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BACTERIOLOGY

A MANUAL
OF
BACTERIOLOGY

CLINICAL AND APPLIED

BY

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PREFACE

TO

THE FOURTH EDITION

In this Fourth Edition the text has been subjected to complete revision, some slight alterations have been made in the arrangement of the sections, and much new matter has been incorporated; otherwise the general scheme and scope of the book remain much the same as in the previous editions. Several new illustrations have been added, for the photo-micrographs of which I am indebted to my friend and colleague Mr. J. E. BARNARD, F.R.M.S., Lecturer on Microscopy in King's College, London.

R. T. H.

KING'S COLLEGE, LONDON;
October, 1911.

PREFACE

In the following manual I have endeavoured to give some account of those portions of Bacteriology which are of especial interest in clinical medicine and hygiene. The preparation of tissues, methods of culture, descriptions of pathogenic organisms and their detection, the examination of water, etc., have therefore been given at some length. As it would be impossible in the space at my disposal to include everything relative to the subject, a selection has had to be made, and such details as the celloidin method, Löffler's stain for flagella, the strictly animal parasitic diseases (with a few exceptions), etc., have, among others, been omitted.

At the end of the sections dealing with the pathogenic organisms which attack man, some directions have been given for the bacteriological clinical diagnosis and examination, but these are in no way exhaustive; in fact, it would not be possible in a short work to give a scheme of examination which would cover every case. These directions will also render the book of service in the laboratory, while I venture to hope that the details given in the Appendix on the use of the remedies and diagnostic agents of bacterial origin may be of value to the practitioner.

I have to thank Mr. Peyton Beale, Dr. Lambert Lack, and Mr. F. J. Tanner, for suggestions and criticism, and the last-named gentleman for the aid he has freely given me in the revision of the proof-sheets. I am also indebted, indirectly, in many ways to my colleagues, Dr. Macfadyen and Mr. Foulerton. My thanks are due to Mr. J. Barnard and to Mr. Frank Stratton respectively for the photo-micrographs and original drawings, while for the eight borrowed illustrations blocks have been kindly lent by Messrs. Baird and Tatlock, and Messrs. Swift & Son.

May, 1898.

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A MANUAL

OF

BACTERIOLOGY

INTRODUCTION.

BACTERIOLOGY is that branch of Biology which deals with the study of Micro-organisms, particularly the minute vegetable organisms known as *Bacteria*. The scope of bacteriology is difficult to define exactly, for the term is often used in a comprehensive sense equivalent to micro-pathology, or even micro-biology, and all investigations connected with micro-organisms, animal and vegetable, may be included under it. So extensive, however, has the subject become that the animal micro-organisms are now being studied as a separate branch, PROTOZOOLOGY. Bacteriology deals with micro-organisms particularly in their relation to processes—disease, fermentation, putrefaction, and the like—while their structure, functions, and life-history are to a large extent left to the botanist and zoologist. There is no space in a work of this kind to enter into the history of the science, but the names of Leeuwenhoek (1675), Müller (1786), Schwann (1837), Cohn, Pasteur, Lister,

and Koch will ever hold an honourable place in its annals.

The study of micro-organisms must always be of considerable importance in general biology, for their vital phenomena are comparatively simple, and throw much light on the more complex processes occurring in the higher orders of living beings. Weismann based his theory of heredity on the fundamental conception of the immortality of these unicellular organisms. Excluding accidents, they are immortal—they reproduce themselves by a process of simple division, an individual dividing, and two daughter forms taking the place of the original parent one, and although the parent has disappeared yet there has been no death, no dissolution; its protoplasm or living material is still existent in its progeny and is immortal, since this process of reproduction apparently may go on indefinitely. Moreover, the study of the mutability and possible transformation of species of micro-organisms is likely to throw light on the theory of evolution. Organisms such as bacteria multiply so rapidly that fifty or sixty generations may be developed in thirty hours, a number which would take years to attain if even the most rapid breeder among mammals were the subject of experiment, and as they occur in vast numbers the opportunity for variation is extensive. These are some of the relations which micro-organisms have to general biology.

In what may be termed the economy of nature micro-organisms are all-important; without them there would be no putrefaction, no decay, and the dead remains of animal and vegetable life would so accumulate as to encumber the earth, which would become sterile for the want of the organic matter originally derived from it, but of which there was no return. In

fact the higher plants, and indirectly, therefore, animals also, are dependent for their existence upon the presence of bacteria in the soil, which break up and render assimilable complex substances presented to them as manures.

The question of life, animal and vegetable, without bacterial activity is an important and interesting one. It would seem from the experiments of Duclaux[1] that the higher plants in ordinary circumstances are unable to obtain nutriment unless the complex compounds, proteins, urea, and other nitrogenous bodies, which form the important constituents of many manures, are broken down into simpler ones through the agency of bacteria. He sowed seeds in sterile soil free from nitrates, nitrites, and ammonia, which was plentifully watered with sterile milk and solutions of sugar and starch. No changes occurred in these substances, the seeds lost weight, and the seedlings dwindled and died. As regards the higher animals various views have been expressed. Pasteur considered that their life also would probably be impossible without the presence of bacteria in the intestinal tract. Nencki expressed the opinion that this idea of Pasteur's was an erroneous one, and his experiments in conjunction with Macfadyen and Sieber[2] showed that any considerable decomposition of the food by bacteria first takes place in the large intestine, and that the digestive juices alone, without the co-operation of bacteria, are able to prepare the constituents of the food for absorption. Nuttall and Thierfelder obtained unborn guinea-pigs by Cæsarian section with antiseptic precautions, and afterwards kept them in a sterile environment and fed them on sterilised food. Not only did the animals live, but

[1] *Comp. Rend.*; t. 100, p. 66.

[2] *Journ. of Anat. and Physiol.*, xxv, p. 390.

they were even in a more thriving condition than those naturally brought up. The intestinal tract was found to be sterile on the eighth day. On the other hand, Schottelius found that chickens reared on sterile food were retarded in development, and experiments by Moro on turtle larvæ lead to the same conclusion, viz. that intestinal bacteria are necessary for normal nutrition. Levin, however, found that the intestinal tract in many Arctic animals—the polar bear, reindeer, seal, eider duck, etc.—is generally sterile, and in these instances, therefore, bacteria are not required for normal nutrition.

Commercially, micro-organisms are of the utmost importance. Without them there would be no fermentation, and the wine, beer, and indigo industries, the ripening of cheese and tobacco, and many like processes would be non-existent. From a financial aspect also micro-organisms cannot be ignored, for many of the so-called "diseases" of beer and wine, which often occasion great loss, are due to the entrance of adventitious forms, while the silk industry and sheep farming in France were once threatened with extinction owing to the ravages of pébrine and of anthrax respectively, but through the genius of Pasteur were restored to their former prosperity. There is no need to emphasise the importance of micro-organisms from a medical and hygienic point of view, but the fact may be recalled that sixty years ago the mortality after operations was very high, and that 40 per cent. of these deaths were caused by pyæmia, septicæmia, and hospital gangrene, conditions which are due to the entrance of micro-organisms, and which are now almost preventable, thanks to the antiseptic system introduced by Lord Lister.

The theory of spontaneous generation or abiogenesis is intimately connected with the study of bacteria.

The putrefaction of animal and vegetable fluids even after boiling, and the growth in them of minute living forms, were held by many to be a sure proof of the development of life from inanimate matter, of the spontaneous generation of the living from the non-living. A succession of investigators, however, showed (1) that if the fluids be boiled sufficiently long, and be then sealed up so as to prevent the access of air, they do not undergo putrefaction; (2) that the sealing up may be dispensed with, provided the air be first filtered through cotton-wool before being admitted to the flasks; (3) that even the cotton-wool is not needed if the air be passed slowly through a long and tortuous channel, so as to deposit its solid particles. Tyndall showed that putrescible fluids may be exposed in open vessels in a closed chamber in which the air has been undisturbed for some time and its solid particles thereby deposited on the walls of the chamber, which had been smeared with glycerin; he also proved that vegetable infusions and the like, which putrefy after having been boiled for ten minutes, do not do so if the boiling be repeated on two or three successive days, and explained this by the supposition, that while the fully developed bacteria are destroyed by the first boiling, their more resistant spores remain alive, but these on being left for twenty-four hours germinate into the less resistant bacterial forms, which are destroyed by the second boiling, and by the repetition of the process complete sterilisation may ultimately be obtained. It is this process of "discontinuous sterilisation," as it is termed, which is employed by the bacteriologist for the preparation of sterile culture media.[1]

[1] The writer believes that this explanation is only partially true, and would ascribe some of the sterilising effect of repeated heatings simply to the injurious action of alternate heating and cooling.

The idea of abiogenesis (or as he prefers to term it, "archebiosis") has recently been revived by Bastian. He claims that certain saline solutions which have been boiled or even heated above the boiling-point in sealed tubes after a time show the development of various living organisms, including bacteria and yeasts.[1]

Dunbar,[2] as the result of a series of experiments conducted over a long period and with every care to prevent contamination, has come to the conclusion that the bacteria are not an independent group of organisms, but that the bacteria, yeasts, and moulds are stages in the life-history of green algæ. The observations were carried out both by culture methods and by microscopical examination. A culture of a single-celled alga belonging to the *Palmellacea* was obtained, but by modifying the culture medium in which a pure culture of the alga was growing, by the addition of acid, of alkali, or of traces of copper salts, other organisms, generally bacteria, occasionally moulds and yeasts, and even spirochætes, made their appearance. Granting that there is no flaw in the experimental methods, and every care seems to have been taken to exclude contamination, etc., the results are susceptible of another explanation, viz. that the secondary growths were derived by transformation of the algal cells, in fact by the phenomenon of heterogenesis which has been claimed by Bastian to occur.

Undoubtedly bacteria exhibit variations and mutations, not only in morphology (see p. 15) but also in function. Thus pathogenic organisms may become non-pathogenic, and Twort has succeeded in training *B.*

[1] See various papers in the *Proc. Roy. Soc. Lond.* and *The Evolution of Life*, Methuen, 1907.

[2] See *Journ. Roy. Inst. Pub. Health*, vol. xv, No. 11, 1907, p. 679.

typhosus to ferment lactose, which ordinarily it does not. Some recent experiments by Horrocks[1] suggest that the *B. typhosus* may, by symbiosis with *B. coli*, be converted into *B. alcaligenes*.

Minchin in a presidential address to the Quekett Microscopical Club points out that syngamy (sexual reproduction, *e.g.* conjugation) is of the greatest importance in preserving differentiation of species, and that without it a species will tend to break up into races. It therefore follows that there are no true species among organisms of the bacterial grade, if it be true, as is usually held, that syngamy does not occur amongst them, and the so-called species of bacteria are to be regarded as mere races or strains capable of modification in any direction.

Doubtless immense progress has been made during the last two or three decades, but a vast amount still remains to be done. We have only touched the fringe of the explanation of the difficult problems of immunity, of the extraordinary variations in virulence and effects of the same organism, and of the important question of cure in, and prevention of, infective diseases, while the chemistry of the products of bacterial activity is still in its infancy.

The literature of Bacteriology is now becoming somewhat extensive. In the following pages a good many references to original papers have been introduced, so that further information may be obtained if required, the aim being so far as possible to refer to easily accessible papers which contain a more or less full bibliography on a particular subject. Kolle and Wassermann's *Handbuch der Pathogenen Mikroorganismen* is the most encyclopædic work on pathological bacteriology yet published.

[1] See *Brit. Med. Journ.*, 1911, i, p. 1073.

CHAPTER I.

THE NATURE, STRUCTURE, AND FUNCTIONS OF THE BACTERIA: THEIR CLASSIFICATION, GENERAL BIOLOGY, AND CHEMISTRY —BACTERIA AND DISEASE.

THE Bacteria or Schizomycetes ("fission fungi") are minute vegetable organisms for the most part unicellular and devoid of chlorophyll, which multiply by simple transverse division or fission; this distinguishes them from the yeasts, in which multiplication takes place by budding or gemmation. A certain number of filamentous forms are also included, serving to connect the unicellular ones with the multicellular true fungi. The "fission plants" may be placed in a sub-kingdom, the Schizophyta, which may be divided into two classes: Class I, Schizophyceæ, the blue-green algæ, and Class II, Schizomycetes, the bacteria.

The unicellular plants are sometimes termed the "Protophyta." It must be understood that there are connecting links between the different groups, and that there is no sharp line of demarcation between them.

The relation of the bacteria to other lower plants is shown in the following scheme:

Relation of Bacteria to Lower Plants.

- Thallophyta (lower plants without fibro-vascular bundles, and with no distinction between root and stem)
  - Forms with chlorophyll (Algæ, desmids, etc.)
  - Forms without chlorophyll
    - Multicellular. Spores in differentiated cells or spore-bearing organs. Frequently a sexual method of reproduction
      - The true Fungi (Eumycetes) including moulds (Hyphomycetes)
    - Unicellular. Spores frequently absent. Spore-bearing cells not, or but slightly, differentiated. No form of sexual reproduction
      - Reproduction by fission
        - The Schizomycetes or Bacteria
      - Reproduction by budding
        - The Blastomycetes or Yeasts

The size of the bacteria is variable, but they are all microscopic, measuring from 0·3 μ to 30–40 μ in diameter or in length.[1] Their shape likewise is very different in the different species; some are spherical, others ovoid, others rod-shaped or filamentous, while in some the rod or filament is twisted into a spiral. The bacterial cell consists of a cell-membrane enclosing the transparent, more or less structureless living matter or protoplasm, the cell-plasma or cytoplasm. Bütschli has described the bacterial plasma as having a reticular structure, but in the young cell this is probably either an artifact or a "false image" due to faulty illumination; the most that can be seen is a fine granulation. The protoplasm frequently contains larger granules composed of fatty or protein matter, pigment, and in some species of sulphur. Occasionally certain granules stain blue with iodine. One to three spherical granules are also present in each cell; these seem to take part in the division of the cell (see below), and if the latter be stained with a very dilute mixture of roseine and

[1] μ = micron = 0·001 mm. See p. 150.

methylene blue are coloured pink, contrasting with the rest of the protoplasm, which is stained blue.

Unless the roseine-staining granules, just mentioned, be regarded as such, no nuclear structure is present in the cell, nor does the suggestion that the bacteria are to be regarded as primitive nuclei almost devoid of protoplasm seem to be tenable. Another and more likely view is that the bulk of the cytoplasm consists of a mixture of nuclear material with non-chromatic substance.

The cell-membrane is usually invisible, but if the cell is treated with salt-solution (2·5 per cent.) *plasmolysis* takes place, the protoplasm shrinking away from the membrane, which then becomes visible. It can also be stained *in vivo* with very dilute solutions of roseine. The cell-membrane sometimes becomes thickened, swollen, and gelatinous on its outer surface, forming a layer or so-called "capsule" around the organism. The clear spaces frequently seen around bacteria in dried and stained preparations, especially in those from blood and lymph, are generally artifacts and not true capsules. In *Cladothrix* and some other forms the cell-membrane becomes hardened, leading to the production of a firm sheath. When bacteria assume the resting stage groups of them adhere together in a kind of jelly-like matrix, forming what is known as a "zooglœa."

The chemical composition of bacteria varies much, not only in different species, but even in the same species when grown on different nutrient media. All bacteria contain proteins, lipoid substances, and salts. Bacterial protein according to Nencki, differs from ordinary protein matter in not being precipitated by alcohol and in not containing sulphur; it was termed by him "myko-protein." This does not appear to be the case with the proteins obtained by grinding

bacterial cells, which seem to agree with other proteins in heat-coagulation, etc.

The proteins are mainly globulins and nucleo-proteins. The cell wall is relatively insoluble, and generally consists chiefly of a material like *chitin*, and not of cellulose; in this respect bacteria resemble animal rather than vegetable cells. Carbohydrates are generally scanty. Spores differ from the parent cells in containing a much larger proportion of solids and less water.

All species of bacteria, but especially the smaller ones, when suspended in a fluid exhibit what is known as Brownian movement, consisting of an oscillation with some amount of rotation about a fixed point, but there is little actual movement of translation, unless due to flotation. This Brownian movement is physical and not vital in origin, and occurs with all fine particles suspended in a fluid, and must be clearly distinguished from a true vital motility. Some bacteria are always motionless, others are more or less motile, but these, too, have a resting stage. For motility to occur the cells must be young, and the conditions favourable to growth and development. Motility is due to delicate protoplasmic threads termed "flagella" connected with the outer layer of the cell protoplasm; these vibrate to and fro and propel the organism through the medium. A cell will, however, move indifferently in either direction; if a motile organism be watched it will often be seen to proceed rapidly in one direction, stop, and then return without turning round. The flagella are not visible in the living state, unless dark ground illumination be used, nor by the ordinary methods of staining, unless previously treated with a mordant, and are extremely liable to be broken off. They vary considerably in number and in length; some organisms have but a single flagellum at one

pole (*monotrichic*), *e.g. Bacillus pyocyaneus*, others have two or more flagella forming a brush or tuft (*lophotrichic*), *e.g. Spirillum rubrum*, while others may be almost entirely covered with them (*peritrichic*), *e.g. B. typhosus* ; in some the flagella are short and straight, and in others long and twisted. The motility of organisms does not necessarily depend directly upon the number of flagella they possess, an organism with a few flagella often being more active than another possessing many, and some are apparently non-motile, though well-marked flagella can be demonstrated. Generally, however, an organism with several flagella will be more motile than a similar form with a few. (See also p. 136.)

Darwin says: "In looking at Nature it is most necessary never to forget that every single organic being may be said to be striving to the utmost to increase in numbers," and in no group perhaps of the animal and vegetable kingdoms is this more marked than among the bacteria. Reproduction is entirely non-sexual, and takes place in two ways—by simple division or fission and by spore formation. Reproduction by transverse fission is common to all bacteria; the bacterial cell becomes constricted at its middle and finally separates into two parts, and thus two young cells take the place of the parent one; reproduction by fission is therefore also an increase in numbers. The fission is always transverse, never longitudinal,[1] the rule being in cell-division that the new membrane is formed in the most economical manner. Longitudinal division, on the other hand, seems to be very common among the Protozoa.

[1] Longitudinal division has been described in a few species, but its occurrence is so rare that a doubt must arise as to whether these forms are true bacteria.

Previous to division the rod-forms become elongated and the spherical ones ellipsoidal, and there is an increase in the number of the roseine-staining granules, partly by division of pre-existing ones and partly by new formation. The constriction in the majority of cases involves and passes through one of the granules. In the monotrichous and lophotrichous bacteria it is always the non-flagellated end of the dividing cell which bears the flagella of the new cell. Under favourable conditions reproduction may be very rapid, fission occurring every twenty or thirty minutes (Klein), so that, the increase being in a geometrical ratio, the number of individuals which might arise from a single bacterium in three or four days is almost inconceivable, and would *en masse* weigh thousands of tons; fortunately there are many checks to such a rapid multiplication. Frequently, although the protoplasm divides, the division of the cell-membrane is incomplete, resulting in a loose union of the cells with the formation of a pseudo-filament. These filaments often become much curved and twisted, forming tangled masses, owing to fission taking place in the cells in the middle of the filament as well as at the ends, so that the filaments have to become curved to make room for the new cells.

Reproduction by spore formation is met with in some species, and is generally described as being of two kinds. In the first, "endogenous" spore formation, a bright refractile round or ovoid body is formed within the bacterial cell, the development of which can be watched under the microscope. Rowland describes the process of spore formation as follows: Refractile, oily-looking droplets, which do not stain with roseine, appear and ultimately coalesce, forming the spore. The cell-plasma at the same time diminishes and retracts from the cell-membrane. The roseine-staining granules increase

in number and aggregate into two spherical masses, which dispose themselves one at each end of the cell. The cell-membrane collapses somewhat, and, when the spore is fully formed, ruptures transversely, leaving two cup-shaped receptacles, in which the granules and remains of the plasma are still recognisable. Only one spore develops in each cell, and the spores seem to fulfil the purpose of perpetuating the race when it is threatened with extinction from adverse circumstances. Each spore consists of a little mass of protoplasm enclosed within a very tough and resisting membrane, which tends to preserve its vitality even under unfavourable conditions; for spores resist the action of desiccation and germicidal agents to a much greater degree than the fully developed organisms. Spores vary much in size and in the position they occupy within the bacterial cell in the different species; their diameter is usually about the same as that of the cell in which they are developed, but may be much greater, and in position they may be central or terminal, and sometimes the spore-bearing cells are swollen or club-shaped; these are termed "clostridia." Endospores are still unknown in a large number of species. The second variety of sporulation, "arthrospore" formation, is of doubtful occurrence, but is stated to take place as follows: Some of the elements formed by fission are slightly larger, more refractile, and more resisting than their fellows, and are stated to have the properties of spores. Placed in favourable circumstances, the spore in either case germinates, it becomes swollen and granular, and loses its refractile appearance; a slight protuberance forms, this increases in size, and an organism similar to the parent one is finally reproduced; the empty spore membrane at first frequently encloses one extremity, and is afterwards

cast off. In certain instances the spore germinates without casting its membrane, the spore membrane becoming the cell-wall of the young organism. The ellipsoidal spores of the *B. anthracis* sprout from the end, those of *B. subtilis* from the side ("polar" and "equatorial" germination respectively).

On the Morphology, etc., of the Bacteria see Rowland, *Trans. Jenner Inst. Prev. Med.*, ii, 1899, p. 143; Fischer, *The Structure and Functions of Bacteria*, 1900; Migula, *System der Bakterien*, i; Dobell, *Quart. Journ. Micr. Sci.*, 1911; Pénau, *Comp. Rend.*, clii, 1911, p. 53.

Classification of the Bacteria.

Many classifications of the bacteria have been proposed, but none up to the present can be said to be strictly scientific, or even satisfactory from the point of view of convenience. In the first place, the bacteria are said to be devoid of chlorophyll, but there are many forms intermediate between those unicellular organisms with and those without chlorophyll, so that a hard and fast line cannot be drawn. In the next place, the bacterial cells are so minute, and their vital phenomena so simple, that only a few broad distinctions can be observed in their morphology and reproductive processes.

One of the most prominent of the older classifications was that of Cohn. He divided the bacteria into four main groups:

I. The Sphærobacteria or spherical forms.
II. The Microbacteria or short rod-forms.
III. The Desmobacteria or long rod-forms.
IV. The Spirobacteria or spiral forms.

Zopf's classification (1885) has many points to commend it, but is largely based on the doctrine of pleomorphism. By pleomorphism is meant a variation in the form of an organism during its life-cycle, a

coccus, for example, growing into a rod, or a straight rod becoming a spiral. In a peach-coloured bacterium examined by Lankester, cocci, rod, filamentous and spiral forms occurred, and the doctrine of pleomorphism received considerable support from his work, though it may be questioned whether he was working with pure cultures. Be that as it may, a certain amount of pleomorphism undoubtedly occurs in some organisms. In the colon, typhoid, and plague bacilli, for example, the rods may sometimes be so short as to be almost cocci, while at others they are well-marked rods and even filaments (see also p. 6). The following is an outline of Zopf's classification, the bacteria being divided into four main groups or families, which again are subdivided into smaller groups or genera :

Family I. COCCACEÆ.—Spherical forms only; division occurs in one or more directions.

Genus 1. MICROCOCCUS (Staphylococcus).—Division in one direction only, but irregular, so that the cocci after division form irregular clusters.

Genus 2. STREPTOCOCCUS.—Division in one plane, but regular, so that the cocci form chains.

Genus 3. MERISMOPEDIA.—Division in two directions at right angles to each other, but in the same plane, so that lamellæ or plates are formed.

Genus 4. SARCINA.—Division in three directions at right angles to each other and in two planes, so that cubical masses are formed.

Genus 5. ASCOCOCCUS.—Cocci which develop in a gelatinous matrix.

Family II. BACTERIACEÆ.—Rods, straight or curved, at some period of the life-history, though cocci and other forms may occur.

Genus 1. BACTERIUM.—Straight rods; endospore formation does not occur.

Genus 2. BACILLUS.—Straight rods; endospore formation occurs.

Genus 3. LEUCONOSTOC.—Cocci and rods; arthrospore formation occurs in the coccoid forms.

Genus 4. CLOSTRIDIUM.—The same as bacillus, but the spore-bearing rods are enlarged and club-shaped.

Genus 5. SPIRILLUM.—Spiral rods; spore formation does not occur.

Genus 6. VIBRIO.—Spiral rods; spore formation occurs.

Family III. LEPTOTRICHEÆ.—These are unbranching thread forms.

Family IV. CLADOTRICHEÆ.—These are thread forms showing true but not dichotomous branching.

There are many features in this classification which are of practical value. The distinction made between a bacterium and a bacillus, for example, is very convenient. Formerly it was the custom to term a short rod a bacterium, and a long rod a bacillus, but such a division is an arbitrary one, and at one stage of its life-history an organism might have to be termed a bacterium and at another a bacillus. The term "bacterium" is now but little used, and any straight rod is termed a bacillus. The term "staphylococcus" is one frequently met with; it is practically synonymous with micrococcus, and refers to cocci which are aggregated into groups or clusters. Of the twisted rods, a simple curved rod is now known as a vibrio, a definitely corkscrew form of three or a few turns is

a spirillum, a long and flexible twisted filament is a spirochaeta.[1]

A later system of classification is that proposed by Migula.[2] The bacteria are divided into two orders: the Eubacteria—bacteria proper—the cells of which contain neither sulphur granules nor a colouring matter, bacterio-purpurin; and the Thiobacteria, the cells of which contain sulphur granules and may be coloured with bacterio-purpurin. The Eubacteria are divided into five families: (1) Coccaceæ, (2) Bacteriaceæ, (3) Spirillaceæ, (4) Chlamydo-bacteriaceæ, and (5) Beggiatoaceæ. These, again, are subdivided into many genera, based partly on the mode of division and partly on the number and on the arrangement of the flagella upon the organisms. The Coccaceæ—globular cells—contain the genera Streptococcus, Micrococcus, Sarcina (non-motile), and Planococcus and Planosarcina (motile); the Bacteriaceæ are defined as long or short cylindrical rods, straight and never spiral; division in one direction only after elongation of the rods; and this family has three genera: (*a*) Bacterium—non-flagellated cells, often with endospore formation; (*b*) Bacillus—cells possessing both lateral and polar flagella, often with endospore formation; (*c*) Pseudomonas—cells with polar flagella only, rarely endospore formation. The Spirillaceæ are curved or spiral rods, and include (*a*) Spirosoma, non-motile forms, (*b*) Microspira, motile forms with one polar flagellum, (*c*) Spirillum, motile forms with two or more polar flagella.

The nomenclature of bacterial species is at present in a chaotic condition. In botanical and zoological nomenclature

[1] Many of the so-called spirochaetæ are probably protozoa and not bacteria.

[2] *System der Bakterien*, 1897. Abstract in *Centr. f. Bakt.* (1te Abt.), xxii, 1897 (September), p. 345

every species has a binomial name, the first being the generic, the second the specific, name. Many bacterial species have received trinomial names, which should be inadmissible. The specific name first given to an organism must stand unless it has been used for some other species.

Conditions of Life of Bacteria.

Bacteria, being living organisms, must be supplied with suitable nutritive substances in order that their life-processes—nutrition, reproduction, and the like—may be carried on in a normal manner. Being devoid of chlorophyll they are mainly dependent upon complex organic compounds for the carbon, hydrogen, and nitrogen which enter into their composition, these elements being derived for the most part from proteins and carbohydrates. Some bacteria, however, are able to obtain the requisite nitrogen from such comparatively simple compounds as ammonia, ammonium carbonate, or nitrates, and one group can make direct use of the atmospheric nitrogen. Certain inorganic salts, sulphates, phosphates, and sodium chloride, also seem to be necessary for normal development. These nutrient substances must be presented to the bacteria in association with water, for without water bacterial activity ceases, though in the dry state many forms, and especially their spores, may retain their vitality for a considerable time; absolute desiccation, however, is rapidly fatal to many.

Temperature is also an important factor. Though the growth of many species occurs through a wide range, there is for almost all an optimum at which growth is best, and of a range not exceeding 5° or 10°. Growth usually ceases below 10° C., but cold does not destroy bacterial life; after exposure to the intense cold produced by the evaporation of liquid oxygen

(– 170° C.) for weeks, or of liquid hydrogen (– 252° C.) for ten hours, bacteria and their spores will grow and germinate, and their chromogenic and pathogenic properties seem to be unaltered.[1] On the other hand, bacterial growth usually ceases when the temperature exceeds 40° C. or thereabouts, and most bacteria without spores are destroyed within half an hour by a temperature of 65° C. The spores are far more resistant; some may even be boiled for a short time without losing their vitality, but prolonged boiling is fatal to both bacteria and their spores. There is, however, a group of so-called thermophilic bacteria, which thrive best at a temperature of 60° to 70° C. They occur in the soil and in water, and are probably of considerable importance in the natural fermentations accompanied by the evolution of heat, such as are met with in manure heaps, the heating of hay, and firing of moist cotton.[2]

Free oxygen is essential to the growth of some organisms; these are termed strictly aërobic. Others will not develop in its presence, strictly anaërobic; others, again, while preferably aërobic or anaërobic, will grow in the absence, or in the presence, of oxygen, and are respectively termed facultative anaërobic or facultative aërobic. Some organisms are strictly parasitic on animals or plants; others live in water, soil, decaying matter, etc.—these are termed saprophytes; and many are able to exist either as parasites or as saprophytes (see also p. 151).

Bacterial development is much influenced by the presence of foreign substances in the nutrient medium.

[1] Macfadyen and Rowland, *Proc. Roy. Soc. Lond.*, February 1st, 1900; April 5th, 1900; May 31st, 1900.

[2] Macfadyen and Blaxall, *Journ. of Path. and Bact.*, November, 1894, and *Trans. Jenner Inst. of Prev. Med.*, vol. ii, 1899, p. 162.

A number of metallic and other salts, chlorine, bromine, and iodine, carbolic acid, salicylic acid, etc., have an injurious effect upon bacterial life, inhibiting or stopping growth, or killing the organisms outright; these are of considerable practical importance and are known as germicides, antiseptics, and disinfectants. The products produced in the nutrient medium by the bacteria themselves also sooner or later inhibit or stop further growth; a familiar instance of this is seen in the alcoholic fermentation of sugar by yeast, which ceases when the amount of alcohol reaches 12 or 14 per cent. The same reason probably accounts for the fact that growths of bacteria in culture tubes do not spread all over the surface of the nutrient medium, and why our cultures sometimes die out more rapidly than might be expected.

Another point affecting bacterial life is the presence of a mixture of organisms in the same nutrient medium. If there be a very vigorous form, it may ultimately grow and multiply to such an extent as to crowd out and finally kill the other forms with which it is associated, and if the nutrient medium equally favour two species, that one which is in an excess at the beginning may outgrow the other. The occurrence of what has been termed symbiosis is of considerable interest in the life of micro-organisms, and too little attention has hitherto been paid to it. This is the co-existence of two or more species which together bring about certain changes. For example, in the well-known ginger-beer plant, Marshall Ward[1] isolated several yeasts, bacteria, and moulds; of these, one of the yeasts and one of the bacteria together induce the particular changes in a saccharine fluid to which ginger has been added, which render the mixture like ginger-beer, and these changes do not occur unless both species develop together.

[1] *Phil. Trans. Roy. Soc. Lond.*, vol. clxxxiii, 1892, p. 125.

Another extraordinary feature exhibited by bacteria is the selective action exerted on certain substances which contain isomerides or right- and left-handed modifications of a substance. The *Bacillus ethaceticus* attacks mannitol but not dulcitol, two alcohols which are very similar in taste and properties and possess the same simple chemical formula.

By a series of most brilliant researches Emil Fischer has succeeded in determining the constitution of the various sugars, and, what is more, has produced them artificially in the laboratory. The natural sugars are all compounds with dissymmetric molecules, powerfully affecting the beam of polarised light, but when prepared artificially they are without action on polarised light, because the artificial product consists of equal numbers of left-handed and right-handed molecules, and the molecules of the one neutralise the molecules of the other, thus giving rise to a mixture which does not affect the polarised beam.

By the action of micro-organisms, however, on such an inactive mixture the one set of molecules is sought out by the microbes and decomposed, leaving the other set of molecules untouched, and the latter now exhibit their specific action on polarised light, an active sugar being thus obtained.

Fructose was one of the principal artificial sugars prepared by Fischer; it is inactive, but consists of an equal number of molecules of oppositely active sugars termed "lævulose." One set of these lævulose molecules turns the plane of polarisation to the right, another set to the left—right- and left-handed lævulose. The left-handed lævulose occurs in nature, while the right-handed lævulose, so far as is known, does not.

Now, on putting brewer's yeast into a solution of fructose, the inactive artificial product, the yeast

organisms attack the left-handed lævulose molecules and convert them into alcohol and CO_2, while the right-handed lævulose is left untouched.

Pressure, unless very great, has little effect on bacteria. Roger investigated the effects of high pressure on certain organisms in bouillon cultures. Pressures of 200 to 250 kilos. per square centimetre had no effect; by raising the pressure to 3000 kilos. per square centimetre one third of streptococci were killed, and of anthrax without spores a good many; while sporing anthrax, *Micrococcus pyogenes*, var. *aureus*, and the colon bacillus were unaffected.[1]

Our countrymen Downes and Blunt first called attention to the injurious effect of light upon bacteria. If plate cultures be prepared and exposed to sunlight, a portion of the plate being protected from its action, as by sticking on a letter cut out of black paper, and the preparation afterwards incubated, it will be found that the colonies develop at the protected portion only, those parts which have been exposed to sunlight remaining sterile. Although this action of sunlight may occasionally be due to chemical changes in the medium, resulting in the production of ozone or other germicidal bodies, the experiments of Marshall Ward and others have conclusively shown that germicidal action may be caused by the direct action of the light, the violet and ultra violet rays being those concerned, and the red end of the spectrum has no effect. The Röntgen rays seem to have little or no influence upon bacteria, but the results obtained are somewhat contradictory.

[1] Bacteria being so minute, the actual pressure on a bacterial cell, even with these high pressures, is small. If, for example, a bacterium measures 1 μ by 5 μ, a pressure of 1000 kgrm. per square centimetre would be but 0·05 grm. ($\frac{3}{4}$ grain) on the cell.

The radium emanations with prolonged exposure and near contact are germicidal to non-sporing organisms.[1]

Electricity, *per se*, has also usually little effect. When the current is passed directly through the cultures electrolysis takes place, and the products formed may destroy the bacteria; currents of high potential, however, may inhibit growth.[2]

Living motile bacilli are very sensible to induced currents of electricity, immediately orientating themselves in the direction of the current, while dead or paralysed bacilli are unaffected.[3]

Bacterial Products.

The chemical changes produced by micro-organisms are chiefly analytic or destructive—the formation of simpler from more complex bodies. This analytic faculty is present to a marked degree in the process known as putrefaction. *Putrefaction* is a term applied to the decomposition of organic, especially protein, matter after the death of the animal or plant. It is usually accompanied by the evolution of foul-smelling gases and by the solution of the solid material. A large number of organisms are concerned in this process, particularly a group to which Hauser gave the name of *Proteus*. The first changes which occur are the formation of proteoses and peptone, then leucin, tyrosin, and glycocol, and basic compounds to which the name of ptomine has been given; next indole, skatole, and phenol, and volatile fatty acids; and lastly, mercaptans, sulphuretted hydrogen, marsh gas, ammonia, carbonic acid, and hydrogen.

1 See Green, *Proc. Roy. Soc. Lond.*, vol lxxiii, 1904, p. 375.

2 Lortet, *Comp. Rend.*, t. 119, 1894, p. 463.

3 *Comp. Rend.*, t. 122, 1896, p. 892.

In view of its practical importance in bacteriological analysis and the identification of species, indole may here be referred to at some length.

Indole.—Indole (C_8H_7N) is a product of the putrefactive decomposition of proteins containing a tryptophane nucleus and is formed during the growth of many organisms, and, since one species may produce it and another allied one may not, the determination of its presence or absence in the culture may be of value in the identification of organisms. The detection of indole is based on the reaction with nitrous acid, with which it gives a fine purplish-red coloration. In order to test for it, the organism is grown in a fluid medium for twenty-four to forty-eight hours or longer, 1 c.c. of a 0·1 per cent. solution of sodium nitrite is added to every 10 c.c. of the culture, and a few drops of pure concentrated sulphuric acid or of hydrochloric acid are allowed to trickle slowly down the side of the test-tube, which is inclined with its mouth away from the operator. As the acid runs down, it is mixed with the fluid; a colour varying from pale pink to pale purple indicates the presence of indole. A control tube, uninoculated, should also be similarly tested to make sure that the reaction is due to the products of the growth of the organism. The culture fluid usually employed is peptone water, preferably 2 per cent., but some samples of "peptone" occasionally fail to give the indole reaction when organisms are grown in media prepared from them; the right kind of peptone must, therefore, be used. As the dilute solution of sodium nitrite is unstable, a stock 5 per cent. solution may be kept; 2 c.c. of this solution are diluted to 100 c.c. with distilled water at the time of making the test, and 1 c.c. of this dilution is added to every 10 c.c. of the culture. The addition of the acid liberates free nitrous acid,

which reacts with any indole present, and yields a pink colour. Sometimes when the reaction is apparently absent or feeble, it may be obtained or intensified by placing the tube in the blood-heat incubator for half an hour. The sulphuric acid should be pure and free from oxides of nitrogen, hence hydrochloric acid is often preferable.

A more delicate method of testing is to run a little hydrochloric acid down the side of the tube, so that a layer forms at the bottom, the nitrite having been previously added to the culture if required. A pink ring at the juncture of the hydrochloric acid and culture indicates the presence of indole. The pink pigment, the product of the reaction, may be extracted by shaking with a little amylic alcohol.

Other delicate and more certain reagents for the detection of indole are para-dimethylamidobenzaldehyde (15 grm., dissolved in water 250 c.c., concentrated sulphuric acid 30 c.c.), which gives a rose to cherry-red, and β-naphthaquinone-sodium-mono-sulphonate (2 per cent. aqueous solution), which gives, when the mixture is rendered alkaline with caustic potash, a blue or blue-green colour or precipitate. The coloured compound may be extracted with chloroform, in which it yields a red solution.

Peptone water is by no means a good culture medium, and broth may therefore be employed, but it should be free from dextrose. Peptone water with the addition of a little rabbit's serum is perhaps the best culture medium for the production of indole.

The presence of dextrose, saccharose, glycerin, or lactose in quantity exceeding about 0·25 per cent. prevents the formation of indole in broth by bacteria. Broth prepared in the ordinary way usually contains a little dextrose derived from the glycogen in the meat,

and this probably explains why the indole reaction is generally much more marked in a peptone water than in a broth culture, although the latter is a better nutrient soil. In order to prepare a soil free from dextrose, T. Smith[1] recommends that the acid beef broth used in the preparation of nutrient broth should be inoculated with the colon bacillus and incubated for twenty-four hours, and the peptone beef broth prepared from it. The dextrose is consumed and no indole is formed.

Some bacteria not only form indole but also produce nitrites in the culture medium by the reduction of the nitrates present in the peptone, etc., used in making the nutrient medium, in which case the addition of pure sulphuric or hydrochloric acid alone suffices to bring out the pink indole reaction. This forms, therefore, an additional means of distinguishing organisms, and is employed especially for the recognition of the cholera spirillum, which, if grown in peptone water, gives the indole reaction (or, as it has been termed, "the cholera red reaction") on the addition of acid alone. The reaction can be obtained as early as twelve hours after inoculation, and becomes very marked in twenty-four to forty-eight hours.

If indole is formed only in small quantities, 100 c.c. of the culture may be distilled; the first 20 c.c. of the distillate will contain the bulk of the indole.

This "indole-reaction" is not necessarily always due to indole; the writer has shown[2] that the indole-like reaction obtained with cultures of the diphtheria and pseudo-diphtheria bacilli is owing to the presence of skatole-carboxylic acid. This substance is distinguished from indole by being non-volatile. To make sure of the

[1] *Journ. of Exper. Med.*, vol. ii, 1897, p. 543.

[2] *Trans. Path. Soc. Lond.*, vol. lii, pt. ii, 1901, p. 113.

presence of indole, the culture should therefore be made alkaline with caustic soda and distilled.

Skatole (methyl indole) seems also to be formed by some organisms. It is volatile like indole, but if a solution containing it be boiled with an acid solution of dimethylamido-benzaldehyde (5 per cent. in 10 per cent. sulphuric acid) it yields a blue colour, which gives a blue solution in chloroform.

Nitrification.—Another important series of changes is that included under the term "nitrification." As mentioned before, protein, albuminoid, and other complex nitrogenous matters and urea, all of which are valuable manures for plant life, cease to be so unless bacteria are present.

It is necessary, in fact, for the nitrogenous matter to be converted into nitrates, in which form alone is it available for the nutrition of plants.

Although so important, extremely small quantities of nitrates are present in the soil; in fertile soils, for example, under some conditions there may be as little as one part of nitrogen in 1,000,000, and there is often less than ten parts. The bodies yielding nitric acid in the soil are: (1) free nitrogen; (2) small quantities of nitrates in rain-water; (3) ammonium salts, applied intentionally or carried to the soil by rain or derived from the decay of organic matter; (4) various nitrogenous organic substances arising from the decay of animal and vegetable matters.

With regard to the production of nitric acid from nitrogenous organic matters very little was formerly known. In 1877 Schloesing and Müntz by an ingenious experiment showed that nitrification (as the production of nitric acid is termed) of nitrogenous organic matter is brought about by living organisms in the soil. Sewage was passed continuously through a tube

containing a mixture of ignited quartz sand and limestone. After three weeks nitrates began to appear in the effluent and increased to such an extent that finally the filtered sewage contained no ammonia. After this had continued for some weeks chloroform vapour was passed at the same time through the tube, with the result that in ten days after the introduction of the chloroform all nitrates disappeared from the effluent.

Subsequently the passage of chloroform vapour was discontinued, but nitrification did not resume until the washings from 10 grm. of garden soil were added. Eight days after this addition nitrates again appeared in the effluent (this was confirmed by Warington). Evidently the chloroform vapour acted as an antiseptic and killed the nitrifying organisms, while the addition of soil washings re-inoculated the material.

Shortly after this Schloesing and Müntz found that exposure of soil to 100° C. for an hour destroyed the power of inducing nitrification. Soils thus treated were exposed to a current of air, purified by ignition, without nitrification taking place ; the addition of a little unheated mould was, however, sufficient to cause nitrification to recommence. They also tried seeding the sterilised soils with various Hyphomycetes, etc., without result.

In 1884 Warington concluded that the factor determining the formation sometimes of nitric acid and sometimes of nitrous acid was a difference in the character of the organisms ; for it is possible to have two similar solutions under identical conditions, and for nitrites to be produced in the one, and nitrates in the other.

In 1886 Dr. Munro showed that the process of nitrification could take place in solutions practically destitute of organic matter.

Nitrification in the soil takes place in three stages :

I. *Ammonisation.*—When complex organic compounds such as albuminoids are applied to the land they are broken up; first they become liquefied, peptone-like bodies being produced; these are then further acted upon and we get alkaloidal substances in small quantity, indole, skatole, leucin, and tyrosin and amino-acids, valerianic acid, volatile fatty acids, lactic acid, etc.

These changes are brought about by numbers of organisms, among which the varieties of *Proteus* (formerly known as *Bacterium termo*), *B. mesentericus*, *B. mycoides*, *B. fluorescens liquefaciens*, and *B. putrificus* are the more important.

The nitrogenous compounds are then further acted upon and ammonium salts are formed. According to Emile Marchal, ammonisation takes place essentially under the influence of microbes living in the upper layers of the soil. The *Bacillus mycoides* is one of the most energetic of these, and seems to play a double *rôle*, being ammonising in the presence both of nitrogenous organic substances and of nitrates. Urea is ammonised especially by the *Micrococcus ureæ*.

