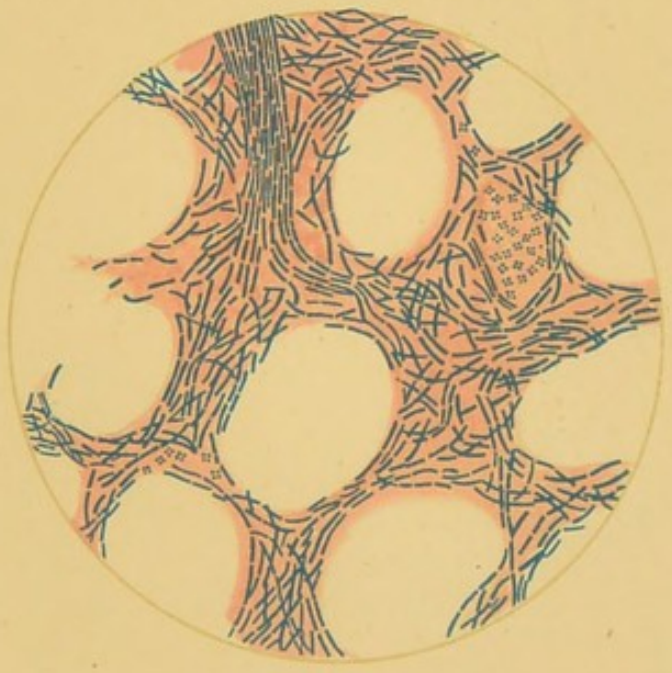
To follow plate XVI.



BACILLUS ANTHRACIS.

Fig. 1. From a section of Liver of a mouse. Weigert's and Orth's methods. (gentian-violet and piero-lithium-carmine.) Zeiss' 1/12. o.i. Oc. 2.



BACILLUS ANTHRACIS AND MICROCOCCUS TETRAGONUS.

Fig. 2. From a section of Lung of a mouse. Gram's method and Eosin. Zeiss' 1/12 o.i. Oc. 2.

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been borne out by experiment.* Bacilli, however, occur in large numbers in the blood and discharges from the nose and mouth of the moribund animals, and in the urine and fæces. They find a nourishing soil in decaying vegetable and animal matter, and having free access of oxygen form copious spores, so that the grass is extensively contaminated.

In warm and marshy districts the spore formation is still more active, and the spores may be carried by floods over adjacent meadows. As to the mode of infection, the animals may be directly infected through buccal wounds caused by siliceous grasses, or by wounds of insects; the intestinal and pulmonary mucous membranes are also regarded as pathways of infection. In animals the disease is known as "splenic fever."

In man the mode of infection is by inhalation of spores, and ingress by the pulmonary or intestinal mucous membrane, or by direct inoculation of a wound or abrasion. The spores are derived from the wool or hides of animals which have died of anthrax, and the resulting disease is known as "wool-sorter's disease," and "pustula maligna." Bacilli are found in the serum of the pustule, and in sputum, urine, fæces, and sweat; and if the disease prove fatal, in the capillaries throughout the body.

Attenuation of the virus.—By cultivating the bacillus in neutralised bouillon at 42°—43° C. for about twenty days, the infecting power is weakened

* Koch, *Mittheil. Gesundheitsamte.* 1881.

and animals inoculated with it (*premier vaccin*) are protected against the disease.* To obtain a still more perfect immunity, they are inoculated a second time with material (*deuxième vaccin*) which has been less weakened. The animals are then protected against the most virulent anthrax, but only for a time. From such a culture, however, new cultures of virulent bacilli can be started, and a culture that is "vaccin" for sheep kills a guinea-pig, and then yields bacilli that are fatal to sheep.† Exposure to a temperature of 55° C., or treatment with ·5 to 1 per cent. carbolic acid, deprives the bacilli of their virulence. The virulence of the bacillus is also altered by passing the bacillus through different species of animals. The bacillus of sheep or cattle is fatal when re-inoculated into sheep or cattle; but, if inoculated in mice, the bacilli then obtained lose their virulence for sheep or cattle; only a transitory illness results, and the animals are protected for a time against virulent anthrax.‡ The possibility of mitigating the virus depends upon the species of animal; rodents cannot be rendered immune by any known "vaccin."

* Pasteur, *Compt. Rend.*, 1861, and *Revue Scientifique*, 1883.

† Klein, *Micro-organisms and Disease*. 1885.

‡ Klein, *Reports of the Medical Officer of the Local Government Board*. 1882.

METHODS OF STAINING THE BACILLUS ANTHRACIS.

Cover-glass preparations of blood, etc., can be stained with a watery solution of any of the aniline dyes. They may be rapidly stained with a drop of fuchsine or gentian-violet (p. 46), but more satisfactorily by floating the cover glasses for twenty-four hours. The preparations may be dried and mounted in Canada balsam, but the typical appearances are best observed in freshly stained specimens examined in water.

The sheath and protoplasmic contents can be differentiated by staining with eosin after the method of Gram.

The spores are not stained by the ordinary methods. The cover-glass preparations must be raised to a high temperature in the incubator, or treated with sulphuric acid (p. 148), or passed about twelve times through the flame of the Bunsen burner.

Tissue sections are best stained by the method of Gram, and *after-stained* with eosin, picrocarminate of ammonia, or picro-lithium-carmine.

A more rapid double stain is obtained by immersing the sections in a watery solution of gentian-violet, rinsing in alcohol, and then staining by the method of Orth (p. 58).

Weigert's Method.—Place the sections for two to five minutes in a 1 per cent. watery solution of gentian-violet. Wash in alcohol, rinse in water, and transfer to picro-carmine solution (Weigert) for from a half to an hour. Treat with alcohol till the colour is almost washed out, and finally clear in oil of cloves and mount in Canada balsam.

Bacillus ulna, Cohn.—Cocci, short rods, long rods, and threads. Diam. of the cocci 1·5—2·2 μ. Spore formation in both short and long rods. No

septic odour is produced by this bacillus in a nourishing liquid. Cloudy masses are found on the surface of the liquid, which later form a thick dry pellicle. The latter consists of bundles of threads matted together. The formation of ellipsoidal spores occurs in the usual way; they measure 2·5—2·8 μ long, and more than 1 μ wide. The bacillus is found on rotting eggs, and can be cultivated on boiled white of egg. It is closely allied to the bacillus subtilis.

Bacillus tumescens, Zopf.*—Cocci, long and short rods. They form a jelly-like disc, ·5—1 cm. in diam., on slices of boiled carrot, with the appearance of a rather tough crumpled skin of a whitish colour. Examination of this pellicle shows that it is formed of rows of rods lying closely together. These rods can be observed to divide into short rods and cocci. Spore formation occurs in two stages of development, viz., in the cocci and in the short rods. A cultivation is obtained by exposing slices of boiled carrot, slightly moistened, to the air at the temperature of the room.

Bacillus megaterium, De Bary.—Large rods 2·5 μ wide, and four to six times as long. They are usually somewhat curved. Transverse division occurs, each segment attaining the length of the original rod. In the fresh state they appear non-articulated, but when treated with a dehydrating agent (tincture of iodine, alcohol), they are seen to be

* Zopf, *Die Spaltpilze*. 1885.

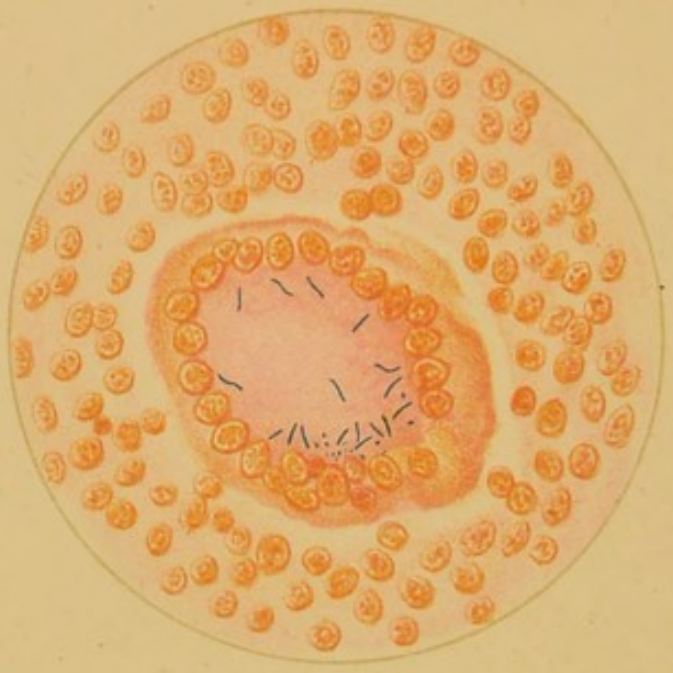
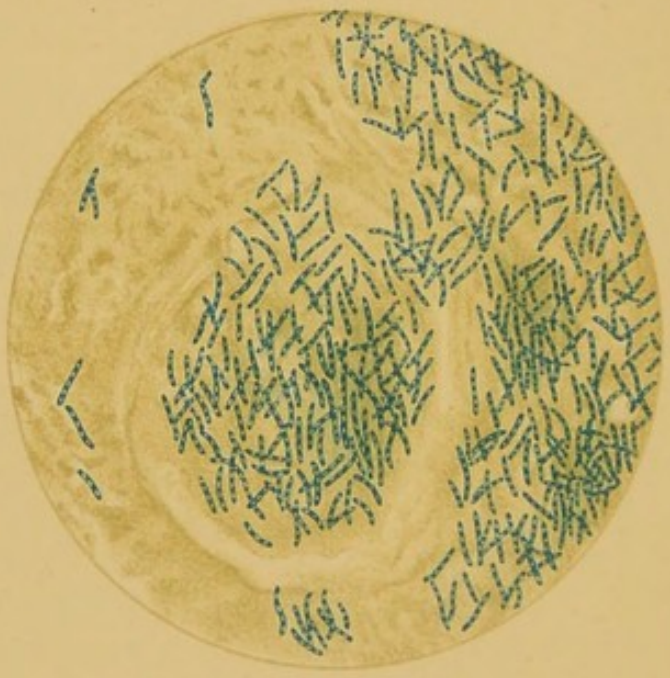


Fig. 1. From a section of a lymphatic gland from a case of tuberculosis in a fœtal calf. Ehrlich-Koch method. (methyl-violet and bismarck brown) and Eosin. Zeiss' 1/12. o.i. Oc. 4.



BACILLUS TUBERCULOSIS.

Fig. 2. From a section of Lung from a case of artificial tuberculosis in a rabbit. Ehrlich-Koch method. (methyl-violet.) Zeiss' 1/8 o.i. Oc. 4.

Edgar Cruickshank fec. et pinx.

Vincent Brooks, Day & Son, Lith.

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composed of short segments. The rods are motile, and form irregular chains, of a disjointed appearance. Spore formation occurs in the usual way. It was first observed on boiled cabbage, and thence cultivated in a solution of grape-sugar and nutrient gelatine.

Bacillus Fitzianus, Zopf.—Cocci, short rods, long rods, and threads. This bacillus, cultivated in meat extract and glycerine at 36° C., causes an active fermentation with the production of ethyl alcohol. Spore formation occurs in the rods. Observed in unboiled hay infusion, accompanying the hay bacillus.

Bacillus tuberculosis, Koch.*—Rods 2—4 μ and occasionally 8 μ long, very thin, and rounded at the ends. They are straight or curved, and frequently beaded, and occur singly, in pairs, or in bundles. They are found in the cells of tubercles, especially in the interior of giant cells. In the latter they are often accompanied with grains which exhibit the same colour reaction (Plate XVIII., Fig. 1). They are non-motile. Spore formation occurs, even within the animal body, the spores having the appearance of clear vacuoles. The bacilli can be cultivated artificially. The best medium is solid blood serum (p. 81) of cow or sheep, with or without the addition of gelatine; and the most favourable temperature for their development is 37°—38° C. The

* Koch, *Berl. Klin. Woch.*, No. 15, 1882; and *Mittheil. aus dem Kaiserlich. Gesundheitsamte; Aetiologie der Tuberkulose.*

growth takes place very slowly, and only between the temperatures of 30° and 41° C. In about eight or ten days the growth appears as little whitish or yellowish scales and grains (Plate XI., Fig. 1).

The bacillus can also be cultivated in a glass capsule on blood serum, and the appearances of the growth studied under the microscope. The scales or pellicles are then seen to be made up of colonies of a perfectly characteristic appearance, which may be still further studied by making a cover-glass impression (p. 49, and Plate XI., Fig. 4). They are then seen to be composed of bacilli, arranged more or less with their long axis corresponding with that of the colony itself, and with an appreciable interval between the individual bacilli. The colonies themselves appear as fine curved lines, the smallest being mostly S-shaped. Longer colonies have serpentine twistings and bendings, which often recall the curves of fancy lettering. The ends of the lines run to sharp points, but the middle of the growth is spindle-formed. The youngest colonies are extremely delicate and narrow, but the older colonies increase in size, are thicker across, and, blending with each other, gradually obliterate the characteristic appearances; a lamellated growth results, which increases, and gives the appearance to the naked eye of the scale or pellicle already described. The blood serum is not liquefied unless putrefactive bacteria contaminate the culture. A fresh tube can be inoculated with one of these little

scales, and a new generation started. The scales gradually increase in size, and consist entirely of bacilli. In about three to four weeks the cultivation ceases to increase, and it is then necessary to inoculate a fresh tube. The virulence is not weakened by carrying on successive cultivations. A relatively small portion of the cultivation inoculated into the subcutaneous tissue, into the peritoneal or pleural cavity, into the anterior chamber of the eye, or directly into the blood stream, produces after three or more weeks artificial tuberculosis in guinea-pigs and rabbits. Dogs and cats can also be infected by experimental inoculation.

The appearances observed at the autopsy are, swollen lymphatic glands in the neighbourhood of the inoculation, followed by softening and abscess; enlargement of the spleen and liver, with formation of caseous tubercles; tuberculosis of the lungs, bronchial glands, and peritoneum. After inoculation of the eye, grey tubercles appear on the iris, and undergo enlargement and caseation, followed by tuberculosis of the eyeball and organs generally. The bacilli appear to be the direct cause of tuberculosis, and the presence of the bacillus in the sputum of patients is regarded as a distinctive sign of the existence of this disease. The detection of the bacillus has, consequently, become a test which is daily applied by physicians in forming clinical diagnoses.

The bacilli are found in all tubercular growths

of man, monkeys, cattle (*Perlsucht*), birds, and many other animals, and in cases of artificial tuberculosis, in rabbits, guinea-pigs, cats, etc. (Plate XVIII., Fig. 2). In man the bacillus can be detected in the tissues, in the sputum, in the blood, and in the urine.*

Tuberculosis may also be produced by inhalation and feeding experiments (p. 92). The channels of infection in man are also most probably the pulmonary or intestinal mucous membranes. The possibility of inoculation of skin wounds is open to doubt. The bacilli or their spores are inhaled from the air, or taken in with food. As a relatively high temperature is required for their growth, they cannot thrive outside the animal body in cold climates.

METHODS OF STAINING THE TUBERCLE BACILLUS.

Numerous methods have been recommended for staining the *bacillus tuberculosis*, each of which will be given in detail.

Ehrlich's and Gibbes' methods may be with advantage employed in staining cover-glass preparations. *Gibbes' rapid double stain* is obviously the best to employ for clinical purposes. For sections both *Ehrlich's and Neelsen's methods* give excellent results.

Koch's original method.—Cover-glass preparations or sections are laid in Koch's solution (No. 23, *c.*) for twenty-four hours, or for one hour if the solution is warmed to 40° C. Rinse in water; immerse in a watery solution of vesuvin for two minutes; rinse again in water, and examine; or, after rinsing in water, treat with alcohol, clove-oil, and Canada balsam.

* Babes, *Centralbl. f. d. Med. Wissensch.*, 1883, p. 145.

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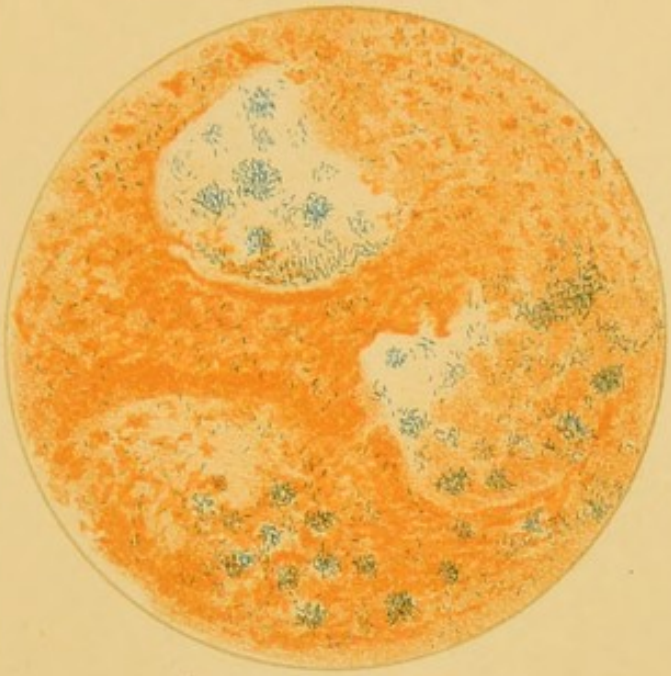

Fig. 1. From a section of liver of a hen.
Ehrlich-Koch method (methyl-violet and bismarck-brown) Zeiss' DD. Oc. 4.

BACILLUS TUBERCULOSIS.

Fig. 2. The same preparation. Zeiss' 18. Oc. 4.

Edgar Crookshank fec. et pinx.

Vincent Brooks, Day & Son, Lith.

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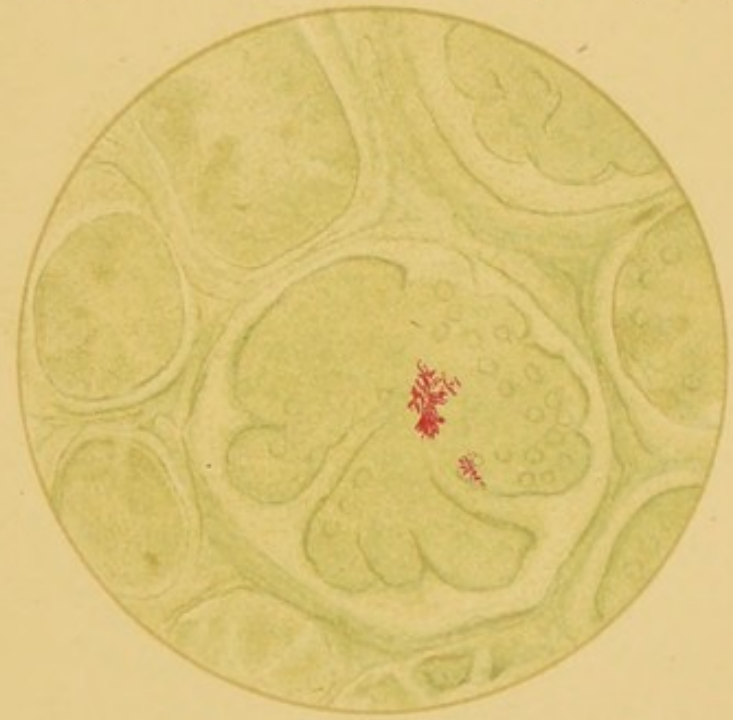
To follow plate XIX.

BACILLUS TUBERCULOSIS.

Fig. 1. From a cover-glass preparation of pus from a tubercular cavity of the human lung. Ehrlich's method. (fuchsine and methylene blue). Zeiss' ft. o.i. Oc. 4.



BACILLUS LEPRÆ.

Fig. 2. From a section of kidney from a case of leprosy. Ehrlich's method. (fuchsine and methylene blue). Zeiss' DD. Oc. 4.

Edgar Crookshank fec. et pinx.

Vincent Brooks, Day & Son, Lith.