II. *Nitrosation.*—The ammoniacal salts are next converted into nitrites. The nitrous organisms can probably attack nitrogenous organic substances such as asparagine and milk, but only feebly, milk being much more rapidly nitrified when the nitrous organisms are mixed with other species. The organisms bringing about this change are short, stumpy, motile bacilli with single polar flagella which are grouped under the generic name of *Pseudomonas*.

III. *Nitratation.*—These nitrites are then converted into nitrates. The "nitric" organisms are minute non-motile bacilli known as *Nitrobacter*.

Stages II and III are brought about by different species, the nitric organisms having no effect whatever on ammonia, but acting only after this has been oxidised into nitrous acid by the nitrous forms.

The discovery of Dr. Munro that organisms will grow in purely inorganic solutions has been made use of for the isolation of the different species. Solutions such as the following have been used :

For the Nitrous Organisms.	For the Nitric Organisms.
Ammonium chloride, 0·5 grm.	Potassium nitrite, 0·3 grm.
Potassium phosphate, 0·1 grm.	Potassium phosphate. 0·1 grm.
Magnesium sulphate, 0·02 grm.	Magnesium sulphate, 0·05 grm.
Calcium chloride, 0·01 grm.	Calcium carbonate, 5 grm.
Calcium carbonate, 5 grm.	Distilled water, 1000 c.c.
Distilled water, 1000 c.c.	

These are seeded with traces of earth, and by carrying on the cultivation for many generations a large number of organisms are eliminated. This method does not lead to a pure cultivation, for several forms besides the nitrifying organisms persistently maintain themselves in these mineral solutions.

So recourse was had to gelatin plate cultivations. Although several organisms were isolated in this manner, none of them possessed the slightest nitrifying power.

Frankland, and later Warington (1890), succeeded in isolating nitrous organisms by the dilution method. Nitrifying solutions were diluted, and traces inoculated into ammoniacal solutions; in some of these nitrification occurred, although no growth could be obtained on gelatin, and they were found to contain the nitrous organism only. A little later Winogradsky isolated nitrous organisms, first by modified gelatin plates, and afterwards by the silica jelly method.

Warington gives the following directions for the preparation of silica jelly plates : Sodium carbonate is fused in the blowpipe, and fine white sand is added as long as effervescence is produced. The mass is allowed to cool, and is then dissolved in water. The solution is poured into an excess of

very dilute hydrochloric acid (silicic acid and sodium chloride being formed). The solution is dialysed and sterilised. Some of this is placed in a sterile dish and is mixed with the following solution and inoculated :

Ammonium sulphate . . .	0 4 grm.
Magnesium sulphate . . .	0·5 ,,
Di-potassium hydrogen phosphate .	0·1 ,,
Calcium chloride	trace
Sodium carbonate	0·6–0·9 grm.
Water	100 c.c.

This mixture sets to a jelly in five to fifteen minutes.

Winogradsky has also made use of agar for plates, but this medium is not so suitable as the silica jelly. A 2 per cent. aqueous agar is prepared and poured into Petri dishes ; the film is then sown with *Proteus*, and allowed to grow for seven to ten days. It is then thoroughly washed, collected, melted, and mixed with the salts mentioned above. The object of growing the *Proteus* upon it as a preliminary is to eliminate the organic matter admixed with the agar.

Nitrification in the soil is thus brought about by two groups of organisms. The first oxidises ammonia into nitrous acid, and is isolated by successive cultivation in solutions of ammonium carbonate. The second group oxidises nitrous acid into nitric acid, and may be separated by successive cultivations in a solution of potassium nitrite containing a little sodium bicarbonate. In the soil the nitric and nitrous organisms are equally active.

Besides the derivation of nitrogen from nitrogenous compounds, the free atmospheric nitrogen is also "fixed" through the agency of certain micro-organisms and rendered available for plant life.

Thus, the Leguminosæ are able to obtain their nitrogen directly from the nitrogen of the air. If the roots of a pea, bean, or vetch be examined, numerous

little nodules will be found upon them; these contain minute irregular and Y-shaped bodies, which have been termed "bacteroids," and seem to be of the nature of involution forms. On inoculation into suitable culture media[1] the bacteroids give rise to a growth of a motile bacillus known as *Pseudomonas radicicola*; this "fixes" the atmospheric nitrogen. The organisms penetrate the young roots through the root-hairs, multiply and form a filamentous zooglœa, which grows into the tissue of the root and penetrates the cells. Large amounts of nitrogen are taken up by the bacteroids, and are converted into nitrogenous compounds which can be assimilated by the plant. Leguminous plants grown from sterile seeds in a sterile soil dwindle and die, but if inoculated with the organisms derived from another plant of the same species growth becomes vigorous; if inoculated with those derived from another species growth still takes place, but not nearly to the same extent. The Leguminosæ thus store up one of the most important elements of plant food, and hence their value in the rotation of crops. There is apparently no increase of nitrogen compounds in the soil, the excess found being due to the root residues remaining. A substance termed "nitragin," consisting of a culture of these root organisms, has been prepared as a fertiliser. Nobbe's "nitragin" did not prove a success, apparently because the organisms soon lose their vitality. A better preparation, "nitro-bacterine," was devised by Moore of the United States Department of Agriculture. Besides the leguminous organisms, other bacteria are present in the surface layers of the soil which fix

[1] Such as wood-ashes maltose agar. Boil 8 grm. of wood-ashes with 500 c.c. of water for one minute; filter. To 400 c.c. of this extract add 4 grm. maltose and 4 grm. agar. Boil until dissolved; filter, tube, and sterilise.

atmospheric nitrogen. The principal of these are ovoid organisms known as *Azotobacter*. This group can be cultivated in a mannite medium, *e.g.* di-potassium phosphate 0·2 grm., mannite 20 grm., water 1 litre. This may be used for isolation by converting into an agar medium by the addition of 2 per cent. agar. Prof. Bottomley has succeeded in obtaining a powder preparation of *Azotobacter*, which retains its vitality for months, and the preparation properly applied to *poor* soils produces astonishing results.

It has been found that partial sterilisation of the soil, *e.g.* by heat, *increases* its fertility, whereas it might have been supposed that such a procedure would *decrease* the fertility by destruction of nitrogen-fixers. Russell and Hutchinson believe that the explanation is that in ordinary soil amœbæ and other protozoa devour and keep down the bacteria; by the sterilisation the protozoa are destroyed and the more resistant bacteria are then free to develop.

Besides nitrifying bacteria many de-nitrifying or-organisms occur in the soil. They may (1) reduce nitrates to nitrites; (2) remove oxygen from nitrates and nitrites and form ammonia; (3) form nitrous and nitric oxides or nitrogen from nitrates and nitrites.

Fermentation.—Another important group of changes produced by micro-organisms is that comprised under the comprehensive title of "fermentation," of which it is difficult to give an accurate definition, for the distinction between it and other chemical changes due to the activity of micro-organisms is conventional rather than scientific. The original conception of the term involved the occurrence of frothing of the fermenting liquid, owing to the escape of gaseous products. Fermentation is brought about by the action of ferments, two classes of which are recognised, viz. the living or

organised ferments, which, in other words, are micro-organisms; and the unorganised or chemical ferments, bodies such as pepsin, which in minute amount produce changes in a considerable quantity of the substance acted upon, without themselves undergoing alteration.

It is better to reserve the term "fermentation" for the changes brought about by the organised ferments or living organisms, and to call the unorganised ferments enzymes, and the changes which they produce zymolysis. As fermentations are investigated more critically, the tendency is to find that they are brought about by enzymes, extra-cellular or intra-cellular, so that in course of time this distinction may no longer hold good.

The following are the chief varieties of fermentation:

The alcoholic fermentation.—This is mainly brought about by the decomposition of sugars of the hexose group ($C_6H_{12}O_6$), principally dextrose and lævulose, by yeasts into alcohol and carbonic acid, but some of the bacteria and moulds also produce appreciable quantities of alcohol. Other carbohydrates by the action of enzymes secreted by the organisms may be converted into hexoses, which are then fermented. The general reaction is as follows:

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2.$$

As a matter of fact small amounts of by-products appear in addition to the alcohol and carbonic acid, viz. glycerin, succinic acid, and higher alcohols. Until 1897 no enzyme had been obtained which would carry out this change; it only occurred when the living yeast-cells were present, but in that year Buchner, by grinding up the living yeast-cells, obtained a juice which decomposed dextrose with the formation of alcohol and

carbonic acid. This "zymase" Buchner claimed to be the alcoholic enzyme of yeast.

The lactic acid fermentation.—This is brought about chiefly by bacteria. Hexoses are converted into lactic acid, the reaction being—

$$C_6H_{12}O_6 = 2(HC_3H_5O_3),$$

but it is probably not actually so simple as this, for carbonic acid is given off at the same time. A familiar example of this form of fermentation is the souring of milk, in which the lactose is acted upon as follows:

$$C_{12}H_{22}O_{11} + H_2O = 4C_3H_6O_3.$$

The butyric acid fermentation.—Butyric acid is formed from carbohydrates by the action of bacteria, mainly the *Bacillus butyricus* and *Clostridium butyricum*, the latter an anaërobic organism, some by-products being formed in addition. Milk which has been just boiled usually undergoes the butyric rather than the lactic fermentation, the spores of the butyric organisms surviving. Lactic acid is first formed, and this is then converted into butyric acid:

$$2C_3H_6O_3 = C_4H_8O_2 + 2CO_2 + 2H_2.$$

The acetic acid fermentation.—The conversion of alcohol into acetic acid is also due to bacteria, familiar examples of which are the souring of beer and wine.

Bacterial enzymes.—Many changes brought about by bacteria and other micro-organisms are due to enzymes, which may be not only intra-cellular but may escape from the cells into the medium in which they are. The most familiar example is the peptonising enzyme produced by bacteria which liquefy gelatin and digest coagulated protein, fibrin, etc. The enzymes differ: an organism

which liquefies gelatin does not necessarily digest blood-serum. The proteolytic enzyme is tryptic in nature and escapes from the cells into the surrounding medium, so that some of the liquefied gelatin free from cells or in which their action is inhibited by an antiseptic, liquefies other gelatin if added to it. Amylolytic enzymes are also produced, such as amylase (digesting starch), maltase, lactase, inulase, and invertase. Lipases and rennet-like enzymes also occur. "Fermentation" of urea takes place by means of an enzyme secreted by the *Micrococcus ureæ*, etc., with the formation of ammonium carbonate. These enzymes do not seem to possess any poisonous action.

Formation of pigment.—Numerous organisms, especially those of air and water, during their growth produce various coloured pigments. They are termed "chromogenic bacteria," examples of which are the *Sarcina lutea* and *Micrococcus cereus*, var. *flavus*, which form citron-yellow pigments; the *Bacillus prodigiosus* and *Spirillum rubrum*, red pigments; the *Bacillus violaceus* forms a rich violet one; and the *Bacillus pyocyaneus*, a blue. A large number of chromogenic organisms require oxygen for the production of the pigment, and potato is often the most favourable culture medium. In some cases the medium may become coloured, and the property of fluorescence be conferred upon it, as is the case with the *Bacillus fluorescens liquefaciens*. Usually the pigment is extra-cellular, occasionally, as in *B. violaceus*, it is intra-cellular.

Phosphorescence, or light-production, is developed by some bacteria, notably by many marine forms, and is well seen in decomposing fish. Some spirilla are also known occasionally to produce phosphorescence.

A necrotic action on the tissues is produced by many pathogenic organisms. For example, the tubercle and

glanders bacilli cause necrosis and caseation of the surrounding tissues.

Gas production.—This is common to many organisms. The gas may consist of carbonic acid, hydrogen, or marsh gas, and in some cases of foul-smelling sulphur compounds, sulphuretted hydrogen, mercaptans, etc.

Sulphuretted hydrogen may be detected by the blackening of lead acetate paper. Methyl mercaptan may be detected by aspirating a current of air through the culture, through a calcium chloride drying-tube, and then through a test-tube or small flask containing isatin dissolved in concentrated sulphuric acid. The red colour of the isatin solution is changed to olive- or grass-green by the mercaptan.

Toxic bacterial products.—Almost without exception the pathogenic action of bacteria is brought about by means of the chemical substances produced in one way or another by their metabolic processes (see also p. 135), The toxic bacterial products may be classified as follows:

(1) *Decomposition products.*—These are substances produced by the decomposition of the medium upon which the bacteria are growing. Thus proteoses appear to be formed by the anthrax bacillus and the pyogenic cocci.

The *ptomines* form another group of these substances. These are a very important group of nitrogenous bodies, analogous to the vegetable alkaloids and mostly solid and crystalline in nature, which are formed by the action of bacteria on protein and albuminoid matter. They often occur naturally in decomposing and putrefying food, meat, fish, etc., and as many of them are virulent poisons they are of considerable practical import. Generally speaking, the poisoning due to tainted food is due to the absorption of toxic ptomines formed by bacterial action. A number of

toxic ptomines were isolated by Brieger from cultivations of pathogenic microbes, and great importance was once attached to them. They are referred to in the chapters describing the pathogenic organisms.

Brieger's work, however, needs revision, for his methods were not such as to exclude alteration by the reagents employed.

Stevenson obtained traces of a highly poisonous crystalline ptomine from some sardines that had caused death. Vaughan has isolated a body, tyrotoxicon, apparently identical with diazobenzene, from poisonous cheese and milk. Mytilotoxin ($C_6H_{15}NO_2$) is the specific poison of toxic mussels. Neurin and muscarin are extremely poisonous and may occur in decomposing flesh. Some of the ptomines produced by putrefaction are very similar to certain vegetable alkaloids and are thus of considerable medico-legal importance. The ptomines are not specific like the true toxins, and toxic ones may be produced by non-pathogenic bacteria.

(2) *Toxins.*—These are the soluble poisons elaborated by the bacteria and excreted by the cells into the surrounding medium. They are regarded by Martin and others as being allied to the proteoses. Roux and Yersin suggested that the diphtheria poison might be an enzyme, while Brieger and Fränkel regard it as albuminous. The toxins are non-basic substances closely related to the proteins and hence have been named tox-albumins, and are considered to be the specific toxic poisons of the pathogenic bacteria. It is difficult or impossible to prepare them in a state of purity and their chemical constitution is therefore unknown, and they are characterised by extreme specificity. Such are the poisons of the diphtheria and tetanus bacilli.

(3) *Endotoxins.*—These are toxic substances elabo-

rated by the bacteria which do not to any extent escape from the cells. They are as specific as the toxins and possess analogous properties (see below).

(4) *Bacterial proteins.*—These are toxic constituents of the bacterial cells which do not diffuse from the cells, are not specific, and which probably usually play little part in the production of the disease symptoms.

Literature.

On Nitrification, see Warington, *Journ. Chem. Soc.*, 1886, *et seq.*; Frankland, *Cantor Lectures*, 1892; *Nature*, 1890, *et seq.*; Löhnis, *Handbuch der landwirtschaftlichen Bakteriologie* (Borntraeger, Berlin, 1910, full bibliography). *On Bacterial Products*, see *Cellular Toxins, by* Vaughan and Novy, 1902 (*Bibliog.*), *Ueber Ptomaïne, by* Brieger, 1885; Macfadyen, *The Cell as the Unit of Life* (Churchill, 1908); Wells, *Chemical Pathology*, 1907. For General Bibliography, see Kolle and Wassermann, *Pathogenen Mikroorganismen.*

Endotoxins.

The majority of pathogenic micro-organisms do not excrete any appreciable amount of toxin; the toxin remains within the cells. To such an intra-cellular toxin the name of "endotoxin" has been given. The toxins of the staphylococci and streptococci, the typhoid-colon group, plague, cholera, etc., are endotoxins. Various methods have been employed to prepare these endotoxins, such as extraction of the cells by the action of weak alkalies and enzymes, and by autolysis or self-digestion.

The late Dr. Allan Macfadyen conceived that if the intracellular toxins (endotoxins) of such organisms as the typhoid bacillus, cholera vibrio, etc., could be obtained free from the bacterial cells, it might be possible to prepare sera (anti-endotoxic sera) of much more therapeutic potency than the ordinary anti-microbic sera.

The disintegration of the bacterial cells in the presence of intense cold, to prevent chemical change in the bacterial juice

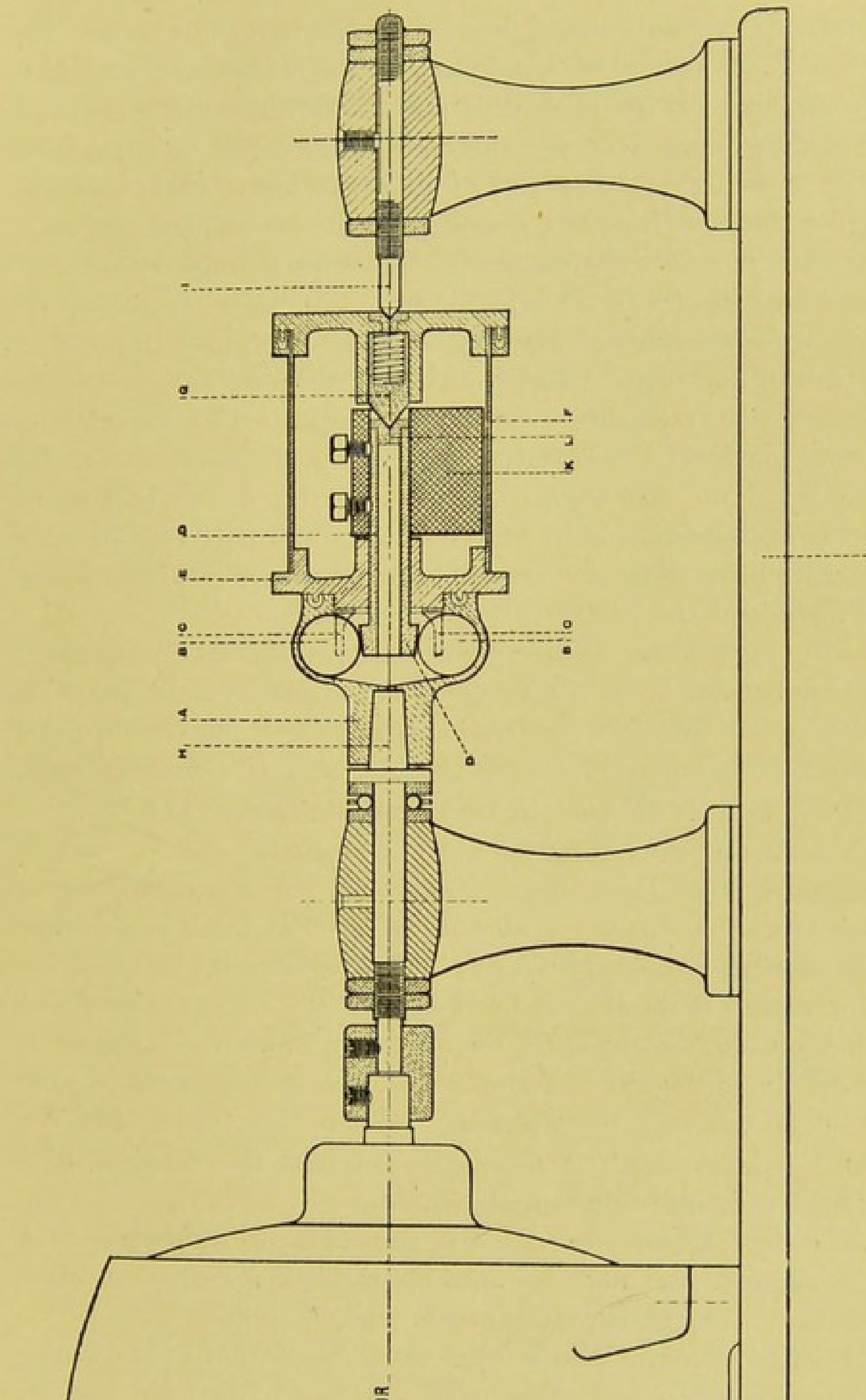


FIG. 1.—Barnard machine for disintegrating bacterial and other cells.

obtained, was the method devised by Macfadyen to attain this end. With the aid of his colleagues, Mr. Rowland and Mr. Barnard, and of his laboratory assistants, Messrs. Burgess and Thompson, apparatus and methods were evolved to effect this.

By growing on the surface of agar or other suitable medium in plate bottles (Fig. 15), scraping off the growth and suspending this in salt solution, centrifugalising at high speed, and collecting the bacterial cell-mass on the walls of the centrifuge vessels, sufficient material is readily obtained to grind or triturate, and thus disintegrate the bacterial cells so as to liberate their contents. This is accomplished by means of a special machine, the essential part of which consists of a metal cone revolving at a high speed in a metal pot, the bottom of which is shaped so as to fit the cone. The pot, with its contents, is immersed in a vessel of liquid air or other freezing mixture, and the bacterial mass is ground.

After grinding, the ground material is made up with distilled water or with 0·1 per cent. sodium hydrate, so as to form a 10 per cent. solution (calculated on the original weight of the moist bacterial paste); this is centrifugalised, and the fluid is filtered through a sterile Berkefeld filter.

The filtrate thus obtained is the endotoxin, and is used to immunise horses and other animals in the same manner as with any other toxin; it should be used as fresh as possible. The amounts of a typhoid or cholera endotoxin employed for immunising must at first be small, 0·2–0·5 c.c., as it produces considerable disturbance on injection, and the amount is gradually increased. After some weeks' treatment a dose of 20–30 c.c. may be injected. When tests show that the serum has attained the necessary potency, the horse is bled and the serum obtained and bottled.

The endotoxins also possess immunising properties to a high degree, and may be used as prophylactic or as curative vaccines; they markedly raise the opsonic index.

Another machine has been devised by Barnard for disintegrating bacterial and other cells. It is supplied by Messrs. Baker, of High Holborn, and is depicted in Fig. 1, p. 41.

The containing vessel consists of a phosphor-bronze body. A, in which five hardened steel balls, B, are placed. The shape of the containing vessel is such that when these balls are at its periphery they accurately fit the inner side of the vessel. The balls are evenly distributed round the vessel by means of a cage, C, and during the time they are running this cage ensures that they are equi-distant and do not collide one with another. At the centre of the metal vessel is a steel cone, D, which is of such a size that it keeps the balls in their proper position in close contact with the periphery of the containing vessel. The vessel is closed by a screw cap, E, through which the steel cone passes, and in which it is free to rotate. Over the whole of this a metal cylinder, F, is placed, and is screwed down, completely sealing the upper opening in the metal vessel. In the top of this metal cylinder a steel bearing, G, is placed, which has freedom of movement in a horizontal direction, but is kept down on the top of the steel cone by the action of a spring. It therefore follows that when this metal cylinder is screwed down the steel cone is pressed on to the balls, and the balls are in their turn forced out to the periphery of the metal pot. The whole appliance is mounted on a cone, H, and a centre, I, which are carried by two uprights attached to the base plate, J; one end of the shaft is attached to the electric motor.

The grinding action is brought about by retarding the revolution of the central cone, D. This has been effected by mounting on the spindle of the central steel cone, D, a semi-cylindrical mass of iron or lead, K, the weight of which must be such that when the whole apparatus is rotated it is sufficient to hold the central cone still.

By retarding the cone in this way a drag is placed on the balls, they slide to a certain extent over the inner surface of the pot and exert a grinding action.

See Hewlett's *Serum Therapy*, 1910; Hewlett, *Proc. Roy. Soc.*, B., 1909 and 1911; *Proc. Roy. Soc. Med.*, vol. iii, 1909–10 (Pathological Section), p. 165; Barnard and Hewlett, *Proc. Roy. Soc.*, B., 1911.

CHAPTER II.

METHODS OF CULTIVATING AND ISOLATING ORGANISMS.

It is necessary for the satisfactory study of micro-organisms in their relation to the various processes of infection and disease, of fermentation, putrefaction, and the like, to separate and isolate the different species occurring in a mixture, and, having done so, to cultivate, grow, or propagate each species on suitable soils through successive generations. A slight consideration will show that unless we work with pure cultures—that is, cultures consisting of a single species—we can never be sure that a particular result is due to a given organism; in a mixture several or all of the forms present may conduce to the effect produced. With regard to the pathogenic organisms, or disease germs, Koch laid down certain conditions which have been termed "Koch's Postulates" (p. 154), which must be complied with before the relation of an organism to a disease process can be said to be completely demonstrated, one of which is that "the organism must be isolated and cultivated outside the animal body on suitable media for successive generations."

In order to isolate organisms in a state of purity it is absolutely necessary to employ vessels, instruments, and culture media which are sterile, that is, free from any living organisms, and to possess the means of

manipulating them in such a way that the entrance of organisms from without is prevented and contamination avoided. Various methods of destroying and of getting rid of organisms are known, such as the use of chemical "germicides," heat, and filtration through porous porcelain. The addition of chemical germicides, such as carbolic acid or corrosive sublimate, is out of

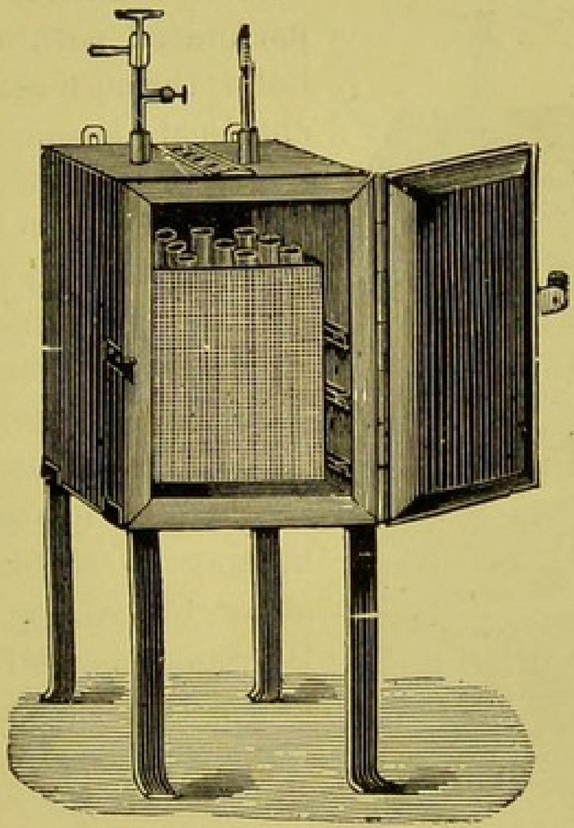


FIG. 2.—Hot-air steriliser.

the question; for although the vessels and media might be rendered sterile thereby, the growth of the organisms which are being investigated would equally be prevented, so that the two last, viz. heat and filtration, are those which are employed, the former being used for vessels, instruments, and culture media, solid and fluid, the latter for fluid culture media only.

Various apparatus are needed for sterilisation and

the preparation of culture media. These will now be described.

Hot-air steriliser (Fig. 2).—This is a rectangular box of sheet iron with double walls, having an air-space of nearly an inch between them, and furnished with a door. The bottom should be protected with a loose piece of sheet iron, which can be renewed as it "burns" away. The top is perforated with a couple of holes, through one of which a chemical thermometer, registering to 200° C., is inserted in a cork, while through the other some form of mercurial regulator can be introduced if required, but is not usually needed. In the hot-air steriliser all thin-glass vessels and cotton-wool are sterilised by heating to a temperature of about 150° C. by means of a Bunsen or a small ring burner under the steriliser, which is supported on a suitable iron stand. If the steriliser is placed on a table or other wooden support, a piece of sheet iron, asbestos cardboard or uralite should be laid over the wood to protect it from the heat. An inexpensive substitute for the hot-air steriliser may readily be devised, any iron box or even a biscuit-tin being used for the purpose.

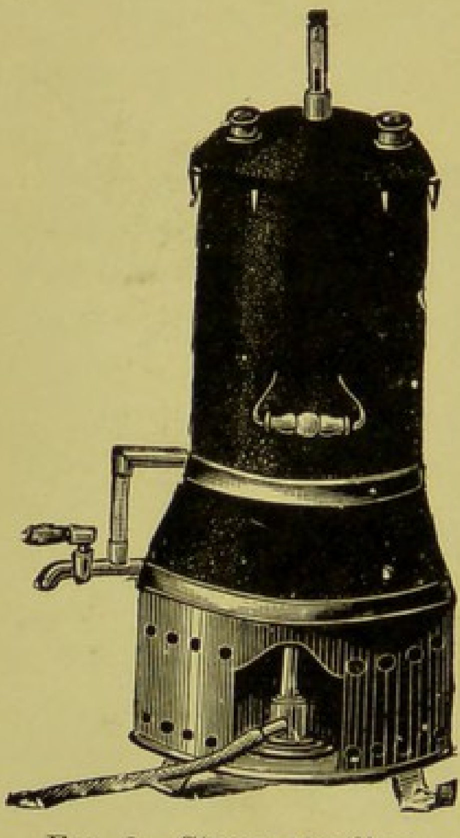


FIG. 3.—Steam steriliser.

Steam steriliser (Fig. 3) —This consists of a cylindrical or rectangular vessel of tinplate, galvanised iron, or copper, covered on the outside with a layer of felt

or asbestos, having a false perforated bottom supported a few inches above the true bottom, and provided with a movable lid. In the steam steriliser or "steamer" the culture media, and thick glass vessels and other apparatus which would crack or be damaged by the high temperature of the hot-air steriliser, are sterilised by steam. The lower chamber of the steamer, below the false bottom, is partly filled with water, which is boiled by means of a Bunsen or ring burner. Above the false bottom the culture media or apparatus are placed, and are sterilised by the steam at 100° C. which fills this space.

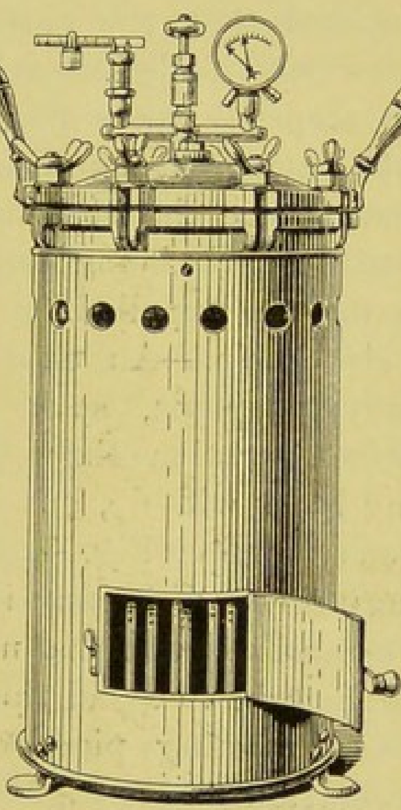


Fig. 4.—Autoclave.

Here again an inexpensive substitute may be devised; the ordinary kitchen saucepan with steamer will do well for many purposes, while a "warren pot" answers admirably.

Autoclave (Fig. 4).—This is most useful for many purposes, but it is expensive and not a necessity, as the steam steriliser can be made to answer almost every purpose for which the autoclave is employed with the expenditure of a little more time and trouble. It consists of a strong boiler of brass or gun-metal with a removable lid, which is attached to the boiler by means of screw-bolts. The lid is provided with a safety valve, a gauge for indicating the pressure and temperature, and a stopcock to relieve the pressure if required. A small quantity of water is placed in the

bottom, and the media or apparatus to be sterilised having been introduced, the lid is screwed down. It is heated by means of one or more Bunsen burners, which are turned down when the required temperature has been reached. The temperature usually employed is about 115° to 125° C. When sterlising media care should be taken that the vessels are not filled too full, and that the autoclave is allowed to cool down below 100° C. before relieving the pressure by opening the stopcock, or a good deal of the contents may be lost by violent ebullition. Also, while raising the temperature the stopcock should always be left open until steam is being freely generated in order that the air may be expelled.

Air-pump.—An exhaust pump is very useful for many purposes, such as evaporating to dryness *in vacuo*, filtration through porous porcelain filters, etc. Any form will do, but of the more elaborate ones the Fleuss pump (Fig. 5, p. 50) made by the Pulsometer Engineering Company is one of the best. In using it care must be taken that no fluid or moisture gains access to the barrel ; to avoid this it is a good plan to intercept the connecting pipe with a vessel containing strong sulphuric acid (D, Fig. 5), over the *surface* of which the exhausted air has to pass. A double-necked Woulfe's bottle does well for this, the inlet and outlet tubes extending nearly down to, but not dipping below, the surface of the sulphuric acid.

For greasing the vessels, etc., to make air-tight joints, beeswax dissolved in the Fleuss pump oil with the aid of heat to a stiff paste is a good composition, or the resin ointment of the Pharmacopœia may be used.

Bell-jars with ground rims and one or two tubules are useful for evaporation *in vacuo*. They should

stand on a square of thick ground glass. To make an air-tight joint the surface of the rim of the bell-jar, which must be quite clean, should be well greased and pressed thoroughly home on the ground-glass plate. A thick ridge of grease should then be plastered all round the angle formed by the rim of the bell-jar and the glass plate. Thick rubber pressure tubing must be used for connections, and all joints should be well greased. For evaporating large quantities of fluid the writer devised a copper stand with shelves, the shelves supporting glass dishes containing alternately strong sulphuric acid and the fluid to be evaporated, the whole being placed under a suitable bell-jar. A mercurial gauge is a useful addition to show the amount of exhaust and the occurrence of leakage. The ordinary glass filter pumps used in chemical work and actuated by a stream of water are also useful for many purposes.

Porous porcelain filters.—The forms which are generally employed are the Pasteur-Chamberland, the Doulton, and the Berkefeld. These consist of "candles" composed in the first two of unglazed porous porcelain, in the last of a specially prepared diatomaceous earth. The filtration through the Pasteur-Chamberland is much slower than through the Berkefeld. All give a germ-free filtrate, but the last should be employed if the fluid is thick or contains many particles ; a preliminary filtration through paper is an advantage. A useful method of conducting filtration is the following : The filter "candle" (B, Fig. 5, p. 50) is connected by a short length of pressure tubing with a piece of glass tubing passing through a rubber cork in the neck of an ordinary filtering flask C. The "candle" is placed in a jar A, such as a glass measure or urine-jar, which is filled up with the solution to be filtered. The lateral

branch of the filter flask is then connected with the air-pump. On exhausting, the fluid passes through the filter "candle" over into the filtering flask, in which it is collected. Before use the "candle" should be well scrubbed and some water or ½ per cent. carbolic run through to clean it, and the whole may be sterilised in the steamer for an hour or two. After use the same process should be repeated to cleanse it.

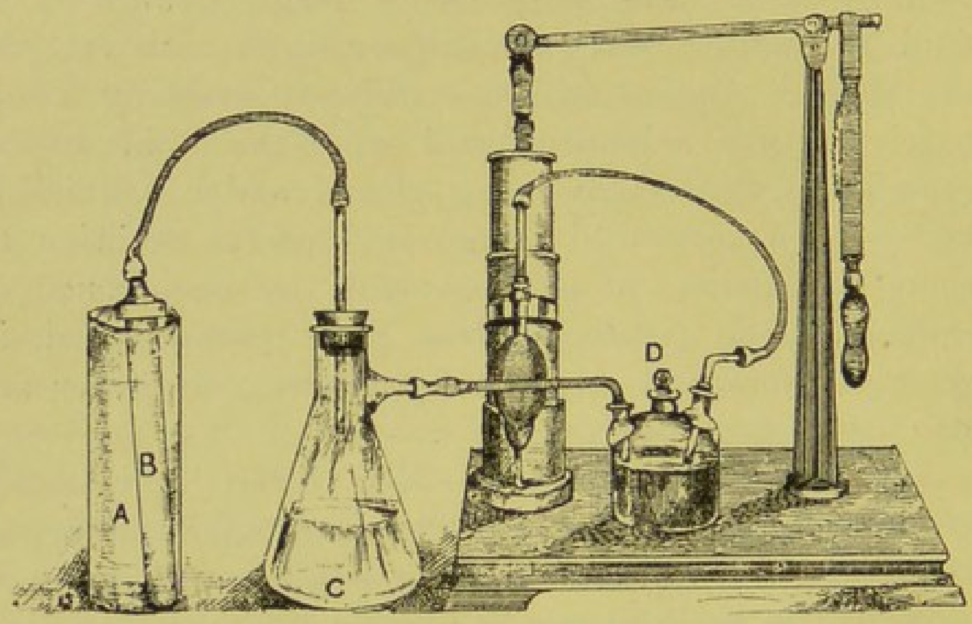


FIG. 5.—Fleuss exhaust pump, arranged for filtration.

Flasks, beakers, and test-tubes.—A good supply of these is required of various sizes: Erlenmeyer and ordinary shapes, tall and short forms of beakers, etc. A few "yeast flasks" are also useful (see Fig. 13, p. 76). Beakers and flasks of "Jena" glass are to be preferred. Enamelled iron ware, jugs, saucepans, mugs, etc., may replace glass for many purposes.

The best size of test-tube is $5'' \times \frac{5}{8}''$; a few larger sizes and "boiling tubes" should also be kept.

Platinum needles (Fig. 6).—Two or three platinum needles are required. They consist of about two

inches of platinum wire in a handle of glass rod. One end of a glass rod is softened in the Bunsen or blow-pipe flame, and about an eighth of an inch of the platinum wire is embedded in it with a forceps, the wire having been first heated to a red heat. The

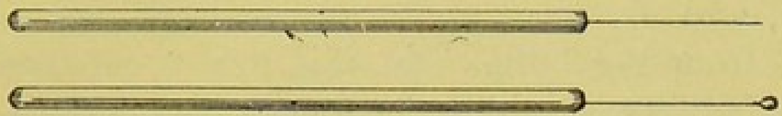


FIG. 6.—Platinum needles.

glass-wire joint is then well annealed in the flame and allowed to cool slowly. Metal handles may also be used. Two thicknesses of platinum wire are desirable, viz. 0·4 mm. (27–28 B.W.G.) for most purposes, but a thicker wire of about 0·7 mm. where stiffness is required, and one or two 3 in. or more in length are useful.

Forceps, needles, etc.—Several forceps are necessary, the ordinary dissecting form in two or three sizes, one or two pairs of fine pointed, two or three small brass ones, and two or three pairs of the "Cornet" pattern. A few ordinary sewing needles of various sizes mounted in wooden handles serve all purposes.

FIG. 7.—Glass pipette.

Glass pipettes and capillary tubes.—These are useful for preserving or storing blood or pus, etc., for examination, for sterile water in making film specimens, and for many other purposes. A piece of glass tubing is heated in the blowpipe flame until quite soft; it is then *taken out of the flame* and the two ends

pulled steadily apart; this forms a capillary tube of greater or lesser length and smaller or larger diameter, and it can be sealed off in convenient lengths. To make a pipette proceed in the same way: seal off the capillary tube two or three inches from the wide tube, then heat this close up to where it was heated before, and draw out again and seal off two or three inches from the bulb. In this way a capillary tube with a bulb at its middle is formed (Fig. 7). "Vaccine tubes," pipettes made of glass tubing drawn out at one end, and Wright's capsules (see Fig. 35, *a* and *d*, p. 225) are also useful.

India-rubber caps.—A few indiarubber caps for capping test-tube or flask cultures are required. They retard evaporation and the desiccation of the medium, and prevent the entrance of moulds. For use they should be soaked in 1–500 corrosive sublimate solution; they should not be *kept* in the solution, as vulcanised rubber absorbs mercuric chloride (Glenny and Walpole). Tinfoil or gutta-percha tissue (sealed down by warming) may also be used to cover the tops of tubes and flasks.

Preparation of Sterile Test-tubes, Flasks, etc., for the Reception or Manipulation of Culture Media.

To sterilise cotton-wool.—Non-absorbent cotton-wool best or No. 2 quality, should be used for plugging purposes. The wool should be pulled apart so as to assist the penetration of heat; in the compressed condition the interior is difficult to sterilise. The separated wool is placed in the hot-air steriliser and the temperature is slowly raised to 145° C. and maintained at this for at least an hour. Above 150° C. cotton-wool becomes brown and brittle. It is a common practice now to use

various coloured wools for the different culture media, especially the carbohydrate ones, so that they are readily distinguishable by the eye. The coloured wools may be purchased, or the ordinary white wool may be dyed with the "Dolly" dyes.

Glass vessels.—The vessels (usually test-tubes, flasks, and dishes) are thoroughly washed and rinsed in water, then rinsed with 25 per cent. hydrochloric acid, and afterwards washed well with tap-water and drained. A final rinse with distilled water or alcohol is an advantage, as no deposit then occurs on drying. The cleansed vessels should be dried before sterilising, either in the air or by placing in the hot-air steriliser for half an hour. When dry, the vessels are plugged with a firm plug of the sterilised cotton-wool, and are placed in the hot-air steriliser, the temperature of which is then raised to about 150° C. They should remain at this temperature for not less than half an hour, after which the steriliser and its contents are allowed to cool slowly.

Petri dishes for plate cultures, graduated pipettes, etc., are cleaned as described for tubes and flasks. They may be sterilised and kept in sheet-iron or copper boxes of appropriate size and shape.

If tubes, flasks, pipettes, etc., are required in a hurry they may be rapidly sterilised as follows: After washing in water they are rinsed with 5 per cent. carbolic, then with absolute alcohol, and finally with ether, and are then well flamed over a Bunsen flame, holding in a suitable forceps or holder. The ether evaporates and burns at the mouth, and when dry, a pledget of cotton-wool is held in the forceps and singed in the flame, and, while burning, the tube or flask is plugged with it.

When thick glass vessels, such as measures, etc., have to be sterilised, it is not safe to do this in the hot-

air steriliser unless the heating and cooling are carried out very slowly, as they are very liable to crack. It is preferable, after cleaning and plugging with sterile wool, to steam in the steam steriliser for three to five hours, the heating and cooling being conducted slowly.

Culture Media.

The ordinary methods of preparing culture media are here given, but "Standard" media, having definite reactions, are now largely employed (for the method of standardisation, see p. 64). Certain special media will be described as required. In all cases the media are filled into the cleansed and sterilised vessels, test-tubes, flasks, etc. (p. 53). For ordinary laboratory cultures test-tubes are generally used. Media which are solid at ordinary temperatures, *e. g.* agar, gelatin, and serum, are prepared either as deep, upright tubes (fig. 8, A), for which 8–15 c.c. of the medium are required for a tube, or as sloping tubes (Fig. 8, C), for which 4–5 c.c. are required for a tube. Of fluid media 7–15 c.c. are used for a tube. The prepared media having been introduced into the test-tubes, etc., sterilisation is effected in the steam steriliser (p. 46) by steaming for twenty to thirty minutes on two or three successive days, or in the autoclave (p. 47) by heating to 115°–120° C. for half an hour on one occasion. Culture media may also be kept in bulk in flasks; these need somewhat longer sterilisation than tubes. Tubes of some of the culture media can also be purchased ready for use. Certain media can be obtained in powder form (Chopping's) from Messrs. Baird & Tatlock, and in tabloid form (Thompson's) from Messrs. Burroughs & Wellcome. These are convenient when small quantities are required for occasional use.

Acid beef-broth.—The basis of the most important culture media, viz. peptone beef-broth, gelatin, and agar-agar, is an infusion of meat prepared usually from beef. In order to prepare this infusion, which may be termed acid beef-broth, proceed as follows: Take 1 lb. of beef ("gravy beef") free from fat, chop fine or mince, add one litre of tap-water, and allow it to simmer in a saucepan for one hour; cool, remove any solidified fat from the surface, and filter through filter-paper into a clean glass flask. If not required for immediate use, plug the neck of the flask with cotton-wool, and steam in the steam steriliser (or boil) for three-quarters of an hour on two successive days. It may then be kept until required.