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Ehrlich's method.—Cover-glass-preparations are allowed to float in a watch-glass, containing a solution of gentian-violet or fuchsine, added to aniline water. A saturated alcoholic solution of the dye is added till precipitation commences (10 ccm. aniline water, and 10—20 drops of the colour solution). The cover-glasses are left in the solution for about half an hour; then washed for a few seconds in strong nitric acid (one part commercial nitric acid to two of distilled water), and rinsed in distilled water. *After-stain* with vesuvin or methylene-blue, rinse in water, dry and preserve in Canada balsam (Plate XX., Fig. 1).

Sections and cover-glass-preparations may be stained by this method, as described by Koch.*

Saturated alcoholic solution of methyl-violet or fuchsine	11
Aniline water	100
Absolute alcohol	10

Preparations are left for twelve hours in this solution (colouring of the cover-glass-preparations can be expedited by warming the solution).

Treat the preparations with (1—3) solution of nitric acid a few seconds.

Wash in alcohol (60 per cent.) for a few minutes (cover-glass-preparations need only be rinsed a few times). After-stain with diluted solution of vesuvin or methylene-blue for a few minutes.

Wash again in 60 per cent. alcohol, dehydrate in absolute alcohol. Clear with cedar-oil, mount in Canada balsam.

Rindfleisch's method.—Prepare a solution composed of

Saturated alcoholic solution of fuchsine	10 drops
Aniline water	2 drams.

Pour it into a watch-glass, and float the cover-glass; warm the watch-glass over a spirit-lamp until steam rises. Remove it from the flame, and set it aside for five minutes.

* *Mittheil. aus dem Gesundheitsamte*, Zweiter Band, 1884, p. 10.

Take out the cover-glass, and transfer it for a few seconds to acidulated alcohol (two drops of nitric acid in a watch-glass full of alcohol). Wash in distilled water, dry, and preserve in balsam. After-stain, if necessary, with bismarck-brown, or methylene-blue.

Weigert-Ehrlich method (*vide* p. 58).

Orth's modification of Ehrlich's method.—Stain by the method of Ehrlich, but decolorise with acidulated alcohol (one of hydrochloric to one hundred parts of 70 per cent. alcohol).

*Gibbes' method.**—Stain cover-glass-preparations in magenta solution (No. 22) for 15—20 minutes. Wash in (1—3) solution of nitric acid, until the colour is removed. Rinse in distilled water. After-stain with methylene-blue, methyl-green, iodine-green, or watery solution of crysoidin, five minutes. Wash in distilled water till no more colour comes away. Transfer to absolute alcohol for five minutes; dry, and preserve in Canada balsam. Leave sections in the stain for half an hour, then treat with nitric acid, and wash with distilled water. Transfer to methylene-blue till deeply stained, wash again in distilled water, and then in spirit. Pass through absolute alcohol and clove-oil, and preserve in Canada balsam.

Gibbes' new method.—Cover-glass-preparations are placed in the double staining solution (No. 16), which has been warmed in a test-tube, and, as soon as steam rises, poured into a watch-glass. They are allowed to remain for five minutes, and then are washed in methylated spirit till no more colour comes away, dried in the air or over a spirit-lamp, and mounted in Canada balsam. If the solution is used without warming, the cover-glasses must be left in it for an hour. Sections are treated on the same principles, but must be left in the solution for several hours. The crumpling of the sections by the action of nitric acid is avoided.

* Gibbes, *Practical Pathology*. 1883.

Baumgarten's method.—Cover-glass-preparations of sputum are made as already described (p. 46), and immersed in a very dilute solution of potash (1—2 drops of a 33 per cent. solution of potash in a watch-glass of distilled water) The cover-glass is pressed down on a slide, and examined with a high power. The bacilli can be thus examined in the unstained condition, and to avoid any mistake from confusion with other species, the cover-glass can be removed, dried, passed through the flame, and stained with a drop of an aqueous solution of fuchsine, or gentian-violet. The putrefactive bacteria are stained, but the tubercle bacilli remain absolutely colourless.

Baumgarten's new method.—A solution is prepared as follows: Drop 4—5 drops of concentrated alcoholic methyl-violet solution into a small watch-glass full of water. (*a*) Stain the sections in this solution, wash them in water, and decolorise in absolute alcohol (five to ten minutes), or, before treating with alcohol, immerse the sections for five minutes in a half-saturated solution of carbonate of potash. Pass through clove-oil, and mount in a mixture of Canada balsam, free from chloroform, and clove-oil (equal parts). The object of this process is to differentiate the tubercle bacilli from chance bacteria, inasmuch as the tubercle bacilli gradually are decolorised by the clove-oil. (*b*) Sections stained in the above solution are placed for five minutes in alcohol, and then in a concentrated solution of bismarck-brown in 1 per cent. solution of acetic acid. The after-treatment may be conducted as already described.

Neelsen's method. — Cover-glass-preparations may be quickly stained in Neelsen's solution (No. 25) warmed in a watch-glass till steam rises. Sections are left for from five to ten minutes in the solution, and then washed in a watery solution of sulphuric acid (25 per cent.) ; rinsed in distilled water, and immersed in methylene blue solution. After two or three minutes they are passed through alcohol and oil of cloves, and mounted in Canada balsam.

Balmer-Fräntzel method.—Dissolve two grammes of freshly-powdered gentian-violet in 100 grammes of aniline-water. Immerse the sections for twenty-four hours, and treat as in Ehrlich's method.

Ziehl's method.—Stain with Ehrlich's method, but omit the nitric acid; after-stain with methylene-blue. The latter replaces the stain of all bacteria except the tubercle bacillus.

Lichtheim's method.—Concentrated solution of fuchsine or gentian-violet is diluted with distilled water, and the sections stained for thirty-six hours.

Peters' method.—Sections are stained for half an hour in fresh aniline-gentian-violet solution. Transfer to 20 ccm. of absolute alcohol for eighteen hours, the alcohol being renewed two or three times. Rinse in distilled water for one minute, and immerse for three minutes in a watery solution of aniline-yellow (aniline-yellow ·2 dissolved in distilled water 10, filter). Wash in absolute alcohol, clarify with clove-oil, and preserve in Canada balsam.

Fränkel's method.—Sputum preparations are rapidly double-stained by the following method: Prepare a solution by adding concentrated alcoholic methyl-violet or fuchsine solution, drop by drop till opalescence arises, to 5 ccm. of aniline-water heated to 100° C. Float the prepared cover-glasses two minutes in the warmed solution. The process of after-staining and decolorisation is effected by placing the preparation for one to two minutes in one of the following solutions: for fuchsine-stained preparations a saturated solution of methylene-blue in a mixture of

Alcohol	50
Distilled water	30
Nitric acid	20

which is filtered before use; for preparations stained in methyl-violet, a saturated solution of vesuvin may be used in

Alcohol	70
Nitric acid	30

which must be filtered before use. The sections are washed in water (or weakly acidified 50 per cent. alcohol), dried and mounted in the usual way.

Pfuhl-Petri's method.—The colouring solution consists of 10 ccm. of a saturated alcoholic solution of fuchsine added to 100 ccm. of water. Float the cover-glasses for two minutes in the solution heated till steam rises. Wash for one minute in glacial acetic acid, rinse in water, and after-stain in an alcoholic or watery solution of malachite green for a half or one minute. Rinse again in water. Dry, and examine in glycerine, or preserve in Canada balsam.

Senkewitsch's method.—Stain cover-glass-preparations in concentrated fuchsine solution. When strongly coloured, wash out the stain for one to two minutes in alcohol, to which one drop of nitric acid has been added for every 10 ccm. Rinse in water, dry, and mount in Canada balsam.

Kaatzer's method.—Place the cover-glass-preparations for twenty-four hours in a solution of over-saturated alcoholic gentian-violet, or, if warmed to 80° C., for three minutes. Decolorise in a solution consisting of

Alcohol 90 per cent. . . .	100 ccm.
Water	20 ccm.
Strong hydrochloric acid . .	20 drops.

Rinse in 90 per cent. alcohol, and after-stain with concentrated watery solution of vesuvin for two minutes; wash again in distilled water, dry, and mount in Canada balsam.

Ehrlich's method and eosin.—The author has found that after sections have been stained with methyl-violet and bismarck-brown by Ehrlich's method, as described by Koch (p. 163), they may with advantage be immersed in a weak alcoholic solution of eosin, then rinsed in clean absolute alcohol, clarified with clove-oil, and mounted in Canada

balsam. The giant cells are then stained pink, while their nuclei are brown and the bacilli blue (Plate XVIII., Fig. 1).

Bacillus cyanogenus, Fuchs. (*Bacterium syncyanum* : *Bacillus of Blue Milk*).—Motile rods 2·5—3·5 μ in length, and double rods 5·5—6 μ. The rods after division may remain linked together, and form chains. Non-motile rods enveloped in a gelatinous capsule, and involution-forms, have also been described.

Cultivated in a test-tube of nutrient gelatine, the bacilli grow principally upon the free surface, in the form of a white layer. The surface of the gelatine becomes cupped, and a peculiar greenish-brown colour develops in the medium, especially in proximity to the growth.

On a sloping surface of nutrient agar-agar, it grows as a white layer, and colours the upper part of the medium a smoky brown (Plate II., Fig. 2).

The bacilli can also be cultivated in milk and on various other substrata, as potatoes (Plate XXI.), boiled rice, and starch. A pure cultivation in sterilised milk develops an alkaline, not an acid reaction. The colouring matter which is formed, varies with the nourishing medium ; for example, in milk a slate-blue coloration is produced, but if the milk has become acid by the growth of the *bacillus acidi lactici*, then the colour is an intense blue.

The micro-organism occurs occasionally in cow's milk, producing a blue colour. It has been

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Fig 1. After three days growth.

Fig 2. After nine days growth.

POTATO CULTIVATION OF BACILLUS CYANOGENUS.

H. Cruickshank Pinxt.

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observed, especially in the north of Germany, during the warm months; and where milk is kept in hot rooms, in the winter also. The bluing was originally attributed to a diseased condition of the cows, or to their eating certain meadow plants.

Bacillus acidi lactici.—Long and short rods, 1—2·8 μ long, 3—4 μ thick, and thread forms; no cocci; spore formation.* Cultivated on nutrient gelatine the breadth of the rods is lessened. They grow best between 35 and 42° C., and cease under 10° C. Cultivated at a temperature over 45·5°, they are no longer able to produce acidity. They are not identical with a *bacterium acidi lactici* which has been described. Probably several micro-organisms are able to produce an acid reaction in milk.

They occur with various other bacteria in sour milk, and a pure cultivation, isolated by plate-cultivations, turns sterilised milk sour.

Bacillus œdematis maligni, Koch. (*Pasteur's Septicæmia*). Rods from 3—3·5 μ long and 1—1·1 μ wide; they mostly lie in pairs, and then appear to be double this length. The rods are rounded at their ends, and form threads which are sometimes straight, but more commonly curved. In stained preparations they have a somewhat granular appearance. The bacilli are distinguished from anthrax bacilli by their being somewhat thinner, by their rounded ends, and by their being motile. Anthrax bacilli also never appear as threads in fresh

* Hueppe, *Mittheil. a. d. Gesundheitsamt*, Zweiter Band, p. 339.

blood, and are differently distributed throughout the body. They are anœrobic, and can be cultivated on blood-serum and on neutral solution of Liebig's meat extract in an atmosphere of carbonic acid. By embedding material containing bacilli in nutrient agar-agar and nutrient gelatine, characteristic cultivations are obtained. The following process may be adopted to obtain a pure cultivation.* A mouse inoculated subcutaneously with dust, as a rule, dies in one to two days. It is then pinned out, back uppermost, on a slab of wood (p. 96), and the hair singed with a Paquelin's cautery from one hind leg up to the neck, across the latter, and down again to the opposite hind leg. Following the cauterised line, the skin is cut through with sterilised scissors, and the flap turned back and pinned out of the way. With curved scissors little pieces of the subcutaneous œdematous tissue, in the neighbourhood of the inoculated spot, are cut out, and sunk with a platinum needle in a 1 per cent. nutrient agar-agar, or 5 per cent. nutrient gelatine. Fragments of tissue may also be embedded by the author's method already described (p. 98).

The inoculated tubes are placed in the incubator. In a few hours a whitish turbidity spreads out from the piece of tissue, and upwards in the needle track. Examined microscopically, the turbidity is found to be due solely to the development of bacilli of œdema.

* Hesse, *Deutsch. Med. Woch.*, No. 14. 1885.

The surface exposed to the air exhibits no trace of the bacilli.

To investigate the tubes microscopically, a sterilised glass tube with a capillary end may be used, with its neck plugged with sterilized cotton wool, and provided at the mouth with a suction ball. The capillary end is thrust into the cultivation, and a small fragment removed by aspiration. In the course of the first day the bacilli spread throughout a great part of the agar-agar in such a way that a more or less equally diffused cloudiness of the medium ensues, with subsequent appearance of strongly marked clouds or lines of turbidity. At the same time gas bubbles develop along the needle track, and a collection of liquid takes place, while spore formation also commences. The following day these appearances are more marked, the opacity is more pronounced, the development of gas increases, and the liquid contains more spore-forming bacilli and numerous free spores.

The nutrient gelatine cultures during the first day show no macroscopic change, but after a few days the piece of tissue is surrounded with a white halo. This gradually spreads in all directions, and is apparently beset with hairs. The gelatine liquefies, and the fragment of tissue, degenerated bacilli, and spores, sink to the bottom. The cultivation is also very characteristic in $\frac{1}{2}$ per cent. nutrient agar-agar. If placed in the incubator, in a few hours a cloudiness forms around the piece of embedded tissue,

which is caused by bacilli gradually spreading in all directions in the nutrient medium. Mice inoculated from these cultivations die more quickly than from the original infection from dust. On potatoes they are cultivated by introducing a piece of liver or other tissue containing the bacilli, into the interior of a sterilised potato (p. 98), incubated at 38° C. The bacillus is not deprived of its virulence by cultivation. The spores of the œdema-bacilli appear to be very widely distributed. They are found in the upper cultivated layer of the soil, in hay dust, in decomposing liquids, and especially in the bodies of suffocated animals, which are left to decompose at a high temperature. From any of these sources animals can be successfully inoculated. If a guinea-pig, for example, be subcutaneously inoculated with earth, putrid fluid, or hay dust, death frequently occurs in from twenty-four to forty-eight hours. At the autopsy the most characteristic symptom is a wide-spread subcutaneous œdema, which originates from the point of inoculation, accompanied with air-bubbles, and contains a clear reddish liquid full of motile and non-motile bacilli. The internal organs are little changed, the spleen is enlarged and of a dark colour, and the lungs are hyperæmic, and have hæmorrhagic spots. Examined immediately after death, few or no bacilli are detected in the blood of the heart, but in that of the spleen, liver, lungs and other organs, in the peritoneal exudation, and in and upon the serous coating of abdominal organs they are

present in large numbers. If, on the other hand, the animal is not examined until some time after death, then the bacilli are found in the blood of the heart, and distributed all over the body.

Bacillus of septicæmia of mice, Koch.— Extremely minute bacilli, ·8—1 μ long, and ·1—·2 broad, often in pairs, seldom in chains of four. On cultivation they do not appear to make threads, but the bacilli lie together in masses. Spores have been observed. The bacilli are probably non-motile. They are most commonly in the interior of white blood corpuscles. In these they increase, and in many cases a white cell is only represented by a mass of bacilli. The bacilli, or rather their spores, occur in putrid liquids. If a number of mice are inoculated with a minimum quantity of putrid fluid, about a third of them die of septicæmia. They rapidly sicken, their eyes inflame, their eyelids stick together, they become soporific, and die in from about forty to sixty hours. At the autopsy one finds slight œdema at the seat of inoculation, and enlargement of the spleen; the bacilli are found both free and lodged in the white corpuscles, in the œdematous tissue, and in the blood capillaries. A minimal quantity of this blood produces the disease if inoculated in house-mice or sparrows. Field-mice have an immunity. Rabbits and guinea-pigs inoculated in the ear suffer from only a local erythema, which disappears after five or six days, and renders them for a time im-

mune. Rabbits inoculated in the cornea suffer from an intense inflammation of the eyes. The bacilli are easily cultivated outside the body on a mixture of aqueous humour and gelatine, and especially on nutrient gelatine rendered slightly alkaline with sodium phosphate. They grow also very well on the ordinary nutrient gelatine, forming in plate cultivations scarcely perceptible cloud-like specks, and in a test-tube of nutrient gelatine they form a delicately clouded cultivation along the needle track (Figs. 38 and 39). A small quantity of a pure cultivation carried through many generations reproduces the disease when inoculated into mice. The organs should be hardened in absolute alcohol, and sections stained preferably by the method of Gram (Plate XXII., Figs. 1 and 2).

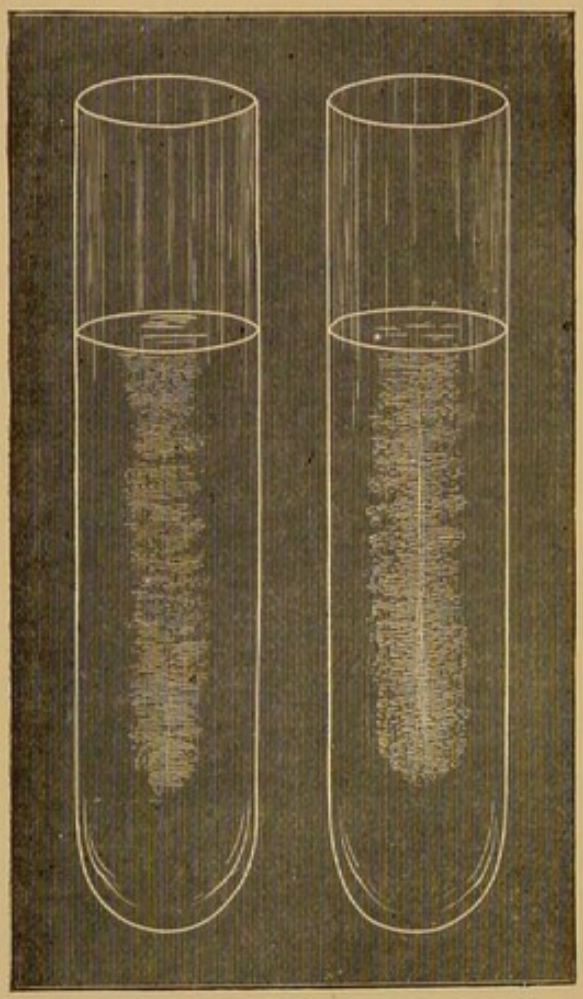

Fig. 38. Fig. 39.

PURE-CULTIVATIONS OF THE BACILLUS OF SEPTICÆMIA OF MICE IN GELATINE-PEPTONE-BROTH.

Fig. 38. In two days.
Fig. 39. In five days.

Bacillus typhosus, Eberth (*Bacillus of typhoid*

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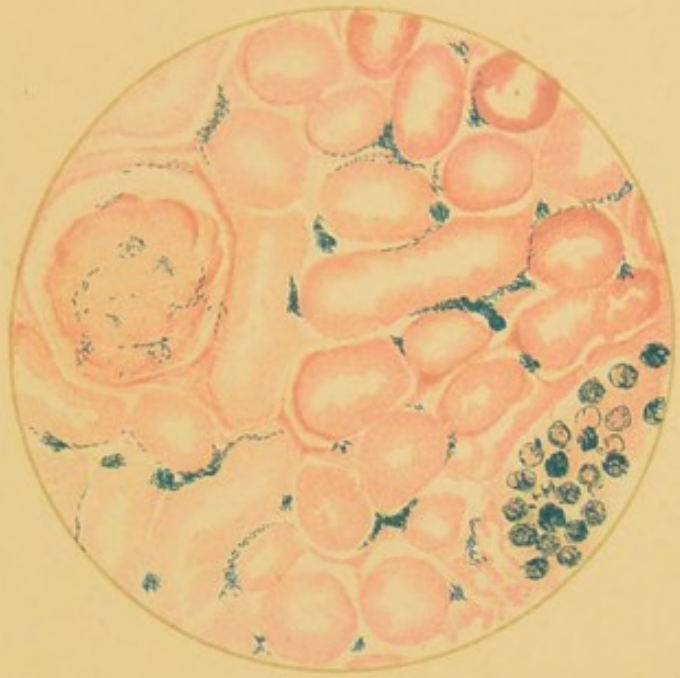
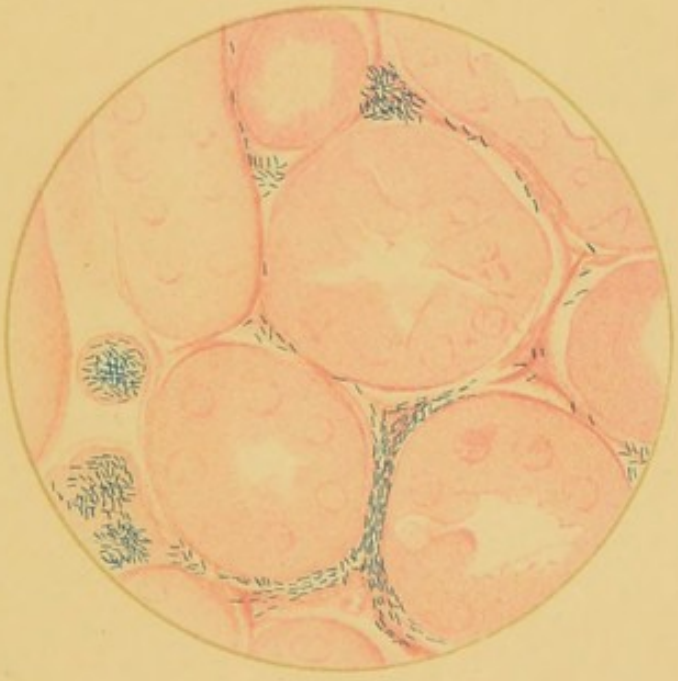


Fig. 1. From a section of Kidney of a mouse. Gram's method and Eosin. Zeiss' DD. Oc. 2.



BACILLUS OF SEPTICÆMIA OF MICE.

Fig. 2. The same preparation. Zeiss' $\frac{1}{18}$. o.i. Oc. 4.

Edgar Crookshank fec. et pinx.

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Vincent Brooks, Day & Son, Lith.

fever).—Rods, ·2 μ broad, and forming filaments up to 50 μ long;* or† rods, short, rounded at their ends, and occasionally constricted in the middle; some exhibiting spore formation. These bacilli have been observed in inflamed Peyer's glands, in the spleen, mesenteric glands, and the lungs in fatal cases of typhoid fever. More recently‡ a bacillus has been cultivated on several plates of gelatine which were inoculated from different spleens. After twenty-four hours the course of the inoculation streak became visible, and in forty-eight hours a distinct whitish growth had developed. With a low power this was found to consist of numerous colonies of a yellow-brownish colour. The gelatine was not liquefied. The rods varied in length, were capable of development into threads, and both forms were motile. They can be cultivated on potatoes at 37° C. They grow well also on blood serum, forming a whitish-grey, somewhat transparent layer. Spore formation occurs at the ends of the rods. Inoculation experiments gave negative results.

METHODS OF STAINING THE BACILLUS OF TYPHOID FEVER.

The bacilli stain badly with most aniline dyes. The method of Gram can be employed, or the sections may be left for twenty-four hours in methylene-blue. Koch recommends bismarck-brown. To colour the spores cover-glass preparations and sections must be left for several

* Kleb's *Arch. f. Experimental Pathol.* 1880.
† Eberth, *Virchow's Archiv*, Bd. 83.
‡ Gaffky, *Mittheil a. d. K. Gesundheitsamte.* 1884.

days in the fuchsine solution employed in the method of Erhlich (p. 163); or the solution may be warmed, and in the case of cover-glasses, even raised to boiling-point. They are then decolorised with nitric acid, and after-stained with methylene-blue.

Bacillus lepræ, Hansen.—Fine slender rods, 4—6 μ long, and less than 1 μ wide, occasionally pointed at both ends, some clearly motile, and others not. Many possess bright oval spores, and others have a beaded appearance. They have been cultivated artificially on blood-serum and alkaline meat extract. Inoculation experiments on monkeys and other animals have failed to produce the disease; though in cats and rabbits there have been indications of success.* The bacilli occur in enormous numbers in tubercular leprosy in the nodules of the skin (Plate XXIII., Figs. 1 and 2), and of the mucous membrane of the mouth, palate, larynx, etc.† They occur also in the liver, spleen, testicles, lymphatic glands, and kidneys (Plate XX., Fig. 2); and in the interstitial tissue of the nerves in anæsthetic leprosy. They probably spread by the lymphatics, and are not found in the blood. In their behaviour to staining reactions they are similar to the bacillus of tubercle, except that they stain much more readily.

* Damsch, *Virchow's Archiv*, Bd. 92, Heft 1.

† Thin, *Med.-Chir. Trans. Lond.*, 1883; *Brit. Med. Journal*, No. 1229, 1884, and Steven, *Brit. Med. Journal*, No. 1281, 1885.

To face page 176.

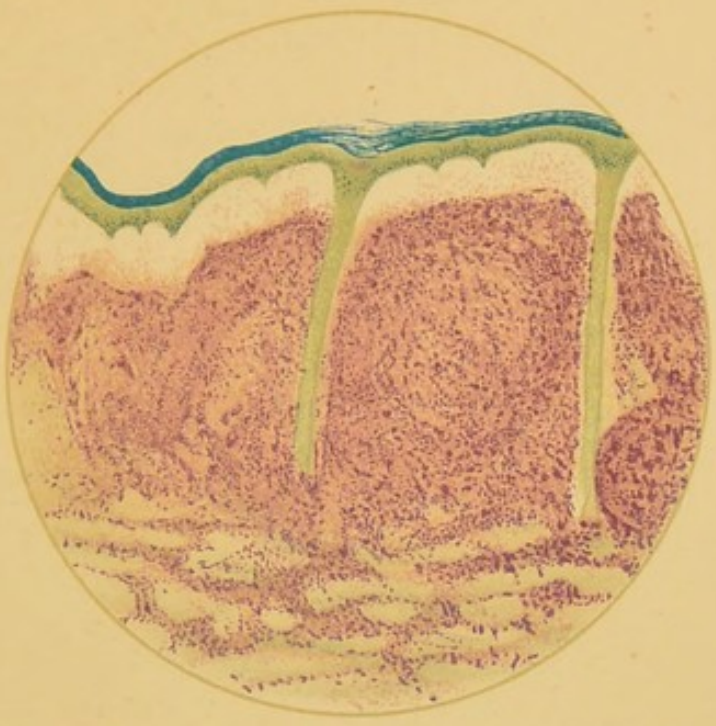
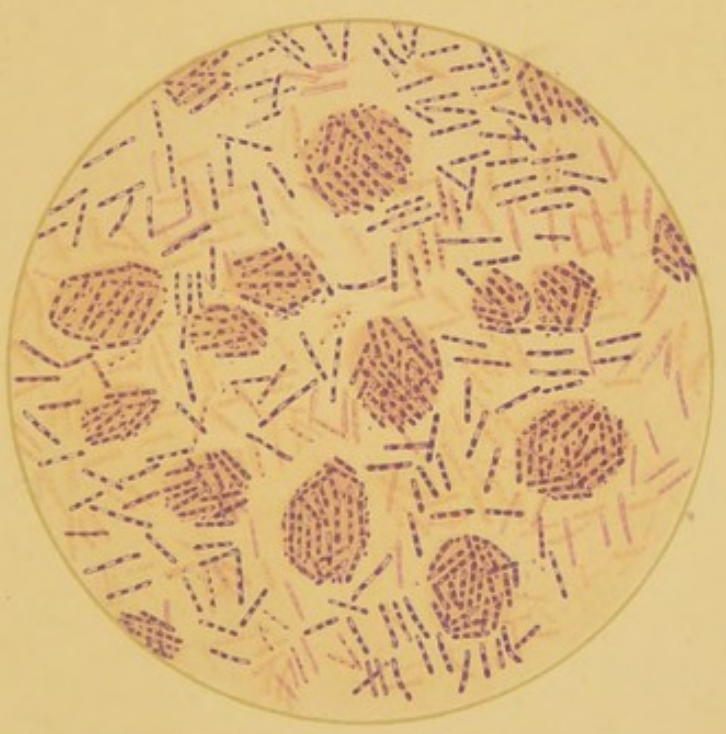


Fig. 1. From a section of skin from a case of leprosy. Ehrlich's method. (fuchsine and methylene blue.) Zeiss AA. Oc. 2.



BACILLUS LEPRÆ.

Fig. 2. The same preparation.-Zeiss' $\frac{1}{18}$. o.i. Oc. 4.

Edgar Cockshank. fec. et pinx.

London, Published by H. K. Lewis, 136, Gower Street.

Vincent Brooks, Day & Son, Lith.

METHODS OF STAINING THE BACILLUS OF LEPROSY.

Cover-glass-preparations may be made in the ordinary way, or by a special method, which consists in clamping a nodule with a pile-clamp, until a state of anæmia of the tissue is produced. On pricking with a needle or sharp knife a drop of clear fluid exudes, from which cover-glass-preparations may be made.* Cover-glass-preparations and sections may be stained by Ehrlich's method (p. 48), or the latter by the following process :—

Method of Babes.†—Preparations are stained in a solution of rosaniline hydrochlorate in aniline-water. Decolorise in 33 per cent. hydrochloric acid, and after-stain with methylene-blue.

Bacillus of diphtheria of man.—Rods about the same length as the tubercle bacillus, but about twice as thick; the longer ones consist of single individuals linked together. Cultivated in 5 per cent. gelatine at 20—22° C., the rods develop into irregular involution-forms. Inoculation results are doubtful. The bacillus was isolated from diphtheritic membrane.‡ In the diphtheria of calves and pigeons a bacillus also has been observed. Inoculation with a cultivation of the latter produced the disease in young pigeons and sparrows. (*Vide Streptococcus diphtheriticus.*)

Bacillus malariæ, Klebs (*Bacillus of intermittent fever*).—Rods, 2—7 μ long, which grow into twisted threads. Spore formation takes place

* Manson, *Lancet.* 1884.

† *Compt. Rend. de l'Acad d. Sc.* 1883.

‡ Löffler, *Mittheil. a.d. Gesundheitsamt*, Zweiter Band.

in the centre, or at either end (Plate I., Fig. 14). They grow in liquids rich in nitrogen, such as albumen, urine, and glue. Deprived of oxygen, they cease to develop, and are, therefore, ærobic.

Inoculated into rabbits they produce a febrile disorder considered analogous to malarial fever,* and in the spleen and marrow the threads and spores of the bacilli were found in abundance. Bacilli with end-spores have been discovered also in the blood of patients suffering from malaria.†

The bacillus was first described as present in the soil of the Roman Campagna.

Bacillus mallei (*Bacillus of glanders*).—Rods about the size of tubercle bacilli. When cultivated on solid sterile blood serum at 38° C., the growth appears in the form of minute, transparent drops consisting entirely of the characteristic bacilli. On sterilised potatoes they form, in a week to ten days at 37° C., a brown gelatinous layer. Pure cultivations after several generations produce the following results when inoculated into horses, rabbits, guinea-pigs, and field-mice. A spreading ulcer with indurated base appears at the site of inoculation, while smaller ulcers break out in its vicinity. The lymphatics become swollen, and general infection follows in the form of nodules in the internal organs, and nodules and ulcers on the nasal septum. In guinea-pigs a characteristic tumour of the testis,

* Klebs and Tommasi-Crudeli, *Archiv f. Exper. Pathol.*, 1879.
† Marchiafava, *ibid.*

or ovary and vulva, frequently results, and should be prepared for microscopical sections. The bacilli are found in the nodules of the nasal mucous membrane, the lung, spleen, liver, and other organs in horses and sheep affected with glanders.

METHODS OF STAINING THE BACILLUS OF GLANDERS.

The bacilli of glanders are extremely difficult to demonstrate. The most satisfactory results are obtained as follows:

Method of Schütz.—The sections are placed for twenty-four hours in a mixture of

Potash solution (1 in 10,000);	Equal parts.
Concentrated alcoholic methylene-blue soltn.;	

Wash the sections in a watch-glass with water acidulated with four drops of acetic acid. Transfer for five minutes to 50 per cent. alcohol, fifteen minutes to absolute alcohol, clarify in clove-oil, and mount in Canada balsam.

Bacillus caucasicus, Kern.—Rods forming two spores, one at each end, otherwise similar to *bacillus subtilis.* They occur in the form of whitish lumps in company with *Saccharomyces mycoderma* in the production of a drink "kephir" from cow's milk. The fermentation is not due to the bacillus.

Bacillus dysodes, Zopf.—Cocci, long and short rods, and spores. They were observed in bread, making it greasy and unfit for food, and generating a penetrating odour resembling a mixture of peppermint and turpentine. A great loss may result

to bakers if the fungus is introduced with the yeast.

Bacillus Hansenii, Rasmussen.—Rods, 2·8—6 μ long, ·6—·8 μ wide. Cultivated on sterilised potato in four days they form a chrome-yellow layer with an agreeable fruitlike smell. Two or three days later the growth dries, and changes to orange-yellow in colour; later it passes to yellowish or brown, and forms at the same time spores 1·7 μ long, 1·1 μ wide. The colouring matter is insoluble in most reagents.

This bacillus occurs on nourishing solutions, malt infusion, broth, wine, which have been kept at 31 to 33° C., as a yellow or whitish skin.

Bacillus erythrosporus, Cohn.—Motile rods and threads; rods exhibiting spore formation. They were found to form a pellicle on meat-extract-solutions and on rotting albuminous liquids.

Bacillus ruber, Frank.—Minute motile rods, singly, in twos, and fours. They were observed on boiled rice, where they develop a brick-red pigment.

Genus V. Vibrio.

Vibrio rugula, Müller.—Rods and threads, 6—16 μ long, about ·5—2·5 thick. The rods are either simply bowed, or possessed of one shallow spiral. They bear a flagellum at each end. The rods form swarms when causing decomposition, and then, or after, grow out into threads, curved

in a screw-like manner. In the next stage of development the rods cease to move, and become swollen with granular contents. One extremity develops an enlargement, giving the rod the appearance of a pin. The spore formed by the contraction of the plasma in the swollen end finally becomes globular. The vibrios appear in vegetable infusions, causing fermentation of cellulose.

Genus VI. Clostridium.

Clostridium butyricum, Prazmowski (*Bacillus amylobacter* Van Tieghem; *Bacillus butyricus. Bacillus of butyric acid fermentation*).—Rods of 3—10 μ long, and under 1 μ wide, often indistinguishable from *bacillus subtilis*. They grow out into long, apparently unjointed threads. They are mostly actively motile, but also occur in zoogloœa. The rods and threads are sometimes slightly bent like vibrios. They are anærobic. The shorter rods as a rule swell in the middle, becoming ellipsoidal, lemon or spindle-shaped; the long rods, and sometimes the short ones, swell at one end; in either case ellipsoidal spores are developed.

If they be cultivated in nutrient gelatine, the medium is liquefied, and a scum formed on the surface. They grow best between 35° and 40° C. The spores are widely distributed in nature, and grow readily on fleshy roots, old cheese, etc. They convert the lactic acid in milk into butyric acid,

and produce the ripening of cheese. They occur also in solutions of starch, dextrine, and sugar, and are the active agents in the fermentation of sauerkraut and sour gherkins.

METHOD OF STAINING THE BACILLUS OF BUTYRIC ACID FERMENTATION.

Treat the bacilli with iodine-solution. At certain stages of the fermentation-process the plasma takes a blue or violet-black coloration. The young rods give the former appearance, and the older ones the latter. It is most easily observed when the bacillus is cultivated in a substance containing starch, or, if starch is wanting, in the presence of cellulose, calcium-lactate, or glycerine; in bacilli cultivated in sugar solutions the reaction seldom appears.

Clostridium polymyxa, Prazmowski.—Threads consisting of rods which vary in length; cocci, involution-forms, and spores are also present; cultivated on nourishing solutions they develop a thick skin on the surface. On boiled beet and other roots it forms a gelatinous scum, which often consists of crinkled, tough masses, several cm. in diam., somewhat similar to the *Ascoccus Billrothii*. They cause fermentation in solutions of dextrine, and more actively in potato or bean paste. Some cells give the iodine reaction weakly, as in *clostridium butyricum*.

Clostridium of symptomatic anthrax (*Rauschbrand, Charbon symptomatique**).—Rods rounded at

* Arloing, Cornevin et Thomas, *Bull. de l'Acad. de Med.* 1881.

the ends, mostly with a shining spore at one end. They are especially distinguished from the bacilli of anthrax by being motile. Cultivated on blood-serum, threads develop, consisting of both rods and cocci. From blood-serum they can be cultivated on nutrient gelatine, and vegetable albumen.

Cultivation does not deprive the micro-organism of its virulence, but heating the spores to 85° C. renders them harmless.

Inoculation in the subcutaneous tissue of guinea-pigs, rabbits, calves, and sheep proves fatal. White rats, dogs, and fowls have an immunity. Injection into the veins in small quantity produces a febrile disorder, in larger quantities death. Animals in the former case suffer an abortive illness, which protects them against further inoculation. The micro-organism is the cause of a disease in cattle, "*blackleg*," "*quarter-evil*," or "*Rauschbrand*." At the autopsy the micro-organisms are found in the subcutaneous connective tissue, in the lymph glands, kidneys, spleen, and lungs. An irregular tumour is formed in the skin, which develops rapidly, and gives crepitus on palpation. The tumour, which is hæmorrhagic effusion, occurring in the extremities, impedes the animal's movements. The cattle infected die in thirty-six to forty-eight hours.

Group III. Leptotricheæ.

Genus I. *Crenothrix.*—Threads articulated; cells sulphurless; habitat water.

Genus II. *Beggiatoa.*—Threads unarticulated; cells with sulphur granules; habitat water.

Genus III. *Phragmidiothrix.*—Threads jointless; successive subdivision of cells is continuous; cells sulphurless; habitat water.

Genus IV. *Leptothrix.*—Threads articulated or unarticulated; successive subdivision of cells not continuous; cells sulphurless.

Genus I. Crenothrix.

Crenothrix Kuhniana, Rabenhorst. — Cocci, rods, and thread-forms. The cocci are globular, 1—6 μ in diam. The threads are colourless, 1·5—5 μ thick, and club-shaped at the extremity, reaching a diam. of 6—9 μ. The threads form colonies with a brick-red, olive-green, or dark-brown to brown-black coloration caused by impregnation with oxide of iron. The threads are distinctly articulated, and ensheathed. The segments are set free when the sheath bursts, and develop into new threads. In other cases the segments remain enclosed, and subdivide into discs, which, by vertical fission, break up into globular forms (cocci). These again develop into new threads, either within the sheath and eventually penetrating it, or, after they are set free.

The micro-organism appears in little whitish or brownish tufts in wells and drain-pipes, and it not only renders drinking-water foul, but may stop up the narrower pipes.

Genus II. Beggiatoa.

Beggiatoa alba, Vauch.—Cocci, rods, spirals, and threads. The threads are longer and thicker than leptothrix, indistinctly articulated, actively oscillating, and colourless; their protoplasm contains numerous, strongly refractive granules consisting of sulphur. They occur as greyish- or chalk-white gelatinous threads, 3—3·5 μ thick, in sulphur springs and marshes.