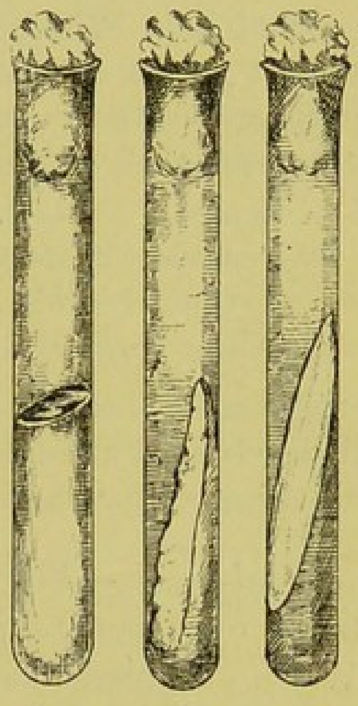


Fig. 8.—Tubes of culture media. A. Upright agar. B. Potato. C. Sloping agar.

Peptone beef-broth.—Take one litre of the acid beef-broth, add to this 10 grm. of peptone (Witte's) and 5 grm. of common salt (*i. e.* 1 per cent. peptone and 0·5 per cent. sodium chloride), mix in a flask, and steam in the steam steriliser until dissolved. When dissolved, remove from the steam steriliser and render slightly alkaline with a 10 per cent. solution of caustic soda (preferably) or of sodium carbonate, *glazed* litmus-paper being used as an indicator. Having done this, return to the steamer for one hour, then filter through two thicknesses of German filter-paper. It should now be quite clear and bright and may be kept in bulk, after sterilising, or be introduced into test-tubes, etc., and

sterilised. Beef-broth, if prepared in this manner, may need no clarifying, but if it should filter at all cloudy, cool to 50° C., add the white of an egg beaten up with the shell, and steam for half an hour, filter, and finally sterilise as before. Other preparations of peptone may be used.

Instead of meat infusion, meat extracts have been much used of late. The general opinion is, however, that meat-extract media are not such good nutrient soils for many purposes as those made from meat. The following is the composition of "Lemco" broth:

Lemco	10–20 grm.
Peptone (Witte)	10–20 grm.
Sodium chloride	5–10 grm.
Water (preferably distilled) . .	1 litre.

The constituents are dissolved with the aid of heat, neutralised, clarified and filtered. Lemco may also be used to make all the other media for which acid beef-broth is employed.

Veal-broth.—For some purposes veal presents advantages over beef, *e. g.* for growing the tubercle bacillus. When obtained from the butcher's the veal is frequently powdered with flour; this should be brushed and washed off as completely as possible, as it renders the broth turbid and difficult to clarify. The veal-broth is made in precisely the same way as peptone beef-broth. It is, however, often slightly alkaline, so that less alkali is required for neutralisation. For the cultivation of the tubercle bacillus about 4 to 6 per cent. of glycerin should be added.

Glycerin beef-broth is prepared in the same manner, 4 to 6 per cent. of the best glycerin being added to the fluid after filtration.

Glucose broth.—For the cultivation of anaërobic organisms the addition of 0·5 to 2 per cent. of grape

sugar is an advantage. It should be added after filtration.

Peptone water.—Add to distilled water 1 to 2 per cent. of Witte's peptone and ½ per cent. of common salt, dissolve by heat, make faintly alkaline, steam for one hour and filter.

For the cholera vibrio it is an advantage to add 1 per cent. instead of ½ per cent. of common salt (Dunham's solution).

Beer-wort.—Procure beer-wort (preferably unhopped) from the brewery. Allow it to stand in a cool place for twelve hours, filter, and then steam for an hour and filter again. Fill into sterile test-tubes and sterilise.

Nutrient gelatin.—Take 1 litre of the acid beef-broth in a large flask and add to it 100 grm. of the best "gold label" gelatin (Coignet's), 10 grm. of peptone, and 5 grm. of common salt. Place in the water-bath or steamer until quite dissolved. Then render faintly alkaline, as for the peptone beef-broth; cool to 50° C., and add the white of an egg, stir well, and return to the steamer for one hour. Filter through two thicknesses of filter-paper in a hot-water funnel (this is best, but it may be done in the steamer at a low temperature, *e.g.* 35° C.) Fill into test-tubes and sterilise. After the third steaming the tubes are allowed to solidify, either in the upright or oblique position, according as they are required for stab or surface cultivation.

In hot summer weather 15 or even 20 per cent. of gelatin (150 grm. or 200 grm. to the litre) are necessary for the product to remain solid, as nutrient gelatin melts at 24° C. or a little under. Prolonged boiling diminishes and ultimately destroys the gelatinising power of gelatin, so the less it is heated the better. It must not be autoclaved.

Glucose gelatin.—Ordinary gelatin with the addition of 1 to 2 per cent. of grape sugar.

Beer-wort gelatin.—This is one of the best culture media for yeasts and some of the fungi (*e. g.* ringworm). Procure from the brewery some beer-wort, preferably unhopped, and add to every litre 100 grm. of gelatin. Dissolve, clarify, and filter, as in the case of ordinary gelatin. It is not neutralised.

Nutrient agar-agar.—This is one of our most valuable culture media, and has the advantage over nutrient gelatin that it remains solid at blood-heat.

Agar is a carbohydrate substance of high melting-point and considerable gelatinising power, obtained from Eastern seaweeds. The powdered form is now generally used. Add 15 grm. (*i.e.* 1½ per cent.) of powdered agar to 1 litre of acid beef-broth, together with 10 grm. of peptone and 5 grm. of common salt in a large glass flask, place in the water-bath until dissolved (half an hour to one hour), and then render alkaline as for peptone beef-broth; allow it to cool to 50° C., and add the white of an egg. Return to the steamer for an hour and a half, then filter through an *agar filter-paper* ("papier Chardin") in a hot-water funnel or in the steamer. By this treatment a litre of agar should pass through the filter in two to three hours. If it does not come through clear, add another white of egg and repeat the process.

If an autoclave is available, a quicker and better method is, after neutralising and adding the white of an egg, to place in the autoclave with a small beaker inverted over the mouth of the flask, and heat to 134° C. (two atmospheres pressure) for half an hour. Turn the gas out, and allow to cool without opening the stopcock. When cool, open, and filter through the *special agar filter-paper* in a hot-water funnel; the agar will pass

through in about ten minutes or a quarter of an hour. Fill into test-tubes and sterilise. Solidify in the upright or oblique position as required.

In the case of bar or stick agar, first steep the agar in 1 per cent. acetic acid for a quarter of an hour, then drain and wash it so as to thoroughly remove the acid. The further procedure is the same as detailed above. This yields a very clear, pale product, and is perhaps preferable when an autoclave is not available.

Glycerin agar.—Add 4 to 6 per cent. of glycerin to the nutrient agar after filtration and proceed as before.

Glucose agar.—One or two per cent. of grape sugar is added to the nutrient agar after filtration.

Litmus media.—The addition of neutral litmus to the various culture media is a useful method of demonstrating the production of acid or of alkali by organisms. To prepare the litmus solution take the lump litmus, powder finely, and boil with distilled water so that a saturated solution is obtained. Filter, and preserve in a flask stoppered with cotton-wool, after sterilising by boiling for half an hour on two successive days. For some purposes a special solution of litmus, the Kubel-Tiemann solution, which can be procured ready for use, is employed. It must not have any antiseptic added to it (as is sometimes done to preserve it for use in the chemical laboratory).

Sufficient of this litmus infusion is added to the nutrient media, after filtration, to tinge them a distinct purplish colour. After steaming the colour has usually disappeared, but returns as the tubes cool.

Milk.—Use separated milk, but failing this, centrifugalise ordinary new milk, or place it in a tall cylinder and allow it to stand overnight in a cool place, preferably in an ice safe. Then pipette off the milk from

the bottom, rejecting the cream. Introduce the separated milk into test-tubes to the depth of about an inch to an inch and a half and steam for one hour on two successive days. The milk is usually tinged with litmus before tubing, forming *litmus milk*.

Potatoes.—Choose sound potatoes, and scrub them well with water to remove dirt. Cut off the ends, and with a cork-borer, slightly smaller than the test-tubes which are used, bore through the potato so that a cylindrical piece is removed. Push this out of the borer, and divide it into two portions by a very oblique transverse cut, so that two wedge-shaped pieces are obtained, and in this manner prepare as many pieces as there are tubes to be filled. Place them in a basin under the tap, and allow the water to flow over them for about two hours. This prevents the darkening of the potato in the subsequent steaming. The test-tubes for the potato-wedges are prepared as follows: After plugging and sterilising in the ordinary way, introduce a small pledget of sterilised wool into each, push to the bottom, and moisten with a little sterilised distilled water. Drop the potato-wedges into the tubes, plug, and sterilise by steaming for three-quarters of an hour on two successive days (Fig. 8, B). The object of the moist wool is to prevent drying, and for the same purpose Roux's tubes (Fig. 9) may be used, the lower bulb being filled with water.

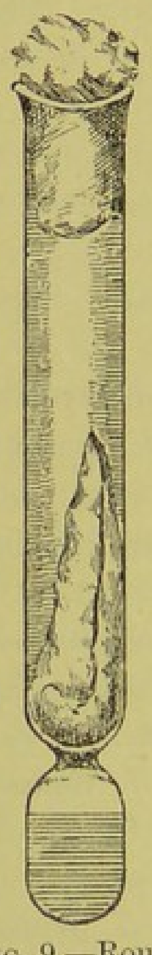


FIG. 9.—Roux's tube for potato.

Blood-serum.—Clean some glass jars of about 2 to 3 litres capacity, plug with wool, and sterilise in the steamer for an hour on three successive days. Bleed

a horse, with aseptic precautions, and catch the blood in these sterilised jars. Allow the jars to stand in a cool place for twelve hours. Then pipette off the clear serum with a sterile pipette, and fill the sterilised test-tubes to the depth of 2–4 cm. The tubes are then arranged in a sloping position on the shelves of the serum inspissator, or failing this in a hot-water oven, the temperature of which should be about 50° C. At this temperature they remain for thirty hours; it is then raised to 65° C., at which temperature the serum coagulates in from four to six hours and the tubes are now ready for use. It is well, however, to place them in the blood-heat incubator for a night, so that any contaminating bacteria may form colonies, and the contaminated tubes may then be rejected.

Loeffler's blood-serum is prepared by adding one part of glucose broth to three parts of the serum before inspissation.

The serum inspissator is practically an incubator (see p. 68) with slightly inclined (10–15°) shelves, on which the tubes rest, and thus the serum is coagulated in a sloping position.

Fluid serum, etc.—Fluid blood-serum, ascitic and hydrocele fluids, etc., are sometimes useful, and may be used alone or mixed with peptone beef-broth in various proportions.

Ascitic or hydrocele fluid may be obtained by using sterile trocars, etc., and carrying out the tapping with aseptic precautions, collecting the fluid in sterilised flasks. It is better to collect in several small flasks than in one large one.

Fluid blood-serum may be obtained by collecting blood with aseptic precautions in sterilised flasks. When the blood has coagulated and the serum separated, the serum is pipetted off with a sterile pipette into sterile flasks.

The flasks of serum, etc., should be kept in a warm place for two or three days to make sure that they are sterile, those in which a growth appears being rejected.

Serum, ascitic fluid, etc., may also be obtained sterile by filtering through a sterilised Berkefeld filter into sterile flasks.

Serum, ascitic and hydrocele fluids, etc., may be preserved in bulk and used as required. The material is collected as aseptically as possible, 5 per cent. of chloroform is added, and the whole is well mixed and kept in a cool place in the dark in a well-stoppered bottle. Subsequently, during the process of sterilisation, the chloroform is volatilised.

Serum agar (Kanthack and Stevens).—Ascitic, pleuritic, or hydrocele fluid is collected in clean (not necessarily sterilised) flasks, and allowed to stand overnight in a cool place to allow the sediment or blood to deposit. The clear fluid is then poured off, and to each litre enough of a 10 per cent. caustic potash solution is added to render it very distinctly alkaline—usually about 2 c.c. to every 100 c.c. of the fluid. The alkaline fluid is heated in the autoclave for two to four hours. To this fluid 1·5 to 2 per cent. of agar is added, and the mixture is heated until the agar dissolves. It is then filtered, introduced into test-tubes, sterilised, and solidified in the ordinary way. The addition of 5 per cent. of glycerin and 1 per cent. of glucose is an advantage.

Serum agar may also be prepared by adding sterile serum or hydrocele or ascitic fluid, warmed to 45° C., to sterile nutrient agar (2 to 3 per cent. agar) melted and cooled to 45° C. Equal parts of the serum and agar may be mixed, or 1 part of serum to 2 parts of agar.

Blood agar.—This may be prepared by smearing the surface of the agar in sloping agar-tubes with blood obtained aseptically from the finger or from a rabbit. Or blood obtained aseptically may be defibrinated by shaking with glass beads or with a coil of fine wire, and the defibrinated blood, warmed to 45° C., is added to sterile agar liquefied by boiling and cooled to 45° C. *Hæmoglobin agar* may be prepared by laking defibrinated blood by the addition of sterile distilled water and adding to the liquid agar as before. Blood agar cannot be sterilised after preparation, and the blood therefore must be sterile.

Alkali albumen (Lorrain-Smith).—To 100 c.c. of fresh serum add 1 to 1·5 c.c. of a 10 per cent. caustic soda solution; mix and introduce into test-tubes in the ordinary way. Place the test-tubes in the slanting position in the autoclave at 115° C. for twenty minutes, or in the steamer on three successive days.

Egg cultures (Hueppe).—These are very useful for some purposes. A hen's egg is taken and one end sterilised by washing with carbonate of soda solution, rinsing in sterile water, soaking in 1–500 corrosive sublimate solution, and washing in alcohol and in ether. A small hole is then chipped in the shell with a sterile needle and the inoculation made through this. The hole is afterwards closed with a little sterilised wool and collodion.

Uschinsky's Fluid.	Parts.
Sodium chloride . .	5–7
Calcium chloride . .	0·1
Magnesium sulphate .	0·2–0·4
Di-potassium phosphate	2–2·5
Ammonium lactate .	6–7
Sodium asparaginate .	3–4
Glycerin	30–40
Water	1000

Pasteur's Fluid.	Parts.
Cane sugar	10
Tartrate of ammonia .	1
The ash of 1 grm. of yeast	—
Water	100

Uschinsky's fluid is a solution of known composition without protein which can be used for investigating the chemical products of bacteria. Pathogenic organisms grow well in it and produce their toxins.

Pasteur's fluid is a good culture medium for yeasts, etc.[1]

Standard Nutrient Media.

Slight variations in the composition of the nutrient media have a marked influence upon the characters of the growths of micro-organisms developing upon them. In order to obtain more uniformity for descriptive purposes, etc., a committee of the American Public Health Association drew up a scheme for the preparation of nutrient media of approximately constant composition and reaction. Eyre[2] has devoted considerable attention to this subject, and the following descriptions are based largely upon his papers.

(1) *Preparation of acid beef-broth.*—1000 c.c. of distilled water are introduced into a large flask, 500 grm. of finely minced fresh lean beef added, and the mixture is heated in a water-bath at 40°–45° C. for twenty minutes with frequent agitation. It is then boiled for ten minutes, strained, and filtered through paper. To the filtrate sufficient distilled water is added to make up to 1000 c.c.

(2) *Standardisation.*—This may be most simply described in the case of acid broth. A 100 c.c. Erlenmeyer flask is rinsed out with boiling distilled water, 25 c.c. of the acid beef-broth are introduced into it, and 0·5 c.c. of phenolphthalein solution is added (0·5 per cent. phenolphthalein in 50 per cent. alcohol). This is kept boiling and decinormal caustic soda

[1] Several formulæ for synthesised media will be found in the *Journal of Experimental Medicine*, vol. iii, p. 666.

[2] *Brit. Med. Journ.*, 1900, vol. ii, p. 921; 1901, vol. ii, p. 788.

solution[1] is run in from 25 c.c. burette, divided into tenths, until a faint pink tinge appears in the boiling fluid. From the amount of soda solution used the amount of normal or deka-normal soda solution required to neutralise a given volume of the acid beef-broth (*e. g.* a litre) can be calculated, and this amount is then added. Although neutral to phenolphthalein, the medium is now strongly alkaline to litmus — too alkaline for the optimum growth of most organisms. The reason for this is that the di-sodium hydrogen phosphate (Na_2HPO_4) present in the medium is alkaline to litmus but neutral to phenolphthalein. To reduce the alkalinity (to litmus) normal hydrochloric acid is then added. The American Committee recommended an acidity of + 1·5—that is, to every 100 c.c. of the medium neutral to phenolphthalein 1·5 c.c. of the normal hydrochloric acid are added. Eyre advises a reaction of + 1·0 (*i. e.* 1 c.c. of normal hydrochloric to every 100 c.c.), while Chester considers that the acidity should not exceed + 0·5. Whatever the reaction adopted, it should be stated. Similarly, if a medium is used which is alkaline to phenolphthalein, this is expressed by the minus sign; *e. g.* a reaction of −1·5 indicates that to every 100 c.c. 1·5 c.c. of normal hydrochloric acid must be added to render it neutral to phenolphthalein, or, what is almost (but not quite) the same thing, that to the *neutral* medium 1·5 c.c. of normal caustic soda solution have been added to every 100 c.c. Various methods are adopted to obtain the

[1] By a "normal" solution is meant the equivalent weight in grammes of a substance dissolved in (*i. e.* made up to) a litre of water; a "deci-normal" solution contains one tenth of, a deka-normal ten times, this amount. A normal solution of caustic soda contains 40 grm. of pure NaOH (NaOH = 40), of sulphuric acid 49 grm. of pure H_2SO_4 $\left(\frac{H_2SO_4}{2} = 49\right)$, per litre.

final reaction; the American Committee recommend first neutralising and then adding sufficient acid (or alkali); Eyre, having calculated the acidity, adds only sufficient alkali to *reduce* the reaction to the required point. Eyre describes the reaction as that represented by the number of c.c.'s of normal alkali or acid per litre, *e. g.* + 10 on Eyre's scale is equivalent to the American + 1·0. In making nutrient broth, agar and gelatin, the salt and peptone and agar or gelatin are added and dissolved, and the titration and neutralisation are carried out as described, on the fluid medium itself, and after neutralisation the whole is heated over a water-bath for half an hour before filtration.

The Cultivation and Isolation of Micro-organisms.

It should be clearly understood that micro-organisms cannot usually be identified by their microscopical characters alone. We can state from a microscopical examination the form of an organism, that it is a bacillus or a micrococcus, or a sarcina, its size, that it is motile or non-motile, sporing or non-sporing, but we cannot as a rule go beyond this. It is necessary in most cases to ascertain the characters of the growths of organisms on the various culture media before species can be identified, and this is the principal reason for having a varied assortment of nutrient soils. It is likewise necessary for the successful cultivation of pathogenic organisms, *i. e.* those connected with disease processes and developing in or upon the bodies of man and of animals, to maintain the cultures at a temperature approximating to that of the host. For this purpose some form of incubator is required. This consists of a box or chamber of copper or iron with double walls (Fig. 10), the space between which is filled

with water, the outside being covered with wood or felt, or some other non-conductor. The water between the wall is heated by means of a small burner, the gas supply for which passes through some form of regulator inserted in the water, so that the temperature, indicated by a thermometer inserted through a hole in the top, can be kept constant. The regulator is usually a mercurial one, such as Page's or Reichert's, the principle of its action being that as the temperature rises the mercury expands and at a certain point cuts off the greater part of the gas supply, only sufficient gas then passing to keep the flame of the burner alight. This point can be varied either by a sliding tube, in Page's, or by a screw, in Reichert's, so that the temperature may be set at any desired point. In Hearson's incubator, which is one of the best forms, the regulator consists of a capsule containing a fluid of a certain boiling point, which when ebullition takes place raises a lever and so partially cuts off the gas supply. While the Hearson regulator is a very constant one, it has the disadvantage that it can only be used for a range of temperature of a few degrees unless the capsule be changed. At least one incubator is required, and it is convenient to have two or three. If there be only one the regulator should be set for a temperature of 37° C.; if more, another should be kept at about 20° C. The incubator at 37° C. is termed the warm or blood-heat, and that at 20° C. the cool or room temperature one. A warm room or cupboard will serve most of the purposes of the cool incubator. A third incubator set for 42° C. is useful for water examination, and a fourth at 25° C. for fermentation work.

A substitute for the large and expensive incubator can readily be devised. An ordinary chemical hot-

water oven may be employed, or simply a smaller tin set in a somewhat larger one, the interspace being filled with water; and, with a little scheming, regulators can be dispensed with by making use of a small gas or

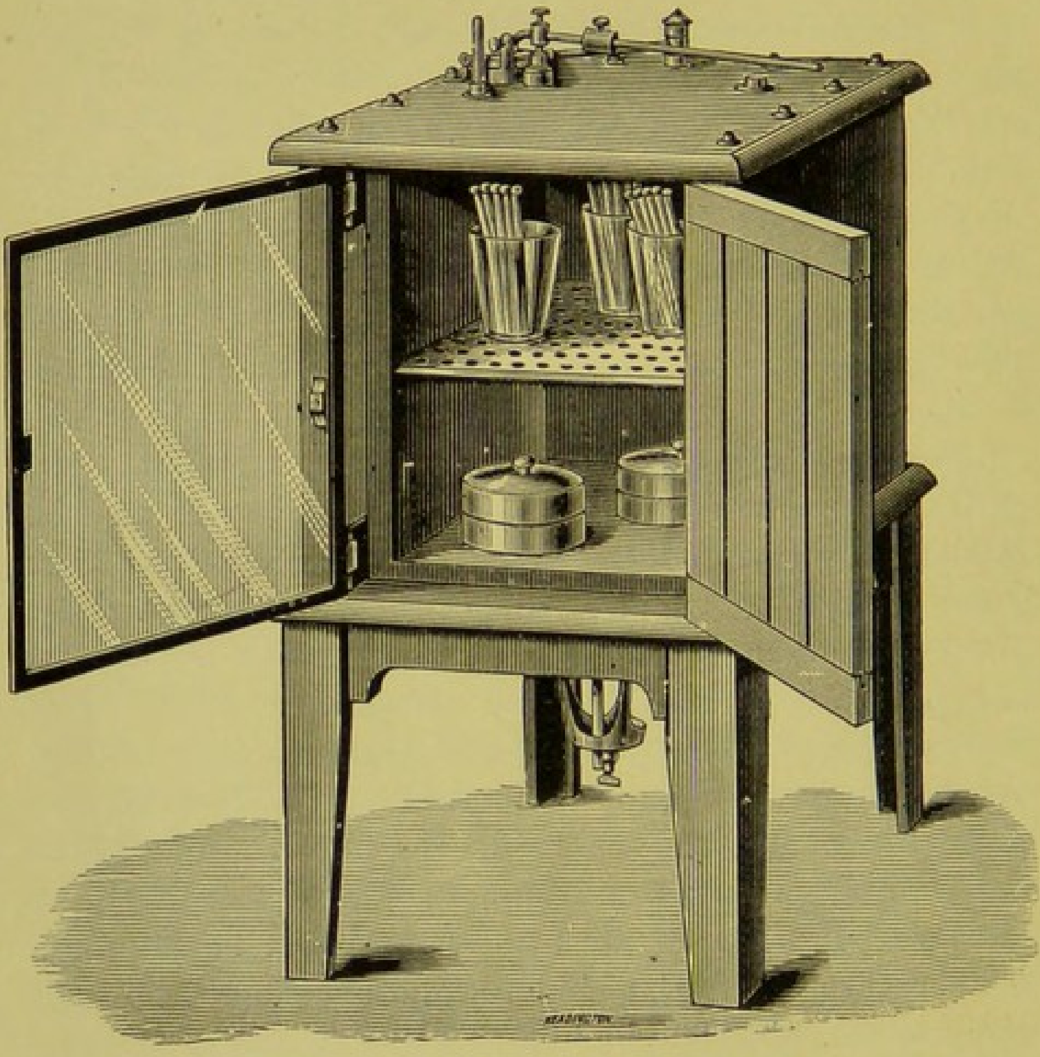


FIG. 10.—Hearson's incubator.

lamp flame, varying its size and distance from the bottom until the right temperature has been attained. Gas is a great convenience, but if not available, regulating oil lamps can be obtained to take its place. Electricity has also been adapted for heating incubators.

Gelatin will remain solid only at temperatures below 24° C., and cannot therefore be placed in the blood-heat incubator without becoming for practical purposes a fluid medium. Agar, however—and this is one of its most valuable properties—does not liquefy below a temperature of 97°–99° C., though when once liquefied it does not set again until the temperature has fallen to about 45° C. Gelatin is therefore usually reserved for use at low temperatures, while agar, blood-serum, potato, and the fluid media can be used indifferently either at low or at high temperatures. Agar is often a better cultivating medium than gelatin, even at low temperatures, probably because it is so much moister. The growths in fluid media are usually of the nature of a general turbidity and are not particularly characteristic, but sometimes an organism produces a film on the surface which another similar organism does not, or the medium remains clear, the growth forming a flocculent deposit, thus affording a distinction. Not only do the characters of the growths of organisms on media differ more or less, but in some instances chemical changes occur in the media which afford valuable information in the differentiation of species. Thus many organisms exert a peptonising effect on gelatin, and render it fluid sooner or later, while others have no such action. Milk is coagulated by some organisms, the coagulation being brought about in one of two ways, either by the production of acids and precipitation of the caseinogen, or by the action of a rennet-like ferment with the formation of a clot of casein. Most organisms which liquefy gelatin coagulate milk, but the converse is not the case. Agar is carbohydrate, not albuminoid, in nature, and only two or three organisms are known which liquefy it. In fluid media, such as broth and peptone water, chemical tests

can be applied, especially for indole, which is formed by some organisms but not by others.

Method of inoculating tubes.—The following is the procedure by which sub-cultures are prepared from an original test-tube or other culture: Tubes of the culture media selected are placed in a test-tube rack. Their mouths are then singed by holding in the Bunsen flame for a few seconds, and with a forceps, also sterilised by heating in the flame, the wool plugs are loosened by a rotatory motion, and then partially withdrawn. The mouth of the original culture-tube is similarly singed and its plug partially withdrawn. A platinum needle is selected and carefully straightened. The original tube is then taken in the left hand between the thumb and index finger with the palm upwards, and is held obliquely, the mouth of the tube pointing to the right, a tube of sterile medium being held side by side with the original culture in an exactly similar manner. The wire of the platinum needle is then heated to redness by holding nearly vertically in the flame, and the lower part of the handle is also carefully heated. Holding the sterilised needle between the finger and thumb of the right hand, the plug of the original culture is now withdrawn by grasping between the ring and little fingers of the right hand, and is held there while the platinum needle is carefully introduced into the tube without touching the mouth or sides, and a trace of the growth is picked up with it, preferably from the margin. To ensure that the needle is cool, it may first be touched on the medium where there is no growth. The needle is quickly withdrawn without touching the sides of the tube and the plug at once replaced. The plug of the sterile tube is now withdrawn in the same manner, and the inoculated needle introduced. If a typical surface culture is

desired, a single light streak is made with the needle from the bottom to the top of the medium without penetrating the surface; if an abundant growth be required for any purpose the whole surface of the medium may be rubbed with the needle; if a stab culture, the needle is plunged steadily into the centre of the medium and withdrawn; if a fluid one, the growth removed is rubbed up on the side of the tube at the margin of the fluid, and the emulsion washed down by tilting the tube. The inoculation having been completed, the plug is quickly replaced, and the needle is again heated in the flame to destroy the remains of the growth upon it. If the original culture is in a deep stab, or a fluid medium, a looped platinum needle may sometimes be used with advantage. The inoculations completed, the mouths of the tubes are singed and the wool plugs pushed in level with the lip. Before replacing the plugs each may, if desired, for greater safety, be taken with the forceps, held in the flame for a second or two, and pushed while burning into the tube, and this procedure must always be adopted if the plug be dropped or brush against anything. If the tubes have to be kept for any length of time, especially in the blood-heat incubator, each should be capped with a rubber cap, tinfoil, or gutta-percha tissue which has been soaked in 1–500 corrosive sublimate solution.

Anaërobic cultures.—Many organisms refuse to grow in the presence of free oxygen, and various expedients have to be adopted to exclude or remove it. The simplest of all is to make the cultivation in a deep stab in glucose agar or gelatin. Narrow test-tubes filled three parts full with the medium are best, and immediately before the inoculation they should be placed upright in a beaker of water, boiled for five minutes, and then

cooled and solidified in cold water. The object of this is to soften the medium so that it does not split, as a dry medium will, when the needle is plunged into it; moreover, the needle track closes up more readily, and the dissolved oxygen is expelled. The tubes being cool, the inoculation is made with a long thin wire, either straight or with a closed loop at the end. It is inoculated and plunged steadily into the centre of the medium, nearly to the bottom, rotated, and then withdrawn, and the wool plug is replaced and singed. The tube is then carefully heated at the upper border of the medium so as to melt this slightly and seal the puncture, and a well-fitting rubber cap is applied while the tube is hot. The heating expels a portion of the air, and, with a well-fitting cap, creates a negative pressure within the tube, so that the residual oxygen is not so readily absorbed, or the tubes may be placed in a Buchner apparatus (see below). The tubes are placed in the incubator at a suitable temperature, and it will be found that the most strictly anaërobic organisms can be cultivated in this way.

When, however, an organism is required to grow anaërobically on the surface of the medium, or in a fluid medium, some other method must be adopted. The tubes may be placed under the receiver of an air-pump and exhausted as completely as possible. This is not very convenient, for it is difficult without great care to maintain a vacuum, and special receivers must be used when the cultures have to be incubated at blood-heat, while with fluid media ebullition causes considerable difficulty.

For fluid cultures Hamilton's method is the simplest of all. The fluid in the tubes is covered with a layer of olive oil 1–2 cm. thick, and the tubes are then sterilised. The layer of oil prevents the access and

entrance of oxygen. The only disadvantage is that the inoculation, or the withdrawal of culture, must usually be performed with a sterile glass pipette; if a wire needle be used the material is very liable to be detached in the oil.

Another method (Buchner's) is that usually adopted, and consists in absorbing the oxygen by means of alkali and pyrogallic acid, and so cultivating in an atmosphere of nitrogen. This can be carried out in two ways—either in a wide-mouthed bottle with well-fitting glass stopper, sufficiently large to contain the test-tubes, or in a Buchner's tube. For the first the inoculated culture tubes are placed in the bottle, into which a few cubic centimetres of a strong aqueous solution of pyrogallic acid have previously been poured. By means of a thistle funnel, an equal volume of 20 per cent. caustic potash[1] or soda solution is then added. As quickly as possible the thistle funnel is withdrawn without mixing the solutions, and the stopper, well vaselined, inserted and twisted well home, and some melted paraffin may be poured all round the joint and melted in with a hot iron. The solutions in the bottle are now well mixed, and the whole is placed in a suitable incubator. The Buchner's tube (Fig. 11)

Fig. 11.—Buchner's tube arranged for anaërobic cultivation.

[1] Thirty-two grm. of pyrogallic acid and 64 grm. of caustic potash dissolved in 100 c.c. of water will absorb 9200 c.c. of oxygen. At the same time some carbon monoxide is evolved (122·5 c.c.) The evolution of CO is a minimum when the potash is in excess and only one fifth of the theoretical absorbable amount of O is absorbed.

is convenient for single test-tube cultures. It consists of a strong glass test-tube, large enough to take an ordinary test-tube, and having a constriction about an inch and a half from the bottom. The constriction supports the test-tube culture, while the mixture of pyrogallic acid and caustic potash fills the portion below the constriction. A well-fitting rubber cork closes the mouth of the tube, and the joint may be paraffined for additional security. If a Buchner's tube is not available, the cotton-wool plug of the culture tube may be pushed into the tube for an inch, some solid pyrogallol is placed on the wool plug, this is just *moistened* with caustic potash solution and the tube is stoppered with a rubber cork.

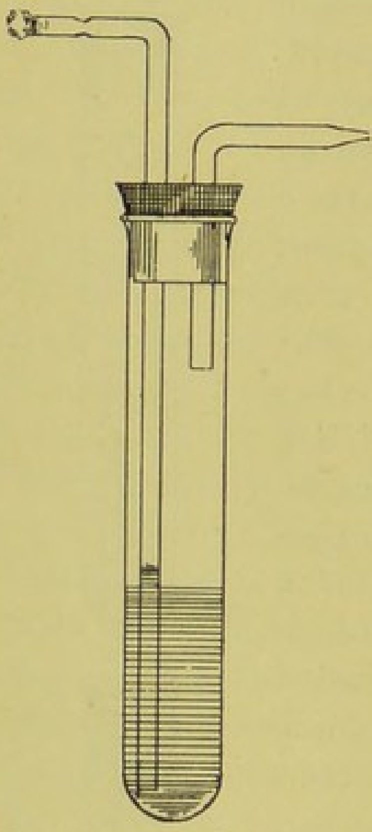


FIG. 12.—Fränkel's tube for anaërobic cultivation.

The displacement of the atmosphere by means of hydrogen may be adopted, and is to be preferred for fluid cultures. Hydrogen does not seem to inhibit the growth of any anaërobic organisms, whereas carbon dioxide gas, which might be still more conveniently used has a very decided inhibitory action on some species. The hydrogen is best generated from zinc and sulphuric acid in a Kipp apparatus, or the compressed gas in cylinders, or even coal-gas, may be used. Care must be taken that all joints are tight, and they may be paraffined with advantage. The gas should be passed through a strong solution of caustic potash, and may be passed through some alkaline pyrogallic acid if the most rigorous condition of anaërobiosis is desired, but for

ordinary purposes this is not essential; it should also pass through two or three fairly firm plugs of cotton-wool to remove organisms; these must be dry, for if moist the passage of the gas may be stopped.

For tube cultures Fränkel's method may be adopted (Fig. 12). The broth or gelatin is introduced into a large strong test-tube, the mouth of which is plugged with a rubber cork pierced with two holes. Through these holes two pieces of glass tubing pass, one to the bottom of the tube, the other just through the cork. Outside the cork these tubes are bent over at right angles, and each is drawn slightly out so as to contract its lumen at about the middle. The long tube is connected with the hydrogen supply, and a current of the gas is passed through and escapes by the shorter tube. After the gas has been passing for twenty minutes to half an hour, and *all oxygen has been expelled*, the distal, *i. e.* shorter, tube is sealed off at the contracted portion in the Bunsen or blowpipe flame, and then the proximal or longer one in the same manner. The rubber cork must, of course, fit well, and the joints should be paraffined. If gelatin be the medium, it must be kept fluid in a bath of warm water while the hydrogen is passing.

For broth or fluid cultures, which are essential for obtaining toxic products, flasks are used which are fitted with an india-rubber cork pierced with two holes. Through the holes two pieces of glass tubing pass, one to the bottom of the flask, the other just through the cork, as in the Fränkel tube described above. The ends of these tubes are plugged with cotton-wool, and the whole—flask, cork, tubes and medium—is sterilised. The medium is inoculated from a recent culture by momentarily removing the cork. Hydrogen is then passed through from a Kipp apparatus, the long tube

being connected with the hydrogen supply. After passing for about half an hour, the tubes are sealed off and the flask is incubated. For convenience of sealing the tubes should be drawn out slightly.

As many organisms produce gas during their growth, it may be necessary to provide for its escape, or the flasks may burst owing to the pressure. This can be done by adjusting a mercury valve, and may be carried out in a simple manner by a method devised by the writer. "Yeast flasks," which can be obtained in various sizes, are made use of, and are filled three parts full with a 2 per cent. grape-sugar bouillon. The neck is corked with a perforated rubber cork (A, Fig. 13), through which a glass tube, B, passes to the bottom of the flask, projecting two inches above the rubber cork and here plugged with cotton-wool. The lateral tube of the yeast flask is also plugged with cotton-wool, care being taken that the plugs are loose enough to allow air to pass freely. The whole is sterilised and inoculated. The glass tube, B, which passes through the rubber cork, is then connected with a Kipp or other hydrogen-generating apparatus by means of a rubber tube, and a current of hydrogen is

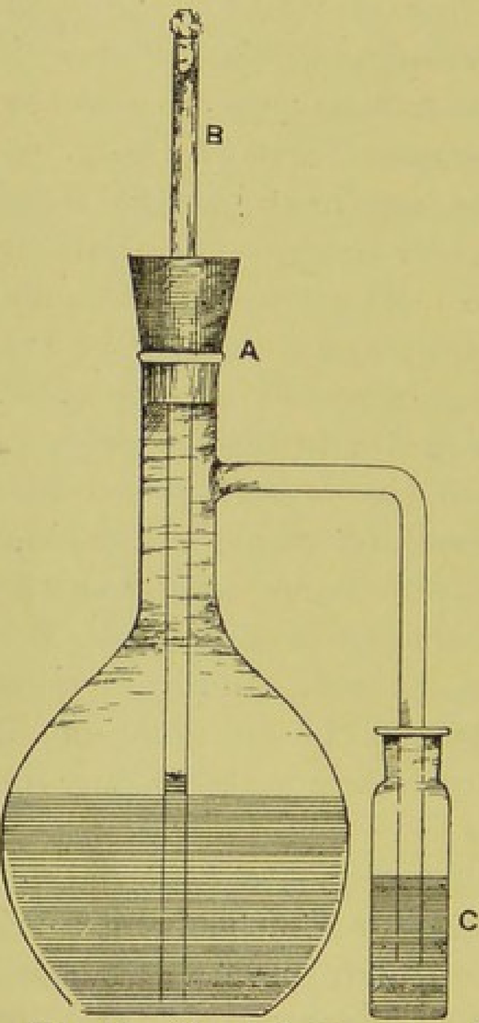


FIG. 13.—Yeast flask arranged for anaërobic cultivation.

passed through the flask. The hydrogen bubbles through the bouillon and escapes by the lateral tube. After the gas has been passing for half an hour a small tube containing mercury, C, is applied to the end of the lateral branch, so that the open end just dips below the surface of the mercury, and the tube B, which passes through the rubber cork, is sealed off in the blowpipe flame, care being taken that all the air has been expelled from the flask by a free current of hydrogen. The flask, with the capsule of mercury applied to the end of the lateral branch, can then be placed in the incubator. The mercury thus forms a valve through which air cannot enter, while gases formed by the growth of the organism have free exit.

For large flasks, the lateral tube may be just bent down and a little capsule of mercury hung on.

The addition of ½ to 1 per cent. of sodium formate to the culture media much simplifies anaërobic cultivation; the tetanus bacillus, for example, can be grown in formate broth in a stoppered bottle without any elaborate precaution for excluding the last traces of air. The sodium formate should be added immediately before the last sterilisation, not previously, or decomposition may occur. Sodium sulphindigotate (0·3 per cent.) may be similarly used.

With such a broth, Dean's bottle may be used for anaërobic cultivation. This consists of a bottle around the neck of which a gutter for mercury is formed. A loose glass cap fits over the mouth of the bottle, and its edge dips into the mercury in the gutter, thus sealing the bottle.

Plate cultivations.—The method of plate culture is one of the most important in bacteriology. It is used for three purposes: (1) for obtaining pure cultivations, *i. e.* cultures containing a single species, from a mixture

of organisms; (2) for the enumeration of organisms; and (3) for ascertaining the characters of the colonies of organisms as an aid in the identification of species.

Before the introduction of plate cultivations pure cultures of organisms could only be obtained by chance, or by the dilution method, which was also by no means certain. The dilution method consisted in estimating approximately the number of organisms in a given volume of fluid by means of an instrument on the same principle as the hæmatocytometer. The fluid is then diluted by the addition of some sterile fluid so that a given volume of the dilution contains a single organism only, assuming the organisms to be evenly distributed throughout the fluid. By transferring this volume to tubes of sterile media pure cultivations can in some cases be obtained, a single organism having been sown in a tube.

It is obvious, however, that this method is at best an uncertain one, but the plate-culture method to a large extent obviates this uncertainty. It depends upon the following principles: Gelatin and agar media, when melted, remain fluid down to 25° and 45° C. respectively, temperatures which will not affect the vitality even of delicate organisms. By inoculating the fluid gelatin or agar, thoroughly mixing, and then pouring on to a level sterilised surface, so that the medium solidifies in a thin film ("plating"), the organisms, wherever they may be situated, are fixed and are unable to wander, and, being in a good nutrient soil, grow and multiply and ultimately form visible growths or colonies. Many of these colonies will have arisen from a single organism; the growth, therefore, is "pure," *i. e.* consists of a single species, and pure cultures can be obtained by inoculating tubes of sterile media from them.

When suitable, sterile nutrient gelatin is usually employed for the preparation of plate cultivations, as it is more easily manipulated than agar. Three tubes of sterile nutrient gelatin are melted at a low temperature in a beaker of water (gelatin melts at 24° C.; the temperature should not exceed about 45° C.) The tubes may be termed respectively 1, 2, and 3. Tube No. 1 is inoculated, by means of a platinum needle, with a trace of the growth from which pure cultivations are desired. The trace of growth is thoroughly mixed up and distributed throughout the melted gelatin. If this mixture be "plated," so many organisms may be present in the film that the colonies which develop will not be separate, but will form a confluent growth. To obviate this difficulty a second and a third dilution are prepared. The second dilution is made by inoculating the tube of melted gelatin No. 2 with one platinum loopful from tube No. 1, and thoroughly mixing up; and to be quite sure that the resulting colonies will be isolated from one another, a third dilution is prepared in the same manner by inoculating the tube of melted gelatin No. 3 with two to four platinum loopfuls from tube No. 2. The organisms having been distributed throughout the gelatin by rolling and gentle shaking, the wool plug is in each case withdrawn from the mouth of the tube, the mouth of the tube is sterilised in the Bunsen burner to prevent contamination, then cooled for a few seconds, and finally the melted gelatin is poured on to a level sterile glass surface. Formerly plates of glass were used (hence the name); but now

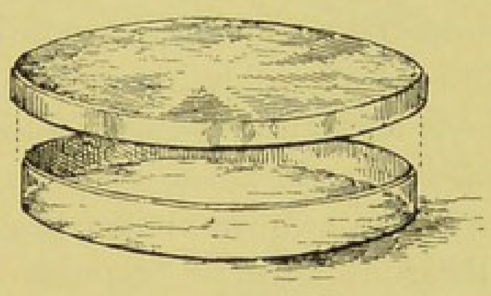


Fig. 14.—Petri dish for plate cultivation.

shallow glass dishes with lids, about three or four inches in diameter, known as Petri dishes (Fig. 14), are almost always employed. They are previously sterilised in the hot-air steriliser in suitable iron or copper boxes holding a dozen or so; the melted gelatin having been poured in, the dish is tilted to diffuse the gelatin over the bottom of the dish, placed on a level surface for the gelatin to set, and then stored in the cool incubator. The plates are examined daily, with a hand lens if necessary, or with a low power of the microscope, the dish being turned bottom upwards on the stage of the microscope for this purpose. When the colonies have developed, inoculations can be made from them by means of a platinum needle on to tubes of sterile media. The colonies, having arisen from single organisms, are pure, and the resulting sub-cultures are therefore also pure (it sometimes happens that the colonies are mixed owing to two or more organisms being close together). Different species of organisms usually form colonies having different appearances, so that the colonies are an aid in diagnosis and enable the various species to be picked out from a mixture. The colonies in gelatin are as a rule much more distinctive than those in agar. Whereas the plate cultivation prepared from tube No. 1 is generally too crowded, plates 2 or 3, or both, can be made use of, and it is apparent that, to make certain of isolating all the organisms from a mixture, several sets of plates should be prepared. Flat bottles (Fig. 15) may likewise be used for plate culturing, and are also very useful for growing organisms in bulk for the examination of the constituents and actions of the bacterial cells.