Beggiatoa roseo-persicina (*Cohnia roseopersicina. Bacterium rubescens. Peach-coloured bacterium*). —Cocci, rods, spirals, and threads. The cocci, globular or oval, reach 2·5 μ in diam. They form at first solid families, bound together by gelatinous substance. Later they become larger, globular or ovoid in shape, and hollow, containing watery fluid in their interior. The families reach a diameter of 660 μ, in which the cocci form simply a peripheral layer. The hollow families or vesicles are often perforated, presenting a delicate reticulated appearance, which finally may become broken up into irregular structures. The red colouring matter can be distinguished from other red pigments, and is designated by the name bacteriopurpurin. It is quite distinct from the pigment

produced by *Micrococcus prodigiosus*, being peach-blossom red, and insoluble in water, alcohol, etc. Examined spectroscopically it shows a strong absorption in the yellow, and a weaker band in the green and blue, as well as a darkening in the more refrangible half of the spectrum. In the cocci, especially of the older vesicles, dark granules are to be seen, which consist of sulphur. The micro-organisms occur on the surface of marshes, or on water in which algæ are rotting. They form a rose-red, blood-red, violet-red, or violet-brown scum; and sometimes in such quantity that whole marshes and ponds may be coloured blood-red by them.

Beggiatoa mirabilis, Cohn.—Threads distinguished from others of this genus by their breadth, which may reach 30 μ. They are motile, bent and curled in various ways, and rounded at the ends. Around the threads isolated cells have been observed, "macrococci," but spiral forms are as yet unknown. The threads are filled with sulphur granules. They occur on sea water, forming a white gelatinous scum on decomposing algæ, etc.

METHODS OF EXAMINING SPECIES OF BEGGIATOA.

The articulation of the threads is best demonstrated by staining with an alcoholic solution of methyl-violet, fuchsine, or vesuvin; or by treating with sodic sulphate, or warm glycerine.

Genus III. Phragmidiothrix.

Phragmidiothrix multiseptata.—Cocci and threads. The latter, 3—6 μ in breadth, are separated by transverse partitions into short cylindrical discs, whose height is a fourth or sixth of their breadth. Repeated transverse and longitudinal division takes place in the discs, resulting in the formation of cocci. The cocci have not been observed isolated from the threads in a free state, but they develop *in situ* into slender threads. In addition to this continuous subdivision, *Phragmidiothrix* differs from *Beggiatoa* in the absence of sulphur, and from *Crenothrix*, by its wanting a sheath. They occur attached to crabs (*Gammarus locusta*) in sea-water.

Genus IV. Leptothrix.

Leptothrix buccalis, Robin. — Long, thin threads, ·7—1 μ broad, colourless, often united in thick bundles or felted together. Masses of cocci occur with the threads, and the threads themselves are composed of long rods, short rods, and cocci. The threads may break up into spiral-, vibrio-, and spirochæte-forms. The last-named occur in large numbers in the mouth, and have been named *spirochæte buccalis*. The leptothrix buccalis is found in teeth slime, and is believed to be intimately connected with dental caries. The threads pene-

trate the tissue of the teeth, after the enamel has been acted upon by acids generated by the fermentation of food. The short rods, long rods, cocci, leptothrix-forms, and screw-forms are found in the dental canals.

METHODS OF STAINING THE LEPTOTHRIX BUCCALIS.

The threads of leptothrix buccalis have a special staining reaction (Leber). They become coloured if in an acid medium with iodine; if the medium is alkaline, it must be acidified with very dilute hydrochloric acid or acetic acid, and the filaments then stained with iodine. The contents are stained violet, and contrast with the sheath and septa, which remain uncoloured.

Leptothrix gigantea, Miller. — Long rods, short rods, and cocci, can be observed in the same thread. There are also screw-threads, which may take the form of spirals, vibrios, or spirochæte. The threads increase in diameter from base to apex, and corresponding with the thickness of the threads, the rods and cocci show different dimensions. They have been observed in the diseased teeth of dogs, sheep, cats, and other animals.

Group IV. Cladotricheæ.

Genus I. Cladothrix.

Cladothrix dichotoma, Cohn.—Threads resembling those of leptothrix; slender, colourless, not articulated, straight or slightly undulated, and

in places twisted in irregular spirals with pseudo-branchings. The development can be traced from the cocci to rods and threads. The latter are at the beginning simple threads, which were formerly described as *Leptothrix parasitica*, or if coloured by impregnation with iron, as *Leptothrix ochracea*. Later they form false branches by single rods turning aside, which by repeated division lengthen into threads. A thread appears to be first composed of long rods, then of short rods, and lastly of cocci. The iodine reaction must be applied to distinguish these forms, especially when the sheath of the threads has a yellow, rust-red, olive-green, or dark-brown coloration. The cocci may grow into rods while still in the sheath, and finally become leptothrix threads, surrounded by a delicate gelatinous sheath, from which the false branching proceeds. Fragments may break off, which are actively motile, and appear as vibrios, spirilla, and spirochæta-forms. They may also occur in zoogloœa.

They are the commonest of all bacteria in both still and running water, in which organic substances are present. They are observed also in the waste water of certain manufactures, such as sugar. Artificially they can be cultivated on infusions of rotting algæ and animal substances, forming on these media small tufts, about 1—3 μ, and floating masses.

Cladothrix Fœrsteri (*Streptothrix Förster*i,

Cohn).—Cocci, rod-forms, and leptothrix-threads. The threads are twisted in irregular spirals, and branch sparingly and irregularly. Screw-forms are produced by the threads breaking up into small pieces. They occur in the lachrymal canals of the human eye, in the form of closely felted masses.

Sphærotilus natans.—Cells 4—9 μ long, 3 μ thick, united in a gelatinous sheath to form threads. The cells comprise rods and cocci-forms; the cocci are set free, and develop into rods, which again form threads. In the last a false branching has been observed. The plasma of the cells breaks up into minute, strongly refractive portions, which develop into round spores, at first of a red, and afterwards a brown colour. They occur in stagnant and flowing water contaminated with organic matter, and form floating flakes of a white, yellow, rust-red, or a yellow-brown colour.

CHAPTER X.

SPECIES OF SCHIZOMYCETES MENTIONED BY WRITERS AND NOT DESCRIBED, OR NOT RECOGNISED AS DISTINCT SPECIES, IN THE PRECEDING CLASSIFICATION.

Micrococcus cyaneus, Cohn (*Bacteridium cyaneum*, Schröter).—Elliptical cells, growing upon cooked potato, and producing a blue colour. In nutrient solutions it forms zoogloœa, which are at first colourless, then bluish-green, and finally intense blue.

Micrococcus candidus, Cohn.—Forms snow-white points and spots, upon slices of cooked potato.

Micrococcus indicus, Koch.—Round cells, which grow upon sterilised potato, forming a vermilion layer (Plate XV., Fig. 1). In nutrient agar-agar the appearances are very characteristic. In a pure cultivation a brilliant, vermilion-coloured reticulated pellicle develops on the surface (Plate II., Fig. 1). In the track of the needle beneath the surface no pigment is formed (Plate XIII., Fig. 1). Cultivated in nutrient gelatine it liquefies the medium, and colours it crimson. The growth, of

a darker crimson hue, subsides to the bottom of the tube. In plate-cultivations on nutrient agar-agar, the colonies have a scarlet tint. They are round, ovoid, or spindle-shaped, and have characteristic granular margins.

Micrococcus crepusculum, Cohn. (*Monas crepusculum*, Ehrenberg. *Mikrokokken in faulenden Substraten*, Flügge). Round or short oval cells, scarcely 2 μ in diam.; singly or in zoogloea. Occurs in various infusions and putrefying fluids in company with *Bacterium termo*.

Micrococcus septicus, Cohn. (*Microsporon septicum*, Klebs).—Cells round, about 5 μ in diam. Singly, in chains and masses of zoogloea. They occur on the mucous membrane of the mouth, in catarrhal exudations, on the surface of intestinal and other ulcerations, in the cavity of the intestines, the secretions of open wounds and ulcers, abscesses and purulent inflammations, the serum of epidemic puerperal fever, and the tissues and vessels in cases of pyæmia and septicæmia (*vide Streptococcus pyogenes*, etc.).

Micrococcus endocarditicus, Klebs.—Cocci 1 μ and ·5 μ in diam., and chains. They have been observed in masses upon the altered valves and in the detritus of the ulcerations of the endocardium in *endocarditis ulcerosa;* as chains also in the muscle of the heart, and forming plugs in the vessels of the heart, spleen, and kidney. Some forms are identical with *Staphylococcus pyogenes*

*aureus.** Micrococci have also been described in connection with chronic and other forms of endocarditis.

Micrococcus of measles.—Round cocci and diplococci have been observed in the catarrhal exudations, in the papules and in the capillary vessels of the skin and in the blood of patients attacked with measles.†

Micrococcus of scarlatina.—Cocci have been described in cases of scarlet fever as being present in the blood,‡ in the scales of the desquamating epidermis,§ and in the discharges and ulcerated tissue of the throat.

Micrococcus of cerebro-spinal meningitis.‖ —Cocci, diplococci, and chains have been observed in the exudation of cerebro-spinal meningitis; all forms were detected in the meninges, and zooglœa in the kidneys.

Micrococcus of typhus. — Actively motile dumb-bell cocci have been described in the blood, and plugs of cocci in the lymphatics of the heart, in cases of typhus fever.¶

Micrococcus of acute yellow atrophy.— Cocci have been observed in the vessels of the liver in this disease.**

* Ziegler, *Patholog. Anat.* 1885.

† Keating, *Phil. Med. Times.* 1882. Cornil and Babes, *Les Bacteries.* 1885.

‡ Coze and Feltz, *Malad. Infect.* 1872.

§ Pohl-Pincus, *Centralblatt f. d. Med. Wiss.* 1883.

‖ *Leyden. Centralblatt f. Kl. Med.* 1883.

¶ Mott, *Brit. Med. Journal.* 1833.

** Eppinger, *Prager Vierteljahrsschrift.* 1875.

Micrococcus of whooping cough.—Elliptical cocci are said to be constantly present in the expectoration of persons suffering from whooping cough.*

Micrococcus of hæmophilia neonatorum, Klebs.—A coccus, which has been named *monas hæmorrhagicum*, is stated to be characteristic of this disease.

Micrococcus of puerperal fever.—Cocci in zoogloœa, and sometimes in chains, are present in all organs affected in puerperal fever, and especially in the endocardium, lung, spleen, kidney, and brain (*vide Streptococcus pyogenes*).

Micrococcus of gangrene.—Oval and round cocci are found, which form zoogloœa in the depth of gangrenous tissues. From gangrene of the lung cocci have been isolated, which form greyish-white colonies in plate-cultivations of nutrient gelatine. In a test-tube of nutrient gelatine a growth results chiefly on the surface; the cultivations yield a penetrating odour.

Micrococcus of yellow fever.—Cocci ·6—·7 μ in diameter have been observed in this disease.† They occur in chains, aggregated in masses, which distend the vessels of the kidney and liver.

Micrococcus of dental caries.—Several species of micrococci are believed to be intimately connected with caries of the teeth.‡

1. Occurs in the form of cocci, diplococci, and

* Bürger, *Berl. Klin. Woch.* 1883.
† Cornil and Babes, *Les Bacteries.* 1885.
‡ Miller, *Deutsche Med. Woch.* 1884.

chains, which develop very rapidly in nutrient gelatine, speedily converting it into a turbid liquid. They are the agents of lactic acid fermentation.

2. Occurs in the form of cocci, rods, and filaments, which develop slowly in nutrient gelatine. The different forms may be observed in the canaliculi of the dentine.

3. Occurs as very small cocci, rarely in chains, which rapidly liquefy nutrient gelatine.

4. Occurs as cocci, which very slowly liquefy the gelatine, the culture retaining for some time the appearance of a thread (*vide Comma bacillus of the mouth*).

Micrococcus of saliva (*Microbe de salive*, Pasteur).—A micrococcus has been cultivated from the saliva of a child which had died of hydrophobia.* The saliva produced a fatal result when injected into rabbits. Micrococci have also been observed in normal human saliva, which produced septicæmia in rabbits.† The virulence of the child's saliva was, therefore, probably independent of the poison of rabies. Hydrophobia is, nevertheless, believed to be due to a micro-organism, and researches in connection with the virus are at the present time exciting keen interest.

Micrococcus of pyæmia in mice, Klein.‡—Certain cocci which were present in pork broth proved fatal to mice in about a week, producing purulent inflammation and abscess in the lungs.

* Pasteur, *Comptes Rendus*, xlii.
† Magnin and Sternberg, *Bacteria*. 1884.
‡ Klein, *Micro-org. and Disease*. 1885.

Fresh inoculations in mice again produced a fatal result with pyæmic symptoms.

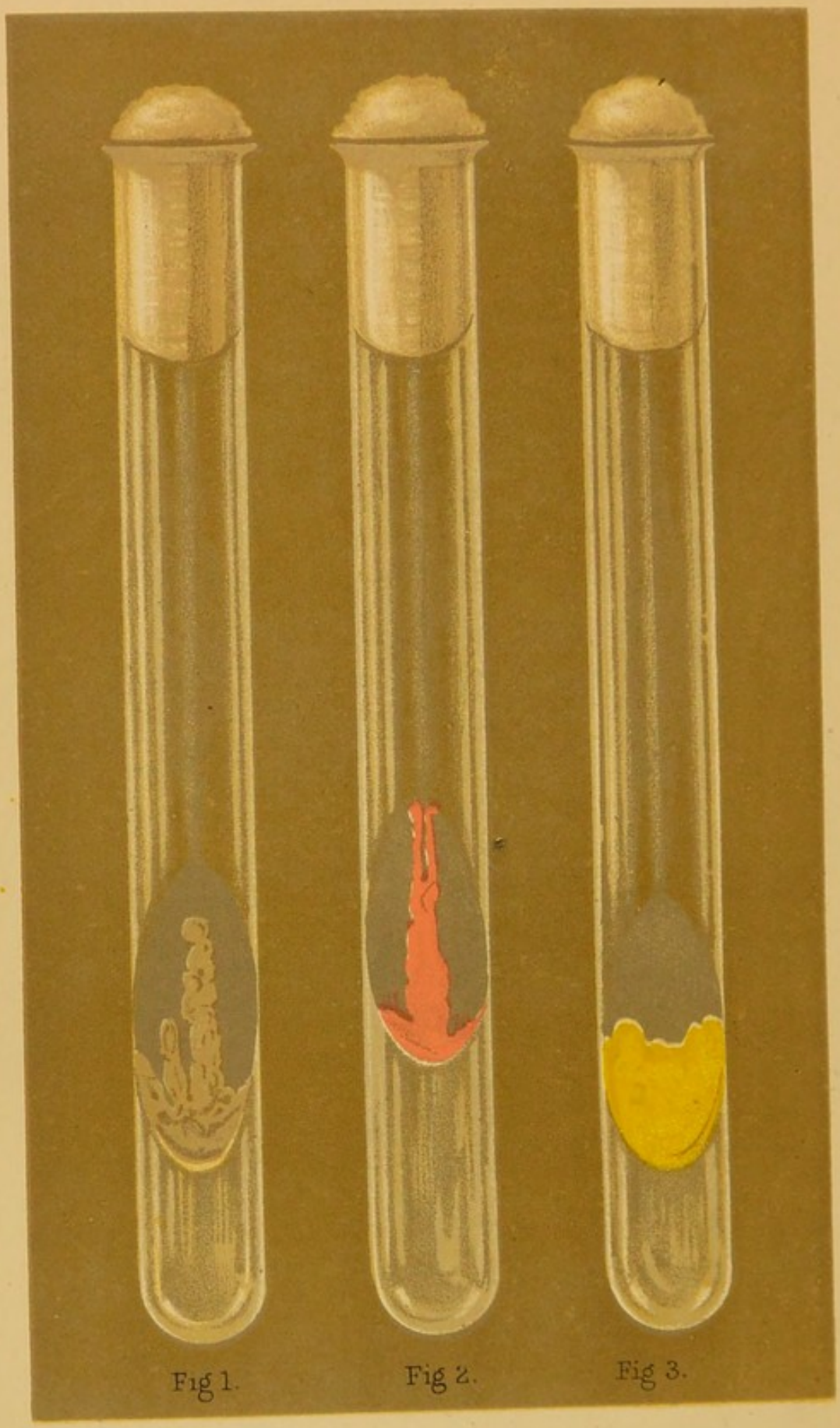
Micrococcus of swine-fever, Pasteur.—Cocci, diplococci, chains, and zooglœa have been described as present in the blood in "*rouget du porc.*" The microbe has no effect upon fowls, but kills rabbits and sheep. Inoculated into healthy pigs it gives rise to the disease, and occasions a fatal result. Inoculation with weakened virus protects against virulent matter.

Micrococcus of septicæmia consecutive to anthrax, Charvin.—In rabbits, some hours after death from anthrax, micrococci have been found in the kidney and elsewhere. Inoculated into rabbits fatal septicæmia is produced without the presence of suppuration.

Micrococcus toxicatus, Burrill.—Globular cells, ·5 μ in diam., singly, in pairs, and rarely in chains. They occur in species of *Rhus* (*Sumach*), and have been credited with being the cause of the inflammation which is produced by the poison of the plant.

Micrococcus insectorum, Burrill.—Obtusely oval cells, ·7—1 μ long and ·55 μ broad, singly, in pairs, chains, or zooglœa. They were detected in the digestive organs of the chinck-bug (*Blissus leucopterus*) when suffering from a certain contagious disease.

Micrococcus amylivorus, Burrill.—Oval cells, 1—1·4 μ long, ·7 μ broad, singly, in pairs, and rarely in fours, never in chains, are found em-



CULTIVATIONS ON NUTRIENT AGAR-AGAR

Fig 1. Bacterium lineola.
Fig 2. Micrococcus rosaceus.
Fig 3. Staphylococcus pyogenes citreus.

H. Crookshank Pinxt.

Vincent Brooks, Day & Son, Lith.

London, Published by H. K. Lewis, 136, Gower Street.

bedded in an abundant mucilage which is very soluble in water. They have been described as producing the so-called "fire blight" of the pear tree and other plants.

Micrococcus of mucoid fermentation, Pasteur.—Globular cells ·2 μ in diam., singly or in chains. These and allied forms have been considered to be the cause of mucoid fermentation in wine and beer * (*vin filant*, *bière malade*).

Staphylococcus pyogenes citreus.†—Cocci singly or in pairs, or zoogloea. If cultivated on nutrient gelatine or nutrient agar-agar, a sulphur or lemon-yellow growth develops (Plate XXIV., Fig. 3). When inoculated under the skin of mice, guinea-pigs, or rabbits, an abscess forms after a few days, from which a fresh cultivation of the micro-organism can be obtained. They are frequently present in pus.

Staphylococcus cereus albus.†—Cocci also obtainable from pus, but distinguished by forming on nutrient gelatine a white, slightly shining layer, like drops of stearin or wax, with somewhat thickened, irregular edge. The needle track develops into a greyish-white, granular thread. In plate cultivations, on the first day, white points are observed, which spread themselves out on the surface to spots of 1—2 mm. When cultivated on blood serum a greyish-white, slightly shining streak

* Pasteur, *Etudes sur le Vin; sur la Bière.* 1866; 1876.
† Passet, *Fortschritte der Medecin*, Jan. 15th and Feb. 1st, 1885.

develops, and on potatoes the cocci form a layer which is similarly coloured.

Staphylococcus cereus flavus.*—Cocci which also occur in pus. If cultivated in nutrient jelly the growth, which is at first white, becomes a lemon-yellow, somewhat darker in colour than *Staphylococcus pyogenes citreus*. Microscopically *Staphylococcus cereus flavus* corresponds with *Staphylococcus cereus albus*, and they both form zoogloœa of medium-sized cocci (diam. 1·16 μ). Inoculation experiments with both kinds give negative results. Among the micro-organisms present in pus a coccus has been described as occurring occasionally, which is almost identical with *Bacterium pneumoniæ crouposæ;* compare also *Streptococcus pyogenes* (p. 114) and *Micrococcus pyogenes aureus* (p. 122).

Bacillus pyogenes fœtidus.†—Small rods, with rounded ends of about 1·45 μ in length, and ·58 μ in width; often in twos, or chains. When cultivated in nutrient gelatine, a greyish, veil-like growth forms on the surface. In plate cultivations white points appear after twenty-four hours, which develop into greyish spots, and these enlarging coalesce into a layer. In nutrient agar-agar the cultivation resembles the growth on gelatine. On blood serum a moderately thick, greyish-white streak develops, and on sterilised potato an abundant, shining, brownish culture. From all these media a putrid odour emanates, but

* Passet, *ibid.* † Passet, *ibid.*

no smell is detected from a cultivation in milk. Inoculated into mice and guinea-pigs abscesses are produced or death from septicæmia results. They are occasionally present in pus.

Bacterium termo, Dujardin.—Short cylindrical or oblong cells, 1·5 μ long, ·5—·7 broad, generally occurring as dumb-bells. The cells have dark contents, invested by a thick membrane, and are provided with flagella, to which the characteristic movements are due (Plate I., Fig. 8). They are the cause of putrefaction, and invariably appear in decomposing albuminous substances and liquids. A growth can be readily started by placing a piece of meat in water in a warm place. Cultivated in broth, they produce a turbidity, and on sterilised potatoes, a slimy grey layer. (Compare *Bacterium æruginosum*).

Bacterium lineola.—Cells 3·8 μ—5·2 μ long, 1·5 μ wide. They occur singly or in pairs, occasionally in zooglœa, but never in chains. The cells are provided with flagella, and contain strongly refringent contents. They resemble *Bacterium termo* in form and in movement, but are considerably larger. They occur in well water and stagnant water, and form slimy heaps on rotting potatoes, and zooglœa and pellicles on various infusions. Cultivated on nutrient agar-agar they form a semi-transparent growth (Plate XXIV., Fig. 1).

Bacterium lactis.—Cells* about 1·5—3 μ long. They are constricted in the middle, and arranged

* Pasteur.

in threads and colonies. They are actively motile. They convert milk sugar into lactic acid ; *Bacterium lactis* has also been described * as consisting of cocci, short rods, long rods, filaments, and saccharomycetoid forms (involution-forms). The cocci are about ·5 μ in diam., in pairs or chains, and threads 1·25 μ in diam. The bacteria readily appear in milk, and produce the lactic acid fermentation.

Bacterium litoreum, Warming.—Cells ellipsoidal 2—6 μ long, 1·2—2·4 μ wide, occur singly in sea water, never as chains or zooglœa.

Bacterium fusiforme, Warming. Cells spindle-shaped, with pointed ends, 2·5 μ long and ·5—·8 μ thick. Observed as a spongy layer on the surface of sea water.

Bacterium navicula, Reinke and Berthold.—Cells spindle-form or ellipsoidal, including motile and non-motile forms. They have one or more dark spots, which may be coloured blue by iodine. They have been observed in rotting potatoes.

Bacterium æruginosum, Schröter —Cells resembling *Bacterium termo.* Cultivated in nutrient gelatine they liquefy the medium, and give it an iridescent green colour by reflected light, and a deep orange by transmitted light (Plate V., Fig. 3). On nutrient agar-agar they form a white layer, and colour the medium a pea-green. They are found in green-blue pus.

Bacterium violaceum, Bergonzini. — Cells

* Lister, *Quart. Jour. Micr. Sci.* 1873.

similar to *Bacterium termo*, ·6—1 μ thick, 2—3 μ long. They occur on white of egg, forming a violet pigment.

Bacterium brunneum, Schröter.—Motile rods, producing a brown colour. They are observed on a rotting infusion of maize.

Bacterium of septicæmia, Davaine.—Cells resembling *Micrococcus of septicæmia in rabbits* (p. 126). They were isolated from putrid ox-blood, and produced septicæmia in rabbits, but differ from the *micrococcus* referred to, in that they produce a fatal result in guinea-pigs, but have no effect on birds.

Bacterium hyacinthi, Wakker.—Cells resembling *Bacterium termo*. Observed in the yellow slime of diseased hyacinth bulbs.

Bacterium fœtidum, Thin.—Cocci, short rods, long rods, and leptothrix. The cocci are 1·25—1·4 μ in diam., and occur singly or in pairs. Spore-formation is observed in the rods. They were isolated from the exudation in a case of profuse sweating of the feet, and the odour was noticeable in the cultivations. (*Vide Bacillus saprogenicus.*)

Bacterium decalvans, Thin.—Cocci, singly or in pairs, 1·6 μ in length. Observed in the roots of the hair in cases of Alopecia areata.

Bacterium photometricum,* Engelmann.—Cells slightly reddish in colour, which react in a very high degree under the influence of light.

Bacillus tremulus.—Rods shorter and thinner

* Engelmann, *Jour. Royal Microscop. Soc.*, 1882, page 656; and 1883, page 256.

than those of *Bacillus subtilis*. They are provided with a flagellum at both ends, and exhibit characteristic trembling and rotatory movements. Spores thicker than the bacillus, and often placed laterally. They were observed on rotting plant infusions, forming a thick slimy skin.

Bacillus of jequirity, Sattler.—Rods 2—4·5 μ long, and ·58 μ thick. They can be cultivated on nutrient gelatine and blood serum. Infusion of jequirity containing the bacilli, or an artificial cultivation of the bacilli, inoculated into the conjunctiva of healthy rabbits produces severe ophthalmia. The poisonous principle is, however, believed to be a chemical ferment, *abrin*, and not the bacillus. Boiling, which does not destroy the spores of the bacillus, destroys the ferment, and cultivations started with these spores, though teeming with jequirity bacilli, are quite harmless.* The bacilli occur in infusions of the beans of *Abrus precatorius*, or jequirity.

Bacillus of potatoes, Koch. — Rods, very frequently in the form of a delicate, wrinkled veil, which later has a brown reticulated appearance. They frequently occur on potatoes which have been prepared as a culture medium, but have not been thoroughly sterilised.

Bacillus fluorescens.—Rods which cultivated on nutrient agar-agar form a white layer, and colour the medium an emerald green (Plate VIII., Fig. 3).

Bacillus figurans.—In plate-cultivations ex-

* Klein, *Micro-org. and Disease.* 1885.

Fig. 1. Cover-glass impression-preparation from a plate-cultivation. (fuchsine). Zeiss' AA. Oc. 2.

BACILLUS FIGURANS.

Fig. 2. The same preparation. Zeiss' ⅛. o.i. (1)

Edgar Crookshank, fec. et pinx.

London, Published by H. K. Lewis, 136, Gower Street.

posed to the air various bacilli are found to develop. One of these has a perfectly characteristic appearance on cultivation. In plate cultivations it causes a cloudy growth, spreading from various points; if a cover-glass impression is made, this is found to consist of parallel rods, varying in length. The chains of rods become twisted at intervals into curious convolutions, from which offshoots are continued in various directions. These long shoots or processes are again twisted at intervals into varying shapes and patterns (Plate XXV., Figs. 1 and 2). Cultivated in nutrient gelatine, the bacillus forms on the surface visible windings, from which fine filaments grow down into the gelatine. It spreads out also in almost parallel lines transversely from the needle track. On an oblique surface of nutrient agar-agar the filaments spread downwards into the substance of the jelly, and outwards from the central streak on the surface, forming a feather-like cultivation * (Fig. 40).

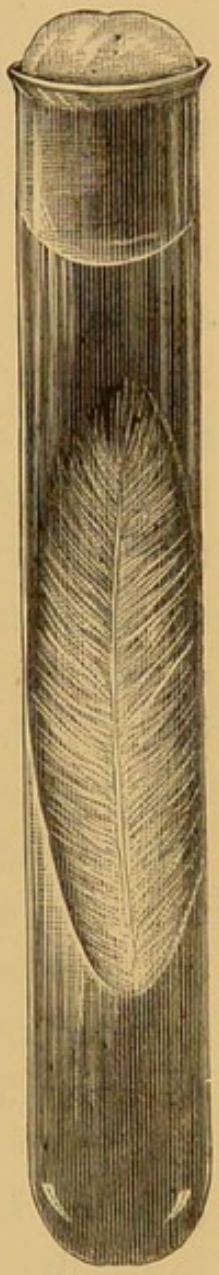


FIG. 40. PURE CULTIVATION OF BACILLUS FIGURANS IN AGAR-AGAR-PEPTONE-BROTH.

Bacillus valei, Cheshire and Cheyne.†—Rods varying in size, and

* Crookshank, *Notes from a Bacteriolog. Laboratory. Lancet*, 1885.

† Cheshire and Cheyne, *Journ. Royal Microscopical Society*, 1885, pp. 582-601.

forming large oval spores. When cultivated in nutrient gelatine in test-tubes a delicate, ramifying growth appears on the surface, and irregular whitish masses arise along the needle track. Processes shoot out from these masses, and extend through the gelatine for long distances. They are thickened at points in their course, and clubbed at the ends. The gelatine is gradually liquefied, and the bacilli form a loose, white, flocculent deposit at the bottom of the tube. The liquid in the tube becomes yellowish in colour after a time, and gives off an odour of stale but not ammoniacal urine. The colour and odour are distinctive also of the disease attributed to the bacilli. In plate cultivations, the bacilli grow out in series of rods in single file, or in rows of several side by side. The processes which are formed, tend to curve, and at a short distance from the track of the needle-streak form a distinct circle, from which another process grows out, and a fresh circle is developed. The gelatine in the vicinity of the bacilli gradually liquefies, and channels are formed in the gelatine in which the bacilli move backwards and forwards. On nutrient agar-agar a whitish layer develops, consisting of bacilli arranged side by side, which in a few days are replaced by rows of spores similarly arranged. On potatoes they form a dryish yellow layer, and in milk a tremulous jelly. A cultivation of the bacillus in milk, sprayed over a honeycomb containing a healthy brood of bee

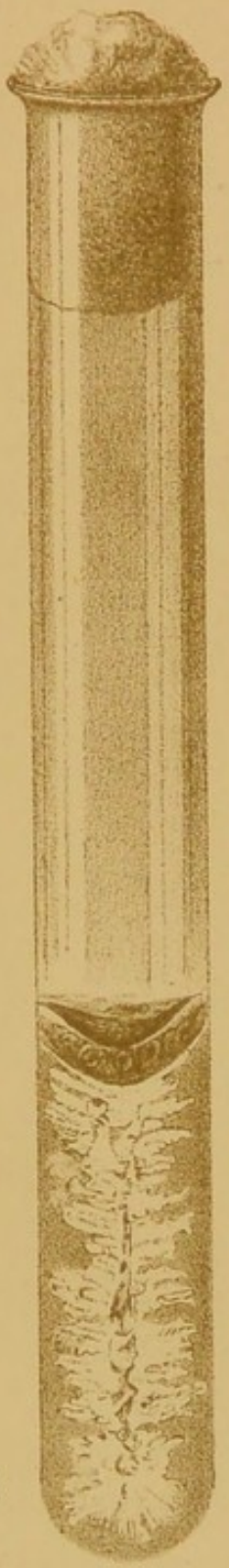


Fig.1.

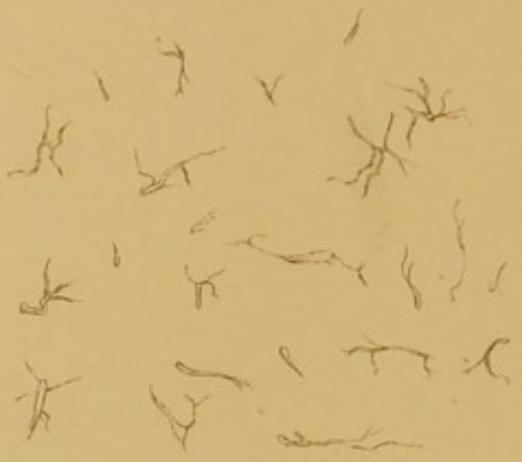


Fig.2.

Fig.3.

BACILLUS OF SWINE-FEVER (SCHÜTZ).

Fig.1. Pure-cultivation in nutrient gelatine.
Fig.2. Colonies on a plate-cultivation.
Fig.3. Cover-glass preparation of blood from an inoculated pigeon.

Vincent Brooks, Day & Son, Lith.

After Schütz Arb. a. d. Kaiserl. Gesundheitsamte Bd. I. London, Published by H.K. Lewis, 136, Gower Street.

larvæ, produced the disease known as "foul-brood." Adult bees fed on material containing bacilli became affected; inoculation of mice and rabbits with the bacillus gave doubtful results. The bacilli were isolated from the diseased larvæ of bees.

Bacillus of swine fever (*Bacillus des Erysipelas malignum. Rothlauf. Bacillus of pneumo-enteritis of the pig*, Klein.*)—Rods 2—3 μ long, actively motile; capable of spore-formation. They can be cultivated in broth and hydrocele-fluid, and carried on through successive generations. A drop of any of these cultivations produces the disease in pigs, mice, and rabbits; and they die with a characteristic swelling of the spleen, coagulative necrosis of tracts of the liver tissue, and inflammation of the lungs; pigs inoculated with artificial cultures are protected against a fatal attack.

The bacillus was observed in the diseased organs of pigs that had died of swine fever, and of animals that had died from the inoculated disease.

Quite recently † fresh investigations of swine fever have been published; in some a figure-of-eight micro-organism apparently corresponding with the *micrococcus of swine fever* was isolated. In others an extremely minute bacillus, bearing a close resemblance to the *bacillus of septicæmia of mice*. In nutrient gelatine the latter develops as a cloudy

* Klein, *Report to Med. Offic. Loc. Govt. Board*, 1877—1878.

† Löffler und Schütz, *Arbeiten aùs dem Kaiserlichen Gesundheitsamte*, vol. i., 1885.

growth in the needle track in test-tubes (Plate XXVI., Fig. 1), and characteristic, thread-like, branching, or star-like colonies form in plate-cultivations (Plate XXVI., Fig. 2). Inoculated into mice and rabbits, a fatal result is produced; but experiments with pigs were unsuccessful. Pigeons were also susceptible, and the bacilli were detected in their blood (Plate XXVI., Fig. 3).

Bacillus of choleraic diarrhœa from meat-poisoning, Klein.*—Rods from 3—9 μ in length, 1·3 μ wide, rounded at their extremities, singly or in chains of two. Spore formation occurs, the spores being 1 μ thick, oval, and situated in the centre or at the end of the rod.

Feeding with the bacilli and inoculation produced positive results. At the autopsy, pneumonia, peritonitis, pleuritis, enlargement of the liver and spleen, and hæmorrhage were observed, and bacilli were present in the blood and exudations of the animal. They occurred in several fatal cases of choleraic diarrhœa at Nottingham and Welbeck. Symptoms affected many people who had partaken of beef and ham sandwiches, and baked pork, which produced languor, giddiness, griping in the abdomen, diarrhœa, nausea, vomiting, and in some cases death. Bacilli were found in the blood and juices, and especially in the capillaries of glomeruli of the kidney.

* Klein, *ibid.*, p. 87.

Bacillus septicus, Klein.*—Rods varying in size, non-motile. They form threads or leptothrix filaments, and are rounded at the ends. They are anærobic, and form spores independently of access of air. In a nourishing fluid they are overcome by the presence of *micrococci*, *Bacterium termo* or *Bacillus subtilis*. They occur in the soil, in putrid blood, and many putrid albuminous fluids, and occasionally in the blood-vessels of man and animals after death.

Bacillus of septicæmia of man, Klein.†—Rods singly or in chains, 1—2·5 μ long, ·3—·5 μ wide, which were observed in the blood-vessels of the swollen lymphatic glands, forming continuous masses in the capillaries and the minute veins.

Bacillus of gangrenous septicæmia, Arloing and Chauveau.—Short rods, possessing spores, were observed around wounds in gangrenous septicæmia, and considered to be the cause of the gangrene.

Bacillus of ulcerative stomatitis in the calf, Lingard and Batt.—Rods 4 μ—8 μ, or more in length; 1 μ in width. Spores are frequently present. Injected into the rabbit or mouse they produce a fatal result. They were observed in ulcerations on the tongue and mucous membrane of the mouth of calves.

* Klein, *Micro-organism and Disease*. 1885.
† *Ibid.*, p. 84, 1885.

METHOD OF STAINING THE BACILLI OF ULCERATIVE STOMATITIS.

Sections through the ulcerations of the calf's tongue, or of the inoculated tissue of the rabbit, were stained by immersion in a mixture of magenta and methylene-blue. They were then washed in spirit, cleared in clove-oil, and mounted in Canada balsam.

Bacillus of syphilis, Lustgarten.*—Rods resembling the bacilli of leprosy and tuberculosis, 3—4 μ long, ·8 μ thick. Two or more colourless, ovoid points in the course of the rod are visible with a high power; it is thought that they are possibly spores. The bacilli are always found in the interior of nucleated cells which are more than double the size of leucocytes. They have been observed in the discharge of the primary lesion, and in hereditary affections of tertiary gummata.

METHOD OF STAINING THE BACILLUS OF SYPHILIS.

Method of Lustgarten :—

Sections are placed for from twelve to twenty-four hours in the following solution, at the ordinary temperature of the room, and finally the solution is warmed for two hours at 60° C.:—

Concentrated alcoholic solution of gentian-violet	11
Aniline water	100

The sections are then placed for a few minutes in ab-

* Lustgarten, *Die Syphilisbacillen. Mit 4 Tafeln.* 1885.

solute alcohol, and from this transferred to a 1·5 per cent. solution of permanganate of potash. After ten minutes they are immersed for a moment in a pure concentrated solution of sulphurous acid. If the section is not completely decolorised, immersion in the alcohol and in the acid bath must be repeated three or four times. The sections are finally dehydrated with absolute alcohol, cleared with clove-oil, and mounted in Canada balsam.

Bacillus of Rhinoscleroma, Cornil and Alvarez.*—Short rods, 1·5—3 μ in length, ·5—·8 μ thick. Deeply coloured points or granules are observed in the course of the rods when stained, and are possibly spores. The rods have rounded ends, and the smaller ones are ovoid in shape. The bacilli are surrounded by ovoid capsules. The capsule is composed of a tough resisting substance; two or more capsules may unite by fusion, enclosing two or three, or a great number of rods. The bacilli were observed in sections of a tumour *rhinoscleroma*, which develops on the lip and on the nasal and pharyngo-laryngeal regions.

METHOD OF STAINING THE BACILLUS OF RHINOSCLEROMA.

Method of Cornil and Alvarez :—

Sections are immersed in a solution of methyl-violet (B) for twenty-four to forty-eight hours, with or without the addition of aniline-water; are then decolorised after

* Cornil and Babes, *Les Bacteries* 1885.

treatment with the solution of iodine in iodide of potassium. If the sections are left to decolorise in alcohol for forty-eight hours the capsule is rendered visible.

Bacillus Saprogenes, Rosenbach. — Three varieties of *Bacillus saprogenes* have been described as intimately connected with *Bacterium termo* in putrefactive processes.*

No. 1.—Large rods which, cultivated on nutrient agar-agar, form an irregular sinuous streak, with a mucilaginous appearance. They grow also very readily on blood serum, and all cultivations yield the odour of rotting kitchen refuse. It is not pathogenic.

No. 2.—Rods shorter and thinner than No. 1. They develop very rapidly on agar-agar, forming transparent drops, which become grey. They were isolated from a patient suffering from profusely-sweating feet. The cultivations yielded a characteristic odour similar to the last. They are pathogenic.

No. 3.—Rods isolated from the putrid marrow of a case of compound fracture. Cultivated on nutrient agar-agar, an ash-grey, almost liquid culture is developed, with a strong characteristic odour of putrefaction. Injected into the knee joint or abdomen of a rabbit, an opaque, yellowish-green infiltration resulted.