In addition to the isolation of species from mixtures and for diagnosis, plate cultures are also used to enumerate organisms. Assuming that every colony arises from a single organism, which is approximately

the case, the number of colonies represents the number of organisms originally introduced into the gelatin, and if a known weight or volume of the material inoculated be used, the number of organisms in it can be calculated. For example, in the bacteriological examination of water a measured volume of the water is added to melted gelatin by means of a sterilised pipette, and by counting the resulting colonies the number of organisms originally present in 1 c.c. of the water can be estimated.

Agar plate cultures may be prepared in a similar way. The agar must, however, be brought to a temperature of nearly boiling before it melts; it is then allowed to cool to nearly 45° C. and the tubes are inoculated in the same manner as for a gelatin plate culture described above. Unless the manipulations be carried out expeditiously the agar will solidify, or the agar film in the Petri dish be lumpy.

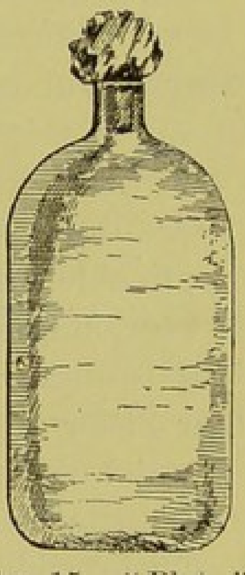


Fig. 15.—"Plate" bottles.

Agar plates should usually be inverted during incubation, or the growth may become confluent owing to the condensation water carrying the organisms all over the film.

The plate-culture method can be modified to suit particular circumstances: for example, the melted gelatin or agar, uninoculated, may be poured into the dishes and allowed to solidify, and the film then inoculated by streaking or painting with the material, or by pouring a few drops of broth containing the organisms upon it. This is practically the only way in which blood-serum can be used, the sterile blood-serum being placed in the Petri dish, solidified in the inspissator in the same manner as for blood-serum tubes, and the coagulated film inoculated.

For many purposes plates are unnecessary, the same result being obtained by rubbing over the surface of two or three tubes of sloping agar or gelatin successively the *once* charged needle, straight or looped. In the second or third tubes isolated colonies generally develop.

The plate-culture method often fails if the organism to be isolated forms but a small minority of the total organisms present in the mixture; the only alternative then is to multiply the number of plates, which, however, may entail great labour in their examination.

Single-cell cultures.—With large cells, such as yeasts, it is not difficult to obtain growths from single cells by making miniature plate cultures on ruled cover-glasses, examining microscopically, and ascertaining the places in the film where single cells are located (see Chapter XVI). But with the minute bacterial cells this method is inapplicable. By the use of Burri's Indian ink method,[1] however, single-cell cultures of bacteria can be obtained. Fluid Indian ink is diluted with 6–10 volumes of distilled water and the mixture is sterilised in the autoclave. Several loopfuls of this are deposited in series on a sterile slide. The first drop is inoculated with the culture which is being investigated, the second drop is inoculated from the first, the third from the second, and so on. A fine mapping-pen, sterilised in the flame, is then dipped into the third, fourth, or fifth drops, and the trace of Indian ink mixture so picked up is deposited on a gelatin or agar plate. The droplet is covered with a sterilised cover-glass and is examined with a $\frac{1}{6}$ in. or $\frac{1}{8}$ in. objective, with a high eyepiece. An organism shows up white on a black background. Many drops are deposited on the plate and examined, and

[1] *Das Tuschverfahren* (G. Fischer, 1909). Günther Wagner's ink (Hanover) is recommended and is supplied by Grübler.

those in which only a single organism can be found are noted and the plate is then incubated so that colonies may form, from which sub-cultures may be prepared.

Esmarch's roll cultures.—Another modification of the plate-culture method is known as Esmarch's roll culture. For this purpose large test-tubes ("boiling tubes"), at least an inch in diameter and six inches long, are sterilised and plugged with cotton-wool. The sterile melted gelatin, about 10 c.c., is poured in and inoculated, the wool plug replaced, and the tube held in the horizontal position and rotated under a stream of cold water, or in warm weather on a block of ice, until the gelatin has set. In this way the gelatin forms a thin film over the inside of the tube, but a little practice is required to get it evenly distributed. The colonies then develop in the film of gelatin, which is quite analogous to a film in a Petri dish.

Anaërobic plate cultivations are sometimes required. The plate culture after preparation as described above, using a *deep* Petri dish, is inverted, and some alkaline pyrogallol is placed in the lid; this absorbs the oxygen within the dish. The preparation must be kept under observation for the next hour or so, and more alkaline pyrogallol is added from time to time to compensate for the rise of fluid within the dish until absorption of the oxygen from the contained air is complete.

In Botkin's method a bell-jar standing in a glass dish is made use of. The Petri dishes are placed on a support within the bell-jar, and mercury or oil is poured into the glass dish. By means of a piece of bent glass tubing a stream of hydrogen is passed into the bell-jar under its rim so as to displace the air, which bubbles out through the oil or mercury. When the air has been entirely displaced the glass tube is removed, the bell-jar weighted, and the whole placed

in the incubator. Bulloch's apparatus is somewhat similar to this. Wide-mouthed jars with well-ground glass lids, which are luted down, are very convenient, the oxygen being absorbed with alkaline pyrogallol placed at the bottom, and the Petri dishes stacked on a glass capsule or other support to raise them above the fluid.

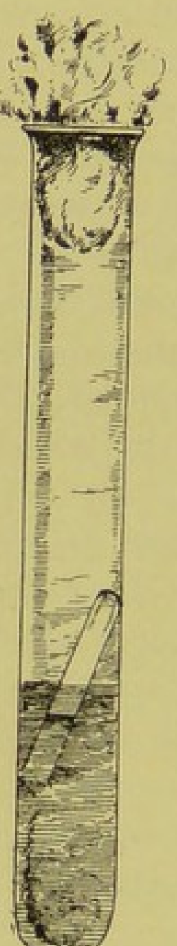


Fig. 16.—Durham's fermentation tube.

The Esmarch roll cultures can be adapted for anaërobic plate cultures. The wool plug is replaced by a rubber cork with two holes, through which inlet and outlet glass tubes pass, as in Fränkel's anaërobic tubes (Fig. 15). The roll culture having been prepared, and the film having set, hydrogen is passed in and the tubes are sealed off, or, better still, the hydrogen is allowed to bubble through the inoculated melted gelatin, the test-tube meanwhile being kept in a little warm water to prevent the gelatin from solidifying, the tubes are sealed off, and the roll culture is then prepared.

For the detection of fermentation and gas production, stab cultures in glucose agar or shake cultures in gelatin may be employed. For the latter a tube of gelatin[1] is melted at a low temperature, inoculated with the organism, and allowed to solidify in the upright position; the organism is thereby distributed throughout the medium. Fermentation with gas production is indicated by the presence of gas bubbles, or even by the disruption of the medium. Durham's fermentation tubes are very convenient for showing

[1] Lemco gelatin frequently gives no gas; a meat-broth gelatin should therefore be used for gelatin shake cultures.

fermentation. These are test-tubes containing suitable fluid media (10 c.c. each) into which small glass tubes closed at the upper end are placed; the latter become filled during the sterilisation. The tubes are inoculated and incubated, and if fermentation occurs the little tube becomes filled with gas (Fig. 16). Einhorn's saccharimeter may also be used (Fig. 17). The tube is filled with the medium, sterilised, inoculated, and incubated.

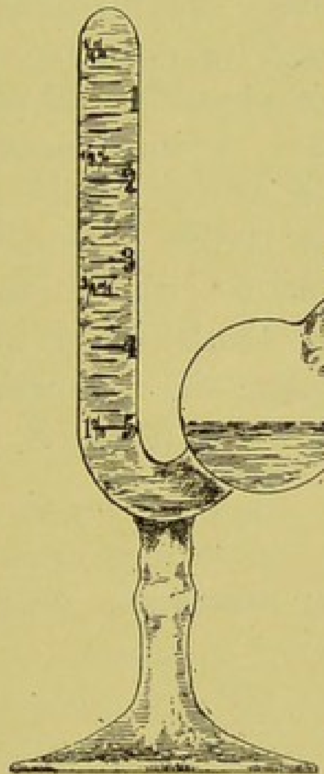


FIG. 17.—Einhorn's saccharimeter.

Any gas produced collects in the closed limb of the tube. When the amount of gas ceases to increase, a little strong caustic potash solution may be added; this absorbs the CO_2, the residue probably being hydrogen, and thus the $H : CO_2$ ratio may be determined. The most suitable media for fermentation are peptone broth, the acid beef-broth for which has been treated with the colon bacillus (see p. 27), 1–2 per cent. peptone water, or a medium which has been

largely used by Houston, Gordon, and others, consisting of a 1 per cent. solution of "Lemco" in distilled water with the addition of peptone 1 per cent., sodium bicarbonate 0·1 per cent.; to either medium is added 1–2 per cent. of glucose, lactose, saccharose, starch, inulin, mannitol, dulcitol, etc., and the mixture is tinged with litmus.

The fermentation tube has been much used of late for the examination of fæces in abnormal intestinal conditions. For this purpose 1 grm. of fæces is thoroughly emulsified in 10 c.c. of physiological salt solution and 1 c.c. of the suspension is introduced into the fermentation tube, the long arm of which is 95 mm. long. The media employed are 1 per cent. dextrose, lactose, and saccharose broths made with "Lemco" (as above) or with sugar-free meat broth (see p. 27). With such tubes normal stools yield the following amounts of gas:[1]

Dextrose.	Lactose.	Saccharose.
26·75 .	. 29·9 .	. 19·5 mm.

[1] See Herter and Kendall, *Studies from the Rockefeller Institute* (Reprints), x, 1910.

CHAPTER III.

THE PREPARATION OF TISSUES AND ORGANISMS FOR STAINING AND MOUNTING. STAINING AND STAINING METHODS.

A SELECTION of the numerous methods devised for the preparation and staining of tissues, bacteria, etc., is here given. Special methods occasionally employed will be described when required.

Preparation of Tissues.

As the demonstration of the bacteria in the tissues is of the primary importance, the elaborate methods which have been described for fixing the tissue elements are not essential in bacteriology, unless of course the relation of the bacteria to the tissue elements is being studied. The tissues should always be obtained as fresh as possible, because within a few hours of death they are invaded by numerous bacteria, derived from the air and from the intestine, which may mask the original bacterial infection and lead to serious mistakes if this source of error be not carefully borne in mind. In all cases the tissue should be cut into pieces of convenient size, not more than about 1 cm. in thickness, and organs if kept *en masse* should be sliced. Having been thus prepared, the material may be treated by one of the following methods :

(*a*) Place directly in methylated spirit[1] for a week or a fortnight.

(*b*) Place in methylated spirit 1 part, water 2 parts, for twenty-four to forty-eight hours, transfer to methylated spirit and water, equal parts, then to methylated spirit, and finally to absolute alcohol for like periods.

(*c*) Place in rectified spirit (86 per cent. alcohol) containing 1 per cent of corrosive sublimate for twelve to forty-eight hours, and pass through increasing strengths of alcohol as in (*b*).

(*d*) Place for six to twenty hours in a saturated aqueous solution of corrosive sublimate. This is prepared by saturating boiling distilled water with the corrosive sublimate, cooling, and filtering. Keep in the dark. When removed from the corrosive sublimate solution the tissues must be washed in a stream of running water for an hour, or, better, placed for a day in 70 per cent. alcohol deeply coloured with iodine, to remove the excess of corrosive sublimate and prevent precipitation. The tissues are then passed through increasing strengths of alcohol, as in (*b*).

(*e*) Formalin, a 40 per cent. aqueous solution of formic aldehyde, is an excellent fixing agent. A solution of 1 part of formalin and 9 parts of water, or better, physiological salt solution, may be used, the pieces of tissue remaining in this for twelve to twenty-four hours. They are then washed in running water for an hour or two and passed through increasing strengths of alcohol, as in (*b*).

[1] Methylated spirit free from mineral naphtha should be used and can be obtained in quantities of five bulk gallons, "for scientific purposes only," by special order from the Inland Revenue Authorities, Somerset House, W.C. If it cannot be procured, absolute alcohol must be employed. Duty-free absolute alcohol can also be obtained under somewhat similar conditions and is much cheaper than the ordinary.

All tissues after fixing and hardening should be preserved in dilute alcohol—water 1 part, absolute alcohol or methylated spirit 2 parts.

The methods (*c*), (*d*), and (*e*) are to be recommended, especially the two last, as the tissue elements are well fixed thereby. In all cases the fixing fluid should be used in considerable excess. Fixing fluids containing potassium bichromate (as in Müller's fluid) and chromic acid seem to prevent the bacteria from staining with any certainty, and should be avoided.

Section Cutting.

In order satisfactorily to demonstrate bacteria in tissues, and their relation to the tissue elements, it is usually necessary to prepare sections. For this purpose either the freezing or the paraffin method should be employed.

(*a*) *Freezing method.*—The tissue, in suitable pieces, must first be soaked in water to remove the alcohol. A convenient way of doing this is to place the material in a wide-mouthed bottle, into the mouth of which an ordinary glass funnel is introduced, and the bottle with the funnel is placed under a stream of running water; the funnel, while allowing the water to flow out, retains the pieces of tissue in the bottle. With running water the alcohol will be completely removed in from one to two hours; in still water, which should be changed two or three times, this result may not be attained for several hours, during which time there is an ever-increasing risk of bacterial contamination from without. *It is essential to remove all the alcohol, or the tissue will not freeze.*

When the alcohol has been removed, which is known by the tissue *sinking* in the water (lung is an exception

—it always floats unless solid from any cause), the pieces are transferred to a strong mucilage of gum acacia:

Gum acacia	5 grm.
Cane sugar	0·5 grm.
Water	100 c.c.

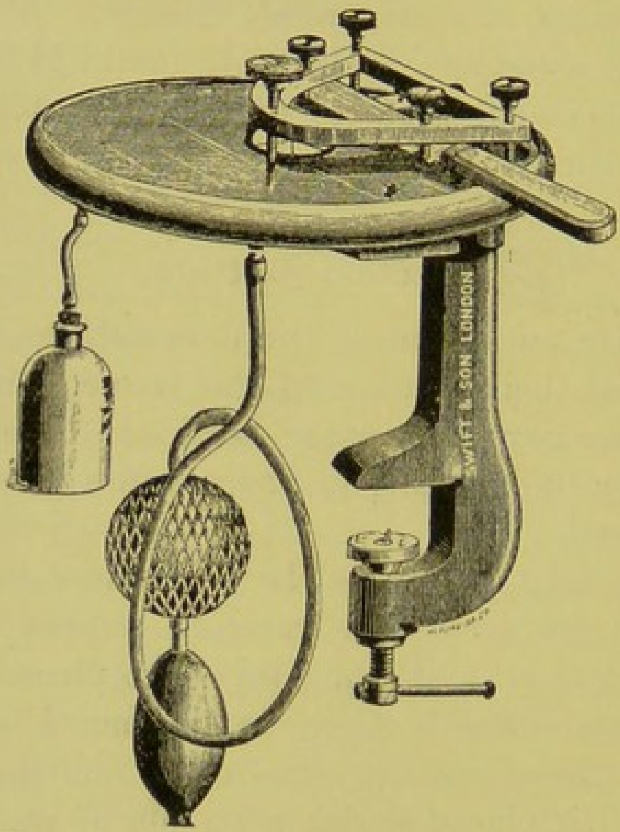


Fig. 18.—Swift's ether-freezing microtome.

Add a piece of thymol or a little carbolic acid to prevent decomposition. Hamilton saturates the solution with boric acid.

In this gum solution the pieces remain for twelve to forty-eight hours, according to their size and the time at the disposal of the investigator, and are then cut on one of the numerous ether-freezing microtomes now to be obtained, such as Swift's (Fig. 18) or Cathcart's. A microtome in which the freezing is effected by

carbonic acid is now largely employed and acts well. Liquid carbonic acid, contained in a cylinder, sprays by its own pressure on to the under surface of the plate on which the block of tissue rests; the tissue quickly freezes and is then cut. This form of microtome works satisfactorily in the hottest weather. The material must not be frozen so hard that the sections roll up and fall off the knife; the sugar in the above solution should prevent this. The sections are transferred successively to two or three lots of distilled water, preferably slightly warmed, to remove the gum, and can then be stained or preserved in equal parts of methylated spirit and water until wanted.

Bacteria seem to retain their staining properties better in the tissue in bulk than in sections. Although the bacteria may stain well in sections for some time after preparation, it frequently happens that in a month or two they refuse to stain. Such is certainly the case with anthrax tissues, but not with tubercle or leprosy, the bacilli in sections of the latter seeming to retain their staining properties unaffected for a considerable time.

(*b*) *Paraffin method.*—Nothing can surpass the paraffin method for the thinness and beauty of the sections obtainable by it, and for some friable tissues, such as actinomycosis, it is almost essential. The tissue, in suitable pieces for cutting, is transferred from the diluted spirit preservative solution to pure methylated spirit for two or three hours, and then to absolute alcohol—which may have to be changed once unless a fairly large volume is employed—for from four to twenty-four hours. It is then removed from the alcohol, lightly dried between the folds of a *dry* cloth or piece of blotting-paper to remove the superfluous alcohol, and placed in an excess of xylol, in which it

remains for from four to twenty-four hours until cleared. This is recognised by the material assuming a more or less semi-transparent condition, and the process may be much accelerated by warming the xylol to from 37° to 50° C. in the blood-heat incubator or paraffin oven, the bottle containing the xylol being well stoppered. When cleared it is ready to go into the bath of melted paraffin. A paraffin of a fairly high melting-point is perhaps the best, viz. 45° to 55° C., and is placed in glass capsules in an oven which can be kept uniformly heated to the required temperature. An ordinary chemical hot-water oven answers the purpose quite well, and is heated by a special form of small Bunsen burner with mica chimney, the temperature being regulated by some form of mercurial regulator, which is set a degree or two above the melting-point of the paraffin employed. The tissue is taken out of the xylol, blotted to remove the excess, and placed in the melted paraffin for six to twenty hours. It is then embedded by pouring a little of the melted paraffin into a watch-glass, or into a small box formed of folded paper or lead-foil, or by bringing together two L-shaped pieces of brass on a glass plate so that a rectangular cavity is produced. The pieces of tissue are then taken out with a small warmed forceps or needle, adjusted to the position they are required to occupy, and more melted paraffin is poured in, so as to cover them. When a film of solid paraffin has formed, the whole is immersed in cold water so as to cool it rapidly.

A new paraffin is frequently crystalline in structure, and acts much better after it has been kept melted for some weeks, or is much improved by heating nearly to its boiling-point for five or six days (P. T. Beale). The xylol for clearing may be used several times and the

paraffin repeatedly, the remains of old tissues being removed. The time which the tissues require to remain in the alcohol, xylol, and paraffin depends upon their size; *very small* pieces may be treated in a few hours, large ones may require two or three days.

Other clearing agents, such as chloroform, turpentine and cedar oil, may be used instead of xylol. The paraffin method is usually straightforward, but *small* pieces of tissue must not be left too long either in

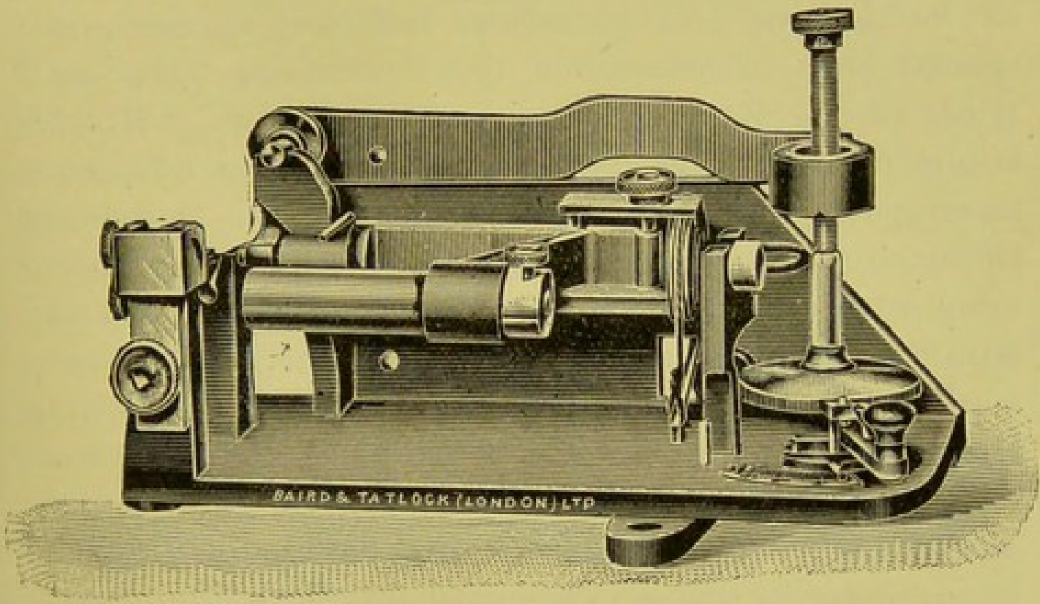


Fig. 19.—Cambridge rocking microtome.

absolute alcohol or in the paraffin bath, for they are liable to become too hard to cut. Thyroid tissue and skin are also rather troublesome; they become very hard unless the whole process is carried out as rapidly as possible. If the pieces of tissue be large, the capsule of melted paraffin containing the tissue may be placed under the receiver of an air-pump, which is then exhausted. This causes the paraffin to penetrate better, and the process may be repeated two or three times during the period of infiltration. A special form of paraffin oven has been devised by Cheatle for infil-

trating under diminished pressure, and is made by Hearson, of Regent Street.

In order to prepare sections from material embedded in paraffin some form of microtome must be employed. An ether-freezing microtome *can* be employed with some manipulation, the paraffin block being placed in a little melted paraffin on the freezing plate so that it is cemented there, and sections are cut with the razor or plane iron, as though it had been frozen (it is *not* to be frozen). It is better, however, to use some special form of microtome, the Cambridge "Rocker" (Fig. 19), or a modification of it, or the "Minot," being perhaps the best. The block of paraffin containing the tissue is trimmed with a knife to remove the excess, and is cemented to the carrier of the microtome with a little melted paraffin, or by melting the paraffin on it with a hot iron (end of a file, etc.) or a match. The union may be made more secure by melting the paraffin around the base of the block with a hot iron.

Having fixed the paraffin block to the carrier, sections may then be cut of any degree of thinness. In order to do this it is essential for the knife or razor to have a keen edge and one of the right nature, for a knife may be perfectly sharp and yet the sections as they are cut roll up in such a manner that it is difficult to flatten them. Though this may be due to a wrong consistence of the paraffin, owing to cold weather or some other factor, in the majority of instances it is the edge of the knife which is at fault. Provided the knife be sharp, stropping on the palm of the hand will usually remedy this difficulty. The paraffin being of the right consistence, and the knife in good order, the sections as they are cut should be flat and should adhere together at adjacent margins so that a ribbon of greater or shorter length is formed.

Satisfactory sections having been obtained, they are transferred with a needle or camel's-hair brush to a tin pan containing a little water, or spirit and water, warmed to about 40° C. The sections float and the paraffin *softens*, so that they spread out perfectly flat (the water must not be hot enough to *melt* the paraffin). A clean slide is then introduced underneath the section, raised so that the section is lifted up on it, and by fixing the section with a needle and tilting the slide the section is deposited in the required position on the slide and allowed to dry. If preferred, the section may be transferred to a slide flooded with water, which is warmed over the Bunsen. The slides can be manipulated in an hour or two if dried at 37° C., but it is best to allow them to dry in the incubator all night. It will be found after this treatment that the sections adhere sufficiently firmly to the slides for all the ordinary methods of staining to be carried out without detaching them, which would be fatal. The sections must be fairly thin, however; if they are at all thick they do not adhere nearly so well.

Should the sections have to be subjected to prolonged treatment during staining, etc., they may be cemented to the slides by the following method. Equal parts of egg-white and glycerin are mixed and filtered, and to every 100 c.c. of the mixture 1 grm. of sodium salicylate is added. The slide is smeared thinly with this, the section is transferred to it in the manner just described, and the slides are dried.

Supposing that, while cutting, the sections, in spite of all precautions, curl up instead of lying flat, it is still often possible to obtain a few that can be mounted. They may sometimes be unrolled by cautious manipulation with a couple of needles after having been softened by warming, or a needle or knife-blade may

be held close to the edge of the microtome knife during cutting, so that the sections, instead of curling, slide up against this guide.

Tissues embedded in paraffin may be kept indefinitely in labelled pill-boxes and cut all at once or from time to time as required, or the ribbons of sections may be preserved in a box in a cool place until wanted. The slides also, with the sections attached, can be kept until it is convenient to stain, if preserved free from dust in a slide box.

Cover-glass and Film Specimens.

The satisfactory preparation of cover-glass and film specimens is one of the most important in bacteriology, for they are used for the examination of cultivations of bacteria, and of blood or other fluids or secretions, organs, etc., for the presence of micro-organisms.

It is necessary in the first place to have clean cover-glasses of the right kind; they must be thin, otherwise the higher powers cannot be employed to examine the preparations, and those described as "No. 1" should be purchased, "¾-in. squares" being a convenient size. These serve both for cover-glass specimens and for covering sections; it is well also to have a few of the same thickness but larger, viz. ⅞-in. or 1-in. squares, for large sections. In order to clean them they should be gently boiled in a porcelain dish with 10 per cent. carbonate of soda solution for a few minutes, well washed, and then treated with strong sulphuric acid, warmed carefully in a porcelain dish, for a few minutes. The acid having been poured off, they are well rinsed in several changes of water, and should be kept in a stoppered glass capsule in absolute alcohol. Slides may be used instead of cover-glasses, and should be cleaned

and manipulated in the same manner as described for cover-glasses.

A clean cover-glass (or slide) is taken, dried with a clean soft linen or silk rag or handkerchief, or with Japanese paper, or it may be momentarily introduced into the Bunsen flame and the spirit burnt off, and placed flat on a convenient support on the work-table —a white glazed tile is excellent—with the corner projecting so that it can be conveniently picked up with the forceps.[1] A droplet (*i. e. small* drop) of water is then placed on it, in the middle, by means of a looped platinum needle, or with a small glass pipette (Fig. 7). Theoretically, sterilised physiological salt solution[2] should be used, a few cubic centimetres being boiled in a sterilised and plugged test-tube for two or three minutes and cooled; but ordinary tap-water may usually be employed. A thin film of organisms has now to be formed on the cover-glass, and the following is the method of procedure with a culture on a solid medium such as agar or gelatin. The culture tube and platinum needle are held and manipulated in precisely the same manner as that described for the inoculation of tubes (p. 70), a trace of the growth being removed from the tube and the wool-plug immediately replaced.

A mere trace of the growth from a culture should be taken, just sufficient to soil the tip of the platinum needle, or the preparation will be too crowded, and this is rubbed up well with the droplet of water on the cover-glass, so as to form an emulsion, which is then spread over the surface. As a general rule the

[1] The writer has devised a useful support for staining. It consists of a square of plate glass, painted half white and half black at the back, and having a narrow strip of thick glass cemented across it on which the covers rest. It is made by Messrs. Baird & Tatlock.

[2] 0·75–0·95 per cent. of sodium chloride dissolved in distilled water.

material should be well emulsified, but in some instances this is inadvisable, as a particular formation or characteristic grouping may be disturbed thereby, in which case, after a slight admixture with the water, the emulsion is gently spread. The thin film on the cover-glass is allowed to dry, or may be dried by gentle warming over the Bunsen flame, preferably holding the preparation in the fingers and moving backwards and forwards over the flame. Having dried the film, it has next to be fixed, which is accomplished by holding the cover-glass, film side up, in the forceps and passing fairly rapidly three times through the Bunsen flame (slides 6–9 times). The object of this "fixing" is to thoroughly dry the film and coagulate albuminous material, whereby the film adheres better to the glass, and is not so likely to be detached in the subsequent processes of staining and washing, etc. Fixing may also tend to diminish the staining capacity of the extraneous matter mixed with the organisms. The preparations are now ready for staining.

When the culture is in a fluid medium, such as broth, the tube is manipulated in the same way, the deposit at the bottom having been shaken up if necessary, and a loopful or two of the fluid removed with a looped platinum needle, transferred to the cover-glass, spread out, dried, and fixed as before, but as the medium is fluid there is usually no need to add a droplet of water.

If a specimen of blood, pus, or sputum is required, the procedure is much the same. A little of the material is taken up with a looped platinum needle and spread in a thin film over the cover-glass or slide, which is then dried and fixed. If necessary, a droplet of water or physiological salt solution may be used to dilute the material so as to obtain a thinner film. If a

specimen is to be made from an organ, a particle of the pulp is picked up and an emulsion made as before, or a small piece of the organ may be held in sterile forceps and the cut surface gently smeared over the cover-glass or slide, which is then dried and fixed; these are termed "smear preparations."

To obtain the best results it is preferable before staining to submit films of blood[1] or pus or smear preparations to the action of some chemical fixing agent, unless the film is stained with Leishman's solution, which both fixes and stains. The simplest method of doing this is to immerse the films, after *air*-drying, in a mixture of equal parts of absolute alcohol and ether for five to fifteen minutes. In hot countries a saturated aqueous solution of corrosive sublimate (five to fifteen minutes) is perhaps as good as anything. Another method, combining both fixing and staining, is to immerse the films as soon as they are prepared and without drying in the following solution:

Absolute alcohol saturated with eosin .	25 c.c.
Pure ether	25 c.c.
Alcoholic solution of corrosive sublimate (2 grm. in 10 c.c.)	5 drops

For four cover-glasses 5 to 10 c.c. of this solution are required, and they should remain in it three to four minutes (it may be prolonged for hours without harm); they are then removed with a forceps and well rinsed in water, stained for not more than a minute in a saturated aqueous solution of methylene blue, washed quickly, dehydrated in absolute alcohol, cleared in xylol, and mounted in xylol balsam. The solution may be used for fixing blood, pus, sputum, etc., if the eosin

[1] For the method of preparing blood-films see the section on "Malaria," Chapter XVIII.

be omitted, and the preparations may then be stained or otherwise treated in any desired manner.[1]

Scott[2] recommends the following as giving the most perfect results with blood films, etc.:

(1) Hold the freshly prepared and still wet film in the mouth of a wide-mouthed bottle half filled with the ordinary formalin solution, film side downwards, for five seconds.

(2) Drop, *while still wet*, film downwards, into absolute alcohol. Leave for fifteen minutes, or, for convenience, for any time up to forty-eight hours.

The preparations may then be stained with methylene blue or hæmatoxylin, and eosin, or with Leishman's stain. (See also under "Malaria" and "Trypanosomes," Chapter XVIII.)

Impression specimens.—These are employed to examine and preserve permanently the colonies or growths of organisms so that their characteristic formation may be observed. With plate cultivations this is very simple. A clean cover-glass is sterilised in the flame and, having cooled, is cautiously lowered on to a selected colony with a sterile needle, avoiding all lateral movement. It is gently pressed on to the colony and then carefully raised by means of a couple of needles; the colony should adhere to the glass, and may be dried and fixed. The colonies in gelatin tube cultures may also be used if the gelatin is removed from the tube. This can be done by dipping the tube for a few seconds into hot water; the gelatin round the walls of the tube will be melted, and the gelatin mass can then be tilted out of the tube on to a glass dish or tile.

[1] Gulland, *Brit. Med. Journ.*, 1897, vol. i, p. 65.

[2] *Journ. Path. and Bact.*, vol. vii, No. 1, p. 131.

Stains and Staining Methods.

Micro-organisms being so minute and transparent, it is usual to stain or dye them, so that they can be more readily examined. In some instances organisms may have a peculiar staining reaction, which may serve as an aid to their identification. But when an organism is being investigated, examination in the fresh and living condition must never be omitted, for it is only thus that its motility and life-history can be studied. Only general methods are detailed here; special ones will be given when they are required.

(1) Löffler's alkaline methylene blue.

Saturated alcoholic solution of methylene blue .	30 c.c.
Solution of caustic potash, 0·01 per cent. .	100 c.c.

A very useful staining solution. Cultures should be quite fresh, or the organisms do not stain well. When the organisms are mixed with extraneous material, as in smears, or there is much *débris*, this is one of the best staining solutions to employ. Methylene blue preparations are, however, not very permanent, and in hot countries rapidly fade. Thionine blue is then preferable.

Cover-glass specimens are stained for three to ten minutes, and sections half to twenty-four hours.

(2) Carbol-methylene blue (Kühne).

Methylene blue	1·5 grm.
Absolute alcohol	10 c.c.
Five per cent. aqueous solution of carbolic acid	100 c.c.

A more intense staining solution than the former, and very useful for sections, which are stained for from half to six hours.

(3) Anilin gentian violet.

Saturated alcoholic solution of gentian violet .	30 c.c.
Anilin water	100 c.c.

The anilin water is prepared by shaking 3 c.c. of anilin with 90 c.c. of distilled water, allowing the mixture to stand for a few minutes, and filtering. Instead of anilin water, 1 per cent. aqueous carbolic may be used (carbol-gentian violet).

This solution is a useful general stain for films, which are stained for two or three minutes, and is employed in Gram's method of staining. It does not keep well.

(4) Carbol-fuchsin (Ziehl-Neelsen solution).

Fuchsin	1 part
Absolute alcohol	10 parts
Five per cent. aqueous solution of carbolic acid	100 parts

The fuchsin is dissolved in the absolute alcohol and then mixed with the carbolic acid solution. It must always be filtered before use.

An intense staining solution. For film specimens it is best diluted with five to ten parts of water; stain for two to five minutes.

(5) Carbol-thionine blue (Nicolle).

Saturated solution of thionine blue in alcohol (90 per cent.)	10 c.c.
One per cent. aqueous solution of carbolic acid	100 c.c.

Sections can be strained in from a few minutes to half an hour. This solution may be used for a modified Gram's method (see p. 109). Can be substituted for methylene blue for all purposes, and is more permanent than the latter.

(6) Chenzinski's solution (after Klein).

Saturated aqueous solution of methylene blue (Grübler's)	50 c.c.
Eosine (soluble in alcohol)	0·5 grm.
Absolute alcohol	70 c.c.
Distilled water	130 c.c.

Film specimens, after fixing, are placed in absolute alcohol for half a minute, dried, and placed, film downwards, in a

watch-glass of the stain, which is then warmed until it steams. The specimen is then taken out and well washed in tap-water, rinsed in distilled water, dried, and mounted.

Sections are immersed in absolute alcohol for several minutes, stained in the cold for six to twelve hours, well washed in distilled water, and passed through absolute alcohol and xylol, and mounted.

(7) Eosin (alcohol-soluble and water-soluble).

A somewhat diffuse stain. Is used for counter-staining the tissues in Gram's method, and for staining red blood-corpuscles and acidophile granules in leucocytes.

A $\frac{1}{2}$ to 1 per cent. aqueous or alcoholic solution may be used, and the staining should not, as a rule, be prolonged for more than about half a minute.

(8) Bismarck brown (Vesuvin).

A saturated aqueous solution should be prepared and diluted somewhat for use. A good counter-stain for the tissues in Gram's method. Stain for two to five minutes.

(9) Orange-rubin.

Prepare saturated aqueous solutions of orange G. and rubin S. Mix equal volumes and dilute with water until of a light port-wine colour. Stain tissues for five to fifteen minutes. A good contrast stain for tuberculosis and actinomycosis.

(10) Picro-carmine.

This is best bought ready prepared. Sections are stained in the solution for half to one hour, washed, then placed in a watch-glass of spirit, to which three or four drops of hydrochloric acid have been added, for two or three minutes, then well washed in water. The section can now be counter-stained with Löffler's blue or by Gram's method.

(11) Hæmatoxylin.

Ehrlich's formula is one of the best and simplest to use,

and can be obtained ready for use. It must be "ripe." It it a histological and not a bacterial stain. Sections are treated as follows:

(1) Distilled water, one to two minutes.

(2) Stain with the hæmatoxylin solution for five to thirty minutes. In some cases the solution is preferably diluted somewhat with distilled water.

(3) Rinse in distilled water.

(4) Rinse in distilled water containing a *trace* of acetic acid.

(5) Treat with distilled water containing a *trace* of ammonia. The sections remain in this until they assume a deep blue colour. (Tap-water, five to ten minutes, may also be used.)

(6) They can then be dehydrated, cleared and mounted, or counter-stained with eosin, orange-rubin, or Van-Gieson, and then mounted.

Hæmatoxylin makes a good contrast stain for the tubercle and the leprosy bacillus and for *Actinomyces*.

Mayer's hæmalum (see section on the '*Amœba coli*') and Delafield's hæmatoxylin are also good hæmatoxylin stains.

(12) Ehrlich-Biondi triple stain.

This is best bought ready for use. It is a good histological stain for tissues and leucocytes. Actinomycosis stains well by it.

Stain for ten to sixty minutes, then treat with methylated spirit until the section becomes greenish. Pass through absolute alcohol, clear, and mount.

(13) Leishman's stain.

Like the Jenner, Wright, and other similar ones, a modification of the Romanowsky stain, a double compound of eosin and methylene blue. The solution will keep for some time, but is best freshly prepared. Grübler's powder or Burroughs Wellcome's soloid may be used, and is dissolved in *pure* (Merck's or Kahlbaum's) methyl alcohol. Failure frequently proceeds from the use of a so-called pure methyl

alcohol, which is not really so. (For method of using, see "Malaria," Chapter XVIII.)

(14) Giemsa stain.

An eosin-azur mixture dissolved in glycerin and methyl alcohol. Useful for blood-films, film preparations, etc., and has been much used to demonstrate the spirochaetes in syphilitic material. (For method of using, see "Syphilis.")

Safranin and acid fuchsin are also used as counter-stains. Malachite green, neutral red, and rosein may be used for *intra-vitam* staining of protozoa, etc.

Eosin, orange-rubin, hæmatoxylin, and picro-carmine keep well in solution; the remainder may or may not, and are best used fairly fresh. All stains should be filtered before use, and may be conveniently kept in bottles having a funnel fitted with a filter-paper, so that they are always ready. Or smaller bottles may be used, fitted with pipettes, and several arranged in a stand.

The best stains are Grübler's, which can be obtained from many agents in this country. Messrs. Burroughs, Wellcome & Co. supply most of the anilin dyes and some other reagents, iodine, etc., in "soloids," which are very convenient and good.

Gram's method.—This is a most useful method, especially for sections, specimens of blood, or smear or impression preparations, as the tissue or ground substance can be counter-stained so that the organisms show up in marked contrast. Ordinary cover-glass specimens do not usually require this method, unless *débris* or ground substance is present and the best result is desired. Unfortunately Gram's method is not applicable for all organisms, as many do not retain their colour by the process. This disadvantage, however, is counter-balanced by the fact that it forms a valuable means of distinguishing organisms, and is always one of the points to be noted in bacteriological diagnosis. Most of the moulds, yeast, streptothrix and

sarcina forms, and cocci stain by it, though there are exceptions; the spirilla and protozoa do not stain by it, but as regards the bacilli no rule can be laid down (see p. 108). Cover-glass specimens are stained for five to ten minutes, and sections for ten minutes to half an hour, in anilin- or carbol-gentian violet solution. Drain off the superfluous stain, and then immerse, without washing, in the following solution for one half to two minutes:

Iodine	1 part
Potassium iodide	2 parts
Distilled water	300 parts

The purple colour of the gentian violet changes to a dirty yellowish-brown, and sections become much like a used tea-leaf. The specimens must not be passed on to the next solution until they have assumed the brown colour. Cover-glass specimens are best *immersed* in the solution in a watch-glass, *film side up*, or they and slide specimens may be flooded with the iodine solution two or three times.

The specimens are removed from the iodine solution, drained, and then immersed in alcohol, preferably methylated spirit. In this the purple colour of the gentian violet returns and is dissolved out, so that they ultimately become colourless; this is aided by moving them gently about, and for sections two or more baths of alcohol may be an advantage, a fresh one being substituted when the first has become deeply coloured. Film specimens decolorise much more readily than sections, and they should be removed from the alcohol when no more colour dissolves out, or the stain may be entirely removed; usually twenty to forty seconds in the alcohol suffices, thick preparations taking

longer than thin ones. It is also important with cover-glass specimens to remember on which side of the glass the film is, for it may be very difficult to ascertain this when the specimen has been decolorised. After decolorising, film specimens are washed in water, dried, and mounted, or, after washing, the ground substance may be counter-stained, if required, with eosin for a few seconds, or Bismarck brown for two or three minutes, washed again in water, dried, and mounted. Sections after decolorising are passed through absolute alcohol and xylol before mounting, or, if required to be counter-stained, are immersed in eosin for fifteen to thirty seconds, or Bismarck brown for three to five minutes, and then passed through methylated spirit, absolute alcohol, and xylol.

Sections frequently are somewhat difficult to decolorise with alcohol alone, in which case it is well to treat them with a slightly acid alcohol (3 per cent. of hydrochloric acid) for a few seconds, and then return to the alcohol (Günther's method).

The iodine in Gram's method seems to act as a mordant, precipitating the stain in a relatively insoluble form in certain species of bacteria. The staining of organisms by Gram is relative; some forms do not stain at all, are *Gram-negative*—*i. e.* the colour is removed by the alcohol with the greatest facility; others stain intensely, are *Gram-positive*, but even these may become decolorised by prolonged treatment with alcohol. The best procedure is to have three watch-glasses of methylated spirit, and to transfer the cover-glass specimen from one to another as the stain is dissolved out; in the last bath, immediately the final trace of colour is seen to be dissolved out, the preparation should be removed, washed in water, dried, and mounted. In order to ascertain whether an organism

is or is not stained by Gram's method, it is sometimes useful to mix with it in making the preparation some undoubted Gram-staining organism—*e. g.* if a bacillus, with the *Micrococcus pyogenes*; if a coccus, with *B. anthracis* or *B. subtilis*. The admixed organism then serves as an index.

The following organisms are Gram-positive: *B. anthracis*, *B. diphtheriæ*, *B. tetani*, *B. Welchii*, *B. botulinus*, *B. tuberculosis*, *B. smegmatis*, *B. lepræ*, *B. murisepticus*, *Actinomyces*, *B. subtilis*, *B. mesentericus*, *B. megaterium*, *B. mycoides*, the pyogenic cocci, the streptococci, including the pneumococcus, most cocci, yeasts, moulds, and streptothrices.

The following organisms are Gram-negative: *B. typhosus*, *B. enteritidis*, *B. dysenteriæ*, *B. coli*, *B. pestis*, *B. influenzæ*, *B. mallei*, *B. pseudo-tuberculosis*, *B. pyocyaneus*, *B. œdematis maligni*, *B. Chauvæi* (usually), *B. prodigiosus*, *B. proteus*, the septicæmic bacilli, such as chicken cholera, the spirilla and vibrios, spirochaetes and protozoa, *M. gonorrhœæ*, *M. meningitidis*, *M. melitensis*, and *M. catarrhalis*.

Gram's method of staining depends upon the formation of an iodine-pararosanilin-protein compound which is not readily dissociable in the case of the Gram-positive organisms. Pararosanilin dyes, such as gentian violet, methyl violet and victoria blue, are alone suitable for the method.

In Claudius's modification of Gram's method,[1] staining is done in a 1 per cent. aqueous solution of methyl violet (films for one minute, sections for two minutes). The preparations are washed, treated with a half-saturated aqueous solution of picric acid for one to two minutes, washed again, and dried with filter-paper. Decolorisation is then carried out in the case of covers with chloroform, in that of sections with clove oil. After decolorising, the preparations are treated with

[1] *Ann. de l'Inst. Pasteur*, xi, 1897, p. 332.

xylol and mounted. By this method the ordinary Gram-positive organisms are stained; also the bacilli of malignant œdema and of black quarter. Counter-staining may be carried out with lithium carmine.

Weigert's modification of Gram's method.—In this process the sections, whether frozen or paraffin ones, should be manipulated *on the slide.* They are stained with the anilin gentian violet and treated with Weigert's iodine solution (iodine 4–5 per cent., potassium iodide 6 per cent.) as in the simple Gram's method. The iodine is then removed with filter-paper and the sections are flooded with anilin oil two or three times. This removes the colour and dehydrates. The anilin oil is removed by flooding two or three times with xylol.

Thionine blue may be used for Gram's method, the carbol solution being employed (No. 5, p. 102). Sections are stained for two or three minutes, then treated with an iodine solution somewhat stronger than Gram's (200 parts of water instead of 300 parts). The sections, after remaining in this for one to two minutes, are decolorised in alcohol containing 1 per cent. of acetone (methylated spirit does very well), and subsequently treated as in Gram's method.

The Staining of Film Specimens.

To stain cover-glass, smear, and impression preparations, after fixing, the film is flooded with a drop or two of the solution, or the preparation, if a cover-glass, may be floated, film side down, on the solution contained in a watch-glass; if it should sink it makes little difference. Various baths or pots can be obtained for staining slides. Warming intensifies the staining properties of all staining solutions, and may be neces-

sary if deep staining is required or if the temperature of the laboratory be low (see also p. 114). When stained sufficiently, the preparation is rinsed in a beaker or tumbler of water, or in a fine stream of water, preferably distilled, to remove the superfluous colour, after which it is dried and mounted on a glass slide, film side down, in a drop of solution of Canada balsam in xylol. The preparation may be dried either by *gentle* warming over the Bunsen flame after the film has been blotted with filter-paper, or the film may be allowed to dry spontaneously in the air, in which case it should always be set up on edge to drain, preferably on a ledge of filter-paper, which is folded into a sort of compressed Z (z) shape. *The preparations must be completely dried before being mounted in balsam.*

To prevent the stain flowing all over a slide, two lines may be drawn across the slide with a grease pencil, one on either side of the area to be stained.

If there be much *débris* or other material which, when stained, would interfere with a clear view of the organisms, various expedients may be adopted. One is to stain for a short time with a solution which does not give a very dense colour, the best for this purpose being Löffler's methylene blue, or Gram's method may be made use of if the organism stains by it, and will give the best result of any. Another plan is to treat the specimen with acetic acid before staining; it may be just dipped in glacial acetic acid and immediately washed in distilled water, or immersed in 20 per cent. acetic acid for five to ten minutes, washed in distilled water, and then stained. A third is, after staining and washing, to rinse the preparation in dilute alcohol (methylated spirit 1 part, water 1 or 2 parts), and immediately wash again in water to stop the further action of the alcohol. If the film be thick, two or

three rinses in the dilute alcohol may be necessary. This process gives excellent results with the sarcinæ, but the staining agent should be anilin gentian violet or dilute carbol-fuchsin and not Löffler's blue, unless it is allowed to act for fifteen to twenty minutes. The treatment with acetic acid before staining may be combined with decolorisation with alcohol after.

Preparations can always be examined in water with the $\frac{1}{6}$ in. objective, after washing and before permanently mounting, in order to see whether they are satisfactory. A drop of water is placed on the slide, the specimen is mounted in this, film side down, and the upper surface of the cover-glass is dried with filter-paper. If satisfactory, the preparation can be slipped off, dried, and mounted in balsam; or if not stained sufficiently, or if stained too deeply, it can be stained again, or further decolorised, as the case may be. If the film is on a slide, a drop of water is put on and covered with a cover-glass.

Treatment of Sections for Staining and Mounting.

(a) *Frozen sections.*—If preserved in spirit they should be rinsed in distilled water before staining, unless the staining solution is an alcoholic one, in which case this is unnecessary. After staining they are well rinsed in water or methylated spirit to remove the excess of stain, and then dehydrated and cleared before being mounted. For dehydrating, if they have been washed in water, they should be well rinsed in methylated spirit[1] to remove the excess of water, and then transferred to absolute alcohol for a few seconds

[1] Absolute alcohol may of course be employed instead of the first bath of methylated (or rectified) spirit, but methylated answers just as well and is less expensive (but see note, p. 88).

to two minutes, the time varying with the size and thickness of the section. In many cases—for instance, when the anilin dyes have been used for staining—the sections must be passed as rapidly as possible, *consistent with thorough dehydration*, through the absolute alcohol to avoid removing too much of the colour. If it is important to avoid any decolorisation, anilin oil may be used for dehydration, as in Weigert's method (pp. 109 and 115). For clearing, xylol or cedar oil is the best agent, for neither dissolves the anilin dyes; they will only clear, however, out of absolute alcohol: hence the preliminary rinsing of water-washed sections with methylated spirit to prevent dilution of the subsequent bath of absolute alcohol. Oil of cloves can also be employed, but has the disadvantage that it dissolves the anilin dyes, and the colour of stained sections treated with it is apt to be less permanent; it has the advantage, however, of clearing out of methylated spirit, absolute alcohol being unnecessary. The alcohol and clearing agents are conveniently placed in watch-glasses or small shallow glass capsules. The section is known to be cleared when it appears quite transparent and almost invisible when the watch-glass or capsule containing it is held over a dark surface. If it appears cloudy and opaque it is not properly cleared, which results from insufficient clearing or dehydrating. If the section does not clear in a minute or two it is evidently not sufficiently dehydrated, and should be returned to a fresh bath of absolute alcohol for a short time, and then transferred again to the clearing agent. Care should be taken that watch-glasses, etc., used for the absolute alcohol and clearing agent are perfectly dry. The clearing agent, especially clove oil, can be used many times before becoming useless.

For transferring the sections from one solution to

another an ordinary needle, fixed in a light wooden handle, suffices, or, better still, a piece of glass drawn out at one end, the section being carefully lifted by one corner to prevent crumpling; but for the final process of mounting it is necessary to use a section lifter or cigarette-paper. The section, spread out with care, is raised by means of the section lifter or cigarette-paper introduced under it, and transferred to the slide, any crinkles are removed by spreading with a needle, the superfluous clearing agent is drained off, a drop of xylol balsam put on, and it is then covered with a clean cover-glass. If clove oil has been used as the clearing agent, the section, after draining, should be blotted with two or three thicknesses of filter-paper to remove as much oil as possible before putting on the balsam. In blotting firm pressure should be used, and the section will then adhere to the glass slide and not to the blotting-paper. With delicate sections all the processes of staining, dehydrating, clearing, etc., may be carried out on the slide.

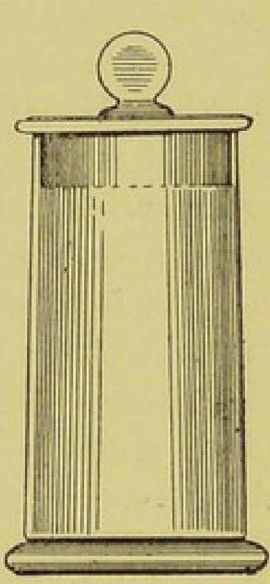


Fig. 20.—Glass pot for clearing, etc.

(b) *Paraffin sections*.—The sections having been safely fixed on the slide (p. 95), it is necessary, in order to stain and mount, to remove the paraffin by solution in xylol. The slides with attached sections are treated as follows: Immerse in (1) xylol for one or two minutes; (2) absolute alcohol one to two minutes to remove the xylol; (3) methylated spirit; (4) distilled water. They are now ready for staining, and are to be flooded with the staining solution or immersed in it, and after staining they are treated in the same manner, but in the reverse order, *i.e.* (1) distilled water; (2)

methylated spirit; (3) absolute alcohol; (4) xylol. On being removed from the xylol the slides are drained for a few seconds, a drop of xylol balsam is then put on, and the section covered with a clean cover-glass. Glass pots (Fig. 20) filled with the alcohol, xylol, etc., are convenient for the treatment of paraffin sections, the slide with the section upon it being immersed in the fluid.

Section Staining.

When Gram's method is applicable it gives by far the best results, and should always be employed. If, however, the organisms are decolorised in Gram's process some other method must be adopted. One of the best is to stain for from ten minutes to six or eight hours in Löffler's methylene blue. Fresh easily staining organisms will be sufficiently stained in ten or fifteen minutes, but when the organism is difficult to stain, as glanders, six to eight hours may not be too long a time. Warming intensifies the staining properties of all staining solutions; for frozen sections the watch-glass of stain may be warmed on a sand-bath or asbestos cardboard, or in the blood-heat incubator. Sections on the slide may be flooded with the stain and warmed on a piece of asbestos cardboard placed over a Bunsen flame, or a penny may be heated in the Bunsen and the preparation laid on it, the coin being re-heated as often as required. The stain may be prevented from flooding the slide by confining it between grease-pencil lines as described for films (p. 110). After staining, the sections are well rinsed in distilled water and then slightly decolorised by rinsing for half a minute or so in a watch-glass of 1 per cent. acetic acid in distilled water.

They are then again washed and passed as rapidly as possible through alcohol, cleared in xylol, and mounted. Carbol-methylene blue or carbol-thionine blue may be used instead of the Löffler's solution, the staining taking from a few minutes to half an hour. If a contrast stain be desired the sections may be treated for a few seconds with the eosin solution after the dilute acetic, but a better method is to use the Chenzinski solution, which is strongly recommended by Klein (see p. 102). If staining be prolonged evaporation must be prevented. In the case of a section mounted on the slide and flooded with stain, the slide should be placed on a piece of wet blotting-paper on a tile and covered with the lid of a Petri dish.

The micro-organisms in sections stained with Löffler's blue are very liable to become decolorised unless the dehydration is expeditiously performed. To avoid this Unna's method may be adopted. After staining and decolorising with acidulated water as described, the sections are placed on the slide (if not already mounted thereon), gently warmed, and so dried; they are then treated with xylol and mounted in balsam. The tissue elements, however, are apt to suffer.

A better method is, after decolorising with the dilute acid, to dehydrate with anilin oil instead of alcohol, the section being treated with fresh anilin two or three times, then with a mixture of equal parts of anilin and xylol, and finally with two or three baths of xylol.

Capsule Staining.

Many organisms, especially in the tissues or body fluids, are invested with a capsule of gelatinous matter, probably derived from the membrane of the bacterial

cell, and differing in composition in different species. The capsule may be as thick as the bacterial cell itself, and appears, in the unstained state or after staining by the ordinary methods, as a clear halo or zone surrounding the organism. Organisms in films of albuminous matter often appear to be surrounded by a clear halo, which must not be mistaken for a capsule. As organisms frequently lose their capsules on ordinary culture media, Moore recommends cultivating in fluid serum to obtain the re-development of the capsule. In order to stain the capsule one of the following methods may be adopted:

1. Stain the preparations by just dipping in the following solution:

Carbol-fuchsin	1 part
Distilled water	1 part

Rinse in water and then stain for fifteen seconds in a very weak aqueous solution of gentian violet (0·1 per cent.). Rinse in water, dry, and mount.

2. *McConkey's method.*—The following solution is prepared:

Methyl green	1·5 grm.
Dahlia	0·5 grm.
Distilled water . . .	100 c.c.

When dissolved, 10 c.c. of a saturated alcoholic solution of fuchsin are added, and the whole is made up to 200 c.c. with distilled water. The stain should not be used for a fortnight, and should be kept in a dark place. Specimens are stained for five minutes or longer, then thoroughly washed in a stream of water, dried and mounted.

3. *Friedländer's method* (for tissues).—Mix,

Concentrated alcoholic solution of gentian violet . . .	50 parts
Distilled water . . .	100 parts
Acetic acid	10 parts

Stain the sections in this solution in the warm incubator for twenty-four hours. Rinse well in 1 per cent. acetic acid, pass through alcohol and xylol, and mount in balsam.

Spore Staining.

When spore-bearing bacteria are stained by the ordinary methods the spores are just tinted, or remain uncoloured with the outlines more or less stained. This seems to be due to the fact that the spores are surrounded with a slightly permeable membrane which inhibits the entrance of the staining agent. By staining by some method which causes the penetration of the stain, and then cautiously decolorising, it is possible to remove the colour from everything except the spores, the impermeable membrane of which in the same way prevents the full action of the decolorising agent.

(*a*) *Simple method.*—A film is prepared in the ordinary way. If a cover-glass, it is floated on a watch-glass, or, if a slide, it is flooded with carbol-fuchsin, and the stain is warmed for twenty minutes. After being washed in water the preparation is rinsed for a second or two in 1 per cent. sulphuric acid and again washed at once in water. If there is still a good deal of the red colour remaining, the film may be once more rinsed in the acid, but if nearly colourless it should be mounted in water and examined with the $\frac{1}{6}$-in. objective. If the spores alone are well stained the preparation may be counter-stained with Löffler's methylene blue for two to five minutes, washed, dried, and mounted. If, however, the bacilli as well as the spores retain the red colour, the preparation must be further decolorised in the acid, while if everything has been decolorised, it may be re-stained with warm carbol-fuchsin.

The spores sometimes stain better if the preparation be fixed by passing through the flame *twelve* times instead of

three, as is usual. To obtain good preparations and ones showing the spores *in situ*, the specimens should be made as soon as spores have definitely developed in the cultures.

Spore staining often requires a good deal of patience, and in many instances it is difficult to obtain a satisfactory preparation by this simple method, in which case that of Moeller should be made use of, and rarely fails.

(*b*) *Moeller's method.*—Prepare the cover-glass or slide specimen in the ordinary way. Treat with absolute alcohol for two minutes, and then with chloroform for two minutes. Wash in water and treat with a 5 per cent. solution of chromic acid for two minutes, wash, and then stain with warm carbol-fuchsin for ten minutes. Wash, decolorise carefully in 1 per cent. sulphuric acid, again wash, and counter-stain with Löffler's methyle e blue for one minute; wash, dry, and mount. Some organisms, such as the *B. mesentericus*, stain better if treated with the chromic acid for five to ten minutes.

Flagella Staining.

Many organisms possess delicate protoplasmic processes—flagella—in greater or less number; but these are not visible when the organism is examined in the living condition (except by the use of dark-ground illumination), nor when the ordinary staining methods are employed. In order to demonstrate them it is necessary to make use of some special method, in which a mordant is essential. One of the earliest devised was that of Löffler, which with care gave fair results. It is not, however, nearly so satisfactory as some more recent ones, so is omitted.

For all methods of flagella staining the cover-glasses or slides must be absolutely clean, the cultures recent, and the growth sufficiently diluted to obtain the organisms in an isolated condition.

(*a*) *Stephens's method.*—This is a modification of the well-

known Van Ermengen method,[1] and has been communicated to the writer by Dr. J. W. W. Stephens.

To clean slides.—Rub the slides with a clean cloth and place on a piece of *clean* wire gauze and heat with a *smokeless* flame for some minutes (by this means grease is completely removed). Remove the slides when cool, not before.

To make the emulsion.—All methods are unsatisfactory. Rub a little of the culture in a small drop of tap-water in a watch-glass. Then transfer a drop with the smallest possible platinum loop to a minute drop of water on the slide. Mix and spread with the platinum wire as quickly as possible. The film thus made should dry *immediately* if a small drop only of water has been used.

Age of the culture.—A twenty-four hours' culture does quite well (a younger one is perhaps better, but flagella can be shown for a week or fortnight or more).

I. *The mordant:*

Osmic acid, 2 per cent. . . .	1 part.
Tannic acid, 20 per cent. watery solution	3 or 4 parts

II. *Silver solution:* Silver nitrate . . 1 per cent.

III. Gallic acid, 2 per cent. solution .	1 part
Ammonia fort.	1 part

To be mixed before using and to be used immediately.

To stain.—Place the mordant on the film for one or two minutes or less (time unimportant).

1. Wash in tap-water thoroughly.
2. Shake off as much water as possible.
3. Place a few drops of silver nitrate on the slide for a few seconds or longer.
4. Shake off all excess.
5. Allow one drop of the ammonia-gallic solution to fall on the *middle* of the slide from a small pipette. A wave spreads away from the centre to each end of the slide. As

[1] *Centr. f. Bakt.*, xv, 1894, p. 969.

soon as the film is seen standing out clearly and black in the centre (in a few seconds), wash off in tap-water.

6. Add again a drop or two of the silver solution and allow it to act for half a minute or thereabouts.

7. Wash in tap-water, blot, and dry over the flame.

8. It is best not to mount in balsam or in cedar-wood oil, as the preparations rapidly fade in these.

If done with any care, the film should now appear black and distinct to the naked eye with no precipitate, and the flagella will be found to be stained distinctly and intensely with hardly any ground substance, or at least insufficient to interfere with a clear view of them.

(*b*) *Pitfield's method.*—Two solutions are freshly prepared:

A.	Saturated aqueous solution of alum .	10 c.c.
	Saturated alcoholic solution of gentian violet	1 c.c.
B.	Tannic acid	1 grm.
	Distilled water	10 c.c.

The solutions should be made with cold water, filtered, and preserved in separate bottles. For use equal quantities are mixed together. The specimens are flooded with the mixture and held over the flame until it nearly boils; they are then laid aside, with the hot stain on them, for one minute, and are finally washed in water. After washing, the preparations are flooded with anilin gentian violet for one second, washed in water, dried and mounted.

(*c*) *McCrorie's method*[1] (*modified by Morton*[2]).—Prepare the following solutions:

A.	Tannic acid	1 grm.
	Potash alum	1 grm.
	Distilled water	40 c.c.
B.	"Night" blue.	0·5 grm.
	Absolute alcohol	20 c.c.

Mix and filter.

[1] *Brit. Med. Journ.*, 1897, vol. i, p. 971.

[2] *Trans. Jenner Inst. Prev. Med.*, vol. ii, p. 242.

The prepared slides are stained with this solution (which should always be filtered before use) for two minutes, the solution being changed two or three times, washed gently in running water, and then counter-stained in anilin gentian violet for one to two minutes, washed, dried, and mounted.

Preservation of Cultures.

Gelatin and agar cultures may be satisfactorily preserved by submitting them to the action of formaldehyde vapour for some hours by soaking the wool plug of the culture tube in formalin and plugging the tube with it. The tube may then be sealed with gutta-percha tissue, sealing-wax, or paraffin wax, or best of all in the blowpipe flame. Plate cultivations may also be exposed to the vapour and the lid of the dish afterwards cemented on, or the cultures may be made in the flat bottles ("Soyka's bottles") devised for the purpose, and after development treated like tube cultures.

Preservation of Pathological Specimens.

These may be preserved in the ordinary way in spirit, but a much better method, by which the natural colour of the specimen is retained, is the following. The specimens are first washed in water, and then placed in the following solution for twenty-four to forty-eight hours:

Formalin	6 parts
Sodium chloride	1 part
Sodium sulphate	2 parts
Magnesium sulphate	2 parts
Tap-water	100 parts

After being taken from the formalin solution the specimens are placed in methylated spirit for ten minutes, and then in a fresh bath of methylated; in this the colour to a large extent returns, and they should be carefully watched and not allowed to remain in it for more than an hour. They are then mounted in the following mixture:

Glycerine	400 c.c.
Potassium acetate	200 grm.
Water	2000 c.c.

A trace of formalin should be added to this.

The writer has preserved meat infected with *B. prodigiosus* very satisfactorily by the following method. Slices were cut off and placed in the formalin solution given above for a few hours. They were then well drained and placed in suitable glass capsules. Ordinary nutrient gelatin was melted and sufficient poured in to cover the specimens, and when it had set a little formalin was poured on and allowed to remain for a few days. It was then poured off and the glass top cemented down.

For further information on preparation of tissues, section cutting, staining methods, etc., see *The Microtomist's Vade-Mecum*, Bolles-Lee; *Practical Histology*, Schäfer; *Methods of Morbid Histology and Clinical Pathology*, Walker Hall and Herxheimer; and *Lehrbuch der Mikroskopischen Technik*, Rawitz.

CHAPTER IV.

METHODS OF INVESTIGATING MICROBIAL DISEASES—THE INOCULATION AND DISSECTION OF ANIMALS—HANGING-DROP CULTIVATION—INTERLAMELLAR FILMS—THE MICROSCOPE.

THE systematic study of a condition dependent on the activity of micro-organisms is in many instances no light matter. When only one or two forms are present and these are readily cultivated it may be comparatively easy, but when there are many the investigation may become exceedingly complicated. The first step to be taken is to ascertain by careful microscopical examination the general characters of any organisms that may be present in the material, and their distribution both in the fresh condition and in stained perparations, and if possible at different stages of the disease. In disease conditions, for example, the blood and secretions may be examined both before and after death, but in the latter it must be remembered that soon after the fatal event adventitious organisms rapidly make their appearance, gaining access from the air and from the intestinal tract. If organisms be detected an attempt should be made to determine whether there is any predominant form and if this is constantly present at different stages. If organisms are found, it simplifies matters; but if not, it cannot therefore be said that they are absent, for they may be few in number, and

consequently be missed in a microscopical examination; or they may be confined to a particular locality or tissue, or are present only at one stage of the infection. In addition to the microscopical examination, cultures must be made on various media, those media being chosen which will probably be suitable for the growth of the organism present in the particular condition; for example, in the examination of animal diseases, media rich in protein, such as blood-serum, peptone-agar, and gelatin, will be the most serviceable. In the examination of plant diseases, vegetable infusions prepared from the plant itself or from other sources, and enriched by the addition of vegetable proteins, and carbohydrates, should be chosen. In fermentations, beer-wort, grape or fruit juice, and saccharine solutions should be made use of; while for the nitrifying organisms, solutions containing nitrates and nitrites, salts of ammonia, urea, and asparagin will have to be employed. In addition, it will in most cases be advisable, and in all safer, in order to isolate the various species, to make plate cultivations, either in Petri dishes (p. 79), or by streaking several sloped tubes of agar, etc. (p. 81). Having obtained pure cultivations it will be necessary to determine the species of organism,[1] if it has been previously isolated and described, or to give a careful description of it, if it be a new one, for the use of subsequent investigators. In the identification or description of an organism all the following features must be carefully noted:

[1] The descriptions of a large number of species of bacteria have been collected and tabulated in convenient form by Chester in *A Manual of Determinative Bacteriology* (Macmillan & Co., 1901). The terms he suggests for describing bacterial growths, etc., might well be adopted by bacteriologists. A committee of the Society of American Bacteriologists has drawn up an elaborate chart for the description of species of organisms.

1. The morphology of the organism under various conditions, its size, form, and motility, the presence of flagella, and their number, arrangement, and character.

2. The presence or absence of spore formation, its nature, the conditions under which it occurs, and any peculiarities in the germination of the spores, and their size and location in the cell.

3. The peculiarities of staining, and the staining reaction with Gram's and the Ziehl-Neelsen methods.

4. The characters of the colonies in gelatin, agar, and other media, both surface and deep.

5. The characters of the growth on a variety of culture media at different temperatures—for example, for a pathogenic organism, on blood-serum, agar, and gelatin (surface and stab cultures), in broth and on potato; liquefaction or not of the gelatin; the growth in milk, with or without curdling, and the reaction therein; and the fermentation reactions on carbohydrates, glucosides, alcohols, etc.; the nature of the gas, if any, formed therefrom, and the H : CO_2 ratio.

6. The behaviour towards oxygen—is it aërobic or anaërobic?

7. The range of growth at different temperatures.

8. The reducing power by growing in litmus broth which becomes decolorised, or by the formation of nitrites in a solution containing nitrates.

9. The production of indole with or without nitrites.

10. The production of pigment and the conditions under which it occurs.

11. The pathogenic action on various animals if it be a disease germ, or the changes which it produces if it be an organism connected with other conditions.

12. The chemical changes which it induces.

13 The thermal death-point and the action of germicides and antiseptics upon it (see Chapter XXII).

For descriptive purposes, "standard" culture media should always be employed, and the acidity or alkalinity of the medium stated (p. 64).

It must never be forgotten that under cultivation the properties of organisms may be considerably modified, and due allowance must be made for this. For example, pathogenic organisms may lose their virulence more or less completely, pigment production be lost, and fermentive action modified. An instance of the latter is given by Percy Frankland; a bacillus isolated by him possessed the power of fermenting calcium glycerate, but after cultivation on ordinary gelatin it completely failed to do so (see also p. 6).

To obviate these difficulties the organisms should be cultivated under as nearly natural conditions as possible and sub-cultivation avoided so far as can be. No general rule can be given as to the duration of life of cultures on artificial media. Most organisms will retain their vitality for at least three or four weeks without being transferred to a fresh soil, some for many months; a few must be sub-cultured every week, or they will die out; while there are still a small number, such as leprosy and relapsing fever, which so far have rarely or never been cultivated. On the whole, organisms retain their vitality best on gelatin.

For an organism to retain its virulence it is, as a rule, necessary to pass it through a susceptible animal at longer or shorter intervals, and to enhance the virulence recourse must be had to a succession of passages through susceptible and then less susceptible animals. In this way the virulence of organisms has been increased to a point far greater than is ever met with naturally, as in the case of the *Streptococcus pyogenes*. If an organism retains its virulence, even slightly, it is generally possible, by employing large doses, to enhance this by passage through a susceptible animal. Another method may also be adopted, namely, to inject along with it some other pathogenic form,

such as the *Streptococcus pyogenes*; the combination will kill the animal, and the slightly virulent organism can be recovered and will be found to have increased in virulence. A third method is to inject the organism into a susceptible animal together with a lethal dose of toxin obtained from a virulent form of the same species, or with some substance, such as lactic acid, which lowers the vitality of the tissues. The slightly virulent organism will then be able to grow under the more favourable conditions, and a form which has become completely non-virulent can be made to regain its lost virulence.

Collodion sacks are now frequently used to study the action upon animals of the dialysable products produced by micro-organisms which do not form any appreciable amount of toxin *in vitro*, for cultivating species which are difficult to grow by ordinary methods, for studying the phenomena of infection when the micro-organisms are protected from the phagocytes, and for other purposes. A glass rod or small test-tube, according to the size desired, is dipped into a beaker containing the ordinary (*not* flexible) collodion, is then withdrawn and allowed to dry, and the process is repeated two or three times. In order to detach the collodion from the glass, the whole is dipped for a few seconds alternately into strong spirit and into water, the collodion loosens, and may be easily peeled off the glass. The sack may be sterilised by placing in a test-tube and heating to 150° C. in the hot-air steriliser.

For the inoculation of animals various methods may be adopted. In some cases, after clipping the hair, the organism may be introduced by rubbing into the skin after scarification, or, a small incision having been made through the skin, a small quantity of a culture may be introduced on a platinum needle; or a broth

culture or an emulsion, made with sterilised water or broth, may be injected with a sterilised syringe subcutaneously, intra-peritoneally, or into the muscular or other tissues or organs as required, since the seat of inoculation may have to be varied for the different species to produce their pathogenic effect. For injection purposes a syringe like an antitoxin syringe, *i. e.* with asbestos or metal piston and glass barrel that can be boiled, may be used. Several sizes are kept in stock, 1 c.c., 2 c.c., and 5 c.c. at least being required. An all-glass syringe is a still better form, but is expensive. For accurate dosage, the piston-rod should be graduated and have a nut travelling on a screw up and down it. Before use the syringe with the needle should be boiled for ten minutes to sterilise it; after use it may be well rinsed and again boiled. The needles should be wiped dry and a wire inserted, or they may be kept in a bottle of xylol.

Guinea-pigs and rabbits are usually inoculated in the thigh or abdomen; mice in the dorsal region or at the root of the tail (dorsally), the hair being clipped, and the skin disinfected, but this is not generally necessary. Numerous mechanical holders have been devised for animals, but are not as a rule required. Rabbits may be inoculated intra-venously by one of the large veins in the ear. The ear is shaved, and the skin is well washed with a little alcohol with vigorous rubbing; the base of the ear is lightly pinched so as to obstruct the venous but not the arterial circulation, and render the vein prominent, and the injection is made with a small syringe fitted with a fine needle, the needle being passed into the vein towards the base of the ear. After the withdrawal of the needle the wound is compressed for a little and may be dressed with some antiseptic wool and collodion.

The phenomena occurring after inoculation must be noted. Usually these are not very obvious in the rodents, but loss of appetite, sluggishness, staring coat, convulsions, etc., may be observed. The weight of the animal is a good index of what is happening. If the infection is serious, the weight rapidly falls; if the animal is to recover, its weight soon begins to increase after the preliminary fall. The temperature in the rectum may also be taken, but is not so valuable, as in the guinea-pig variations occur from mere handling or other slight causes. The temperature of the guinea-pig averages 38·6°, but varies between 36° and 39° C. (Eyre).

The examination of the dead animal should be carried out with as little delay as possible. For dissection, the body should be pinned out on the back on a board, which may stand in a shallow enamelled iron pan, by pins or nails through the feet, and the abdomen well soaked with antiseptic solution, not so much to sterilise the skin as to prevent the hair from getting into the incision; to obtain complete sterilisation of the skin, it is preferable to clip or shave the hair and then sear with a red-hot iron. Knives, forceps, scissors, etc., should be well boiled in an enamelled-iron mug or pie-dish, the water being kept boiling during the progress of the dissection and the instruments rinsed from time to time in it. A little sodium carbonate may with advantage be added to the water. A small enamelled-iron fish-kettle with perforated strainer forms an excellent steriliser for instruments, or a surgical instrument steriliser may be used. An incision is made and the skin well reflected and pinned out; the knife and forceps should then be re-sterilised, or fresh sterile instruments taken, for the deeper incision and opening the body cavities; these again must be re-sterilised, or a third set of instruments employed for incising the organs.

During the progress of the dissection the condition of the tissues at the seat of the inoculation should be noted, and likewise the conditions of the serous membranes and the various organs. In many diseases the organism is met with most abundantly in the spleen, in others in the blood, and in some at the seat of inoculation. When a systematic examination is made, film specimens and cultures on two or three media, aërobic and anaërobic, should be prepared from the seat of inoculation, the spleen, liver, lungs, and heart-blood, and in some cases from the serous membranes, muscles, or central nervous system in addition, the carcase being in the intervals covered with a bell-jar which has been rinsed in, or with filter-paper moistened with, antiseptic solution. An assistant is often useful or even necessary. The greatest care must be taken to avoid dropping or splashing or otherwise disseminating infective material, any stains being immediately swabbed up with antiseptic solution; and the operator must exercise every precaution to prevent the infection of himself and others. It is convenient to have some efficient antiseptic solution near at hand; it may be kept in a large bottle on a wall bracket and drawn off as required by a syphon tube provided with a tap or spring clip. The most generally used antiseptics are 5 per cent. carbolic, and 1–500 corrosive sublimate, but 2 per cent. cyllin or kerol or 3 per cent. lysol is cheaper and more efficient. The access of flies to the carcase must also be guarded against, as they might carry infection. When finished with, the carcase should be efficiently disinfected and disposed of without delay, preferably by burning it, together with the board on which it has been pinned out.

If the carcase be left, especially in warm weather, for even a few hours before the examination is carried

out, the tissues are liable to become invaded and infected by organisms from the respiratory and digestive tracts. In the *post-mortem* room, infection of the tissues is very common; out of fifty cases, Symes[1] found only seventeen to be sterile. Ford states that even in normal animals, killed and immediately examined, bacteria are present in 70 per cent. of the internal organs.[2]

When the blood of an animal is required several expedients may be adopted. If the animal is not needed for further experiment, it may be decapitated and the blood collected in a porcelain dish; but if a sample only is wanted, and the animal has to be further treated, as in antitoxin work, it may be bled from the carotid, the vessel being afterwards ligatured. In the rabbit a small artery passes superficially across the inner aspect of the thigh; this permits of the withdrawal of a small quantity of blood without the necessity of an operation such as is required to expose the carotid. The simplest method of all is to introduce the fine point of a piece of glass tubing, drawn out and bent to a convenient angle, or the needle of a syringe, into one of the ear veins and aspirate the blood into it. Or the vein may be punctured and the blood allowed to drip into a small tube.

Blood may be obtained from a patient for the agglutination reaction, for microscopical examination, or for culture experiments, by pricking the finger or the lobe of the ear with a sterile needle, preferably a flat one of the "Hagedorn" type, or with half a steel pen (nib) or a glass point; for disinfection, the skin may be rubbed with a little alcohol or ether. The blood may be collected in vaccine tubes, small bulbous tubes (Fig. 7, p. 51), or Wright's tubes (Fig. 35, p. 225).

[1] *Lancet*, 1899, vol. i, p. 365.

[2] *Journ. of Hygiene*, vol. i, No. 2, 1901, p. 277.

Organisms, in natural infections in man, are usually present only in small numbers in the blood, and for demonstrating them by culture methods it is necessary to withdraw 2–5 c.c. from a superficial or deep vein by means of a sterile syringe under aseptic conditions, and to inseminate broth tubes or agar plates each with ½ c.c. of the blood.

Although the modern methods of isolation and cultivation have rendered immense service to bacteriology, they have also had the effect of diminishing the attention paid to the exact morphology and biology of organisms. At the present time there is a tendency to investigate bacteria *en masse* rather than to study them as individual living forms, and the following remarks by the late Marshall Ward[1] may be aptly quoted in this connection :

" We must remember that De Bary and Brefeld had aimed at obtaining a single spore isolated under the microscope, and tracing its behaviour from germination continuously to the production of spores again; and when we learn how serious were the errors into which the earlier investigators of the mould fungi and yeasts fell, owing to their failure to trace the development continuously from spore to spore, and the triumphs obtained afterwards by the methods of pure cultures, it is not difficult to see how inconclusive and dangerous all inferences as to the morphology of such minute organisms as bacteria must be unless the plant has been so observed. As a matter of fact, the introduction and gradual specialisation of Koch's method of rapid isolation of colonies encouraged the very dangers they were primarily intended to avoid. It was soon discovered that pure cultures could be obtained so readily that the characteristic differences of the colonies in the mass could presumably be made use of for diagnostic purposes, and a school of bacteriologists arose who no longer thought it necessary to patiently follow the

[1] *Nature*, vol. lvi, 1897, p. 455 *et seq.*

behaviour of the single spore or bacillus under the microscope, but regarded it as sufficient to describe the form, colour, markings, and physiological changes of the bacterial colonies themselves on and in different media, and were content to remove specimens occasionally, dry and stain them, and describe their forms and sizes as they appeared under these conditions. To the botanist, and from the point of view of scientific morphology, this mode of procedure may be compared to what would happen if we were to frame our notions of species of oak or beech according to their behaviour in pure forests, or of a grass or clover according to the appearance of the fields and prairies composed more or less entirely of it, or—and this is a more apt comparison, because we can obtain colonies as pure as those of the bacteriologist—of a mould fungus according to the shape, size, and colour, etc., of the patches which grow on bread, jam, gelatine, and so forth."

Examination in the Fresh State.

One essential procedure in the investigation of an organism is its examination in the fresh and living condition. This may be done by placing a droplet of sterile water, broth, or salt solution on the slide, inoculating with a trace of the organism or growth, and covering with a cover-glass and examining microscopically. The action of stains and reagents on the organisms may be observed by the irrigation method. A drop of the stain or reagent (C, Fig. 21) is placed on the slide, A, just in contact with one margin of the cover-glass, B, and is drawn through the preparation by means of a small piece of filter-paper, D, placed on the other side, a torn margin touching the film of fluid at one edge of the cover-glass.

The filter-paper absorbs the fluid from under the cover-glass, leaving the cells and other particles behind, and at the same time the reagent on the opposite side

flows under the cover-glass to take the place of the absorbed fluid. Afterwards the excess of the reagent or stain may be washed away by running in water under the cover-glass in a like manner. Care must be taken that no fluid gets on to the upper surface of the cover-glass, which must always be kept dry. The advantage of this method is that it may be applied while the specimen is being examined under the microscope, and the action of the reagent on a particular cell or granule can, with a little care, be watched. If the cells be large and it is desirable to avoid pressure of the cover-glass, a fine hair or bristle may be so placed

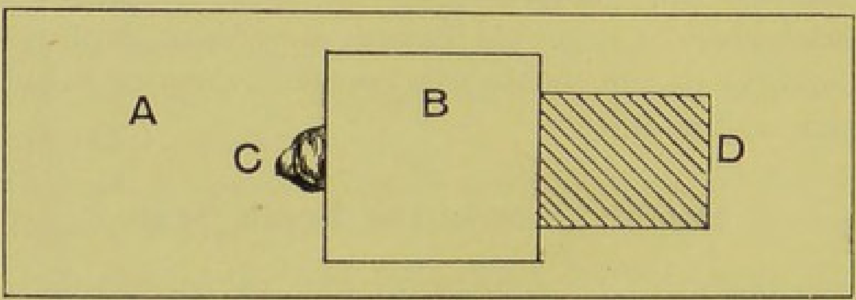


Fig. 21.—Method of irrigation.

on the slide that when the cover-glass is lowered one edge rests on it. If the specimen has to be kept for any length of time, the film of fluid will before long evaporate and the preparation become dry. To prevent this a ring of oil or vaseline may be painted round the margin of the cover-glass so as to seal it to the slide.

A simple method for keeping organisms under examination for a lengthened period of time, and of watching their growth and development, is by the use of hanging-drop preparations. To prepare a hanging drop, a ring of vaseline is painted round the margin of the hollow of a hollow-ground slide (or other cell, see below). A cover-glass is sterilised by flaming in the Bunsen, care being taken not to heat sufficiently to

melt it. A droplet of some sterile fluid medium—water, broth, wort, sugar solution, etc.—is then placed in the centre of the cover-glass with a sterile platinum loop. This droplet is then inoculated with the organism which is to be observed, care being taken not to add too many organisms—a few isolated organisms and small groups in each field is what should be aimed at. The vaselined cell is now taken and turned over, so that the ring of vaseline is downwards, and is then placed on the cover-glass, in such a way that the droplet is situated in the middle of the hollow, but not touching the slide at any point. The cover-glass adheres to the slide by means of the vaseline, and on quickly inverting the whole, so that the fluid has no time to run, it will be found that the droplet is hanging

Fig. 22.—Hanging-drop preparation.

from the under surface of the cover-glass in a cell which is hermetically sealed by the vaseline, and evaporation is thus rendered impossible (Fig. 22). Such a preparation, in fact, can be kept for a week or ten days in a warm incubator without drying up. Great care must be exercised in examining a hanging-drop specimen microscopically, especially with the immersion lenses, for the slightest pressure breaks the unsupported cover-glass. It often saves time first to centre the drop with the low power before examining with the immersion lens; an ink or pencil dot at the margin of the drop aids focussing. The light must be diminished by closing the diaphragm, lowering the condenser, etc. (p. 140), and artificial light is generally preferable to daylight. The central parts of the drop only should be examined, not the margin.

Instead of hollow slides, various devices may be employed to form the cell. Metal, glass, or vulcanite rings, or rings cut out of thin sheet lead, tin-foil, card-board, or two or three thicknesses of paper or filter-paper may be cemented on to slides with vaseline, Hollis's glue, gold size, or Canada balsam, or even a thick ring of vaseline may be used.

The only certain method for ascertaining whether an organism is motile or not—often an important clue to its identification—is by the use of hanging-drops. Actively motile organisms may frequently be met with in a resting stage, although still alive, and various factors may bring about this condition, such as old age, exhaustion of nutriment, excessive heat or cold, electric shocks, and the like. The absence of movement of an organism in a specimen prepared from an ordinary culture, particularly if more than a day old, does not necessarily prove that it is non-motile. A hanging-drop should be prepared with a nutrient medium (the best, perhaps, is glucose broth) and placed under conditions of temperature, etc., favourable to the growth of the organism, and examined after an interval of an hour or so, or better still at intervals of half an hour for three or four hours. In this time the old cells will revivify, and new ones will have been produced, and if the organism be a motile one, more or less active movement of some of the cells is almost sure to be observed. It is necessary to beware of two fallacies in connection with motility—not to mistake for it the so-called Brownian movement, which is a vibratory one backwards and forwards about one point, and common to all fine particles suspended in a fluid; and not to be misled by a flotation of the cells due to currents set up in the fluid from some cause or other—all the particles then tending to move *in the same direction*.

Another purpose for which the hanging-drop cultivation may be employed is that of obtaining a permanent record of the various phases through which an organism may pass during its development. If a number of these cultivations be made, say twenty, in an exactly similar manner, and afterwards kept under identical conditions, and if at the end of every half-hour one of the preparations be taken, its cover-glass carefully removed, and the droplet dried and stained, a permanent record of the life-history of the organism is obtained extending over ten hours.

Various more elaborate forms of cells for hanging-drop preparations can be obtained, some being provided with inlet and exit tubes for the passage of various gases. For anaërobic preparations cells are made having a groove at the bottom into which a mixture of pyrogallic acid and potash is introduced.

The observation of hanging-drop cultivations at blood-heat can be carried out on some form of warm stage.

Interlamellar films.[1]—Another method of investigating the life-history of organisms, especially moulds and protozoa, is by means of interlamellar films. A glass slide 1½ by 3 in. is sterilised in the Bunsen flame, and while hot three small drops of sealing-wax are placed on it, so arranged that they form the apices of an equilateral triangle, the side of which measures about one inch, and a drop of sterile nutrient medium is deposited between them. A cover-glass of about 1¼ in. in diameter is then sterilised in the Bunsen flame, a droplet of a suitable nutrient medium is placed upon it and inoculated with the organism to be observed, and the prepared cover-glass is picked up with sterilised forceps, inverted, and lowered on to the slide. The

[1] Delépine, *Lancet*, 1891, vol. i, June 13th.

nutrient medium is thus contained between the slide and the cover-glass, and by using a hot wire, and so softening the sealing-wax, it can be spread out to form as thin a layer as desired. The preparation is kept in a moist chamber to prevent evaporation, and can be studied when required.

The Microscope.

A bacteriological microscope should be of the monocular form, and have a rack-and-pinion coarse adjustment and an efficient fine adjustment. The stage, preferably of vulcanite, should be large and roomy and quite plain, with two or more holes at its margin to receive spring clips for fixing the slide. For the ordinary examination of specimens a mechanical stage is not needed; in fact it hampers that freedom of manipulation which is so useful for the rapid examination of a specimen. For some purposes a mechanical stage is very useful, and for a critical survey of the whole of a specimen, *e. g.* a blood-film, it is essential. A detachable form is to be preferred (Fig. 23), so that, if required, the stage may be free for the examination of plate cultivations, etc.