Comma-bacillus of the mouth. — Curved rods, spirilla, and threads have been described, in connection with other bacteria, in relation to caries of the teeth. Curved bacilli are also found in the

* Rosenbach, *Microrg. bei Wund-Infections-Krankh.*

saliva, in old cheese, and sometimes in water. They differ materially from the spirillum of Asiatic cholera in their behaviour to nutrient media. Many have failed in the attempt to cultivate the comma-bacillus of the mouth, others have only succeeded by employing an acid nutrient jelly, in which the appearances differed completely from the characteristic growth of the *comma bacillus of cholera*.

Spirillum serpens, Müller (*Vibrio serpens*).—Threads 11—28 μ long, ·8—1·1 μ thick, with three or four windings. They are actively motile, often united into chains, or forming swarms, and are abundant in stagnant liquids.

Spirillum tenue.—Very thin threads, with at least $1\frac{1}{2}$, usually 2—5 spirals. Height of a single screw is 2—3 μ, and the length of spiral, therefore, 4—15 μ. They are very swiftly motile, and often occur in felted dense swarms in vegetable infusions.

Spirillum undula.—Threads 1·1—1·4 μ thick, 9—12 μ long; spirals 4·5 μ high; each thread has $1\frac{1}{2}$—3 spirals. They are actively motile, and possess at each end a flagellum. They occur in various infusions.

Spirillum volutans, Ehrenberg.—Threads 1·5—2 μ thick, 25—30 μ long; tapering towards their extremities, which are rounded off. They possess dark granular contents. Each thread has $2\frac{1}{2}$—$3\frac{1}{2}$ windings or spirals, whose height is 9—13 μ. They have a flagellum at each end, and are some-

times motile, sometimes not. They are found in various infusions and water of marshes.

Spirillum sanguineum, Cohn (*Ophidomonas sanguinea*).—Threads 3 μ and more in thickness, with 2—$2\frac{1}{2}$ spirals, each 9—12 μ high, with their ends provided with flagella. Their colour is due to the presence of reddish granules contained in the cells. They were observed in brackish water with putrefying substances.

Spirillum rosaceum, Klein. — Resembles *Spirillum undula*, but is reddish in colour; the colouring matter is insoluble in water, alcohol, or chloroform.

Spirillum violaceum, Warming. — Threads, crescent-shaped, or possessing 1 or $1\frac{1}{4}$ spiral, with their ends broad, rounded, and provided with flagella. The colour is due to the contents, which are violet.

Spirillum Rosenbergii.—Threads with 1—$1\frac{1}{2}$ windings; 4—12 μ long; 1·5—2·6 μ thick. They are colourless, but the contents include strongly refractive sulphur granules. Also spirals 6—7·5 μ in height, which are actively motile, are found in brackish water.

Spirillum attenuatum, Warming. — Threads much attenuated at the ends, which consist usually of three spirals. The middle spiral is about 11 μ high, and 6 μ in diameter; and the end ones 10 μ high, and 2 μ in diameter. They are found in brackish water.

Spirillum leucomelaneum, Koch. — A rare form observed in water covering rotting algæ. Dark and glass-like spaces alternate in the spirillum, resulting from a regular arrangement of the dark granular contents.

Myconostoc gregarium, Cohn.—The threads are very thin, colourless, unarticulated, but fall apart into short cylindrical links when dried. They form gelatinous masses 10—17 μ in diam. : singly, or heaped into slimy drops on water in which algæ are decomposing. They have been considered to be the zoogloœa form of *Spirillum undula*, and by others a phase in the development of *Cladothrix dichotoma*.

Spiromonas volubilis, Perty. — Colourless, transparent cells, 15—18 μ long. Rapidly motile, and revolving round a longitudinal axis. They occur in marsh water, and putrefying infusions.

Spiromonas Cohnii.—Colourless cells, consisting of $1\frac{1}{4}$ spirals, with both ends acutely pointed and provided with a flagellum. Breadth of the cells 1·2—4 μ. They occur in water containing decomposing matter.

Monas vinosa.—Round or oval cells of about 2·5 μ in diam., often united in pairs. Their motion is slow and tremulous, and the cell substance pale-red, with dark grains interspersed. Flagella have not been observed. They were observed in water with decaying vegetable matter.

Monas Okenii.—Short cylindrical cells, 5 μ

wide, 8—15 μ long, with rounded ends. They undergo lively movements, each end being provided with a flagellum twice as long as the cell itself. They have pale-red cell-substance with dark grains. They occur in stagnant water.

Rhabdomonas rosea.—Spindle-form cells, 3·8—5·0 μ broad, 20—30 μ long. They exhibit slow, trembling movements, having at each end of the cell a flagellum. The cell-substance is very pale with dark grains interspersed. In brackish water.

Monas Warmingii.—Cylindrical cells, rounded at the ends; 15 μ long, 5—8 μ broad. They are possessed of a flagellum at each end of the cell, and exhibit rapid, irregular movements. The cell substance is pale-red, and studded at the rounded ends with dark-red grains.

Proteus vulgaris.—This and the two following species have been recently described * as present in putrefying meat infusion, and as being intimately connected with the process of putrefaction. In the history of their development coccoid, bacterioid, spindle-form, spirulinar, and involution forms have been described, and the name "Proteus" has been suggested for this order. In *Proteus vulgaris* the bacteria vary in size; some measure 4 μ in length, and are almost as broad as long, and others vary from ·94—1·25 μ long and ·42—·63 wide. They are actively motile, and cultivated on nutrient gelatine they convert it into a turbid, greyish-white

* Hauser, *Ueber Fäulniss-Bacterien*. 1885.

liquid. If cultivated in a capsule containing 5 per cent. of nutrient gelatine, a few hours after inoculation the most characteristic movements of the individual bacilli are observed on the surface of the nutrient gelatine, although at this early stage no superficial liquefaction can be detected. Others have stated that the movements occur in a thin liquid film. The movements are not observed if the nutrient medium contains 10 per cent. of gelatine.

Proteus mirabilis.*—Cocci ·4 μ—·9 μ. They occur singly and in zoogloœa, and sometimes in tetrads, pairs, chains, or as short rods in twos resembling *Bacterium termo*, in fact, in all conceivable transition-forms. Cultivated on nutrient gelatine they form a thick, whitish layer in concentric circles, which in time liquefies the medium. Similar movements are observed in capsule-cultivations as with *Proteus vulgaris*.

Proteus Zenkeri.—Cocci, ·4 μ and short rods in twos like *Bacterium termo;* 1·65 μ long. Cultivated on nutrient gelatine no liquefaction results, but a thick, whitish-grey layer is formed. The bacilli are motile, and the same phenomena are observed on the solid medium, as in the other forms. In cover-glass impressions most varied groupings of the bacilli are seen, and also developmental and involution-forms.

Actinomyces. — Actinomycosis is a disease

* Hauser, *ibid.*

occurring in animals* and occasionally in man.† It is caused by a parasite known as *Actinomyces*, or the "ray-fungus." The parasite appears in the form of a rosette of pyriform or club-shaped elements (Plate XXIX., Fig. 1). The little masses are colourless, pure white, or of a yellowish or yellowish-green tinge, and visible to the naked eye.

The fungus is believed to effect an entrance to the animal by the mouth, being taken in with the food, possibly through the medium of a wound of the gum or a carious tooth. In whatever manner it has gained access to the living organism, it sets up inflammation in its neighbourhood, resulting in the formation of a neoplasm, composed chiefly of round cells, resembling a tuberculous nodule. The nodules may break down and suppurate, or may go on increasing in size. Fibrous tissue develops between the nodules, and large tumours eventually result containing purulent cavities and excavations. In the slimy detritus the little pale-yellow grains of fungus can be detected. In cattle the lower jaw is usually affected, and then the upper jaw and neighbouring parts. The organism may also occur in nodular tumours in the lung, subcutaneous and intermuscular tissue. It is the cause of "wooden tongue," and also of diseases which have been variously described before their true nature

* Bollinger, *Centralbl. f. Med. Wiss.* 1877.

† Israel Virchow's *Archiv*, vols. 74—78. Pontick, *Die Actinomycose des Menschen*, 1882; and *Beitr z. Kenntn. der Actinomycose des Menschen*, 1885; *Lancet*, May 2nd, 1885.

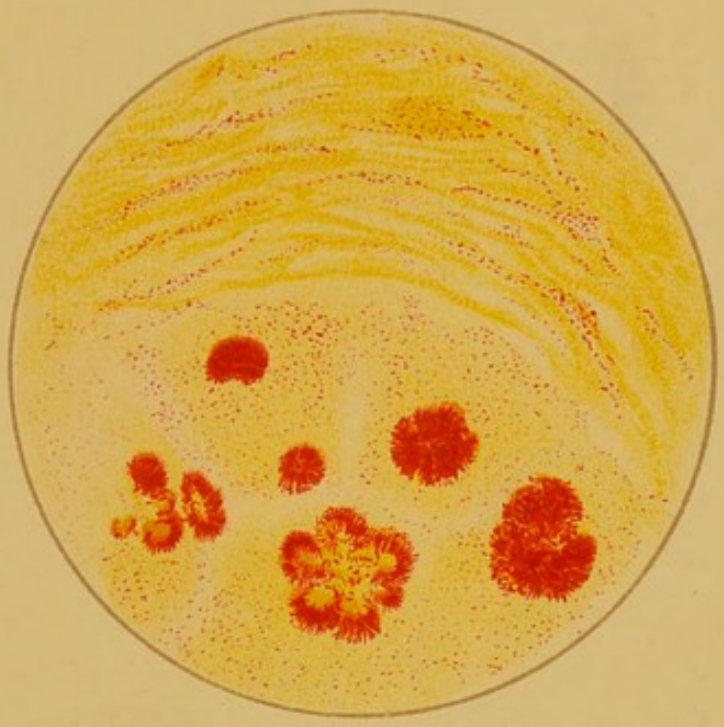


Fig. 1. From a section of a maxillary tumour in a cow. Plaut's method (Magenta and picric acid). Zeiss' AA. Oc. 4.

ACTINOMYCES.

Fig. 2. The same preparation. Zeiss' ⅛. o.i. Oc. 2.

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Vincent Brooks, Day & Son, Lith.

To follow plate 27.

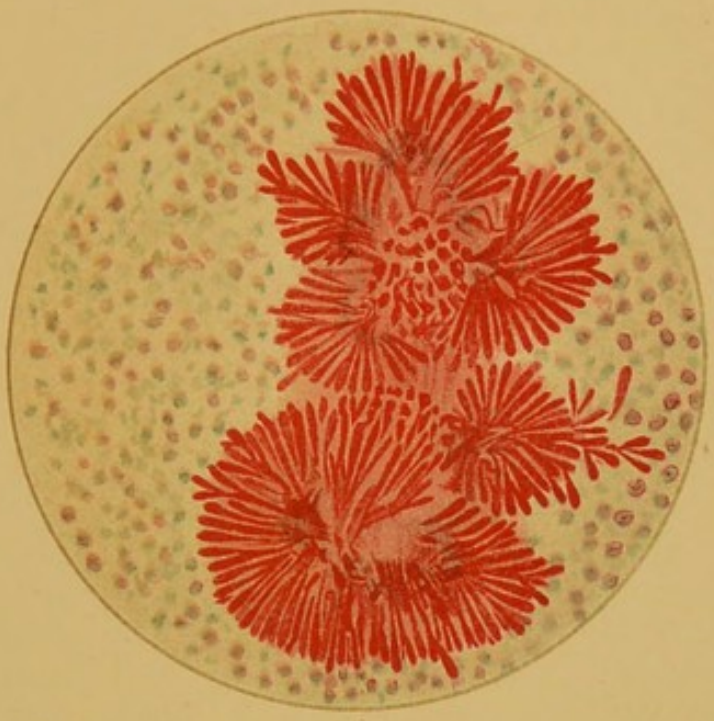
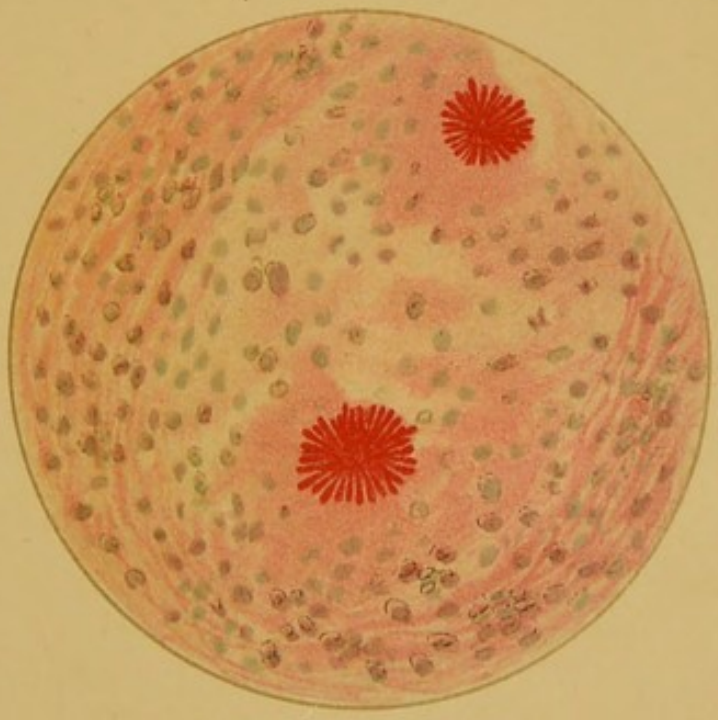


Fig. 1. From a section of a maxillary tumour in a cow.
Weigert's method, (Orseille and gentian-violet). Zeiss' 1/12. o. i. Oc. 4.



ACTINOMYCES.

Fig. 2. From a section of the lung of a cow.
Weigert's method, (Orseille and gentian-violet.) Zeiss' 1/12. o. i. Oc. 2.

Edgar Cruickshank fec. et pinx. Vincent Brooks, Day & Son, Lith.

London, Published by H. K. Lewis, 136, Gower Street.

was understood as bone-canker, bone-tubercle, osteo-sarcoma, etc.

In man the pulmonary formations tend to break down early, forming fistulæ and sinuses, with the clinical character of empyema. In one case there were the symptoms of chronic bronchitis with fœtid expectoration. In other cases the disease, originating in the lung, spread to the præ-vertebral tissues. If the actinomyces invade bones, as has been especially observed in the bodies of the vertebræ, caries results. In another group of cases the organism has been said to produce disease of the intestinal canal. The fungus has also been detected in the crypts of the tonsils of healthy pigs, and a similar, if not identical one in the spermatic duct of the horse.*

The disease has been transmitted from cattle to cattle by inoculation,† and a rabbit has been infected by means of a piece of actinomycitic tumour from a human subject, introduced into the peritoneal cavity.

Until quite recently actinomyces has been classed as a *hyphomycete*, and the flask-shaped structures regarded as gonidia. By recent‡ cultivation-experiments we are led to regard the latter as a result of a degenerative stage in the life-history

* Johne, *Bericht über das Veterinärwesen im Königreich Sachsen für das Jahr* 1884.

† Johne, *Deutsche Zeitschr. cf. Thiermedicin.* 1881.

‡ Boström, *Ueber Actinomycose. Verhandlungen des Congresses für Inn. Med.* 1885.

of the fungus accompanied by the development of involution-forms. Inoculations of nutrient gelatine, in the form of plate-cultivations, and inoculations on blood serum and nutrient agar-agar were made, it is stated, with success. The cultures developed on the latter in from five to six days, growing best at a temperature of 33°—37° C. Nutrient gelatine was not liquefied. The appearances of the cultivation were described as quite characteristic; it has at first a whitish, granular appearance, followed after a few days by little yellowish-red spots which coalesce in the centre, and finally a whitish downy layer results with a golden-red centre; in time the periphery also becomes dotted with little yellow-centred masses. The fungus thus cultivated has been described as corresponding on examination with the form found in man and animals, and at one stage to consist of thread-forms, short rods, and cocci. As a result of these observations actinomyces has been relegated to the bacteria, forming one of the cladothrix group, and possibly closely allied to the *Streptothrix Fœrsteri* of Cohn.

METHODS OF EXAMINING AND STAINING ACTINOMYCES.

In the fresh state a little of the tissue of a tumour, or the purulent detritus, may be transferred to a clean glass slide, and teased out with needles. The little specks are easily recognised, and can be isolated with the needles and transferred to a drop of glycerine upon a fresh slide. A cover-

glass must then be gently pressed down upon the preparation, which is then examined. To stain the fresh tufts, the little fungus masses are teased out, and transferred to a watch-glass containing alcohol, to which a few drops of concentrated alcoholic solution of eosin are added. They can then be mounted and preserved in glycerine.

Sections can be stained by either of the following methods.

Weigert's Method :—

Immerse the sections for one hour in orseille, rinse with alcohol, and after-stain with gentian-violet (Plate XXVIII.).

Plaut's Method :–

Sections are left for ten minutes in Gibbes' solution (No. 22), warmed to 45° C. They are then rinsed in water, and after-stained in concentrated alcoholic solution of picric acid for five to ten minutes; immersed in water five minutes, 50 per cent. alcohol fifteen minutes, passed through absolute alcohol and clove-oil, and preserved in Canada balsam (Plate XXVII.).

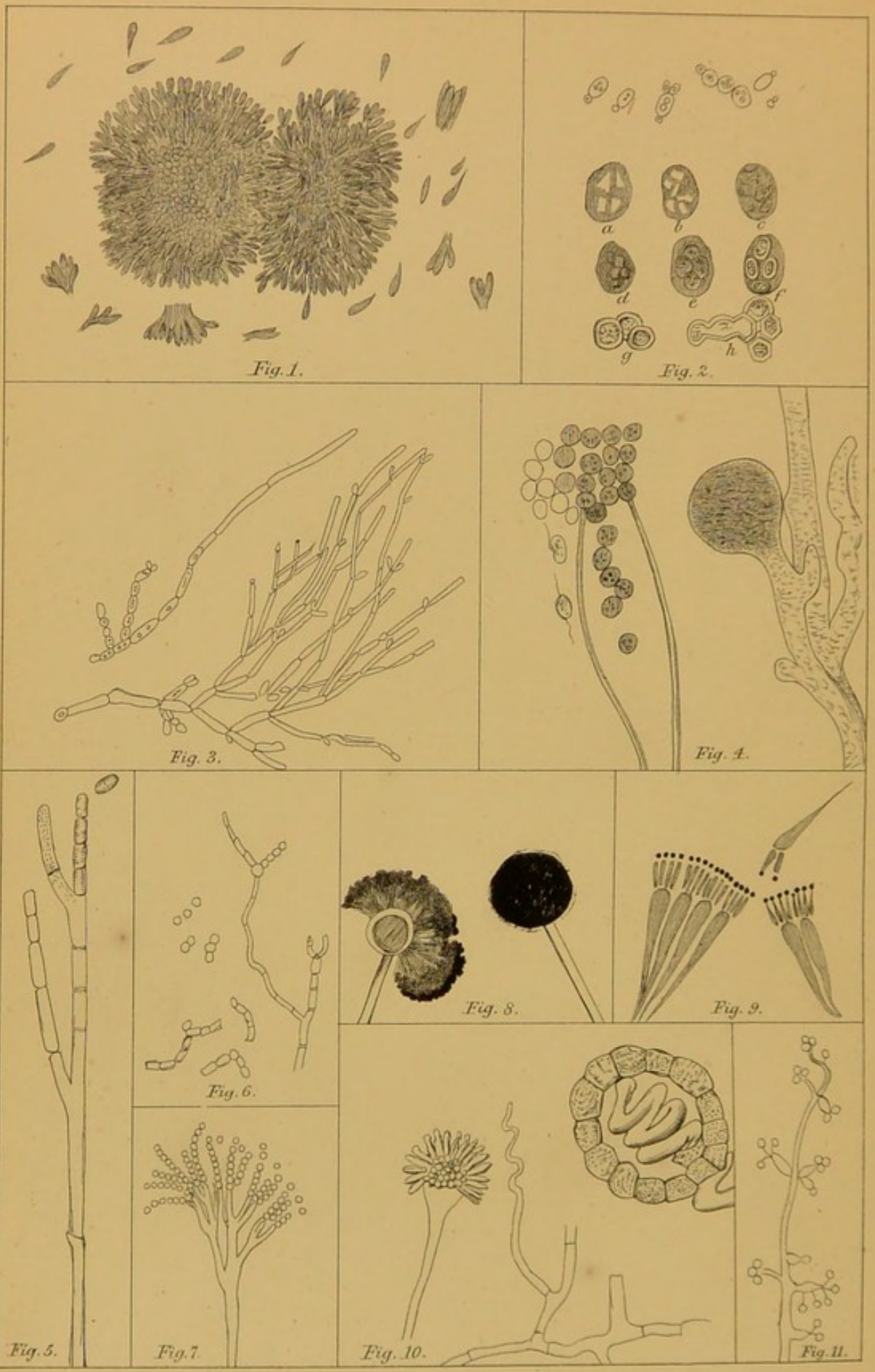


Fig. 1. *Fig. 2.* *Fig. 3.* *Fig. 4.* *Fig. 5.* *Fig. 6.* *Fig. 7.* *Fig. 8.* *Fig. 9.* *Fig. 10.* *Fig. 11.*

YEAST-FUNGI OR SACCHAROMYCETES AND MOULD-FUNGI OR HYPHOMYCETES.

Edgar Crookshank del. *Vincent Brooks, Day & Son. Lith.*

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DESCRIPTION OF PLATE XXIX.

FIG.

1.—*Actinomyces* from maxillary tumour of cow ; stained with eosin.
2.—*Torula cerevisiæ* (after Rees).
3.—*Saccharomyces mycoderma*, or *oidium albicans*, from an artificial cultivation (after Grawitz).
4.—*Saprolegnia* (after Sachs).
5.—*Oidium lactis* (after Flügge).
6.—Fungi of *favus*, or *oidium lactis* (after Neumann).
7.—*Penicillium glaucum* (after Flügge).
8.—*Aspergillus niger*, from a preparation mounted in glycerine.
9.—*Aspergillus niger*, from the same preparation (Zeiss $\frac{1}{12}$ o. i.).
10.—*Aspergillus glaucus* (after De Bary).
11.—*Botrytis Bassiana* (after De Bary).

APPENDIX A.

YEASTS AND MOULDS.

Yeast-fungi and *mould-fungi*, like *bacteria* or *fission-fungi*, are *achlorophyllous Thallophytes*. They belong to two separate orders, the *Saccharomycetes* and *Hyphomycetes*, which are intimately related to each other, but quite distinct from *bacteria*. Their germs occur widely distributed in air, soil, and water, and are constantly encountered in bacteriological investigations. In addition many species are of hygienic and pathological interest or importance in being either accidentally associated with, or actually the cause of various morbid processes. For a complete account of all the described species and full details of the various forms of development,* reference must be made to botanical treatises. A description of certain species is appended here, and may afford some useful information to the worker in a bacteriological laboratory.

YEAST-FUNGI OR SACCHAROMYCETES.

Saccharomyces cerevisiæ (*Torula cerevisiæ*).—Cells round or oval, 8—9 μ long, singly or united in small chains. Spores occur three or four together in a mother-cell, 4—5 μ in diameter (Plate XXIX., Fig. 2).

Sacch. ellipsoideus.—Elliptical cells, mostly 6 μ long, singly or united in little branching chains. Two to

* Sachs, *Text-book of Botany*. 1882.

four spores found in a mother-cell, 3—3·5 μ in diam. It is widely distributed, and is the principal agent in accidental fermentation.

Sacch. conglomeratus.—Cells round, united in clusters, consisting of numerous cells produced by budding from one or a few mother-cells. There are 2 to 4 spores in each mother-cell. They occur on rotting grapes and in wine at the commencement of fermentation.

Sacch. exiguus.—Conical or top-shaped cells, 5 μ long, and reaching 2·5 μ in thickness, in slightly branching colonies. Spore-forming cells are isolated, each containing 2 or 3 spores in a row. Present in the after-fermentation of beer.

Sacch. pastorianus.—Cells oval or club-shaped. Colonies consist of primary club-shaped links, 18—22 μ long, which build lateral, secondary round or oval daughter-cells, 5—6 μ long. Spores 2 to 4. In the after-fermentation of wine, fruit-wines, or fermenting beer.

Sacch. apiculatus.—Cells lemon-shaped, both ends bluntly pointed, 6—8 μ long, 2—3 μ wide. Budding occurs only at the pointed ends. Rarely united in colonies. Spores unknown. They occur with other yeasts in various accidental fermentations.

Sacch. sphæricus.—Cells varying in form; the basal ones of a colony oblong or cylindrical, 10—15 μ long, 5 μ thick; the others round, 5—6 μ in diam. United in ramified families. Spores unknown.

Sacch. mycoderma (*Mycoderma cerevisiæ et vini*). —Cells oval, elliptical, or cylindrical, 6—7 μ long, 2—3 μ thick, united in richly-branching chains. Spore-forming cells reaching 20 μ long. Spores 1 to 4 in each mother-cell. Forms the so-called "mould" on fermented liquids, and develops on the surface without exciting fermentation. When forced to grow submerged, a little alcohol is produced, but the fungus soon dies. They occur on wine, beer, fruit-juices, and sauerkraut.

Sacch. albicans (*Oidium albicans*).—Cells partly

round, partly oval or cylindrical, 3·5—5 μ thick, the cylindrical cells 10—20 times as long as they are thick. The bud-colonies mostly consist of rows of cylindrical cells, from the ends of which oval or round cells shoot out. Spores form singly in roundish cells. They occur on the mucous membrane of the mouth, especially of infants, in greyish-white patches which consist of epithelium, bacteria, yeasts, and the mycelia of various moulds. They can be easily cultivated in a nutrient solution containing sugar and ammonic tartrate.* The cells germinate according to the richness of the fluid in sugar ; they either grow into long threads, or, in a very strongly saccharine solution, many daughter-cells are formed, budding out in various directions (Plate XXIX., Fig. 3).

Sacch. glutinis.—Cells round, oval, or short cylinders, 5—11 μ long, 4 μ wide, isolated, or united in twos. Cell-membrane and contents are colourless in the fresh state, but when dried and remoistened possess a pale-reddish nucleus in the middle. Spore formation unknown. Forms rose-coloured, slimy spots on starch, paste, or on sterilised potatoes. The colouring matter is not changed by acids or alkalies.

Sacch. rosaceus (*Pink Torula*).—Cells 9—10 μ in diam. Forms a coral-pink growth in nutrient gelatine, nutrient agar-agar (Plate XIII., Fig. 3), or on sterilised potatoes (Plate X., Fig. 2). They are present in the air.

Sacch. niger (*Black Torula*).—Cells also present in the air. Cultivated in nutrient gelatine they form a black crust (Plate III., Fig. 3).

MOULD-FUNGI OR HYPHOMYCETES.

The mould-fungi have been divided into five orders :† *Hypodermii*, *Phycomycetes*, *Ascomycetes*, *Basidiomycetes*, and

* Grawitz, *Virch. Archiv*, vol. 70.

† Flügge, *Fermente u. Mikroparasiten*. 1883.

Myxomycetes. The following species, with the orders to which they belong, are of especial interest.

HYPODERMII.

Ustilago carbo (mildew, smut).—Spores, brown, circular; episporium smooth; sporidia, ovoid cells. The spores or conidia occur as a black powder in the ears and panicles of wheat, barley, and oats.

Tilletia caries.—Spores round, pale, brown; episporium with reticulated thickenings. In germinating sporidia grow out radially from the end of the promycelium; these, at their lower part, conjugate by a cross branch, and separate from the promycelium, and at some point of the pair, a hypha grows out on which abundant secondary sporidia develop. The latter are long, oval cells, which can in turn germinate. The fungus occurs in the form of a stinking powder in grains of wheat, which renders the meal impure, and gives it a disagreeable smell.

Urocystis occulta.—The spores consist of several cells united together; partly large, dark-brown cells in the interior, and outside several flat semi-circular, colourless cells. Spores ·024 mm. Promycelium germinates as in *Tilletia*, but the cylindrical cells produce a hypha, without, as a rule, previous conjugation. Occurs as a black powder in rye straw, in long disintegrated stripes, which are at first greyish. The affected plant produces abortive ears.

Empusa muscæ.—Spores, ·011 mm. in diam. A spore or conidium alighting upon the white area of the under surface of the body of the house-fly, germinates into a hypha. The latter, penetrating the skin, forms toruloid cells, which multiply by germination, and are disseminated in the blood throughout the body of the fly. These cells again grow into hyphæ, which penetrate the skin, each forming a conidium, which is cast off with considerable force. The parasite is fatal to flies, especially in the

autumn. They are often observed attached to the walls or window-panes, surrounded by a powdery substance, consisting of the extruded conidia.

Empusa radicans.—The spores form long hyphæ, which pierce the transparent skin of the caterpillar of the cabbage-white butterfly. The terminal cells ramify, and fill the body of the caterpillar with a network of mycelial filaments. The caterpillars attacked become restless, then motionless, and death ensues.

Tarichium megaspermum.—The spores are ·05 mm. in diam., black in colour, and provided with a thickened episporium. They occur at the sides and ends of mycelial threads, attacking caterpillars (*Agrotis segetum*).

PHYCOMYCETES.

Saprolegnia.—Colourless threads, forming dense radiating tufts, occur on living and dead animal and vegetable matter in fresh water. The filaments penetrate into the substratum, and branch more or less in the surrounding water. The cylindrical ends of threads are shut off by a septum forming zoosporangia, or mother-cells, in the interior of which a number of spherical spores, zoospores, develop. These are set free through an apical opening in the thread, and, after a time coming to rest, give rise to new plants (Plate XXIX., Fig. 4). In the sexual mode of reproduction, a spherical bud, the *oogonium*, develops at the end of a mycelial thread ; from the thread, small processes or *antheridia* sprout out laterally towards the oogonium, and blend with its protoplasm (Plate XXIX., Fig. 4). The latter breaks up into a number of *oospores*, which clothe themselves with a membrane, while still within the mother-cell, and eventually being set free, grow into fresh mycelial filaments. The parasite attacks fish and tritons, and produces a diseased condition of the skin, which may be ultimately fatal. In salmon it produces the common disease of salmon.

Peronospora infestans.—Mycelium, ·005 mm. in

thickness. Twigs with as many as five branches, each bearing an egg-shaped conidium. The contents of the conidia falling off and reaching a drop of moisture, break up into a number of swarming zoogonidia, which in turn develop upon plants. Fixing themselves to the cuticle of the host, they throw a germinating filament into an epidermal cell ; piercing first its outer wall, and then its inner wall, the filament reaches an intercellular space, where the mycelium develops. This continues to grow and spread throughout the plant. In tubers it can hibernate and develop in the young shoots in the following spring. The parasites appear in the form of brown patches on the green parts of the plants, especially the leaves. The attacked parts wither and turn yellow or brown in colour. If the under surface of a diseased leaf be examined, a corresponding dark spot may be observed, accompanied with a faint greyish-white bloom which covers it. The latter consists of the conidia-bearing branches of the fungus.

Pilobolus.—Hyphæ, 1—2 mm. high. Fruit-hyphæ, possessing spherical receptacles containing conidia. When ripe the receptacles with their conidia are detached at their bases and spring by their elasticity to some distance. The mould occurs as glassy tufts on the excrement of cows, horses, etc. A cultivation can generally be obtained by keeping fresh horse-dung under a bell-glass.

Mucor mucedo.—Hyphæ, colourless, simple or branched, 1—15 cm. long, sporangia are yellowish-brown or black. Spores ovoid, ·008 mm. long, and ·0037 wide. Occurring as the familiar white mould on fruits, bread, potatoes, excreta, and penetrating into the interior of nuts and apples. A network of fibrils develops in the substance of nutrient gelatine, with formation of sporangia on the free surface. The germination of the spores and development into hyphæ can be observed in a few hours, if the fungus be cultivated in a decoction of horse dung.

Mucor racemosus.—Hyphæ, at most 1·5 cm. long

sporangia, yellowish to pale-brown ; spores round. By continued cultivation in liquids saturated with carbonic acid, the hypha becomes shorter, and exhibits a yeast-like sprouting. These yeast-like or toruloid cells can, when the carbonic acid is withdrawn, germinate into normal mycelium.

Mucor stolonifer, Lichtheim.—Mycelium grows in the air, and then bends down and re-enters the nutrient substratum ; sporangia black, and spores globular. The mycelium can penetrate through the shell of eggs, and form conidiophores within them.

Mucor aspergillus, Lichtheim. Fruit hyphæ, thinned at the base, and with many fork-like divisions, dark-brown spores.

Mucor phycomyces, Lichtheim.—Mycelium thick-walled, olive-green fruit-hyphæ, black sporangia, and oblong spores.

Mucor macrocarpus, Lichtheim.—Spindle-formed, pointed spores.

Mucor fusiger, Lichtheim.—Ovoid spores.

Mucor mellittophorus, Lichtheim.—Spores elliptical. Found in the stomach of bees.

Mucor corymbifer, Lichtheim.—Forms branched fruit-hyphæ ; sporangium has a smooth membrane. Found in the external auditory meatus ; occurring also upon bread. Pathogenic in rabbits.

Mucor rhizopodiformis, Lichtheim.—Occurs on bread. The spores of *Mucor rhizopodiformis* and *Mucor corymbifer*, when introduced into the vascular system of rabbits, can germinate in the tissues, especially in the kidneys, where they set up hæmorrhagic inflammation. Dogs are immune, and only artificial mycosis is known.*

* Lichtheim, *Zeistchr. f. Klin. Med.*, vii. ; Hückel, *Beitr. z. Anat. u. Phys.*, herausgeg. v. Ziegler u. Nauwerck. 1885.

ASCOMYCETES.

Oidium Tuckeri.—Fruit hyphæ, bearing single ovoid conidia. Observed in the form of brown patches, covered with a white mildew-like layer on the leaves, branches, and young fruit of the vine, producing a "grape-disease."

Oidium lactis.—Fruit hyphæ, simple, erect, and colourless, bearing at their ends a series or chain of conidia (Plate XXIX., Fig. 5). In some cases the fruit hypha branches beneath the chain of spores. Spores are short cylinders, ·0077—·0108 mm. long. The fungus is sometimes found as a whitish mould on milk, bread, paste, potato, and excrement, and is believed to be identical* with the fungus of certain human skin diseases, *Favus* (*Achorion Schoenleinii*), *Herpes tonsurans* (*Tricophyton tonsurans*) and *Pityriasis versicolor* (*Microsporon furfur*) (Plate XXIX., Fig. 6). Cultivated artificially in nutrient gelatine, the conidia germinate into filaments of varying length, which by subdivision form septate mycelial hyphæ; these and their branches give rise in turn to spores or conidia. The differences observed in various diseases are attributed to differences in the nutrient substratum. Others† maintain that, in artificial cultivations of the spores of *Tricophyton tonsurans*, the fructification is identical with Penicillium.

Oidium albicans.—Vide *Saccharomyces albicans.*

Aspergillus glaucus (*Eurotium aspergillus glaucus*).—Mycelium, at first whitish, becoming grey-green or yellow-green. Spores grey-green, thick-walled, ·009—·015 mm. in diam. Sometimes found on various substances, chiefly cooked fruit (Plate XXIX., Fig. 10).

Aspergillus repens (*Eurotium repens*), De Bary.—Fruit heads fewer than in the above, which are at first pale and then blue-green to dark-green in colour; conidia mostly oval, smooth, ·005—·008 mm. long, colourless or pale to grey-green.

* Grawitz, *Virchow's Archiv*, vol. 70.

† Morris and Henderson, *Journ. Royal Microsc. Society*. 1883.

Aspergillus flavus.—Gold-yellow, greenish and brown tufts; fruit heads round; yellow, olive-green, or brown. Conidia round, seldom oval; sulphur-yellow to brown in colour, ·005—·007 mm. in diam. Saprophytic in man, pathogenic in rabbits.

Aspergillus fumigatus.—Greenish, bluish, or grey tufts. Fruit heads long and conical. Conidia round, seldom oval, smooth, mostly pale and colourless. Diam. ·0025 to ·003 mm. Observed saprophytically in human lungs, external auditory meatus, and middle ear. The spores introduced into the vascular system of rabbits, or into the peritoneal cavity, establish metastatic foci in the kidneys, liver, intestines, lungs, muscles, and sometimes in the spleen, bones, lymphatic glands, nervous system, and skin (Plate XXX.).

Aspergillus niger (*Eurotium aspergillus niger*, De Bary).—Dark chocolate-brown tufts. Conidia round, black-brown, or grey-brown, when ripe; ·0035 to ·005 mm. This mould can be cultivated readily on bread moistened with vinegar, on slices of lemon, and on acid fruits and liquids. It flourishes best of all, according to Raulin,* in a liquid of the following composition:—

	Grammes.
Water	1500
Sugar-candy	70·
Tartaric acid	4·
Nitrate of ammonia	4
Phosphate	·6
Carbonate of potassium	·6
„ „ magnesium	·4
Sulphate of ammonia	·25
„ „ zinc	·07
„ „ iron	·07
Silicate of potassium	·07

It was also found that the fungus grew best when the liquid was spread out in a layer 2 or 3 cm. in depth in a

* Duclaux, *Health Exhibition Handbook*, London, 1884.

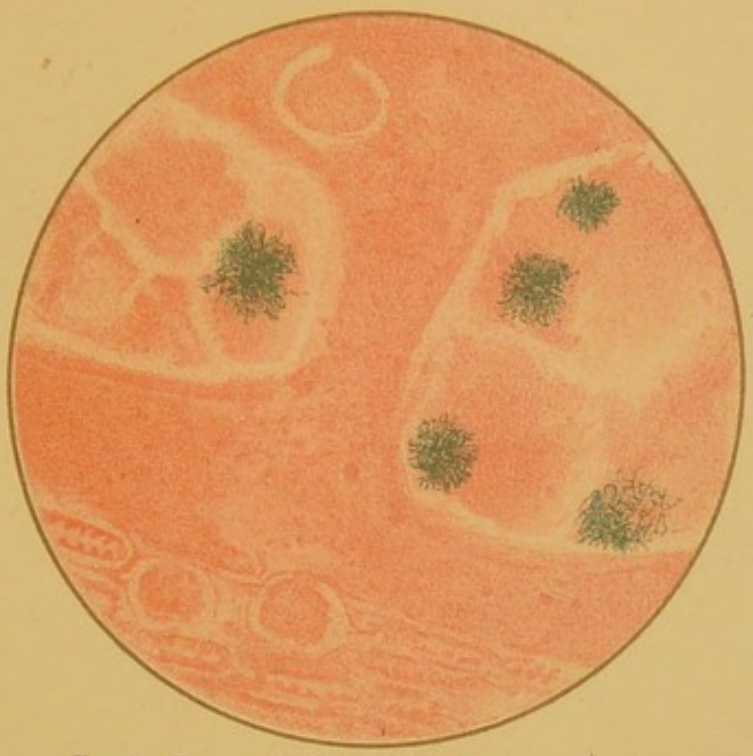
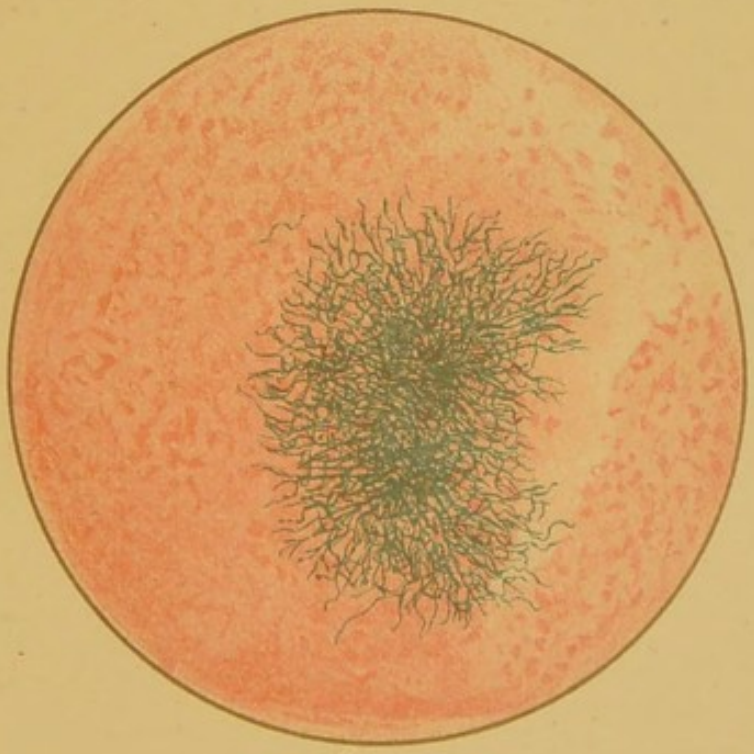


Fig. 1. From a section of the Kidney of a rabbit. Gram's method (gentian-violet) and Eosin. Zeiss' AA. Oc. 4.