A sub-stage condenser is essential for all work in which high powers are employed, and also enhances the value of low powers. It consists of a system of lenses below the stage, by means of which the light is concentrated on the object. It should have a rack-and-pinion, or a screw, adjustment for focussing, and be provided with some form of diaphragm for modifying the light, preferably an "iris." The condenser must be centred—that is, adjusted so that its optical axis corresponds with the optical axis of the objective; and for this purpose it is usually provided with two lateral

screws working at right angles to each other, by means of which its position relative to the optical axis can be altered. In order to centre, a diaphragm with small aperture is used, and the hole in the diaphragm is focussed with a low power; then, by means of the lateral screws, this hole is brought into the centre of the field. Below the sub-stage condenser a mirror with concave and plane surfaces should be fitted, the *plane* surface being used with the condenser, as a general rule. The

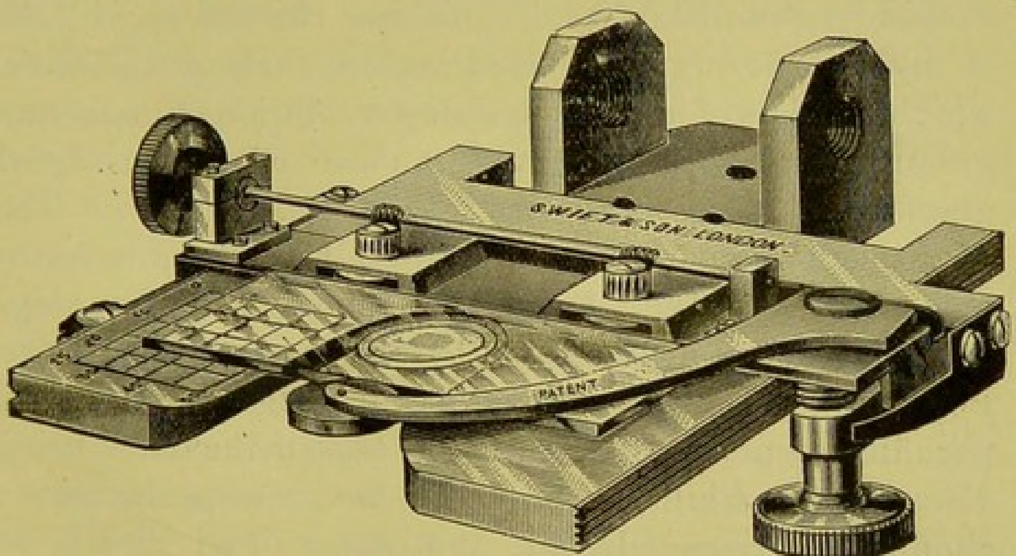


Fig. 23.—Swift's detachable mechanical stage.

concave mirror may be used for illumination with low-power objectives, the condenser being detached or swung out of position. The necessity for careful illumination must be insisted upon; in fact, to obtain the best results the light should be readjusted for every specimen by mirror, diaphragm and condenser, *i. e.* "critical" illumination should be aimed at. A good specimen may be utterly spoilt, visually, by faulty illumination; while an indifferent one may be made to look passable by proper illumination. In the examination of micro-organisms in the fresh or living and unstained condition, it is necessary, *as a rule*, to

diminish the light by means of a small diaphragm, or by racking down the condenser, or by both; while for stained or opaque objects the full aperture of the diaphragm, or thereabouts, may generally be employed. It must be remembered, however, that the resolving power of a lens (see below) is diminished by closing the diaphragm and by throwing the condenser out of focus; the illumination then becomes "non-critical." For fine work, if the illumination is too intense, this should be diminished by diminishing the source of light or by interposing a coloured screen, such as Gifford's, which consists of a cell containing a solution of malachite green in which is inserted a piece of green signal glass. Coloured glass may also be interposed. The microscopist should accustom himself to examine specimens both by daylight and by artificial light; hanging-drop specimens are usually best seen with the latter. For artificial light, probably nothing surpasses a paraffin lamp with flat wick, the *edge* of the flame being always used, while to obtain the best results the mirror should be removed, and the flame used direct by elevating and tilting the microscope somewhat. For the finest work, daylight illumination is inadmissible. An admirable form of electric lamp is the "Barnard," made by Messrs. Swift & Son, the source of illumination being a Nernst lamp. For ordinary routine work, an incandescent carbon or metal filament electric lamp, a Nernst lamp, or an argand or incandescent gas burner may be used. Various devices have been introduced for the employment of monochromatic illumination, *e. g.* the quartz mercury vapour lamp by Barnard.

With the filament, Nernst, or incandescent gas, lamps, the image of the filament or mantle is troublesome when the condenser is in focus; this may be obviated to

some extent by the use of frosted bulbs or by interposing a screen of fine ground glass, by the use of Gordon's glass rod illuminator, or by interposing a spherical flask filled with water or dilute copper sulphate solution. Incandescent bulbs may be frosted by dipping in a 15 per cent. solution of caustic soda and allowing to dry.

Two eyepieces are sufficient, and the lower-power ones are to be preferred, such as the B and C of the English, or the 2 and 3 of the Continental, makers. Although increased magnification can be obtained by the use of a high-power eyepiece, it is at the expense of definition, the image losing its sharpness, because the eyepiece magnifies the image formed by the objective, and any imperfections in the latter are made more apparent, so that the use of very high eyepieces is not to be recommended, except with the finest lenses; moreover, as will be pointed out later, it is useless to increase the amplification beyond a certain point.

With regard to the length of the tube of the microscope, this differs in the English and Continental systems. The standard English tube-length is 8·75 in., the Continental is 6·3 in., and is usually adopted, but the longer tube gives greater amplification. The tube of the microscope is generally provided with an inner, or draw-tube, by means of which its length can be nearly doubled; this gives increased amplification, but at the expense of definition, at least with the higher powers which are corrected or adjusted for a definite tube-length.

The lenses or objectives must next be considered.

For powers higher than the $\frac{1}{6}$-in., or thereabouts, it is advisable, for many reasons, to employ the immersion system of objectives. With these lenses a drop either of water, in the water-immersion system, or of

cedar oil, in the oil-immersion one, is placed on the cover-glass, and the objective is racked down so that its front lens touches and is immersed in either the water or oil, as the case may be. It is a good plan then to raise the objective very slightly by means of the coarse adjustment, still, however, keeping it in contact with the drop of water or oil. The observer then, looking down the microscope, very cautiously and gradually racks down again with the coarse adjustment until the object comes into view, and finishes the focussing with the fine adjustment. The fine adjustment should only be used after the object has been brought into view by means of the coarse adjustment. After the examination has been concluded for the day, the lens should be carefully wiped with a soft rag, or preferably with a piece of soft Japanese paper, to remove the water or oil. If the oil should happen to dry on the lens, it may be removed by wiping with a soft rag or Japanese paper moistened with xylol, quickly drying with another rag or paper. Instead of cedar-oil, a liquid paraffin has also been used.

The $\frac{1}{12}$ in. (2 mm.) oil-immersion lens is the one usually selected. It combines sufficient magnification for most purposes, with adequate working distance for convenience in using. If expense is not an object, the Zeiss $\frac{1}{8}$ in. (3 mm.) apochromatic oil-immersion lens is a very fine one for general use. By means of the compensating oculars sufficient magnification can be obtained, while the working distance is greater, the field is larger, and the penetrative power is greater than with the $\frac{1}{12}$ in. lens.

The immersion system of objectives has many advantages: the loss of light is less, the distance between the cover-glass and the front of the objective—the working distance, as it is

termed—is greater, and more can be seen with an immersion lens than with a dry lens of equal magnifying power. This can be best illustrated by means of two simple diagrams.

In Fig. 24 let *c d* represent the surface of a fluid, either water or oil, and let *a b* be drawn perpendicular to this surface, and cutting it at *y*. Let *r y* represent a ray of light proceeding from a rarer medium, such as air, into a denser one, water or oil. As is well known, this ray when it enters either the water or the oil does not continue in the same direction, but is "refracted" or bent nearer the perpendicular

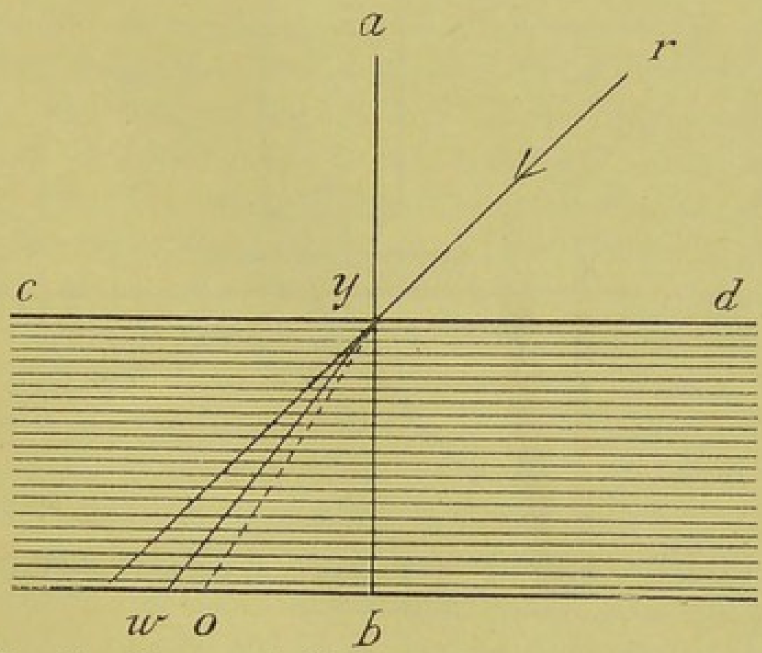


Fig. 24.—Diagram to illustrate the refraction of light.

a b, the bending being more marked with oil than with water. Thus we may suppose that the direction of the ray in water would be represented by the line *y w*, and in oil by the dotted line *y o*. Conversely, a ray of light proceeding from a denser medium into a rarer is bent away from the perpendicular, and the rays *w y* in water, and *o y* in oil, would, on emerging into air, proceed in the direction *y r*.

In Fig. 25 (which for convenience is drawn somewhat out of proportion) let S represent an ordinary glass micro-slide, X a layer of Canada balsam in which the object is mounted, and covered with the cover-glass G, while L is the objective

with its front lens. Let the object be illuminated by the ray of light *Y y*; this on entering the glass of the slide and the Canada balsam will be refracted or bent nearer the perpendicular and will proceed in the direction *y t*. Canada balsam, and also cedar oil, produce about the same amount of "refraction," or bending of a ray of light, as crown glass, and hence these three substances—crown glass, Canada balsam, cedar oil—are said to have the same "refractive index," and, consequently, the glass of the slide, the Canada balsam,

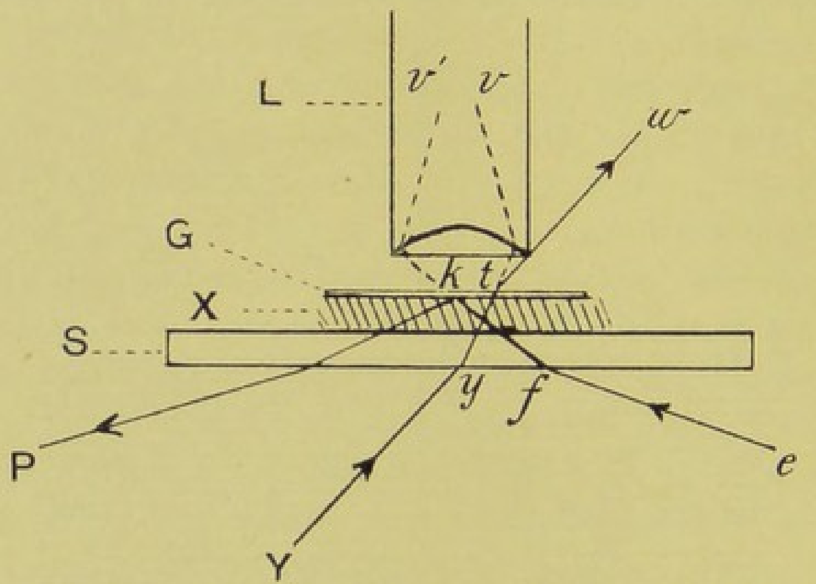


FIG. 25.—Diagram to illustrate the course of rays of light through an objective.

and the cover-glass act as one homogeneous medium, and the line *y t* is a straight one. In the first place, let us suppose that the objective L is a dry one, having a layer of air between its front lens and the cover-glass; then the ray of light, on emerging from the cover-glass into the air, is now bent away from the perpendicular and pursues a direction practically parallel to its former one, represented by the line *t w*, and misses the lens altogether—the lens is unable to take it up. If, however, we suppose that our objective is an oil-immersion one, and that a drop of cedar oil takes the place of the layer of air between the cover-glass and the front lens in the foregoing example, then the glass slide, Canada balsam,

cover-glass, cedar oil, and the front lens of the objective form practically one medium; they all have the same refractive index and produce the same amount of refraction or bending of a ray of light. Therefore the direction of the ray forms a straight line in all these, and the ray passes into the objective as is represented by the broken line *t—v*. More important still, however, is that which happens to rays which fall on the slide at a very oblique angle. In the same figure (Fig. 25) let *e f* represent such a ray; on entering the slide it will be refracted, and its passage through the slide, balsam, and cover-glass may be represented by *f k*. As before, let us suppose that in the first place our objective is a dry one, and that we have a layer of air between the cover-glass and its front lens. In this case, if the angle which *f k* makes with the perpendicular is greater than about 39° or 40°, the ray, instead of emerging from the cover-glass into the layer of air, is totally reflected by the cover-glass and pursues a course roughly represented by *k* P, so that it never enters the objective. If, however, we employ an oil-immersion objective, with oil instead of air between the cover-glass and its front lens, then, as before, the slide, balsam, cover-glass, oil, and front lens of the objective form practically one homogeneous whole, and the ray *e f k*, instead of being totally reflected, continues its course in a straight line, and is taken up by the objective, as is represented by the dotted line *k—v'*. Hence we see that the same rays which are unable to enter a dry objective are admitted by an oil-immersion one, and that an oil-immersion lens can take up rays which fall on the slide at a very oblique angle.

In order that these oblique rays may be present, ready to be taken up by the oil-immersion objective, it is necessary to employ a sub-stage condenser. It is only by means of a sub-stage condenser that a "wide-angled cone of rays," as it is termed, is obtained. Hence to make full use of an oil-immersion objective—to "get most out of it"—it is absolutely essential to employ a sub-stage condenser, and for the finest work a special "oil-immersion condenser" is employed.

It will also be obvious that although a water-immersion objective admits more rays than a dry one, it does not admit so many as an oil-immersion. It must be pointed out, however, that Canada balsam, or some medium having the same or a higher refractive index, must be used for mounting to obtain the full advantage of the oil-immersion system. The oil-immersion can of course be used for examining objects mounted in water, etc., cedar oil being still used between the cover-glass and the lens. It is to be noted that a dry objective cannot be used as an immersion one, nor an immersion objective dry, as the construction differs in the two cases.

Of late "dark ground illumination" has been much employed, particularly for the examination of living objects. In this special condensers are used, the central rays passing through which are "stopped out," so that the object is illuminated only by very oblique rays and appears white on a dark background. A stop has also to be introduced into the objective, and slides and cover-glasses of special thickness together with brilliant illumination are necessary

The lenses in the objective are formed by cementing together different kinds of glass in order to correct for "spherical" and for "chromatic" aberration. The rays passing through the margin and the centre of a simple lens are not focussed at the same point, and a distorted image is the result; this is known as "spherical aberration," while the violet and red ends of the spectrum, being of different refrangibility, and a simple lens acting like a prism, coloured fringes are observed; this is termed "chromatic aberration." The apochromatic system of objectives and eyepieces has these defects very perfectly corrected by the use of special glass and fluorite, correction being partly effected in the objective, and this is completed by combination with the special eyepieces. The latter, termed "compensating oculars,"

are therefore essential for perfect correction with apochromatic objectives, but can also be used with ordinary lenses. For photographic purposes apochromatic lenses are far superior to achromatic ones. Apochromatic objectives are, however, expensive, and though *advantageous* are not really *necessary* for *ordinary* bacteriological work.

In consequence of certain optical principles, the "diffraction" theory, for details of which the reader must refer elsewhere,[1] it is useless to increase the magnifying power of objectives beyond a certain point; for, although the object viewed appears *larger*, no more *details of structure* can be made out.

The use of the immersion system increases the "resolving power," or the amount of detail which can be seen. Thus, if a number of fine equidistant parallel lines be ruled on a glass plate, it is impossible to see with a dry lens, using white light, more than about 90,000 lines to the inch as isolated lines. If more are ruled they will not appear, and practically nothing is visible. With a water-immersion objective it is possible to see about 120,000 lines to the inch, and with an oil-immersion as many as 146,000 lines to the inch, as separate lines—a clear gain in resolving power in the latter case of about one half over a dry lens.[2] As it is necessary, in order to see such fine structures as lines ruled 50,000 or more to the inch must be, to have considerable amplification in addition to resolving power, not much is gained, in ordinary work at any rate, by adopting the immersion system for the lower power objectives, such as the $\frac{1}{6}$-in.

By the physical theory of microscopical visibility, it can

[1] See *Carpenter on the Microscope*, edited by Dallinger. (Churchill.)

[2] These figures refer to lenses having a numerical aperture of 1·0 (dry), 1·33 (water), and 1·4 (oil).

be shown that objects having a diameter of less than about 0·16 μ cannot be seen with the best optical appliances. If, then, a micro-organism is less in size than this it could not be seen microscopically, and this fact may explain why it is that in certain undoubted infective diseases no micro-organism has yet been isolated. Of the existence of such "ultra-microscopic" organisms we have proof. The finest porcelain filters, such as the Chamberland B, do not allow visible particles to pass through, yet in several instances, if the infective material be filtered through such a filter, the filtrate is still infective. This is the case with the blood serum in yellow fever, Cape horse sickness,[1] dog distemper, hog cholera and swine fever, in bird and cattle plagues, and with the juice of bird molluscum. The organism of cattle pleuro-pneumonia is just on the limit of visibility. The rabic and vaccine viruses also seem capable of passing through a Berkfeld V. These experiments do not necessarily prove that the organism *in all stages* is invisible.[2] Siedentopf and Zsigmondy have devised a method whereby ultra-microscopical particles may be rendered visible, bnt inasmuch as they appear merely as luminous points, it is questionable whether the method will be of great service in bacteriology.

There is no real necessity in bacteriological work for the immersion objective to be provided with a "correction collar." The "correction collar" is an additional screw in the objective by means of which the distance between some of its constituent lenses can be altered to "correct" for varying thicknesses of cover-glass, etc., and though necessary with the higher power dry lenses, it is theoretically unnecessary with the immersion system. Nevertheless, as slight variations do occur in the various media, glass, oil, etc., and they may not form a truly homogeneous whole, for the finest work the correction collar is still desirable. So much

[1] *Journ. Comp. Path. and Therap.*, vol. xiii, 1900, p. 1.

[2] See Roux, *Bull. de l'Inst. Past.*, vol. i, 1903, pp. 1 and 49 Remlinger, *ibid.*, vol. iv, 1906, pp. 337 and 385.

for the high-power objectives. As regards the lower powers, which, of course, are dry, a $\frac{2}{3}$-in. and a $\frac{1}{6}$-in. are generally selected. The $\frac{2}{3}$-in. is a more serviceable lens than the 1-in. which is often recommended. A very useful accessory is a "double" or "triple nosepiece." This consists of a light metal framework, which is attached to the lower end of the tube of the microscope, on to which two or three objectives can be screwed. The framework can be rotated, thus bringing each objective in succession into the optical axis of the instrument, and the necessity for unscrewing and screwing on each time an objective is changed is obviated. A microscope such as described, with sub-stage condenser, two eyepieces, a $\frac{2}{3}$-in. and a $\frac{1}{6}$-in. dry and a $\frac{1}{12}$-in. oil-immersion objectives, triple nosepiece, etc., complete in case, can be obtained for about £15, and it is well to add another sovereign or two for superior finish. Both British and Continental firms supply microscopes arranged as indicated, and in this department the English makers hold their own.

The measurement of micro-organisms is carried out by means of a stage micrometer, alone, or in combination with an eyepiece micrometer. The former consists of a scale of tenths and hundredths of a millimetre or hundredths and thousandths of an inch ruled in fine lines on a glass plate, by means of which the measurements can be made by focussing the scale under the microscope. The stage micrometer is placed in position on the stage and the scale is focussed with the particular ocular, objective, and tube length which are to be used. A drawing of the scale is made with a camera lucida; the micrometer is then removed and the object placed in position and a second drawing is made of the object on the scale already drawn. A

simpler and less expensive arrangement is to make use of a disc of glass ruled with equidistant fine lines, which can be placed in the eyepiece by unscrewing the top lens and dropping it on the diaphragm below. The value of the divisions in the eyepiece scale is first ascertained by means of the stage micrometer. The stage micrometer is then removed and the object to be measured put in its place, and its dimensions are determined by means of the eyepiece scale. With the eyepiece micrometer, the value of the divisions is first ascertained by means of the stage micrometer, which is then replaced by the object. If the objective or the eyepiece be changed the value of the divisions of the eyepiece scale in both cases will be altered, and must again be determined by means of the stage micrometer. The unit for microscopical measurement is the micron (sometimes erroneously termed a micro-millimetre), which measures one thousandth of a millimetre, or $\frac{1}{25000}$ of an inch, nearly, and is designated by the sign μ.

If a micrometer is not available, rough measurements may be carried out by comparison with a red blood-corpuscle. The majority of the red corpuscles of normal human blood measure 7·5 μ in diameter.

CHAPTER V.

INFECTION—VEGETABLE AND ANIMAL PARASITES—THE INFECTIVE PROCESS — ANTI-BODIES — ANTI-SERA AND ANTITOXINS—IMMUNITY.

Infection.

By the term INFECTION is meant the invasion of the living tissues by living micro-organisms which grow and multiply at the expense of the host. A disease produced by the growth and multiplication of micro-organisms is termed an *infective disease*, and is transmissible in most instances by inoculation. If the micro-organisms are from time to time discharged from the body of the host, either with the excreta, secretions, desquamated particles, or in some other way, the disease becomes *infectious* or *contagious*, according to the ease with which another individual becomes *infected*, and the material which conveys the infection is often termed the *contagion*. Thus, in scarlatina and smallpox the contagion is very readily conveyed from person to person even for a distance through the air, and these are infectious diseases. Ringworm and syphilis, as a rule, require more or less close contact for infection to take place, and these are, therefore, contagious diseases; while malaria is neither infectious nor contagious, since persons in the neighbourhood never directly contract the disease, though it can be conveyed by inoculation, and it is therefore infective

only. But the distinction between *infectious* and *contagious* is mainly one of degree, and these terms have now to a large extent been discarded. Excluding individual susceptibility, the relative infectivity of a disease probably depends on three factors: (1) the contagion is freely given off aërially and is not destroyed thereby; (2) the contagion gains access by the respiratory tract; and (3) the relative virulence of the contagion; in some instances the smallest amount of the contagion is sufficient to infect. If the contagion can gain access only through a wound or the digestive tract, the chances of infection may be largely reduced. In certain instances infection is conveyed by an intermediary, *e. g.* the mosquito in malaria, and in such cases infectivity will obviously depend on the presence and abundance of the intermediary. Infection is manifestly a part of the whole subject of parasitism, which includes the animal and vegetable parasites which develop in the animal body. If, however, the subject of parasitism is considered more closely, it will be seen that there is a vast difference between, say, a condition caused by the echinococcus or by the round worm, in which the effects are largely mechanical and in which relatively little poison is produced by the parasite, and the disease diphtheria caused by the diphtheria bacillus, in which the diphtheria bacilli have little or no action mechanically, but elaborate virulent chemical poisons which cause a general *intoxication*. Some parasites also may produce a *general* infection, *e. g.* anthrax, others only a *local* infection, *e. g.* ringworm.

Parasites may therefore be divided into infective and non-infective, though there is a series of connecting links between these, and the two groups cannot be sharply separated. The *infective parasites* are: (1) vegetable micro-organisms, chiefly bacteria, a few yeasts

and some moulds; (2) many protozoa; and (3) a few metazoa, generally worms. The *non-infective parasites* are the animal parasites generally, particularly many worms.

The production of the phenomena of disease by pathogenic organisms has been ascribed to (1) the using up of the oxygen which should go to the tissues; (2) the using up of the proteins of the body and of the food; (3) the effects of plugging of the vessels by the microbes; and (4) the effects of substances or "toxins," having a poisonous action, formed by the microbes. Of these, the first three are quite subsidiary, embolism and thrombosis being perhaps the most important, and the toxins are the chief factors which induce the pathogenic effects. These toxins are substances of a very complex composition, probably allied to the proteins; in some instances they seem to be of the nature of enzymes or ferments, and they are direct products of the bacterial cells. The toxins of most pathogenic organisms, *e.g.* typhoid, cholera, plague, etc., are more or less integral parts of the bacterial cells; they are "endotoxins," and are not excreted to any extent into the surrounding medium, but may gain access to it by autolysis of some of the organisms. A few organisms, notably *Bacillus diphtheriæ* and *Bacillus tetani*, produce extra-cellular toxins which are found in the culture liquid. The toxins are classified by Sidney Martin,[1] as follows (see also p. 38):

(1) Poisons produced by the digestive or the destructive action of bacteria on proteins in the culture medium. Examples of these are the poisons of the *Bacillus anthracis* and of the pus-producing staphylococci.

(2) Poisons which are the result of the digestive or

[1] *Manual of General Pathology*, p. 76.

destructive action of bacteria on proteins, but formed as an excretion (the toxin) of the bacterium. The *Bacillus diphtheriæ* is the best example of this. A similar combination of poisons is found in snake-venom.

(3) Poisons which are excretions only, such as those produced by the tetanus bacillus.

(4) Poisons which are typically intra-cellular, but which may also be excretory. The poisons produced by the typhoid bacillus, the *Bacillus coli*, the *Bacillus enteritidis* of Gaertner, and the cholera vibrio belong to this group.

The Infective Process.

With regard to the pathogenic micro-organisms, or disease germs, Koch laid down the following conditions, which have been termed "Koch's postulates," which must be complied with before the relation of an organism to a disease process can be said completely to be demonstrated :

(1) The organism in question must be present in the tissues, fluids, or organs of the animal affected with, or dead from, the disease.

(2) The organism must be isolated and cultivated outside the body on suitable media for successive generations.

(3) The isolated and cultivated organism, on inoculation into a suitable animal, should reproduce the disease.

(4) In the inoculated animal the same organism must be found.

To these may be added :

(5) Chemical products with a similar physiological action should be obtainable from the artificial cultures of the micro-organism, and from the tissues of man or animals dead of the disease.

(6) Specific serum and other reactions, agglutinative, bacteriolytic, complement fixation, etc., generally obtainable, under certain conditions, if the blood of the infected person or animal be allowed to act on the specific organism producing the infection.

It is true that one or more of these conditions may not be fulfilled in all cases, but on general evidence the disease is classed as infective. Thus the *Treponema pallidum* of syphilis cannot be cultivated, and the organism of rabies is quite unknown.

The modes of infection, or entrance of the infective agent into the body, are varied. The infective agent may enter by (1) the gastro-intestinal tract, *e. g.* typhoid, cholera, and glanders; (2) the respiratory tract, *e. g.* pneumonia and influenza, and occasionally typhoid, plague, etc.; (3) by inoculution, not necessarily only of the skin, but also of the mucous membranes, *e. g.* the septic diseases, glanders, tetanus, etc. The extreme infectivity of some diseases—*e. g.* variola, scarlatina, influenza, etc.—may be due to the fact that infection takes place by the respiratory tract. In certain instances the infection is conveyed in some special way, *e. g.* by mosquitoes in malaria and in yellow fever. Nor is infection necessarily confined to one mode of entrance; in plague, for example, infection by the skin is commonest, but it is not infrequent by the respiratory, and may occur by the digestive, tract. The infecting agent may remain localised, giving rise to a *local infection*, or it may be widespread through the body, a *septicæmia*[1] or *general infection*. The absorption of chemical products from a local site of infection may produce general symptoms; this is *intoxication*, as

[1] "Septicæmia" and "a septicæmia" have different meanings. The former is applied to a general infection with the so-called *septic* organisms, the latter to a general infection with *any* organism.

occurs in cholera, in which the microbe is limited to the bowel, in the early stage of diphtheria, in which the diphtheria bacillus is limited to the membrane, and in a local abscess. Fever is usually one of the results both of intoxication and of general infection.

Infection, if recovery ensues, is usually followed by remarkable alterations in the blood and tissues. One of these is the production of immunity or insusceptibility to the same infecting agent; this will be considered later (p. 204). Agglutinins, substances which cause clumping of the infecting organism, are also generally produced (p. 193).

Anti-bodies.[1]

Another remarkable property, and one of considerable importance in immunity, conferred by the injection into an animal of complex substances, such as bacterial toxins, bacteria, blood-corpuscles, cells and cellular proteins, ferments, etc., is the development of *anti-bodies*. Thus an animal injected with sub-lethal doses of a bacterial toxin, *e. g.* diphtheria toxin, acquires a tolerance towards the toxin, becomes immunised, and a substance is developed in the blood that antagonises the toxin which was injected; this substance is known as *antitoxin*. If bacteria be injected, the fresh blood *in vitro* has a solvent action on the bacteria (bacteriolysis); if blood-corpuscles be injected, the fresh blood has a solvent action on the same kind of blood-corpuscles (hæmolysis); if cells be injected, the blood has a solvent action on the cells (cytolysis), and so on. If ferments be injected, anti-ferments are formed and will prevent the specific action of the

[1] All the subjects dealt with in the subsequent portion of this chapter are discussed in detail by Emery, *Immunity and Specific Therapy*, 1909.

ferment. With doubtful exceptions,[1] it is only complex bodies of protein nature, or allied to the proteins, which give rise to the production of anti-bodies on inoculation; alkaloids, carbohydrates, mineral poisons, etc., do not give rise to anti-bodies, though some insusceptibility to them may be produced (see also p. 216). Any substance which gives rise to an anti-body may be termed an "antigen." These anti-bodies, etc., may first be considered, after which immunity will be discussed.

Anti-bodies are probably formed for the most part in the spleen, lymph-glands and bone-marrow by leucocytes, or by endothelial cells, or by both.

ANTITOXINS.—The anti-bodies produced by the inoculation of an animal with bacterial toxins or toxic proteins (*e. g.* ricin, abrin, and snake-venom) are known as antitoxins, and are of considerable practical importance. An animal injected with increasing amounts of the toxin acquires a high degree of immunity, and its blood-serum injected into a second animal confers on the latter a similar immunity against the toxin with which the first animal was injected, but not against other toxins; the serum is specific. The anti-serum formed by the injection of toxin is antitoxic and not anti-microbic, and the diphtheria bacillus will grow and multiply in diphtheria antitoxin. Since, however, such an organism as the diphtheria or the tetanus bacillus produces its pathogenic effects through the toxin which it forms, the antitoxin will counteract the effects of the *micro-organism* as well as of its toxin. The neutralisation of the micro-organism, however, may not be quite complete, a certain amount of local reaction or necrosis ensuing.

[1] Ford has described the formation of an anti-body by the injection of a poisonous glucoside derived from fungi.

Antitoxins are prepared by injecting animals--preferably horses, but goats, rabbits, etc., may also be employed—with bacterial toxins or with cultures.

With those organisms which produce powerful toxins, such as diphtheria and tetanus, it is customary to grow the organism in a fluid medium so that an active and virulent toxin is obtained. The culture is then filtered through a Berkefeld or Pasteur-Chamberland filter and the toxic filtrate inoculated subcutaneously into an animal, generally a horse, commencing with sub-lethal doses.

The dose of toxin can be gradually increased, and concurrently with the increase in insusceptibility the blood-serum acquires antitoxic properties. The treatment is tedious, and the activity of the antitoxic serum is largely dependent upon the amount and activity of the toxin injected. The requisite degree of strength having been attained, the horse is bled with aseptic precautions, the blood is allowed to coagulate, and the serum is bottled for use. Antitoxin may be obtained in a concentrated form by "salting out" the globulin constituents of an antitoxic serum (p. 175), and a dried product may be prepared by evaporating the serum to dryness *in vacuo* at 40° C. (10 c.c. serum = 1 grm. dry residue).

The mode of production of the antitoxin by the injection of the toxin has been the subject of various theories. By some it has been supposed that the antitoxin is modified toxin, the modification being brought about by the vital activities of the cells. But the amount of antitoxin produced does not necessarily bear any relation to the quantity of toxin injected. Woodhead records instances in which the amount of antitoxin formed amounted to 40,000 times the equivalent amount of toxin injected, and substances which increase the

secretive properties of glandular cells, such as pilocarpine, enormously increase the output, so to speak, of antitoxin.

In view of these facts Ehrlich has elaborated his "side-chain theory," a theory which, whether it be the real explanation or no, has received a considerable amount of experimental support, and has had far-reaching effects in stimulating research. Ehrlich believes that the chemical activities which are the manifestations of the vital activities of the living cell are due to a very large nucleus or chemical molecule

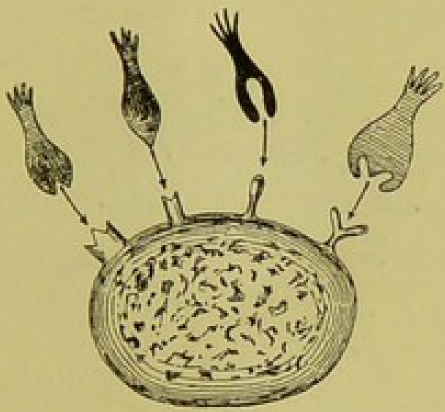


FIG. 26.—Diagram to represent the cell with its various combining groups or side-chains. (After Ehrlich.)

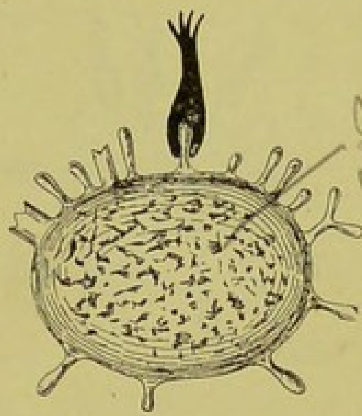


FIG. 27.—First stage in antitoxin formation. (Black = toxin molecule. (After Ehrlich.)

having a ring structure, analogous to the benzene ring, and having attached to it a number of atomic groups or "side-chains." A "side-chain" is an atomic group, a carbon atom of which is linked to one of the carbon atoms in a ring. These atomic groups or side-chains are unstable in nature, and enter freely into combination with other suitable groups should these be presented to them, and thus the physiological activities of the cell, assimilation, nutrition, etc., are carried out (Fig. 26). Now Ehrlich supposes that antitoxin is merely an excess of certain side-chains which are

normally present and subserve some of the ordinary functions of the cell and which have become free in the blood. The antitoxins being specific, by this assumption the difficulty is obviated of supposing that special chemical groups or molecules exist preformed ready to combine with a number of different toxins on the remote chance that some one of these may at some time or other come within the particular sphere of action of one of those groups. Moreover, small amounts of anti-bodies, such as antitoxin, bacteriolysin, agglutinin, etc., are met with in normal untreated

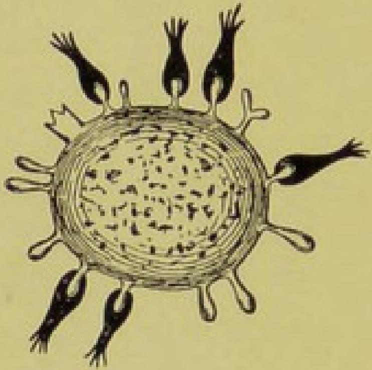


Fig. 28.—Second stage in antitoxin formation. (After Ehrlich.)

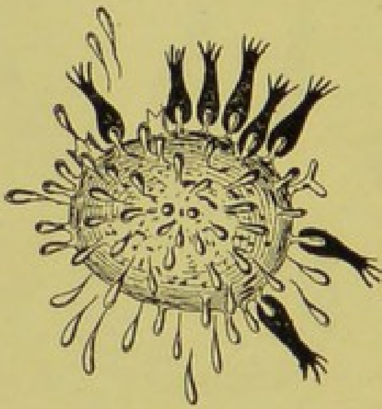


Fig. 29.—Third stage in antitoxin formation. Side-chains beginning to be produced in excess. (After Ehrlich.)

animals and in man. While some have supposed that the small amount of diphtheria antitoxin (equivalent to half a unit or so) present in human blood-serum is due to an infection with the diphtheria bacillus (not necessarily an attack of diphtheria), it seems more rational to suppose that this antitoxin is due to a natural liberation of such side-chains from the protoplasm and that artificial antitoxin production is merely a very great stimulation of this natural process.

The toxin molecule, according to Ehrlich, possesses at least two fixative atomic groups or side-chains. One

of these, the "haptophore group," conditions the union of the toxin molecule with cell-protoplasm; the other, the "toxophore group," conditions its toxic action. Similarly, in order that the cell may suffer the full effect of the action of the toxin, it also must possess two receptive groups or side-chains having a maximum affinity for the haptophore and toxophore groups of the toxin; these may be termed the "receptor" and "toxophile" groups respectively (see Fig. 31). The relationship of each fixative group of the corresponding groups—viz. that of the toxin and that of the side-chain of the cell—must be most intimate, and analogous to the relations to each other of a male and a female screw (Pasteur) or of a lock and its key (E. Fisher).

The genesis of antitoxin on the "side-chain theory" takes place in the following manner: Toxin being introduced, the haptophore groups of the toxin molecules unite with the particular receptor side-chains of the protoplasm for which they have an affinity (Fig. 27). By this combination the physiological activities of the cell are interfered with, a defect is created, the cell is damaged (it is only necessary to consider the case of one cell, or, more strictly of one molecular group of the cell-protoplasm). But through its recuperative powers the cell soon recovers by the formation of new receptor side-chains to take the place of those which have been put out of action. On injecting more toxin, the toxin combines with these new receptors and a defect is again created (Fig. 28). Once more the cell responds, and a fresh series of receptors is developed (Fig. 29). But by this continual stimulation, as it were, the cell commences to form the particular receptors *in excess of that needed to repair the defect created*, and ultimately these receptors are reproduced in such numbers that

they can no longer remain attached to the cell but become free in the plasma (Fig. 30). *These receptor side-chains, detached from the cell and floating free in the blood-stream, constitute the antitoxin.* This excessive production of side-chains after stimulation by repeated injections of toxin is not a phenomenon confined to antitoxin formation, but is a general physiological law enunciated by Weigert; as a result of repeated stimulation, over-production or hyper-compensation is the rule. Ehrlich has termed the diverse free receptors which occur in the body fluids in various circumstances "haptines."

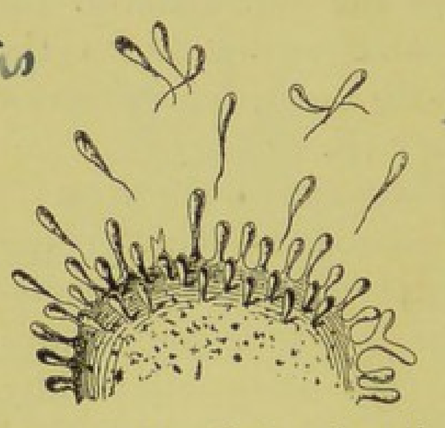
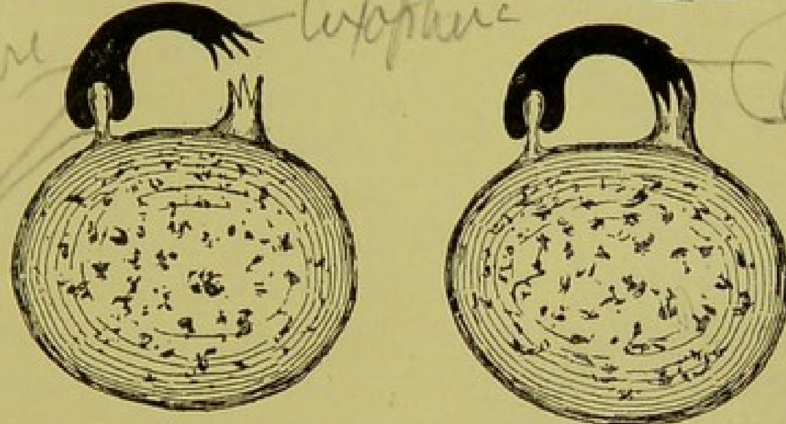


Fig. 30.—Fourth stage in antitoxin formation. Side-chain, *i. e.* antitoxin, free in the blood. (After Ehrlich.)

The existence of both haptophore and toxophore groups in the toxin molecule is suggested by the following experiments. If tetanus toxin be injected into the blood-stream of an animal it rapidly disappears, within a few seconds of the injection, and even if the animal be at once bled, the blood withdrawn being replaced by fresh blood, tetanus ensues, but not till after the lapse of an incubation period of some hours. The tetanus toxin, therefore, immediately becomes fixed or anchored to the tissues of the central nervous system. Evidently the toxin molecule enters at once into combination with the nerve-tissues by means of its haptophore group; this after a time brings the cells within the sphere of influence of the toxophore group, and after a certain incubation period toxic symptoms ensue. The affinity of tetanus toxin for nerve tissues may be shown in another way. If tetanus toxin be emulsified

with fresh guinea-pig brain, the emulsion will be found to be innocuous on injection, owing to a combination between the two having taken place. The cerebral cortex of a highly susceptible animal (*e. g.* mouse) has a marked neutralising power, of a less susceptible animal (*e. g.* rabbit, fowl) a feebler, and of an insusceptible animal (*e. g.* frog, tortoise) no neutralising power.[1] Moreover, both diphtheria and tetanus toxins may be converted into non-toxic modifications ("toxoids") which



A B

Fig. 31.—Diagrammatic scheme to represent the union of toxin (black) with the cell. In A the toxin is attached to the protoplasm by the union of the haptophore and receptor groups. In B the toxophore and toxophile groups have also united, and poisoning now ensues.

to some extent retain the power of immunising and of producing antitoxin on inoculation, and of combining with antitoxin: that is to say, according to Ehrlich, the toxophore groups have been destroyed while the haptophore groups remain unaffected. It is the presence of the haptophore group which conditions the union of toxin with antitoxin. Thus, if toxin be injected into blood containing antitoxin, the haptophore groups of the toxin unite with the free receptor groups, *i. e.* with

[1] The combination of brain matter with tetanus toxin seems to be specific and of the same order as that between antitoxin and toxin. See Noon, *Journ. of Hyg.*, vol. vii, 1907, p. 101, and Besredka and Bordet, *Ann. de l'Inst. Past.*, xvii, 1903.

the antitoxin (Fig. 32), and therefore the toxophore groups cannot exert their influence because the toxin is now unable to unite with the protoplasm, its haptophore or binding groups being already occupied.

In a poisonous toxin, such as diphtheria or tetanus toxin, the toxophore group is more readily destroyed than the haptophore group, and by heating a toxin for some time to 60°–70° C. its toxicity is destroyed, but it still retains an affinity for antitoxin. If some antitoxin be mixed with such heated toxin it will be found that the capacity of the former for neutralising active toxin is much diminished—in other words, although the toxophore groups of the heated toxin have been destroyed, the binding or haptophore groups still remain. Toxin which has been kept for some time decreases in toxicity, but retains the power of combining with antitoxin, again showing that haptophore or binding groups are present (such derivatives of toxin possessing haptophore groups are termed "toxoids"). Wassermann and Bruck have obtained presumptive evidence of the existence of the second stage in antitoxin formation, viz. the increased production of receptors by the cells. Using tetanus toxin which had been kept for some time and had lost its toxicity, but which still combined with antitoxin—that is, toxoids with haptophore groups were still present—they found that on injecting it into animals *no* antitoxin was formed as a result of the injection. They then performed some experiments based on the following line of reasoning: If the old non-

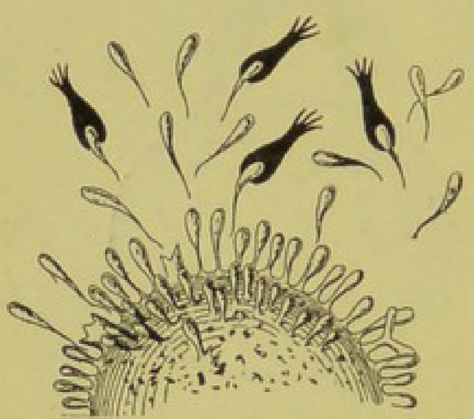


Fig. 32.—Neutralisation of toxin by antitoxin in the blood. (After Ehrlich.)

poisonous tetanus toxin containing these toxoids be first injected into an animal, and after a short interval, some fresh, actively poisonous tetanus toxin, more of the active toxin ought to be required to kill this animal than a normal one, because, owing to the previous toxoid injection, part of the cell receptors susceptible to tetanus toxin are already occupied. Provided Ehrlich's theory be correct, so that this binding of the toxoid really occurs, the conditions should be entirely different, when, instead of injecting the toxin shortly after the toxoid, a longer time elapsed—one to three days—before the injection of the active tetanus toxin. For in that case Weigert's law should come into play and the receptors should have increased in number—*i.e.* the organism would now possess *more* sensitive groups than before. This should be manifest by the fact that, in contrast to the first experiment, the fatal dose of active tetanus toxin ought now to be smaller than previously; in other words, a smaller dose should now tetanise the animal in a shorter time. The experiments yielded results which were exactly in accordance with these theoretical considerations. A guinea-pig was injected with some of the non-poisonous toxoid, and then, one hour later, with the active tetanus toxin. It was found that much more toxin was required to kill this animal than a normal guinea-pig of equal size. If, on the contrary, an interval of one to three days were allowed to elapse, it was then found that a dose of tetanus toxin which would not even tetanise a normal guinea-pig was sufficient to kill this one.