ASPERGILLUS FUMIGATUS.

Fig. 2. The same preparation. Zeiss' 1/12. o. i. Oc. 2.

Edgar Cruikshank fec. et pinx.

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Vincent Brooks, Day & Son, Lith.

shallow dish, and a temperature of 35° C. proved to be the most favourable. The abstraction of zinc from the nutritive liquid reduced the weight of a crop from 25 (the average) to 2 grammes, and the presence of $\frac{1}{100000}$ part of nitrate of silver, or $\frac{1}{50000}$ part of corrosive sublimate, stopped the growth altogether. It is saprophytic in the living body.

METHOD OF EXAMINING ASPERGILLUS NIGER.

Species of aspergillus stain intensely with carmine, fuchsine, or *methyl-violet*, but to examine *Aspergillus niger* with a high-power, a little special technique is employed, as follows:—A drop of glycerine is placed on a clean slide, and a drop of alcohol on a cover-glass. With a fine pair of forceps a few of the fruit hyphæ with their black heads are immersed in the alcohol. The cover-glass is then turned over on to the drop of glycerine, and the slide held in the flame of a Bunsen burner till the spores or conidia are dispersed. To make a permanent preparation, remove the cover-glass, and transfer the fruit hyphæ so treated to a mixture of glycerine and water (1 to 5); a drop may be conveniently placed ready on a slide provided with a ring of Canada balsam. The specimen is then permanently mounted by employing a circular cover-glass, and surrounding it with a ring of cement in the usual way (Plate XXIX., Figs. 8 and 9).

Aspergillus ochraceus.—At first flesh-coloured, and then ochre-yellow heads.

Aspergillus albus.—Pure white fruit heads.

Aspergillus clavatus.—Club-shaped fruit heads on long stems.

Penicillium glaucum.—Occurs as a white, and later a blue-green mould, on which dewlike drops of liquid may appear (Plate IX., Fig. 2). Its spores are present in large numbers in the air, and are liable to contaminate cultivations. Diam. of the spores ·0035 mm.; threads vary in diameter between ·004 and ·00071 mm.,

according to the nourishing material; the fruit hypha bears terminally a number of branched cylindrical cells, from which chains of greenish conidia are developed (Plate XXIX., Fig. 7). It is the commonest of all moulds.

Botrytis Bassiana.—Hyphæ and spores colourless. Hyphæ usually simple, but sometimes united in arborescent stems (Plate XXIX., Fig. 11). It is the cause of muscardine, a fatal disease of silk-worms, and occurs also in various other caterpillars and insects.

UNCLASSED.

Chionyphe Carteri.—Mycelium, penetrating the skin and subcutaneous tissue, sets up suppuration and ulceration. Described as the cause of a disease known in India as "madura-foot."

APPENDIX B.

EXAMINATION OF AIR.

THE air, as is well known, contains in suspension mineral, animal, and vegetable substances. The mineral world is represented by such substances as silica, silicate of aluminium, carbonate and phosphate of calcium, which may be raised from the soil by the wind, and particles of carbon, etc., which gain access from accidental sources. Belonging to the animal kingdom we find the *débris* of perished creatures as well as sometimes living animals. The vegetable world supplies *micrococci*, *bacilli*, and other forms of the great family of *bacteria*, spores of other fungi, pollen seeds, parts of flowers, and so forth. The air of hospitals and sick rooms has been found to be especially rich in vegetable forms, *e.g.*, fungi and spores have been observed as present in particularly large numbers in cholera wards, spores of Tricophyton have been discovered in the air of hospitals for diseases of the skin, and achorion in wards with cases of favus. The tubercle-bacil-

lus is said to have been detected in the breath of patients suffering from phthisis.

These points indicate that, in addition to the interest for the microbiologist, considerable importance from a hygienic point of view must be attached to the systematic examination of the air. Especially a knowledge of the microbes which are found in the air of marshy and other unhealthy districts, and in the air of towns, dwellings, hospitals, workshops, factories, and mines, will be of practical value.

Miquel,* who has particularly studied the bacteria in the air, has found that their number varies considerably. The average number per cubic metre of air for the autumn quarter at Montsouris is given as 142, winter quarter 49, spring quarter 85, and summer quarter 105. In air collected 2,000 to 4,000 metres above the sea-level, not a single bacterium or fungus spore was furnished, while in 10 cubic metres of air from the Rue de Rivoli (Paris) the number was computed at 55,000.

The simplest method for examining the organisms in air consists in exposing plates of glass or microscopic slides coated with glycerine, or a mixture of glycerine and glucose which is stable, colourless, and transparent. Nutrient gelatine spread out on glass plates (p. 71) may be exposed to the air for a certain time, and then put aside in damp chambers for the colonies to develop. Sterilised potatoes prepared in the usual way (p. 77) may be similarly exposed. In both the last mentioned methods separate colonies develop, which may be isolated as already described, and pure cultivations carried on in various other nutrient media (p. 74). Nutrient gelatine has also been employed in the special methods of Koch and Hesse.

Koch's Apparatus.—This consists of a glass jar about six inches high, the neck of which is plugged with cotton wool. In the interior is a shallow glass capsule, which can be removed by means of a brass lifter. The

* Miquel, *Organismes vivants de l'atmosphère.*

whole is sterilised by exposure to 150° C. for an hour in the hot-air steriliser. The nutrient gelatine in a stock-tube is liquefied, and the contents emptied into the glass capsule. The jar is exposed to the air to be examined for a definite time, the cotton wool plug replaced, and the apparatus set aside for the colonies to develop.

Hesse's Apparatus (Fig. 41).—The advantage of

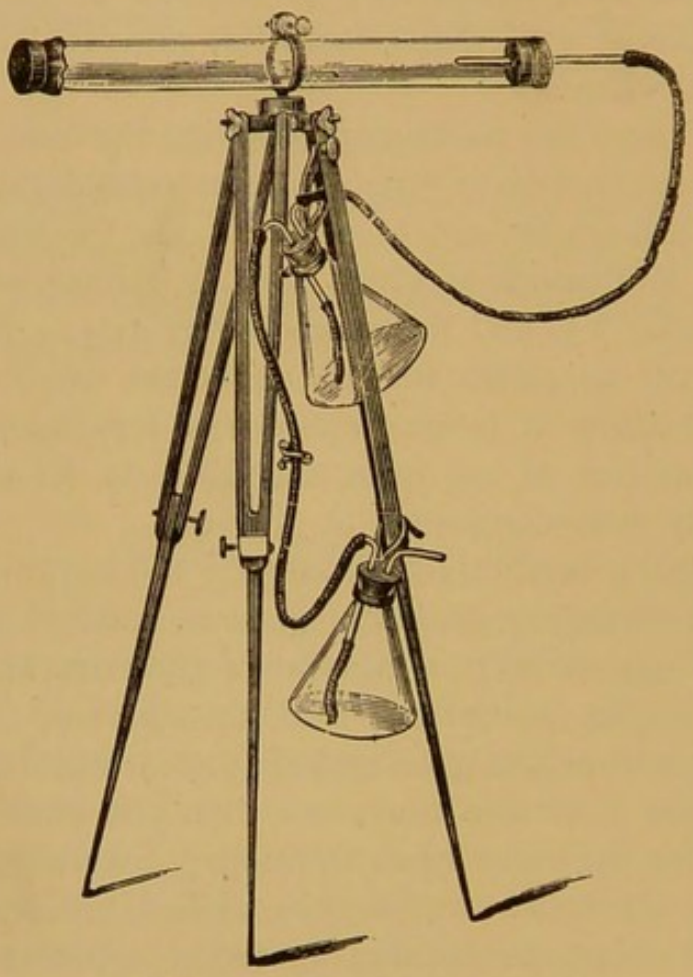

FIG. 41.—HESSE'S APPARATUS.

this apparatus consists in that a known volume of air can be examined. A glass cylinder, 70 cm. long and 3·5 cm. in diameter, is closed at one end by an india-rubber cap, perforated in the centre. Over this fits another cap, which is not perforated. The opposite end of the cylinder is closed with a caoutchouc stopper, perforated to admit a glass tube plugged with cotton wool. The tube can be connected by means of india-rubber tubing with an

aspirating apparatus. This apparatus consists of a couple of litre-flasks, suspended by hooks from the tripod stand which supports the whole apparatus. The cylinder, caps, and plug, are washed with solution of corrosive sublimate, and then with alcohol. After being thus cleansed, 50 ccm. of nutrient gelatine are introduced, and the whole sterilised by steaming for half an hour for three successive days. After the final sterilisation the cylinder is rotated on its long axis, so that the nutrient medium solidifies in the form of a coating over the whole of the interior. When required for use, the cotton-wool plug is removed from the small glass tube, and the latter connected with the upper flask by means of the india-rubber tubing.

The apparatus is placed in the air which is to be examined, the outer india-rubber cap removed from the glass cylinder, and the upper flask tilted until the water begins to flow into the lower one. The emptying continues by syphon action, and air is drawn in along the cylinder to replace the water. When the upper flask is empty, the position of the two is reversed, and the flow again started. When a sufficient volume has been drawn through the cylinder the outer cap and the cotton-wool plug are replaced, and it is set aside for the colonies to develop. As an example, twenty-five litres of air from an open square in Berlin gave rise to three colonies of bacteria and sixteen moulds; on the other hand, two litres from a school-room just vacated by the scholars gave thirty-seven colonies of bacteria and thirty-three moulds.

Various forms of "aeroscopes" and "aeroniscopes" have from time to time been employed. Pouchet's aeroscope consists of a small funnel, drawn out to a point below which is a glass slip coated with glycerine. The end of the funnel and the glass slip are enclosed in an air-tight chamber, from which a small glass tube passes out connected by india-rubber tubing with an aspirator. The air passing down the funnel strikes upon the glycerine,

which arrests any solid particles. For a description of the more exact apparatus employed by Maddox, Cunningham, and Miquel reference should be made to the writings of these authors, and particularly to the treatise published by the last-named.

APPENDIX C.

EXAMINATION OF SOIL.

SURFACE-SOIL, or mould, is exceedingly rich in bacteria. Miquel, *e.g.*, has computed that there exists in a gramme of soil an average of 750,000 germs at Montsouris, 1,300,000 in the Rue de Rennes, and 2,100,000 in the Rue de Monge. As agents of putrefaction and fermentation they play a very important *rôle* in the economy of nature, but there exist in addition bacteria in the soil which are pathogenic in character. Pasteur has succeeded in isolating from the earth the bacillus of anthrax, and sheep, sojourning upon a plot of ground where animals which have died of anthrax had been buried, succumbed to the disease. Pasteur considered that the spores were conveyed by worms from buried beasts to the surface soil. The bacillus of malignant œdema is also present in soil, and Nicolaier has cultivated a bacillus from earth which produced tetanus in mice, rabbits, guinea-pigs, and other animals.

To obtain a cultivation of the microbes in soil a sample of the latter must be first dried and then triturated. It may then be shaken up with distilled water, and from this a drop transferred to sterilised bouillon. The employment of solid media is, however, much more satisfactory: A sample of earth is collected, dried, and triturated, and a small quantity sprinkled over the surface of nutrient gelatine prepared for a plate-cultivation. In another method the gelatine is liquefied in a

test-tube, the powder added, and, in the usual way, distributed throughout the medium, which is then poured out upon a glass plate. Just in the same way the dust which settles from the air in houses and hospitals, or food substances in powder, may be distributed over nutrient gelatine, and the micro-organisms which develop studied, both as to their morphological and biological characteristics.

APPENDIX D.

EXAMINATION OF WATER.

As in the case of air, so, too, in that of water a knowledge of the micro-organisms which may be present is not only of interest to the microbiologist, but of the greatest importance in practical hygiene. Common putrefactive bacteria and vibrios may not be hurtful in themselves, but they indicate the probability of the presence of organic matter in some of which there may be danger.*

The Microzyme Test, which was introduced for their detection, consisted in adding three or four drops of the sample of water to 1 or 2 ccm. of Pasteur's fluid, the nourishing fluid having been previously boiled in a sterilised test-tube. If the microzymes or their germs existed in the water, the liquid in a few days became milky from the presence of countless bacteria. This test is of no real value, for it does little more than indicate that bacteria were present, which we may accept as being present in ice and all ordinary water. On the other hand, the bacteriological test of Professor Koch is a most valuable addition to the usual methods of water-analysis. It enables us not only to detect the presence of bacteria, but to ascertain approximately their number, and to study very minutely their morphological and biological charac-

* Parkes, *Manual of Practical Hygiene*. 1883.

teristics. The importance of a thorough acquaintance with the life-history of the individual micro-organisms cannot be too strongly insisted upon. For example, by such means the spirillum of Asiatic cholera can be distinguished from other comma-shaped organisms, and inasmuch as its presence may be an indication of contamination with choleraic discharges, such water should be condemned for drinking purposes, even though we may not yet be in a position to affirm that the microbe is the cause of the disease. The test, in short, consists in making plate-cultivations of a known volume of water, counting the colonies which develop, isolating the micro-organisms, and studying the characters of each individual form.

Collection and Transport of Water Samples.—Erlenmeyer's conical flasks of about 100 ccm. capacity may be employed with advantage for collecting the samples of water. They are cleansed, plugged, and sterilised in the hot-air steriliser. When required for use, the plug is removed and held between the fingers, which must not touch the part which enters the neck of the flask. About 30 ccm. of the water to be examined are introduced into the flask, and the plug must be quickly replaced and covered with a caoutchouc cap. If collected from a tap, the water should first be allowed to run for a few minutes, and the sample should be received into the flask without the neck coming into contact with the tap. From a reservoir or stream the flasks may be filled by employing a sterilised pipette. During transport contact between the water and cotton-wool plug must be avoided, and if likely to occur the sample must be collected and forwarded in a Sternberg's bulb (p. 78).

Examination by Plate Cultivation.—The apparatus for plate-cultivation should be arranged as already described. Crushed ice may be added to the water in the glass dish to expedite the setting of the gelatine, so that the plate may be transferred as quickly as possible to the damp-chamber. The caoutchouc cap

is removed from the flask, and the cotton-wool plug singed in the flame to prevent contamination from adventitious germs on the outside of the plug. The flask is then held slantingly in the hand, and the plug twisted out and retained between the fingers. With a graduated pipette a drop of the sample is transferred to a tube of liquefied nutrient gelatine, and the plug of the flask and tube quickly replaced. If the water is very impure, it may be necessary to first dilute the sample with sterilised water. The inoculated tube must be gently inclined backwards and forwards and rolled as already explained, to distribute the germs throughout the gelatine (p. 70), and the gelatine finally poured on a plate.

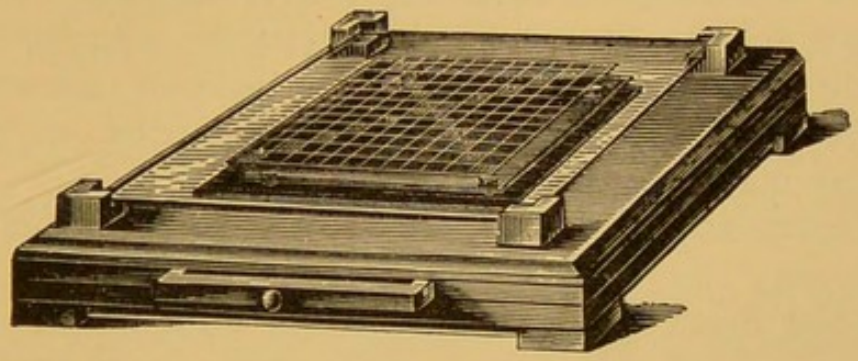

FIG. 42.—APPARATUS FOR ESTIMATING THE NUMBER OF COLONIES IN A PLATE-CULTIVATION.

When the gelatine has set, the plate is transferred to a damp chamber, which should be carefully labelled and set aside in a place of moderate temperature. In about two or three days the cultivation may be examined. In some cases the colonies may be counted at once ; more frequently they are so numerous that the plate must be placed on a dark background, and a special process resorted to. A glass-plate, ruled by horizontal and vertical lines into centimetre squares, some of which are again sub-divided into ninths, is so arranged on a wooden frame that it can cover the nutrient gelatine-plate without touching it (Fig. 42). A lens is added to assist in discovering minute colonies. If then the colonies are very numerous,

the number in some small divisions is counted, if less in some large ones, and an average is obtained from which the number of colonies on the entire surface is calculated. A separate calculation of the *liquefying* colonies should be also made, and their number, as well as the total number of colonies present in 1 ccm. of the sample, recorded. Any peculiar macroscopical appearances, colour, etc., should be noted, and then the microscopical appearances of the colonies studied. Lastly, examination of the individual organisms should be made by cover-glass-preparations, and by inoculation of nutrient gelatine, potatoes, and other media.

Examination by Test-Tube Cultivation.—A drop of the sample of water may also be added to liquefied nutrient gelatine in a tube, the organisms distributed as already explained (p. 70,) and the gelatine allowed to solidify in the tube. A rough comparison of water samples may be made in this way.

Microscopic Examination.—A drop of the water may be mounted and examined in the way described under drop-cultivations (p. 89), or a drop is allowed to evaporate on a cover-glass placed under a bell-glass. This is then passed three times through the flame, and stained in the usual manner. The examination of rain water, drinking water, tap water, sea water, various liquids and infusions, etc., by these methods opens up a wide field for research. Pettenkofer has shown that impregnation with carbonic acid of water containing many bacteria diminishes the number of the latter. The examination of waters before and after filtration, or after addition of chemical substances, are matters which require further investigation.

APPENDIX E.

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APPENDIX F.

TABLE SHOWING THE MAGNIFYING POWER OF ZEISS' OBJECTIVES.

Ocular:	1	2	3	4	5	
a_1	7	11	15	22		a_1
a_2	12	17	24	34		a_2
a_3	20	27	38	52		a_3
a*		4—12	7—17	10—24		a*
aa	22	30	41	56	75	aa
A, AA	38	52	71	97	130	**A, AA**
B, BB	70	95	130	175	235	**B, BB**
C, CC	120	145	195	270	360	**C, CC**
D, DD	175	230	320	435	580	**D, DD**
E	270	355	490	670	890	**E**
F	405	540	745	1010	1350	**F**
G	260	340	470	640	855	**G**
H	320	430	590	805	1075	**H**
J	430	570	785	1070	1430	**J**
K	570	760	1045	1425	1900	**K**
L	770	1030	1415	1930	2570	**L**
$\frac{1}{8}$	260	340	470	640	855	$\frac{1}{8}$
$\frac{1}{12}$	380	505	695	950	1265	$\frac{1}{12}$
$\frac{1}{18}$	605	810	1110	1515	2020	$\frac{1}{18}$
	1	2	3	4	5	

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