The fact that no antitoxin is formed—*i. e.* no receptors are thrust off—by the single injection of the non-poisonous toxin or toxoid Wassermann ascribes to the lack of stimulus which he suggests resides in the toxophore groups.

The slow combination of the haptophore and receptor groups has been proved by Wassermann in another way. The researches of Meyer and Ransom have shown that tetanus toxin is absorbed by the nerve-trunks, not by the blood and lymph-channels, while tetanus antitoxin is absorbed by the latter—the blood and lymph-channels. Adrenalin is a substance which strongly contracts the capillaries, and thus tends to block absorption in a particular area. The following experiment was devised: Tetanus toxin and antitoxin were mixed in such proportions that the mixture was innocuous to animals, *i. e.* it was just neutral. If this mixture be injected into the hind paw of a guinea-pig no tetanus develops. When, however, some adrenalin is injected into the hind paw of a similar-sized guinea-pig, and a few minutes are allowed to elapse so that the capillaries may contract, and then the mixture of toxin and antitoxin is injected, typical tetanus ensues. The explanation of this is that the channel of absorption for the tetanus *antitoxin*, the vessels, is blocked by the adrenalin, while that for the *toxin*, the nerve path, remains open. The toxin and antitoxin had not yet combined, or such combination as had occurred is a loose one and becomes dissociated, and, therefore, the toxin travelled along the nerves to the central nervous system, with the production of tetanus.

The experiment, however, succeeds only within a certain period, not exceeding an hour after mixture of the toxin and antitoxin, because after this the toxin-antitoxin combination becomes a stable one.

If a longer time—say three or four hours—is allowed to elapse, it will be found that, even in the adrenalin animal, no tetanus is produced, because by this time the combination, previously a loose one, is so firm that the substances can no longer be dissociated. This union can be hastened by employing more tetanus

antitoxin, for with an excess of antitoxin, even after only half an hour, it is impossible by means of adrenalin to free the tetanus toxin. This experiment, therefore, shows that the combination of tetanus toxin with antitoxin takes place slowly and is at first a loose one, and that the union becomes firmer and firmer with time. It also suggests the possibility of hastening the combination by increasing the amount of antitoxin—a point of considerable practical value in serum therapy.

The above considerations are of importance in the antitoxin treatment of disease. Antitoxin, in the strict sense, is not anti-microbic, and therefore antiseptic treatment of the throat in diphtheria, and of the wound in tetanus, should be pursued. The fact that the toxophore group of the toxin does not come into action as a rule for many hours at least (an exception is snake-venom) is a fortunate coincidence, for the antitoxin may, therefore, act before tissue damage has occurred. Antitoxin cannot repair tissue damage if this has been produced by the toxin, but it can, and does, prevent the occurrence of further damage by neutralising any fresh amounts of toxin that may be absorbed. Hence the necessity for early treatment. Toxin already anchored to the tissues by its haptophore group may for some time be dissociated from them if a *multiple* of the simple neutralising dose of antitoxin be injected, and the quantity necessary to accomplish this rises rapidly as the interval between the introduction of the toxin and of the antitoxin increases; hence the necessity for the use of antitoxin in large excess. Probably the union between tissue and toxin at first is a loose one, and a large amount of antitoxin by mass action transfers the affinity of the toxin from the tissue to itself.

An essential condition in antitoxic treatment is the administration of a sufficient amount of anti-serum, and this does not depend on the actual volume of serum injected. The anti-serum may be regarded as a solution containing a variable amount of the antitoxic or anti-microbic constituent, and for

therapeutic use its strength must be ascertained, and is for convenience described in arbitrary units.

The dose of antitoxin is dependent upon the gravity of the disease, and not on the age of the patient, for evidently just as much toxin may be formed in a child as in an adult. The antitoxins are strictly specific; diphtheria antitoxin, for example, has not the slightest influence in tetanus.

To obtain an immediate reaction to antitoxin it should be administered intra-venously. A subcutaneous injection may not be completely absorbed in less than thirty-six hours.

In cases of mixed infection, *e. g.* where diphtheria bacilli are associated with streptococci or staphylococci, the diphtheria antitoxin will have no influence on the streptococcic or staphylococcic infection.

The complications and accidents of antitoxin treatment are few and usually unimportant. Abscess and other local troubles at the seat of inoculation should not occur if proper antiseptic precautions be taken. Urticaria or other rashes and joint pains are by far the most troublesome complications. These are due to the injection of foreign serum, and not to the antitoxin, for the serum of an untreated horse produces a like effect. Repeated injections of serum at short intervals may be continued for a long period without inducing more disturbance than that caused by one or two or a few injections, but if twelve days or more elapse between two injections a condition of "supersensitation," due to anaphylaxis, ensues (see p. 176). This consists in the rapid appearance of rashes, joint pains, pyrexia, etc., or even of grave symptoms, faintness, vomiting, dyspnœa, convulsions, collapse, etc.

Anti-sera may be used as prophylactics, but the immunity produced by them does not last more than three weeks.

Various hypotheses[1] have been advanced to explain

[1] See Craw, *Proc. Roy. Soc. Lond.*, B. vol. lxxvi, 1905, p. 179; *Journ. of Hyg.*, vol. vii, 1907, p. 501; and *ibid.*, vol. ix, 1909, p. 46; Arrhenius, *Immuno-chemistry*, 1907; Madsen, *Brit. Med. Journ.*, 1904, vol. ii, p. 567; Bordet, *Ann. de l'Inst. Pasteur*, xvii, p. 161; McKendrick, *Proc. Roy. Soc. Lond.*, B, vol. lxxxiii, 1911, p. 493.

the manner in which toxin is neutralised by antitoxin. Roux and Buchner suggested that the antitoxin in some way renders the cells and tissues insusceptible to the toxin and Buchner performed experiments showing that while mice are more susceptible than guinea-pigs to tetanus toxin, a tetanus toxin-antitoxin mixture which is just neutral for mice is distinctly toxic for guinea-pigs.

To explain this Ehrlich suggested that there may be present in a toxin solution, several toxic substances, some of which exert a toxic action on the guinea-pig but not on the mouse. Madsen and Dreyer showed that a mixture of diphtheria toxin and antitoxin which is innocuous to guinea-pigs on subcutaneous inoculation is lethal to rabbits on intra-venous injection, and in order to explain this Ehrlich made a similar assumption. Morgenroth, however, has shown that the difference in the latter case depends on the mode of injection. The reaction between the toxin and antitoxin takes time to complete: there is an interval probably of some hours at 20° C. before equilibrium is reached (see also p. 171). When a recently prepared mixture of toxin and antitoxin is injected subcutaneously, absorption is slow, and in the meanwhile the toxin and antitoxin combine, but when the mixture is injected into the veins, the toxin is fixed by the tissues before it has had time to combine with the antitoxin, and poisoning ensues. If the mixture be kept for some hours before injection, intravenous injection is then innocuous.

Ehrlich concluded that diphtheria toxin is neutralised by diphtheria antitoxin much in the same way as a strong base is neutralised by a strong acid, and that the course of neutralisation suggests the presence in the toxin of several toxic and atoxic substances (toxoids and toxones), all of which combine with, though they have different affinities for, the antitoxin.

Arrhenius and Madsen, however, believe that the toxin-antitoxin reaction is analogous to the action of an acid on an alcohol, and that the chemical laws of mass action apply equally to the two. The chief reaction is considered to be between two substances only, toxin and antitoxin, that it is reversible, and that when the system has reached equilibrium, a fraction of toxin and also of antitoxin remain free, this fraction of toxin producing the "toxone effect" (see p. 172). If equivalent quantities of acetic acid and alcohol are mixed, the reaction is never complete; the acid and alcohol never entirely disappear, because the water formed reacts with the ethyl acetate, re-converting it into acid and alcohol. Such a reaction is termed reversible, and this particular case could be thus represented:

$$CH_3.COOH + C_2H_5.OH \rightleftarrows CH_3.COOC_2H_5 + H_2O.$$

Bordet has suggested that the fixation of toxin by antitoxin is an adsorption phenomenon, similar to the fixation of a dye by a tissue.

These hypotheses may now be examined more in detail. Ehrlich's experiments[1] on diphtheria toxin seemed to show that the neutralisation of toxin by antitoxin follows the laws of simple chemical combinations, such as the neutralisation of a strong base (NaOH) by a strong acid (HCl). If so, it would be expected that antitoxin would neutralise proportionate amounts of toxin; but this is not so, and Ehrlich was forced to the conclusion that toxin is a complex mixture of proto-, deutero-, and trito-toxin, and toxone, with different toxicities and different avidities for antitoxin. Moreover, when toxin is kept it decreases in toxicity, though still retaining much of its avidity for antitoxin. Ehrlich

[1] See *Trans. Jenner Inst. Prev. Med.*, vol. ii, p. 1; *Croonian Lect., Roy. Soc. Lond.*, 1900; and p. 293.

assumed, therefore, that the toxin becomes transformed into substances termed toxoids, which are non-toxic but retain their affinity for antitoxin. This he explained as due to destruction of the unstable toxophore groups, with the retention of the more stable haptophore groups. That the neutralisation of toxin by antitoxin is due to a chemical combination between the two seems to be proved by the work of Martin and Cherry. Brodie,[1] and Martin and Cherry,[2] making use of a Chamberland filter, the pores of which had been rendered very fine by saturating with gelatin, found that toxin would pass through such a filter but that antitoxin would not, presumably because the molecule of the latter is larger. By mixing diphtheria toxin and antitoxin in such proportion that the latter was in sufficient quantity to neutralise the toxin, and subjecting the mixture to filtration through a gelatin filter, the filtrate was found to be non-toxic. Now since toxin can pass through such a filter, the inference is that the toxin has combined with the antitoxin. Using snake-venom and its anti-serum or anti-venin, another method was employed. The anti-venin is destroyed by heating to 68° C. for ten minutes, while the toxic properties of the venom are unaltered by this treatment. By making mixtures of venom and anti-venin, and, after a certain time has elapsed for the interaction to take place, heating to 68° C. for ten minutes, it was found that the mixture is non-toxic, pointing to the combination of the toxin (venom) with the antitoxin (anti-venin). Calmette had performed the same experiment but with a different result, finding his mixtures still toxic after heating. Calmette, however, treated his solutions almost immediately after mixing, and Martin and Cherry point out

[1] *Journ. of Path. and Bact.*, 1897, p. 460.
[2] *Proc. Roy. Soc. Lond.*, vol. lxiii, 1898, p. 420.

that a certain *time* must be allowed to elapse for the interaction to take place, and noted that moderate warming hastens it, as is the case with all chemical interactions. For instance, they found that one mixture of venom and anti-venin allowed to interact for two minutes, five minutes, and ten minutes before heating, killed the animals in thirteen hours, fifteen hours, and twenty-three hours respectively (the control animal with the same dose of venom died in nine hours), but after fifteen minutes the same mixture rendered the animal ill but it survived, while after thirty minutes no toxic symptoms ensued.

At one time it was stated that by electrolysis of toxin small amounts of antitoxin are formed, but this is very questionable. Electrolysis destroys the toxicity of toxins by the production of acids, chlorine, and hypochlorites.

Ehrlich's views have been opposed, principally on physico-chemical grounds. Thus, Danysz observed that if ricin or diphtheria-toxin be brought into contact with its corresponding anti-body, the degree of neutralisation depends on the manner of mixture. If the toxin be added to the antitoxin in two fractions, allowing a considerable time to elapse between the additions, the mixture contains a much larger amount of free toxin than is the case when the whole (and same) amount of toxin is added at once to the antitoxin. This phenomenon, known as the "Danysz or toxone effect," seems inexplicable if toxin and antitoxin have relations the same as a strong base and a strong acid.

Arrhenius, Dreyer, and Madsen maintain that the phenomena observed in the toxin-antitoxin reaction are explicable on the hypothesis that the rate of reaction—avidity—of the toxin decreases as antitoxin is added, that the interaction is a slow one, and that different fractions of the toxin are progressively neutralised by the added antitoxin, but more and more slowly. On these grounds they consider that there is no reason to regard the diphtheria poison as a highly com-

plicated body. Whereas Ehrlich considers the toxin and antitoxin to combine with great avidity, analogous to the combination of a strong base with a strong acid, *e. g.* NaOH with HCl, these critics believe the avidity of antitoxin for toxin to be feeble, analogous to the combination of ammonia with boric acid, in which as more and more acid is added, the amount of free ammonia decreases, but more and more slowly, in correspondence with a hyperbolic curve. The phenomena can be calculated according to the law of "mass action," there being an equilibrium between—

$$\frac{\text{Free } NH_3}{\text{vol.}} \cdot \frac{\text{Free } H_3O_3B}{\text{vol.}} = K \frac{(NH_4H_2O_3B)^2}{\text{vol.}}$$

where K is the constant of dissociation. The curve of the neutralisation of tetanolysin by anti-tetanolysin corresponds almost exactly to the ammonia-boric-acid curve.

Whereas on Ehrlich's views the combination of toxin and antitoxin would be represented by a straight line, and the crude toxin seems to be composed of a whole series of different toxins and substances having an avidity for antitoxin, on this hypothesis, although the greater part of the toxicity of toxin is removed by the antitoxin, the latter must be added in large excess before the toxicity completely disappears, and the course of neutralisation would be represented by a hyperbolic curve. In fact, as the antitoxin is added, the amount of free toxin diminishes but never completely disappears. There comes a point, of course, when the amount of free toxin is so small as to be negligible and cannot be recognised by the ordinary indicators (blood-corpuscles, animal tests, etc.). This hypothesis would explain the fact that while a certain amount, V, of a mixture of toxin and antitoxin is innocuous to an animal, a multiple of the dose, $n.V$, of the *same* mixture may be toxic; it would also explain Buchner's experiments alluded to above (p. 169), and Roux's experiments in which a toxin-antitoxin mixture innocuous to normal guinea-pigs was toxic to guinea-pigs whose resistance had been reduced by injections of the Massowah vibrio.

Nernst has questioned from the mathematical standpoint the validity of the views of Arrhenius, and so has Craw from much experimental work on agglutination and on the interaction between megateriolysin and anti-megateriolysin; Craw also considers that there is some doubt attaching to Arrhenius's calculations. According to Craw, the two substances most thoroughly investigated by Arrhenius and Madsen, diphtheria toxin and tetanolysin, do not admit of sufficiently exact determination, the former because of the uncertainty attaching to animal experiments, the latter because tetanolysin is a most unstable body. Working with a more stable substance, megateriolysin, he holds that the Arrhenius and Madsen equation does not apply. Again, on the addition of a small amount of antitoxin to toxin there is no decrease in toxicity (as noted by Ehrlich and attributed by him to the presence of toxoid) as there should be, and Arrhenius was thus forced to the conclusion that a second substance, epitoxonoid, is present with the toxin in diphtheria toxin. Craw denies that the toxin-antitoxin reaction is reversible, believes that antitoxin must be regarded as a colloid (and is not in true solution), that the mixture therefore is heterogeneous, not homogeneous, and that the chemical law of mass action is not applicable.

On the other hand, Craw maintains that the phenomena of the toxin-antitoxin reaction, including the Danysz effect, have their counterpart in adsorption phenomena, such as occur in the staining of paper, porcelain, etc., with anilin dyes, in the absorption of substances by colloids, etc. Thus, when solutions of arsenious acid are shaken up with colloidal ferric hydroxide, a portion of the arsenic is taken up by the ferric hydroxide and a portion remains in solution. Moreover, more arsenious oxide is taken up by the ferric hydroxide from dilute than from concentrated solutions; this has its counterpart in agglutination. Again, when an antitoxin is added to a toxin in just sufficient amount to produce a non-toxic solution, the amount of toxin which must then be added to constitute a fatal dose is greater than the minimum lethal dose without antitoxin. This is also found to be the case with ferric

hydroxide and arsenious acid; if ferric hydroxide and arsenious acid are mixed so as to form just a non-toxic mixture, the amount of arsenious acid which must then be added to render the mixture toxic is greater than the toxic dose of arsenious acid.[1]

Arrhenius[2] replied to Craw's criticisms maintaining the correctness of his own interpretation, and Craw[3] has again maintained the validity of his views, so that the final settlement of these divergent opinions must be left for future research.

The antitoxic constituent of antitoxin seems to be a protein body, probably allied to globulin, and, as already mentioned, the globulin content of the blood of an animal treated for antitoxin production increases in some cases. Tizzoni, by precipitating the antitoxic serum by saturation with magnesium sulphate at 30° C., obtained the antitoxin in the precipitate. By partial saturation of antitoxic serum with ammonium sulphate, the antitoxin is carried down with the second precipitate, that is, with the pseudo-globulin fraction. It is thus possible to concentrate antitoxic serum and to make use of a weak serum, which would otherwise be inconvenient on account of the volume necessary to inject in order to introduce the requisite amount of antitoxin. For this purpose various salts have been employed for saturation, ammonium sulphate (Pick and others), magnesium sulphate (Dieudonné), mixtures of sodium and potassium chlorides (Atkinson), etc.

Dzergowski and Predtétchensky[4] have elaborated a very exact method by which they state that the whole of the antitoxin can be concentrated and recovered from a comparatively weak serum by means of precipitation with ammonium sulphate.

[1] See Findlay, *Physical Chemistry and its Applications in Medical and Biological Science*, 1905.

[2] *Journ. of Hygiene*, vol. viii, 1908, p. 1.

[3] *Ibid.*, vol. ix, 1909, p. 46.

[4] See Hewlett's *Serum Therapy*, 1910, p. 68.

Anaphylaxis.—An animal usually becomes more and more tolerant to injections of an antigen, *e. g.* to diphtheria and tetanus toxins in the preparation of the corresponding antitoxins. Sometimes, however, the opposite effect is produced, *viz.* increased sensitiveness. This has been noticed in the preparation of tetanus antitoxin; after the animal has received a few doses of the toxin without ill-effect, a smaller dose of toxin may cause fatal tetanus. The tuberculin reaction (p. 321) is another example; tubercle toxins circulating in the tuberculous individual render him peculiarly sensitive to a minute dose of tuberculin (*i. e.* tubercle toxin) which in a normal person produces no effect. This condition of hypersensitiveness is known as 'anaphylaxis' (*i. e.* the opposite of 'prophylaxis'). Probably any antigen under particular conditions may induce anaphylaxis, but the phenomenon has been especially studied in connexion with serum injections, though other proteins, *e. g.* egg-white, similarly cause it. The injection of an anti-serum usually produces no ill-effect other than the rashes, joint pains, and pyrexia already mentioned, even if large amounts of the serum be given extending over days or even two or three weeks, but a second injection of serum given after a first injection with an interval of twelve days or more between the two injections is liable to be followed by effects which may be more or less serious, constituting the so-called "serum disease," or immediate or accelerated reactions, "supersensitation," may ensue (see p. 168).

The symptoms of the serum disease are nausea and vomiting, small and rapid pulse, faintness or more serious heart failure, dyspnœa with rapid and shallow respiration and feeling of suffocation, collapse, rigors, convulsions, and even coma. The severity of the symptoms varies in different cases, and the symptoms

usually pass off in the course of an hour or two; but a few fatal cases have been recorded.

In the immediate reaction, rash, pyrexia, joint pains, vomiting, rigors, and occasionally convulsions and collapse occur, generally within six hours after the second injection of serum. In the accelerated reaction, these phenomena appear between the eighteenth hour and the fifth day after the second injection of serum.

The immediate and accelerated reactions may occur a long time after the first course of serum treatment if more serum be given. Goodall records one case in which over four years elapsed between serum treatments for first and second attacks of diphtheria, an accelerated reaction occurring after the reinoculation for the second attack.

The amount of serum given does not definitely influence the result. The remarkable features of the phenomenon are—(1) they do not occur unless an interval of about twelve days or more elapses between the two injections of serum; (2) the long period which may intervene between the two injections of serum and still be accompanied by symptoms; (3) the serious nature of the condition in some instances.

The explanation of the phenomenon is difficult. Undoubtedly the symptoms are due to some substance in the serum which has a toxic action, and have nothing to do with the antitoxic constituent, for *normal* serum produces the same effects.

In experimental anaphylaxis produced in animals by the injection of normal serum, it is found that the condition only occurs if the two doses of serum are separated by an interval of about twelve days or more. Moreover, the two injections must be of the same serum or other protein; thus a first injection of horse serum followed by a second injection of rabbit serum would not produce it. Extremely small doses of serum will

also bring it about; and lastly, anæsthetisation, when the second dose of serum is given, prevents the development of the symptoms—a very extraordinary result.

The Arthus phenomenon occurs when a guinea-pig receives several doses of normal horse serum at intervals of some days. Another injection of horse serum then causes an œdematous mass, an aseptic abscess, or an area of necrosis at the site of the new inoculation, which may be far removed from the region of the previous inoculations, and the animal becomes cachectic and dies.

The Theobald Smith phenomenon occurs when a guinea-pig has been sensitised by a very small single dose of normal horse serum, 0·01 c.c., 0·001 c.c., or even 0·000001 c.c.; if, then, after an interval of twelve to fourteen days a somewhat larger dose of serum, 0·1 c.c., be given, the serious symptoms of hypersensitiveness develop within a few minutes, viz. respiratory failure, paralysis, clonic spasms, and frequently death. The symptoms are generally much more serious when the primary dose of serum is minute than when it is larger, *e. g.* one or more cubic centimetres.

Various hypotheses have been advanced to account for anaphylaxis.

Besredka believes that anaphylaxis is caused by the presence of two substances in the serum, one thermostable and having the properties of an antigen (see p. 157), which he terms "sensibilisogen," and which on injection produces its anti-body, "sensibilisin." The other substance is thermolabile, and is termed "anti-sensibilisin," and combines with sensibilisin whenever it meets with the latter. Sensibilisin is particularly fixed by the cells of the nervous system, and, according to Besredka, it is the violent reaction between anti-sensibilisin and sensibilisin in the nerve tissues which causes the serious disturbance characteristic of ana-

phylaxis. When, therefore, a small dose of serum ($\frac{1}{100}$–$\frac{1}{50}$ c.c.) is administered, the sensibilisogen slowly forms sensibilisin. If a second dose of serum is given twelve days or more after the first injection, the anti-sensibilisin in it combines with the sensibilisin formed by the first injection, and disturbance results. The reason why a large primary injection of serum (*e. g.* 3–5 c.c.) gives rise to much less disturbance with the second dose of serum than a small primary injection does is that the large amount of antisensibilisin present in the serum combines gradually with the sensibilisin as this is in process of being formed (*i. e.* in the pre-anaphylactic stage), and therefore there is comparatively little sensibilisin left for the antisensibilisin present in the second dose of serum to combine with, hence the disturbance caused is much less.

The reason why anæsthetisation with ether when the second injection is given prevents the symptoms of anaphylaxis developing is, according to Besredka, that the anæsthetic renders the nerve cells insensitive to the reaction between the sensibilisin and antisensibilisin.

Anaphylaxis, supersensitation, or hypersensitation may be of considerable importance in serum treatment.

On the serum disease, supersensitation, and anaphylaxis, see Hewlett, *Serum Therapy*, ed. 2, 1910; Rosenau and Anderson, *Journ. Amer. Med. Assoc.*, 1906, p. 1007; Von Pirquet and Schick, *Die Serum-Krankheit*, 1905; Richet, *Ann. de l'Inst. Pasteur*, xxi, p. 497; Besredka, *ibid.*, p. 950, and *Bull. de l'Inst. Pasteur*, vii, 1909, p. 721; Currie, *Journ. of Hygiene*, vol. vii, 1907, pp. 35, 61, and vol. viii, 1908, p. 457; Grünbaum, *ibid.*, vol. viii, 1908, p. 9; Goodall, *ibid.*, vol. vii, 1907, p. 607.

Anti-microbic Sera.—If an animal be injected with increasing doses of bacteria, care being taken to keep below a lethal one, the animal gradually becomes accustomed to the microbe, and ultimately acquires a

high degree of immunity, so that it is unaffected by amounts which would infallibly kill an untreated animal. Moreover, the blood-serum of such a treated animal, if injected into a second animal, will protect the latter against a few lethal doses of the microbe, but not against a large amount. Nor is the protection afforded proportional to the amount of serum injected; for example, if 0·005 c.c. of anti-cholera serum will protect against 5 mgrm. of living cholera culture, three times as much, or 0·015 c.c. of the serum, will not protect against 15 mgrm. of cholera culture, and when a certain dose of the culture is reached no amount of serum will save the animal. The mode in which the serum acts may be studied microscopically. If cholera anti-serum and cholera culture be injected into the peritoneal cavity of a guinea-pig, and if the peritoneal contents be examined at short intervals afterwards, it will be found that the vibrios first lose their motility, then become distorted and globular, undergo solution, and finally disappear. The protection afforded by the anti-serum is therefore due to the destruction of the microbes by solution, the process being known as bacteriolysis,[1] and the bodies which bring it about being termed "bacteriolysins." The reaction is known as "Pfeiffer's phenomenon" or reaction, from its discoverer. If the serum and the microbes be mixed *in vitro* the latter are unaffected; apparently, therefore, some constituent of the *living* body in addition to the anti-serum is necessary for the solution of the microbes. But in 1895 Metchnikoff showed that the reaction will take place *in vitro* provided that some of the *fresh* peritoneal exudate of a normal guinea-pig be added to the mixture of anti-serum and microbes. The same year Bordet found that the addition of the peritoneal exudate

[1] See Gruber, "Harben Lectures," *Journ. State Med.*, 1902.

is unnecessary provided the anti-serum be perfectly fresh. These experiments prove that the solution of the microbes is brought about by the interaction of at least two substances, one of which is present in all fresh serum and in the living body, but is unstable, disappearing on keeping or heating the serum, the other is a relatively stable body produced during the process of inoculation. The former, the unstable normal body present in all animals, is usually termed "complement" (Ehrlich and Morgenroth), "alexin" (Buchner and Bordet), or "addiment"; while the stable constituent produced by immunisation is known as the "amboceptor" (Ehrlich), "immune body," "intermediary," "preparer" (Grüber), "fixateur" (Metchnikoff), or "substance sensibilisatrice" (Bordet). These considerations suggest an explanation why anti-microbic serum neutralises but a limited amount of living culture, viz. the amount of complement present in the body at one time is limited, and when this has been used up bacteriolysis ceases. Anti-microbic sera are relatively inefficient in practice, insufficiency of complement being suggested as the reason. Attempts have been made to supplement the complement present by injecting *fresh normal* serum with the anti-serum, but without success, and some anti-microbic sera, *e.g.* anthrax serum, are not bacteriolytic; this explanation is, therefore, unsatisfactory. Deflection of complement (p. 185) may occur in some instances, or the complement may not be of the right kind. In other cases, the organisms in certain situations may be inaccessible to the blood-stream and to the anti-serum, *e.g.* in the bowel in cholera.

The amboceptor or immune body seems to link the complement to the bacterium (Fig. 33); complement remains free if the appropriate amboceptor or immune body is not present, and bacteriolysis does not ensue (see also p.

188). Complement is *thermolabile, i.e.* it is destroyed by heating to 56° C. for thirty minutes; while the amboceptor is *thermostable, i. e.* it is not destroyed by this treatment.

According to Ehrlich, fresh serum contains numerous complements which are more or less specific for different amboceptors (see also note, p. 189). When the complement is destroyed by heating it is converted into "complementoid" (analogous to toxoid). Both complement and complementoid on injection give rise to anti-complement. The amount of complement in different sera varies considerably; horse serum contains very little, guinea-pig serum much. Complement itself probably consists of two portions.

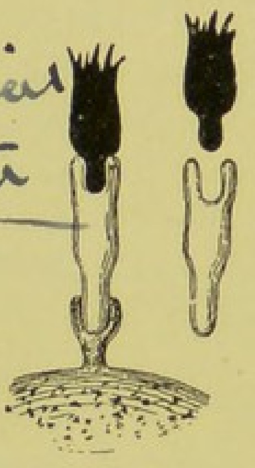


Fig. 33.—Diagram to show the union between complement (black) and protoplasm of cell by means of the amboceptor (white). (After Ehrlich.)

Pfeiffer's reaction is of considerable value in practical bacteriology for the exact recognition of bacterial species. A mixture of an emulsion of the organism to be tested with a small quantity of serum from a highly immunised animal is injected into the peritoneal cavity of a normal guinea-pig. The fluid in the peritoneal cavity is then examined microscopically half to one hour after the injection, and if the reaction be positive the organisms will be found in all stages of degeneration, being mostly converted into spherules. In this case, according to Pfeiffer, the organism is to be regarded as belonging to the same species as that by means of which the immunisation of the animal, from which the blood-serum was obtained, was carried out. If, on the other hand, the reaction be negative, the organisms are unaffected after being in the

Note Pfeiffer's reaction is similar to agglutination - but done in perit. cavity + with bacteriolysis not agglutination e.g. clumping

peritoneal cavity for an hour or so, and the organism is then considered to be a species different from that used for the immunisation. Thus, Pfeiffer's reaction may be made use of to differentiate the cholera-like vibrios from true cholera vibrios and the members of the typhoid-colon group from one another.

The destruction of the bacteria by bacteriolysis is regarded by some as being brought about by osmotic changes, by others by processes analogous to digestion. During bacteriolysis the specific immunising substances and anti-bodies are used up, and for the lysis of a given quantity of bacteria a certain amount of immune serum is necessary, while after lysis has taken place the latter loses the power of dissolving bacteria. The same holds good for hæmolysis, and the facts relating to bacteriolysis and hæmolysis are almost interchangeable.

Anti-endotoxic sera.—The comparative inefficiency of anti-microbic sera, particularly typhoid, led Macfadyen to attempt to prepare sera with microbial endotoxins, and the work has been continued by Südmersen and the writer. The method was to immunise horses with the endotoxin obtained by the method described at p. 40. With a typhoid serum so prepared Goodall and the writer obtained promising results.[1]

Method of applying Pfeiffer's reaction.—For Pfeiffer's test, the organism must be virulent, and a high-grade immune serum is necessary. If the organism is not virulent, it is spontaneously destroyed in the peritoneal cavity without the addition of immune serum. The method may be best explained in the case of a vibrio supposed to be the cholera vibrio. The cholera-immune serum (obtained from a horse repeatedly injected with cholera culture) should possess a titre of not less than 0·0002 c.c., *i.e.* this amount of serum mixed with one loop (2 mgrm.) of an eighteen-hour agar cholera culture (virulent), suspended in 1 c.c. of broth, and injected into the peritoneal cavity of a small guinea-pig

[1] *Proc. Roy. Soc. Med.*, vol. ii, 1907–8, Med. Sect., p. 245 *et seq.*

should cause granular degeneration and bacteriolysis of the vibrios within one hour.

Four mixtures are made—(*a*) one loop of an eighteen-hour agar culture of the vibrio to be tested, 0·001 c.c. cholera-immune serum, suspended in 1 c.c. of broth; (*b*) the same as (*a*), but 0·002 c.c. cholera serum; (*c*) the same as (*a*), but 0·001 *normal* serum of an animal of the same species as that furnishing the cholera serum; (*d*) one quarter loop of the vibrio in 1 c.c. of broth, as a control of the virulence of the culture. These mixtures are then injected into the peritoneal cavities of four guinea-pigs each of about 250 grm. weight. At intervals of thirty and sixty minutes hanging-drop preparations are made of the peritoneal fluid of each animal, the fluid being obtained by inserting a capillary pipette through a minute incision in the skin. In the guinea-pigs injected with (*a*) and (*b*), if the organism be cholera, the vibrios should show marked degenerative changes within sixty minutes, while (*c*) and (*d*) will show plenty of active vibrios. If the organism be non-virulent, two methods may be adopted for applying the Pfeiffer reaction. The first, a microscopical or *direct* method, is carried out by microscopical examination of hanging-drop specimens of the organism suspended in a drop of the immune serum to which a trace of fresh peritoneal fluid (complement) is added. If the organism is homologous with the immune serum, the bacteria are soon transformed into granules. Controls are put up at the same time with a known strain of the organism with (1) its homologous immune serum + complement; (2) non-immune serum of the same animal + complement; also of the organism being tested with non-immune serum of the same animal + complement. The peritoneal fluid may be obtained by injecting 3–4 c.c. of broth into the peritoneal fluid of a guinea-pig and four hours later withdrawing the fluid (now turbid with leucocytes) and centrifugalising, or allowing it to stand on ice for twenty-four hours.

In the second, *or indirect,* method, the organism is used to prepare an immune serum by injecting an animal (*e. g.* a rabbit) with it, and the immune serum so prepared is tested

on a known virulent stain in the peritoneal cavity of guinea-pigs in order to ascertain whether or no it brings about bacteriolysis, *i. e.* the Pfeiffer phenomenon.

Deflection, deviation, diversion or blocking of complement. —Pfeiffer in 1895 observed that a *large* amount of immune serum might not protect an animal from the cholera vibrio, while a smaller amount with the same dose of vibrio did so. In 1901 Neisser and Wechsberg demonstrated an analogous reaction *in vitro*. They studied the effect of a bacteriolytic immune serum when varying amounts of the inactivated serum were employed. The quantity ranged from 0·0005 c.c. to 1 c.c. To each of these amounts constant volumes of normal serum and bacterial suspension were added. No bacteriolysis occurred when large and small amounts of immune serum were used, but with medium amounts bacteriolysis was complete. They explained this anomalous reaction, the absence of bacteriolysis with *large* amounts of immune serum, as follows: When the amboceptors are in large excess, a portion combines with the complement, leaving some amboceptors free, and these free amboceptors then unite with the receptors before the activated amboceptors (amboceptors + complement) do, and thus the complement-amboceptor groups are rendered inert. The reaction is represented diagrammatically in Fig. 34. Arrhenius, however, does not accept this explanation. He says: "If we have the compounds *ea* and *ab* which may combine to form the compound *eab*, the formation of the latter depends wholly upon whether *e* has a greater affinity for *ab* than for *a*. If not, then *eab* is not formed, even if *a* is not present in excess." ($a =$

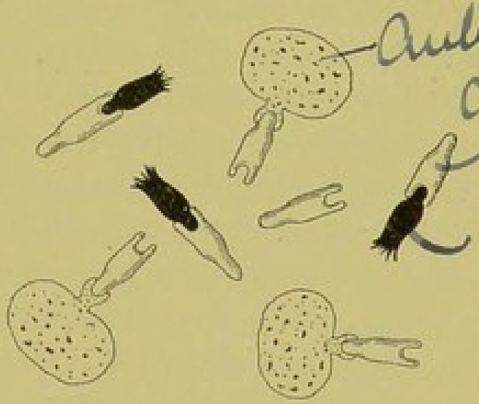


FIG. 34.—Diagram to represent the condition of the blood in which there is an excess of amboceptors. The amboceptors (white) unite with both complement (black) and receptors (dotted), so that the receptors cannot combine with the amboceptor-complement groups.

amboceptor, e = microbe, b = complement.) The phenomenon may be quite analogous with the inhibition met with in agglutination (p. 196).

Aggressins.

Bail has discussed the question of the relationship between bacteriolysis and immunity. He argues that there is apparently little relationship between the bactericidal properties of the body fluids and the immunity of an animal to infection through bacteriolytic processes; and points out that in rabbits immunised against anthrax there is no bacteriolytic power, the bacteria disappearing gradually as the result of phagocytic action of cells, chiefly marrow-cells; that a comparison of the sera of sheep, rabbits, and cattle shows great variation in their content of immune body, though the animals are almost equally susceptible to anthrax; and that in test-tube experiments a bacteriolytic serum is blocked when the conditions are approximated to those in the body by the addition of body cells to the mixture; the bactericidal properties of the serum disappear or are greatly inhibited. Kruse suggested that for infection to take place the invading bacteria must elaborate chemical substances which so act on the cells and fluids of the invaded animal that they overcome its natural resistance against infection. These substances are considered by him and Bail to be distinct from the toxins, and are termed by these writers "aggressins."[1] The aggressins are supposed to be secreted by the living uninjured bacteria and not to be extracts, nor derived by solution, of the bacteria; they occur particularly in the fluids of pathological œdemas and exudates, and may be obtained from these by centrifugalisation and sterilisation at low temperatures. Bail believes that the aggressins cannot be anti-complements, anti-immune bodies, etc., but are substances heretofore unrecognised and the active substances of the infection, and

[1] See *Centr. f. Bakt.*, Orig., xlii, 1906, pp. 51, 139, 241, 335, 437, and 546. Also an excellent summary by Marshall, *Philippine Journ. of Science*, vol. ii, 1907, p. 352.

he considers that in order to produce true immunity in disease anti-aggressin sera must be prepared. The following are some of the properties of these supposed aggressins: (1) Sterilised aggressin with a non-lethal dose of the corresponding organism renders the latter fatal; (2) aggressin alone is only slowly toxic, producing a prolonged illness with emaciation preceding death; (3) inoculation of aggressin with bacteriolytic serum into the peritoneal cavity suspends the action of the latter; (4) aggressin with bacteria blocks phagocytosis. Bail believes that the aggressins promote infection by interfering with the protective mechanism of the infected animal, particularly, if not solely, by inhibiting phagocytosis. Upon the power to produce aggressin Bail has classified bacteria into (1) true parasites which always produce aggressin, *e.g.* anthrax and chicken cholera; (2) half-parasites, the aggressin-producing power of which is variable, *e.g.* typhoid, cholera, dysentery, and plague; (3) saprophytes. The virulence of an organism does not coincide with aggressivity, and extremely virulent bacteria may be half-parasites.

Bail's hypotheses have been much criticised, and Wassermann and Citron believe that the supposed aggressins are derivatives of the bacterial protoplasm which have the power of combining with the specific protective substances of the animal and so inhibit the action of the latter; they are, in fact, endotoxins of feeble toxicity.

Hæmolysis.[1]—Some blood sera possess marked powers of dissolving the red blood-corpuscles of another species, and of setting free their contained hæmoglobin (*e.g.* goat serum dissolves rabbits' and guinea-pigs' corpuscles, and human serum usually dissolves sheep's corpuscles), and if an animal be injected with the blood-corpuscles of another species its blood-serum generally acquires the property of dissolving the blood-corpuscles with

[1] See Bulloch, *Practitioner*, December, 1900, p. 672, and *Trans. Path. Soc. Lond.*, vol. lii, Part 3, 1901, p. 208; Gruber, "Harben Lectures," *Journ. State Med.*, 1902, February, March, and April; Ehrlich, *Collected Studies on Immunity*; Muir, *Studies on Immunity*.

* An aggressin is an "anti-opsonin" E.g. inhibits Phagocytosis

which it has been injected. For example, the serum of a normal rabbit has no hæmolytic action upon the red corpuscles of the sheep; but if a rabbit receive a few injections of defibrinated sheep's blood, its blood-serum acquires hæmolytic properties and dissolves the red corpuscles of the sheep. This solution of the blood-corpuscles is termed "hæmolysis," and the substances which produce hæmolysis are "hæmolysins." If the active serum be heated to 56° C. it loses its hæmolysing power, but can again be rendered hæmolytic or "activated" by the addition of *fresh* normal serum; normal serum, however, rapidly loses its activating properties on keeping. It will thus be seen that there is an almost complete analogy between bacteriolysis and hæmolysis, the latter being brought about by the interaction of two substances, one specific and stable produced by the injections, the hæmolytic "amboceptor" or "immune body," and the other an unstable body present in fresh normal serum, the "complement" or "alexin."

Hæmolysin formed by the injection of corpuscles of another species is termed "heterolysin." If corpuscles of the *same* species be injected, hæmolysin is formed ("isolysin"), but the injection of the animal's own corpuscles does *not* give rise to hæmolysin, *i. e.* "autolysin" is *not* formed.

Blood-corpuscles are more tangible entities than bacteria, and are far easier to work with than the latter, and hæmolysis has been the subject of a large amount of experimental work by Bordet and Gengou, Ehrlich, Morgenroth, Gruber, Bulloch, Muir, and others, and the results obtained have shed considerable light upon the complex phenomena of immunity and of the actions of anti-bodies in general. Moreover, the globulicidal material in hæmolysis *seems* to be identical with the bactericidal one in bacteriolysis—that is to say, it is

the complement or alexin.[1] According to Ehrlich's view, whether it be normal or "immune" serum (*i. e.* serum of a treated animal), bacteriolysis or hæmolysis takes place only when the complement and amboceptor unite (Fig. 33, p. 182), complement by itself having little affinity for the bacterium or erythrocyte, the combination forming the "lysin," which then acts. According to Gruber, however, neither bacteriolysin nor hæmolysin exist as a chemical entity, the specific bacteriolytic or hæmolytic action being due to the fact that the cells first absorb the amboceptor and so become accessible to the complement, for the two substances do not combine in definite proportions—the more the blood-corpuscles are laden with the amboceptor the smaller the quantity of complement required to bring about their solution.

Many bacteria—*e. g. B. pyocyaneus, B. typhosus,* staphylococci and streptococci—produce hæmolysins, and the hæmoglobin staining occurring in septic diseases, etc., is probably partly due to the action of bodies of this nature elaborated by the infecting organisms.

Practical Uses of Hæmolysis, etc.

1. *Hæmolysis test.*—Some micro-organisms produce non-specific hæmolysins, others do not; this may constitute a difference between allied organisms. For instance, as a rule true cholera vibrio do not hæmolyse, while many cholera-like vibrios do so. The test can be applied in two ways: (*a*)

[1] As previously stated (p. 181), numerous complements undoubtedly exist, yet bacteria will absorb both bacteriolytic and hæmolytic complements. Bordet and Gengou suppose that while a particular amboceptor has a maximum avidity for its homologous complement (which may be termed *dominant*), it is also able to take up other "non-dominant" complements, and thus bacteriolytic amboceptor is able to absorb both bacteriolytic (dominant) and hæmolytic (non-dominant) complements.

Defibrinated rabbits' blood may be mixed with melted agar cooled to 45° C. The mixture is poured into Petri dishes, allowed to set, and when cool inoculated with the organism to be tested in such a manner that separate, well-defined colonies are obtained. After twenty-four hours' incubation at 37° C., colonies when hæmolytic are surrounded with a clear, well-defined halo contrasting sharply with the dark opaque colour of the agar. If blood-agar is not available, a substitute may be devised by smearing some sterile human or rabbits' blood on a sterile agar plate. (*b*) A young agar culture is emulsified in 4–5 c.c. of physiological salt solution; 0·1 c.c. of this suspension is mixed in a tiny test-tube with 0·9 c.c. of sterile salt solution and one drop of a sterile suspension of well-washed rabbit or other corpuscles. After twelve to twenty-four hours hæmolysis will be apparent if the organism forms hæmolysins.

2. *Fixation or absorption test.*[1]—A hæmolytic serum may be used as a delicate reagent for complement, and may thus serve as a test for an organism or an immune serum. As an example take the case of a supposed cholera vibrio. If an immune serum (previously heated to 56° C. so as to destroy complement)—hæmolytic for the corpuscles of an animal, or bacteriolytic for a given micro-organism, *e. g.* cholera vibrio—be mixed with the red corpuscles of the same animal, or with the cholera vibrio, the corpuscles or the vibrios respectively absorb the corresponding amboceptor or immune body.

Bordet showed that if corpuscles or microbes that have absorbed the corresponding amboceptor be added to fresh non-heated complement (*e. g.* fresh guinea-pig serum), the corpuscles or the microbes absorb the complement, so that none remains free in the liquid.

But if fresh guinea-pigs' serum be added to cholera vibrios which have not absorbed any cholera amboceptor, the complement will not be absorbed and remains free in the liquid. The proof of this is that if "sensitised" corpuscles (*i. e.* corpuscles which have taken up hæmolytic amboceptor) be

[1] Often termed "deviation of complement" test.

added to such a mixture, the globules are quickly hæmolysed. If, on the other hand, vibrios which have already taken up the cholera amboceptor be added to the same quantity of fresh serum, the microbe-amboceptor complex absorbs the complement; and provided the amount of fresh serum is not too great, the complement is absorbed so completely that "sensitised" corpuscles when added to the mixture are not dissolved. If vibrios other than cholera be added to cholera serum, the amboceptor is not fixed, the complement added remains free, and the sensitized corpuscles are dissolved. These facts constitute the "Bordet-Gengou phenomenon." The mixture of an inactivated hæmolytic serum (*i. e.* heated to 56° C.) with the homologous corpuscles (*i. e.* those with which the hæmolytic serum was prepared) is known as a "hæmolytic system." The method of carrying out the test is as follows: The cholera-immune serum is heated to 56° C. for half an hour. An eighteen hours old agar culture of the organism to be tested is suspended in 2 c.c. of sterile physiological salt solution. The complement is fresh rabbit or guinea-pig serum; a portion of this is also heated to 56° C. (= non-immune serum). The following mixtures are prepared in three small test-tubes:

Tubes 1 and 2 each contain 0·2 c.c. microbic suspension + 0·6 c.c. heated immune serum + 0·1 c.c. complement.
Tube 3 contains 0·2 c.c. microbic suspension + 0·6 c.c. heated non-immune serum + 0·1 c.c. complement.

These are well shaken to mix their contents, and are kept for half to one hour at 37° C. At the end of this time 0·1 c.c. of the following mixture is added to tubes 1 and 3: two volumes of heated (to 56° C. for half an hour) serum hæmolysing sheep's red corpuscles + one volume of washed sheep's corpuscles. To tube 2 is added 0·1 c.c. of a mixture of two volumes of physiological salt solution + one volume of washed sheep's corpuscles. The tubes are kept for a further hour or so at 37° C., and at the end of that time the occurrence of hæmolysis is noted. If the organism is homologous with the immune serum, the immune body will fix the complement in

tube 1 and *no* hæmolysis will occur; in tube 3 hæmolysis will occur because the complement remains free. Tube 2 serves as a control, and should show no hæmolysis in three hours (though if kept for eighteen to twenty-four hours hæmolysis will occur *if the organism produces hæmolysins*, apart from any action of complement). If the organism is not homologous with the immune serum, hæmolysis will occur in tube 1, because the complement does not become fixed, tubes 2 and 3 being the same as before.

The hæmolytic serum may be obtained by injecting rabbits with a 10 per cent. suspension of well-washed sheep's red corpuscles. Four doses of 5 c.c. intra-peritoneally or 2 c.c. intra-venously are given at intervals of a week, at the end of which period the rabbit's serum should be strongly hæmolytic. The sheep's blood should be obtained as aseptically as possible from the slaughterhouse; the blood, as it runs, is caught in a sterile wide-mouthed bottle containing a coil of fine wire with which it is defibrinated by shaking. The fluid blood is then mixed with sterile physiological salt solution (0·9–0·95 per cent.) and centrifugalised, and the deposited corpuscles are again washed with salt solution two or three times.

3. *Antigen Test.*—See "Syphilis."

Cells

CYTOTOXINS.[1]—Anti-sera, analogous to the hæmolysins or hæmotoxins, may be prepared which have a destructive action upon cellular elements; these are termed "cytotoxins." If a rabbit be injected with bull's semen, its serum ("spermotoxin") acquires the property of immobilising the spermatozoa of the bull. The reaction is specific, but spermatolysis does not seem to occur. Similarly, by injecting ciliated epithelium into the peritoneum of a guinea-pig an anti-epithelial serum, or "trichotoxin," is developed. With liver, kidney, and nerve cells anti-bodies having a destructive action upon these cells are developed as a result of their injection. Nephrotoxin, the serum of an animal inoculated with an emulsion of kidney, when injected into a second untreated animal, produces albuminuria and uræmia with disintegration of the epithelium of the convoluted tubules; hepatotoxin, the serum of an

[1] See Bulloch, *Practitioner*, May, 1901, p. 499. (Bibliog.)

animal treated with emulsions of liver, produces fatty and inflammatory changes in the liver resembling phosphorus poisoning; neurotoxin, the serum of an animal treated with emulsions of nerve tissues, produces paresis, paralysis, depression, convulsions, etc.; a leucotoxic serum obtained by injecting leucocytes agglutinates and dissolves the leucocytes, and so on. The formation and mode of action of these cytotoxins resemble those of the hæmolysins. It was hoped that the study and preparation of cytotoxins would open up possibilities in the way of treating such diseases as carcinoma and sarcoma, but so far this hope has not been realised.

AGGLUTINATION.—If an animal be injected with cultures of typhoid or cholera, its serum soon acquires the property of agglutinating or of aggregating into clumps the typhoid bacilli or cholera vibrios respectively when mixed with a broth culture of these organisms. The reaction may be observed microscopically in a hanging-drop preparation; the organisms first lose their motility and soon become aggregated into large masses or clumps. Macroscopically, the reaction may be followed in a narrow test-tube into which the mixture of culture and serum has been introduced; after some hours the micro-organisms become aggregated into masses so large as to form visible flocculi. The substances which bring about this agglutination are known as agglutinins. Agglutinins seem to be present in small amount in normal serum; for instance, most normal human sera up to a dilution of 1 in 2 or 1 in 4 will agglutinate the typhoid bacillus and still more powerfully the glanders bacillus They are also present in bacterial cultures; if an old broth culture of typhoid be filtered, the filtrate agglutinates the bacilli in a fresh broth culture; hence *young* cultures should always be used for agglutination tests. Agglutinin is formed

by the action of antigen derived from the bacterial cell, but may also be naturally present. Agglutination is brought about by the action of the agglutinin on the antigen; the agglutinin first unites with the antigen, and this may occur at 0°C., and afterwards exerts its specific action, which takes place only at higher temperatures and in the presence of certain salts. The agglutinable substance is known as agglutinogen. Agglutinin is converted into agglutinoid at 70°–75° C.; the latter does not agglutinate, though it unites with bacteria and then prevents the subsequent action of agglutinin.

The agglutination of organisms by anti-sera, though hardly specific, is usually very special; given proper precautions as to dilution, time-limit, condition of test culture, etc., an anti-serum will generally only agglutinate the homologous organism or closely allied species—that is, it is a group reaction. The anti-serum may agglutinate both the organism with which it has been prepared, and also allied species, though usually not to the same extent; anti-typhoid serum, for example, may agglutinate not only the typhoid bacillus, but also, though to a less degree, members of the paratyphoid group. As the result of infection or of inoculation with an organism, agglutinins may, however, be produced which agglutinate not only the organism of the infection, but also other organisms—*e. g.* typhoid serum may agglutinate the *B. coli* as well as the *B. typhosus* and typhus serum *B. typhosus* and *M. melitensis*. The agglutinins acting on the infecting organism may be termed primary or homologous, those acting on other organisms secondary or heterologous. In a case of double infection each organism may produce its own primary agglutinin, so that the agglutination of two species by a serum may be due to the presence either of a primary and a secondary agglutinin or of two primary

agglutinins. Castellani,[1] by applying the saturation test (p. 201), found that an organism would absorb both its primary and secondary agglutinins, but would not absorb two different primary agglutinins. This test, therefore, would distinguish a double infection from a single one. Thus, if a typhoid serum agglutinated both the *B. typhosus* and the *B. coli*, and the serum after saturation with typhoid culture still agglutinated the *B. coli*, this would point to an infection with the latter as well as with typhoid. The formation of primary and secondary agglutinins may be brought about as follows: In the bacterial cell there are several substances, each of which forms its own agglutinin. The cells of two bacterial species we can imagine both contain three or four substances capable of producing agglutinins, and it may happen that one of these in each species is the same and will produce the same agglutinin—the secondary agglutinin—and, therefore, the serum produced by each bacterium will agglutinate the other.

The agglutination reaction is made use of in bacteriological tests and in clinical diagnosis. The "Bordet-Durham" reaction consists in testing an unknown organism with a specific anti-serum prepared by injecting an animal with a known microbe; if the organism tested becomes agglutinated, it is regarded as being of the same species as that with which the anti-serum was prepared. With certain precautions the "Bordet-Durham" reaction is one of the most delicate and certain for the recognition of bacterial species. The converse of this is the agglutination reaction proper (frequently termed the Widal reaction), and consists in testing an unknown serum upon a known microbe. It is especially used in the diagnosis of microbial diseases; for example,

[1] *Zeitschr. f. Hyg.*, xl, 1902, p. 1.

in typhoid fever the blood of the typhoid patient powerfully agglutinates the typhoid bacillus, that of Malta fever the *Micrococcus melitensis*, that of bacillary dysentery the dysentery bacillus, etc.

A remarkable phenomenon observed in connection with agglutination, which the writer has particularly noticed in the case of Malta fever, is the occurrence of what may be termed a zone of no reaction or of inhibition with some particular dilution. Thus, dilutions of 1 in 10 and 1 in 20 may agglutinate strongly, a 1 in 30, however, may hardly agglutinate at all, while dilutions of 1 in 40 and upwards to 1 in 100 or more may agglutinate well. A similar phenomenon has been observed with non-specific agglutinating agents, and also in the action of coagulating agents on colloid emulsions. Thus orthophosphoric acid agglutinates a certain volume of a suspension of *B. coli* when present to the extent of between 118 cgrm. and 4 cgrm., and between 1·1 mgrm. and 0·001 mgrm., but not in intermediate amounts between 40 and 1·1 mgrm.

Anti-serum, prepared by injecting erythrocytes, also agglutinates the red blood-corpuscles, and in certain diseases, *e. g.* pneumonia, chromocyte clumping may be a marked feature.

Various theories have been propounded to account for the phenomena of agglutination:

1. Pfeiffer and Emmerich and Loew regarded agglutination as a vital paralysis of the bacilli due to the action of a bacteriolytic enzyme. Agglutination, however, is not a vital phenomenon, for dead bacilli agglutinate, and bacteriolytic enzymes seem to be destroyed by temperatures at which agglutinins remain unaffected.

2. Gruber, Dineur and Nicolle supposed that a glutinous substance, "glabrificin," is absorbed from the serum by the bacilli causing the cell membranes or the

flagella to become adhesive; but this explanation will hardly account for the aggregation of non-motile organisms.

3. Paltauf and Duclaux considered that a precipitate is produced in the medium, which during flocculation mechanically carries the bacilli with it; but there is no demonstrable evidence that such precipitation occurs.

4. Bordet separated the mechanism of agglutination into two stages—(1) fixation of agglutinin, and (2) aggregation. The fixation of agglutinin by the organisms he considers to be analogous to the adsorption of a dye by a tissue; and once the agglutinin is fixed, the organisms obey the laws of inert particles, aggregation being caused by changes in surface tension, in the molecular attraction, between the organisms and the surrounding medium, a view supported by Craw.[1] Ohno[2], however, believes that the union of agglutinin and agglutinable substance is not analogous to the fixation of a dye by a tissue, but that it is a chemical combination, as maintained by Ehrlich.

Agglutinated bacteria are not injured by agglutination; they will, in fact, grow and multiply in an agglutinating serum. The amount of agglutination does not bear any constant ratio to the intensity of an infection; on the whole, if the patient is reacting satisfactorily to an infection, the agglutination reaction tends to be marked; if not, it may be feeble or absent. Thus, in severe typhoid infections with fatal issue, agglutination may be absent. Ruffer and Crendiropoulo[3] regard the agglutinins as being formed in the polymorphonuclear leucocytes.

[1] *Journ. of Hygiene*, vol. v, 1905, p. 113. See also Joos, *Zeitschr. f. Hyg.*, xxxvi, p. 422, and *ibid.*, xl, p. 203.

[2] *Philippine Journ. of Science*, vol. iii, 1908, p. 47.

[3] *Brit. Med. Journ.*, 1902, vol. i, p. 821. (Bibliog.)

The Agglutination Reaction.

A. *For Clinical Diagnosis* (*" Widal " Reaction*).

This is principally made use of in typhoid and paratyphoid fevers, Malta fever, and bacillary dysentery.

Collection of blood.—Blood is collected (p. 131), preferably in a Wright's capsule (Fig. 35, *d*, p. 225), or in a capillary bulbous pipette (Fig. 7, p. 51), or in a vaccine tube. The ends of the tube are sealed, the *dry* end always being sealed first; the blood is allowed to coagulate (which may be hastened by placing in the blood-heat incubator), and then centrifugalised to separate the serum, care being taken that the dry sealed end of the tube, which will be perfectly sealed, is distal when spinning.

If tubes are not available, the blood may be spotted on to a piece of glass, cover-glass, or slide, glazed paper, tinfoil, etc., and allowed to dry. For use, a drop of distilled water is placed on the dry blood to dissolve it, and the solution used like serum.

The culture.—For the *microscopic* test a *young* broth culture is to be preferred. A hanging drop should be examined to ascertain that clumps are absent; this specimen is kept as a control. If clumps are present they may be removed (in the case of typhoid) by filtering the culture through filter-paper. A suspension of an agar culture may also be used, likewise dead cultures: a broth culture or suspension of an agar one being heated to 65° C. for ten minutes and preserved in sterilised glass pipettes; dead cultures are, however, unsatisfactory in tropical climates. For the *macroscopic* test a thick suspension of an agar culture in salt solution is to be preferred, the suspension being allowed to sediment for half to one hour before use. Some strains of an organism are better than others, and old laboratory strains are generally much more sensitive to agglutination than recently isolated ones.

Dilution of the serum.—This may be carried out in various ways, with the hæmocytometer pipette, with a pipette with rubber teat as used for opsonin work (Fig. 35, *a*, p. 225), or with a platinum loop. With the pipette a little serum is

aspirated up so as to occupy $1\frac{1}{2}$–2 cm. of the stem, and the upper limit is marked with a grease pencil or ink. A bubble of air is then admitted so that an air-space is left between the end of the pipette and the lower end of the column of serum. The end of the pipette is then immersed in a watch-glass of salt solution, and the salt solution is aspirated up to the mark, another bubble of air is admitted, and the process is repeated again and again ; so that, finally, the pipette contains 1 volume of serum and 4–14 volumes of salt solution, each volume being separated from the next one by an air-bubble. The contents of the pipette are then expelled into a watch-glass and thoroughly mixed, and further dilution of this dilution is performed in the same manner. Two or three dilutions are usually made—*e. g.* 1 in 15, 1 in 25, and 1 in 50. A platinum loop may also be employed as a measure ; a loopful of the serum is deposited in a watch-glass, and by spotting round it nine or fourteen loops of salt solution a dilution of 1 in 10 or 1 in 15 is prepared, or any other dilution in a similar manner.

The microscopic test.—Two or three hanging-drop slides are vaselined, and two or three cover-glasses cleaned. One loopful of a dilution of serum is placed on each cover-glass, and to each is added a loopful of the broth culture of the organism —*e. g.* typhoid—and well mixed up, and the specimens are mounted as hanging drops. Starting with three dilutions of serum—*e. g.* 1 in 15, 1 in 25, and 1 in 50—the dilutions in the specimens will be 1 in 30, 1 in 50, and 1 in 100 respectively. Should only one dilution of serum have been made—*e. g.* 1 in 15—if on each cover-glass one loopful of this be placed, and to the first be added one loopful, to the second two loopfuls, and to the third three loopfuls of typhoid culture, then the final dilutions in the three specimens will be 1 in 30, 1 in 45, and 1 in 60 respectively.

Care should be taken that the hanging-drop cultures are quite sealed with the vaseline, so that evaporation is prevented. The hanging drops are then examined microscopically, a $\frac{1}{6}$-in. objective sufficing for typhoid. In the case of

typhoid the following phenomena will be observed: The motility of the majority of the bacilli is instantaneously or very quickly arrested, and in a few minutes they begin to aggregate together into clumps, and by the end of the half hour there will be very few isolated bacilli visible. In less marked cases the motility of the bacilli does not cease for some minutes, while in the least marked ones the motility of the bacilli may never be completely arrested, but they are always more or less sluggish as compared with the control hanging drop made from the culture, while clumping ought to be quite distinct by the end of one hour (with a 1 in 30 to 1 in 50 dilution).

The central portions of the drop should be examined, not the margins. With blood which has been dried and dissolved, organisms may become entangled in *débris*, and must not be mistaken for clumps.

In all cases two or three different dilutions should be made to exclude the possibility of a "zone of no reaction" with some particular dilution (see p. 196).

Macroscopic, or sedimentation method.—The *serum*, having been diluted by means of a pipette with four times its volume of salt solution, is mixed with five to twenty times its volume of culture suspension containing plenty of micro-organisms in the same manner as described in the previous section. The mixture is sucked up into a fine, but not capillary, bore tube. This is sealed at the lower end and allowed to stand in the upright position for eight to twenty-four hours at 20° C., or six hours at 37° C.; the reaction is often distinct within an hour at 37° C. When the reaction is positive the organisms become agglutinated, and form flocculi, which are easily seen with the naked eye or with a hand-lens and stick to the sides or sink to the bottom of the tube. The dilution usually employed is 1 in 30 to 1 in 50. Whole blood is not suitable for the sedimentation test; clear serum should always be used. It is well to set up at the same time a control tube with saline solution, or, preferably, with normal serum.

If sufficient serum is available the mixture may be put up

in little test-tubes, such as the inner tubes of Durham's culture-tubes (p. 84).

B. *For the Recognition of Bacterial Species.*

1. *Bordet-Durham Reaction.*—This is carried out in much the same manner as for clinical diagnosis, but an immune serum of high agglutinating value or high "titre" (at least 1 : 1000) is required, and the serum from a patient is not applicable. The immune serum may be obtained from a horse or other animal immunised with killed cultures (and living also if a high titre is required). In the laboratory the serum may be prepared by giving a rabbit three to five intravenous injections at intervals of seven days of killed culture of a virulent strain of the organism, *e. g.* typhoid or cholera. The culture is killed by heating to 60°–65° C. for half an hour, and the dose is increased from one loop to ten loops of an agar culture. Seven days after the last dose the animal is bled from an ear vein, and the serum obtained. The agglutinating value of the serum must be determined, and controls should always be put up with normal serum of an animal of the same species as that from which the immune serum has been obtained. A series of dilutions of both sera is made with salt solution and a twenty-four-hour agar culture of the organism to be tested used. Both the macroscopic and microscopic methods should be employed. The dilutions may be made with a 1 c.c. pipette graduated in hundredths, with the hæmocytometer pipettes, or by the method used clinically.

2. *Saturation test.*—Bordet first noticed that a suspension of a microbe added to the homologous agglutinating serum absorbs most, if not all, the specific agglutinin, whereas an organism not homologous with the serum absorbs little or only a portion of the agglutinin. The test may be carried out as follows :

(*a*) Ten loopfuls of a young agar culture of the organism to be tested are mixed with 10 c.c. of a 5 per cent. solution of a highly agglutinating serum. After standing for two or

three hours at room temperature, the mixture is centrifugalised and the clear supernatant fluid decanted.

(*b*) The agglutinating power of the decanted liquid is then tested on the organism with which the serum was prepared. If the organism treated in (*a*) is homologous with the organism with which the agglutinating serum was prepared, the decanted fluid will have lost most, or a considerable proportion, of its agglutinating power for the latter.

The Meiostagmin Reaction.—Ascoli has found that if an immune serum be mixed with an alcoholic extract of the homologous antigen and the mixture incubated at 37° C. for two hours the surface tension is reduced; if the serum and antigen extract are not homologous the surface tension is unaltered. For example, in the case of typhoid the following is the procedure. An alcoholic extract of typhoid bacilli is prepared; this is diluted with saline solution to 1-1000—1-1,000,000. The typhoid serum is similarly diluted, 1-10. To 9 c.c. of the diluted serum 1 c.c. of the diluted antigen extract is added. By means of some form of viscosimeter or stalagmometer the number of drops yielded by a given volume of the mixture is ascertained, immediately after the mixture is made and after the mixture has been incubated at 37° C. for two hours. If the surface tension has been reduced, the number of drops counted in the second determination will be greater than in the first.[1]

Anti-ferments.[2]—By the injection of rennin or other enzyme the blood-serum of the treated animal acquires the property of neutralising the action of the enzyme with which the inoculation has been performed. Thus if rennin and anti-rennin (the serum of an animal injected with rennin) be mixed with milk no curdling takes place. Similarly, the serum of an animal inoculated with pancreatin inhibits the action of this ferment, and if coagulated egg-albumen, pancreatin, and anti-pancreatin be mixed, the egg-albumen undergoes no digestion.

[1] See Ascoli and Izar, *Münch. med. Woch.*, lvii, 1910, pp. 62, 182, and 403.

[2] See Dean, *Trans. Path. Soc., Lond.*, vol. lii, 1901, Part 2, p. 127.

Precipitins.[1]—Kraus was the first to demonstrate the presence of specific precipitins in blood by adding typhoid, cholera, and plague anti-sera to filtrates of the cultures of the corresponding microbes. If to such a filtrate in a test-tube a little of the corresponding anti-serum be added by running in carefully, so that it forms a layer at the bottom, an opalescent ring makes its appearance at the line of junction of the two fluids. So also if an animal be injected with milk, its serum, when added to milk of the same kind as that with which it has been injected, causes precipitation of the casein. This reaction is specific, and it is thus possible to distinguish various milks from one another. Similarly, anti-sera which produce precipitates, each with the homologous substance, are obtained by the injection of peptone, of egg-albumen, blood-serum, and other proteins. The latter reaction has an important medico-legal application, for by means of it the blood and flesh of different species of animals can be distinguished. Thus the presence of horseflesh in sausages can be detected. The method employed is to inject a rabbit intraperitoneally with four to six injections of defibrinated blood or of blood-serum (or with a solution of the particular substance, e.g. horseflesh), commencing with about 5 c.c. and increasing to 10 c.c. at intervals of a few days. After treatment the animal is bled from an ear vein, and the serum is obtained. The blood to be tested may be dried on filter-paper, pieces are then cut up, a solution is made in 1·6 per cent. sodium chloride solution, and to this the specific serum is added. Tested in this way human blood anti-serum reacts—*i. e.* forms a precipitate—markedly with human blood, less so with ape's blood, not at all with other blood; ox blood anti-serum reacts with ox blood, less so with sheep, feebly with horse, hardly at all with dog. Mixtures of bloods may also be tested. Precipitins are also formed naturally *in vivo*. Thus the serum of a patient the subject of hydatid disease gives a precipitate with

[1] See Nuttall, *Journ. of Hyg.*, vol. i, 1901, p. 367 (Bibliog.), also *Brit. Med. Journ.*, 1902, vol. i, p. 825; Welsh and Chapman, *Journ. of Hygiene*, vol. x, 1910, p. 177; *ibid.*, *Australasian Med. Gazette*, December 21st, 1908 (hydatid disease).

hydatid fluid, and the reaction may be used diagnostically. The production of the anti-body seems to be due to the globulin constituent of the injected serum.

It will thus be seen that the anti-bodies which result from the injection into an animal of different substances are extremely numerous and have varied properties, their most notable characteristics being their extreme specificity and the extraordinary delicacy of the interactions produced by them. It is important to note that these anti-bodies are produced only as the result of inoculation with complex compounds allied to the proteins. The tolerance established by the ingestion or inoculation of simpler compounds, such as arsenious acid and morphine, is of a different nature, and is not coincident with the development of anti-bodies. According to Ehrlich, the latter kind of tolerance may be due to the exhaustion or using up of certain receptors ("chemo-receptors") of the protoplasm (see p. 216).

Immunity.[1]

No fact in biology is more striking than the differences in susceptibility to infection exhibited by different races and different animals. For example, the natives in many parts of the world are comparatively insusceptible to yellow and typhoid fevers and malaria, the dog and goat are rarely affected with tuberculosis, and tetanus is never met with in the fowl; and to come nearer home, while some individuals are lucky enough to escape most of the commoner infectious fevers, others seem to

[1] See Metchnikoff, *Immunity in Infective Diseases*, 1905. Also *Brit. Med. Journ.*, 1902, vol. i, p. 784; 1904, vol. ii, pp. 557–582; and 1907, vol. ii, pp. 1409–1425; *Journ. of Hygiene*, vol. ii, 1902; Emery, *Immunity and Specific Therapy*, 1909.

contract them on every possible occasion, and to suffer from all the ills that flesh is heir to. These instances show that there is often a natural insusceptibility to infective disease, or a natural immunity, as it is termed. This may be complete or partial, or it may appertain only to a race—"racial immunity"; or, varying in different individuals and at different ages, it constitutes "individual immunity," as in the case of diphtheria and scarlatina, which become more and more rare as age advances.

Still more striking, perhaps, is the fact that an insusceptibility may be acquired after an attack of infective disease or be conferred in certain instances by inoculation. Thus second attacks of smallpox and scarlatina are rare, inoculated smallpox and vaccinia protect against variola, and bacterial vaccines confer considerable protection.

With regard to the immunity of native races to certain diseases, this is probably due to natural selection and heredity; during long periods of time, the individuals being all exposed to the same risks, the susceptible ones are weeded out, while the survivors transmit their insusceptibility to their descendants; but this, of course, does not explain the reason for the relatively greater immunity of the insusceptible individuals. Immunity is generally not absolute either to infection or intoxication; that is, infection can usually be induced under certain conditions. Thus fowls, which are highly refractory to tetanus and tolerate considerable doses of tetanus toxin with impunity, can be tetanised with large doses of an active toxin; white rats, which are insusceptible to anthrax, become susceptible after fatigue, or when fed on an exclusively vegetable diet. Immunity is therefore either (1) natural, or (2) acquired, and it is evinced against either (*a*) toxins,

or (*b*) micro-organisms, and these different phases must be considered.

1. *Natural immunity against toxins.*—There are various non-specific reactions in the body by which toxins may be eliminated or destroyed. Thus the dilatation of the vessels and the acceleration of the blood-stream which take place in an inflamed area dilute and eliminate the toxin, and the proteolytic enzymes produced by the organisms and as a result of tissue disintegration may have a destructive action on the toxins. Oxidation, hydration and dehydration and various analytic and synthetic processes which go on in the body, and particularly in the liver, are other agencies whereby toxins may be destroyed. These non-specific processes by which toxin is destroyed or eliminated, though of the greatest importance, can probably deal with only *small* amounts of toxin; if *large* amounts are present, specific reactions have to be evoked.

Another cause of natural immunity to toxins may be the absence of suitable receptors for the toxin. As already stated (p. 161), in order that a bacterial toxin or endotoxin may produce intoxication, it must become anchored to the cells by its haptophore group, and that this may occur the cell molecules must possess atomic groups or side-chains ("receptor groups") which have a special affinity for the haptophore groups of the toxin. Should these be wanting the toxin cannot become anchored to the cells, its toxophore groups cannot exert their influence, and natural immunity is the result.

This has been proved to be the case in several instances. Thus in the lizard and turtle, if tetanus toxin be injected no effect is produced, but the toxin is not eliminated and remains in the body for months, as may be proved by withdrawing a little of the blood

and injecting it into a mouse; the animal dies of tetanus.

In other instances, for some reason or other, the cells of the animal are insusceptible to the toxophore group of the toxin. Thus, if an alligator be injected with tetanus toxin, no effect is produced, but the toxin rapidly disappears from the blood. If the animal be kept at ordinary temperature (20° C.), although the toxin disappears, antitoxin is not formed, but if it is kept at 30°–37° C. antitoxin is rapidly produced. The two experiments together suggest that the toxin is fixed by the cells, but has no effect upon them; if the toxin were not fixed, it would be possible to detect it, and presumably it would not produce antitoxin.

2. *Natural immunity against micro-organisms.*—A number of factors are doubtless concerned in preserving the body from invasion by micro-organisms, and while non-specific reactions may suffice when the number of organisms is small, specific reactions have to be evoked if the number of organisms is large. The unbroken surfaces of the body have a considerable protective action in preventing the entrance of micro-organisms. The flushing-out action of accelerated circulation will exert some action in eliminating organisms from a localised focus of infection just as it does with toxins. The body temperature may be of some importance, and the febrile condition so generally induced by infection is probably to some extent protective and curative. Thus frogs, fish, and chickens are naturally immune to anthrax. In the one case the body temperature is low, 18° C. or thereabouts; in the other it is high, 40° to 41° C., and this may influence the growth of the anthrax bacillus, preventing the full and rapid development which may be necessary for the production of the disease. That such is the case would seem

to be shown by experiments in which when the temperature of the medium is raised or lowered, infection takes place; frogs and fish kept in water raised to a temperature of 35° C., and chicken refrigerated so as to reduce their temperature, all perish from anthrax after inoculation. It is clear, however, that this is not necessarily the only factor, for sparrows, which have a temperature as high as that of the chicken, can be infected with anthrax without refrigerating. Behring would ascribe the immunity of white rats to anthrax to the high alkalinity of their blood, and claims to have shown experimentally that a vegetable diet reduces this, and fatigue is said to act similarly.

In some cases the animal, after invasion by the organism, becomes gradually tolerant to its presence (*immunitas non sterilisans*). This is particularly the case in protozoan infections, *e. g.* piroplasmosis. The animal, after a period of ill-health, gradually recovers, though the organisms may still be present, as can be demonstrated by injecting some of its blood into a susceptible animal. Conceivably the receptors necessary for the intoxication become gradually used up, and when this state is attained the animal becomes insusceptible.

The blood, lymph, and other fluids and tissue juices undoubtedly exert a more or less germicidal action on bacteria experimentally *in vitro*, and to some extent probably also in the body. But in this respect there is often a marked difference between the circulating blood and the blood *in vitro*.

Lewis and Cunningham (1872), Traube and Gscheidlen (1874), Fodor (1877), and Wysokowicz showed that bacteria injected into the circulation rapidly disappear, and were inclined to attribute this result to the bactericidal properties of the blood. In the main, however,

this disappearance is due to lodgment in the capillaries, phagocytosis, and excretion by the excretory glands.

Halliburton prepared from the lymphatic glands a protein, cell-globulin β (really a mucleo-protein). Hankin found that this substance had marked germicidal properties, and concluded that it was probably the germicidal constituent of the blood-serum. Bitter, who repeated Hankin's experiments, failed, however, to confirm them. To the germicidal constituents of the cells and body fluids Buchner gave the name "alexins."

Grohmann performed the first experiments with extra-vascular blood. He found that anthrax bacilli, after being kept in plasma, became less virulent. Fodor, adding anthrax bacilli to blood and plating at intervals, found there was a progressive diminution in the number of organisms.

Nuttall, in 1888, used the defibrinated blood of several animals, rabbits, mice, pigeons, sheep, and found that it destroyed the *B. anthracis*, *B. subtilis*, *B. megaterium*, and *M. pyogenes* var. *aureus*. He confirmed Fodor's results, which also showed that after a while the blood loses its germicidal properties and becomes a suitable culture medium. The blood or serum similarly loses its bactericidal properties on heating, and serum that has once been used loses its bactericidal properties. Nissen continued this work, and also found that fresh serum is germicidal for a variety of organisms.

In 1890, Buchner with Voit, Sittmann, and Orthenberger came to the conclusion that the germicidal action of cell-free serum is due to the protein constituents.

Christmas prepared a germicidal substance from the spleen, and Bitter, who examined the method, in the main confirmed Christmas.

Behring and Nissen, however, found that the serum

of the white rat, dog, and rabbit destroys the *Bacillus anthracis*, but serum from the mouse, sheep, guinea-pig, chicken, pigeon, and frog has no action. Thus, while the rabbit is highly susceptible to anthrax, its serum is germicidal; the chicken, on the other hand, is immune to anthrax, but its serum is inactive. Hence there is a considerable difference between the action of circulating and of extra-vascular blood.

Vaughan, Novy and McClintock, in a series of papers, ascribed powerful bactericidal properties to the nucleins, and surmised that in serum the nucleins set free by the disintegration of leucocytes and other cells are the germicidal agents. Forrest and the writer[1] found, however, that all the germicidal properties ascribed by Vaughan to the nucleins are probably due to the weak alkali in which the nucleins were dissolved, and came to the conclusion that Vaughan's results are at least not proven.

Gengou also found that the *plasma* collected in vaselined tubes is often almost devoid of bactericidal power, whilst the corresponding *serum* may be capable of destroying large numbers of micro-organisms.

We therefore see that while the blood, lymph, and other fluids and tissue juices undoubtedly exert more or less germicidal action on bacteria experimentally *in vitro*, there is often a marked difference in this respect between the circulating blood and the blood *in vitro* and it may be doubted if this factor is of great importance in the production of natural immunity. At the same time, it is to be noted that directly infection has started more or less cellular disintegration and serous exudation occur, and thus the germicidal action of the body fluids and tissues may be exerted *in vivo*, though such substances may act rather by stimulating

[1] *Journ. Roy. Army Med. Corps.*

the leucocytes or by rendering the bacteria more phagocytosable, as will be referred to later (p. 220).

Thus Kanthack and Hardy found that the coarsely granular oxyphile leucocytes in the frog are first attracted to the site of a bacterial invasion, there discharge their oxyphile granules, the bacteria then show signs of degeneration, and polymorphonuclear leucocytes and other "phagocytic" cells now approach and ingest the degenerate bacteria. The observations, however, do not seem to have been confirmed. Wooldridge also protected animals from anthrax by injections of "tissue fibrinogen" (nucleo-protein). For some micro-organisms a bacteriolytic mechanism exists, the amboceptor-complement complex, whereby they may be digested and got rid of. Thus normal serum has a marked bacteriolytic action on *B. typhosus* and *B. coli*. In many cases, however, *e. g.* for staphylococci, such a bacteriolytic mechanism does not naturally exist, but may be evoked as a result of infection.

The hypothesis which ascribes immunity to the germicidal and bacteriolytic action of substances in the fluids of the body has been termed the "humoral theory."

Another important theory of immunity is the doctrine of phagocytosis, so ably supported by Metchnikoff. This is the "cellular" theory of immunity. It has as its basis the following fundamental facts: Firstly, the leucocytes in the circulating blood ingest and destroy any foreign particles present therein; secondly, an injury to the tissues is immediately followed by an inflammatory reaction, in which the leucocytes emigrate from the vessels and congregate at the injured spot. Similarly, in many instances the leucocytes rapidly congregate at the seat of a bacterial infection, and approach and engulf the bacteria in the same manner as they

do other foreign particles, and so rid the body of the unwelcome guests (Plate I., *a* and *b*).

The migration of the leucocytes towards the scene of action is explained by Metchnikoff on the hypothesis that the chemical substances elaborated by the bacteria attract the latter and exert what he termed "positive chemotaxis." In this case the bacteria are removed by the leucocytes, and general infection and death do not occur. But, unfortunately, in other cases the bacterial chemical products repel, or perhaps it is more correct to say do not attract, the leucocytes, and "negative chemotaxis" occurs, so that the bacteria are free to grow and multiply, and general infection ensues. Positive and negative chemotaxis can be shown to occur by a simple experiment. If a fine capillary tube containing some peptone solution be introduced into a suspension of bacilli, *e. g. B. fluorescens liquefaciens*, under a cover-glass, and watched microscopically, the bacilli will be attracted to the tube and soon invade its lumen. If, however, a weak acid be substituted for the peptone water, the bacilli will be repelled. The process by which the bacteria are ingested by the leucocytes can be similarly watched. The leucocytes which act in this manner are termed phagocytes, and they are of two classes—the macrophages, the large mononuclear leucocytes, and the smaller microphages, or polymorphonuclear leucocytes. Certain of the tissue cells and endothelial cells also possess phagocytic properties. The importance of phagocytosis is also shown by the fact that, while in ordinary susceptible rabbits infection with anthrax is followed by a feeble phagocytosis and the animals succumb, in rabbits vaccinated against anthrax phagocytosis is very active. Moreover, in an animal refractory to anthrax, such as the frog, anthrax bacilli grow and multiply if they be enclosed in paper

or collodion sacs, so as to prevent the access of the phagocytes.

Phagocytosis, *in vitro*, and probably also in the normal body, is extraordinarily active, so that it might be expected always to be sufficient to deal with any number of bacteria that might be introduced. If, however, the bacteria be virulent, negative chemotaxis will occur. Moreover, the presence of substances which render the bacteria phagocytosable, "opsonins," is necessary, and it seems likely that the amount of opsonin becomes diminished in infection (see p. 221).

Metchnikoff admits that the destruction of bacteria in phagocytosis is brought about by chemical bacteriolytic substances, which he terms "cytases," and which he regards as being derived from the leucocytes, and as identical with the alexins. He believes that there are two kinds of cytases, one "macrocytase," obtainable from tissues, such as the spleen and lymph-glands, rich in macrophages, which acts specially on elements of animal origin, the other "microcytase," derived from the microphages, and which acts principally on micro-organisms. He considers the alexic action to be of the nature of a digestive process (but this is doubtful), and as regards the complex nature of a cytolytic serum, which contains amboceptor and complement, believes that the amboceptor is formed within the macrophages in intra-cellular digestion, and that a portion of it escapes from them into the serum. All the facts point to the leucocytes and leucocytic tissues being the great defensive mechanisms against parasitic invasion, either by the production of alexins, or of bacteriolysins, or by phagocytosis, or probably by a combination of these (the "cellulo-humoral" hypothesis of immunity). It is probable that the greater

part of phagocytosis takes place in the spleen. This organ acts as a sort of filter, and phagocytosis may be active in it when none can be discerned in the blood. Phagocytosis is also active in the bone-marrow.

Experiments by Tizzoni and Cattani seemed to show that rabbits could not be rendered refractory to tetanus by injection of tetanus antitoxin after extirpation of the spleen; and although Benario and other observers have not confirmed this, the manner in which the spleen is attacked in such diseases as tuberculosis, plague, etc., points to this conclusion. The discordant results obtained after splenectomy may be due to the rapid regeneration of spleen tissue, and to other structures, such as the hæmolymph glands, taking on its functions after ablation.

Although small amounts of antitoxin may occasionally be met with in the normal animal (*e. g.* diphtheria antitoxin in man and in the horse, see pp. 160 and 286), this substance plays little or no part in natural immunity against either toxin or micro-organism. Thus the blood-serum of the fowl, which is highly refractory to tetanus, does not exert the slightest antitoxic or neutralising action on tetanus toxin.

3. *Acquired immunity.*—Acquired immunity may be induced in several ways:

(1) By an attack of the disease ending in recovery.

(2) By vaccinating with a modified and less virulent form of the living infective agent (Pasteur's method).

(3) By treatment with sterilised cultures, or with bacteria-free toxins.

(4) Occasionally by treatment with sterilised cultures or toxins of a different species. Thus, *B. pyocyaneus* protects from anthrax (p. 250), and Klein[1] showed that an injection of one of the six following organisms—(1)

[1] *Trans. Path. Soc. Lond.*, 1893, p. 220.

Koch's comma, (2) Finkler-Prior's comma, (3) *B. coli*, (4) *Proteus vulgaris*, (5) *B. prodigiosus*, (6) *B. typhosus*—will protect an animal against any one of the remaining five. He therefore concluded that there is an immunising agent common to all these six organisms, and that this substance is intra-cellular and a constituent of the bacterial cells themselves. In this case, however, the immunity is probably one against certain bacterial proteins and not against the specific endotoxins of the organisms.

(5) By injection of the blood-serum derived from an animal treated or immunised by method 3—that is to say, antitoxins or other anti-bodies (*e. g.* amboceptors) are introduced.

The immunity acquired by methods 1–4 is known as "active immunity," because the animal's cells and tissues are altered by the process, so that they are no longer susceptible to the microbe or its toxin. The immunity conveyed by method 5—the injection of an immune serum, is known as "passive immunity," because the immunity lasts only so long as the anti-bodies remain; there is no active participation of the animal's cells and tissues in the process. Active immunity is generally of long duration—some months at least—and is not transmissible to the fetus; but passive immunity is of short duration—two to four weeks—and is transmissible to the fetus and nursling. Acquired immunity to toxins may be due to the elimination of the receptors concerned in the fixation of the toxin by the cells, or to the production of the neutralising antitoxin. The leucocytes are probably the active agents in destroying and eliminating toxin, whether neutralised by antitoxin or not.

Passive

Various explanations have been given of the production of acquired immunity against the organisms.

Pasteur suggested that the organism, by its growth in the body, exhausts some specific pabulum necessary for its development, so that it cannot again grow in the animal which has been attacked. This hypothesis, therefore, presupposes that in the body there is some nutrient material necessary for the growth of each species, which is difficult to believe, and is negatived by the fact that an organism will grow in the blood and tissues removed from an animal vaccinated against, and insusceptible to, the disease produced by itself.

Pasteur's "exhaustion" theory has been revived by Ehrlich[1] in a modified form, under the name of "atrepsy," to explain certain cases of immunity. Thus, for a chemical poison to act, Ehrlich assumes that particular receptors in the protoplasm for binding the poison are necessary; these he terms "chemo-receptors." Bird-pox, virulent for both fowl and pigeon, if passed through the pigeon becomes completely avirulent for the fowl. To explain this Ehrlich suggests that the parasite in passing through the pigeon has to assimilate substances different from those assimilated during its passage through the fowl; therefore that part of the receptors which deals with the nutritive substances of the fowl's organism is not in use during the passage through the pigeon, and may become atrophied, so that on the parasite being transferred back to the fowl it will not be able to thrive owing to the loss of the receptors necessary to assimilate the fowl's nutritive substances. Ehrlich suggests that the majority of non-pathogenic micro-organisms, if introduced into the animal body, perish by this mechanism. In the case of mouse carcinoma inoculated into rats, the tumour-cells proliferate for a few days, then atrophy and disappear. Ehrlich suggests that some specific substance is necessary for the proliferation of mouse carcinoma-cells which is not present in the rat, and as soon as the traces of this specific substance carried over by the inoculation are

[1] "Harben Lecture," ii, *Journ. Roy. Inst. Public Health*, 1907.

used up, the cancer-cells cease to proliferate and finally atrophy and disappear. These are examples of Ehrlich's "atrepsy" and "atreptic immunity."

Chauveau, in his retention theory, suggested that the bacteria during their growth in the tissues form substances which ultimately inhibit their growth, and, if the animal recovers, prevent a subsequent development of the organism. The same objections may be urged against this hypothesis as against Pasteur's exhaustion hypothesis.

Bacteriolysis and phagocytosis are probably the two main factors which bring about the refractory condition in acquired immunity against bacteria, as well as recovery from an infection. After immunisation it may be shown that phagocytosis is increased, and that positive chemotaxis takes place towards the organism, whereas previously negative chemotaxis occurred; the leucocytes have been "educated," as it were, to be attracted, instead of repelled, by the bacterial invasion. According to Andrewes,[1] the defence against the pyogenic cocci is not only essentially phagocytic, and dependent upon the polynuclear lencocytes, but is also, in the main, opsonic. In tuberculosis and syphilis the polynuclear leucocyte takes little part in bodily defence, which is essentially a function of the endothelial and fixed tissue-cells. With the colon group of organisms certain humoral responses, notably agglutination and bacteriolysis, are better marked than with most other bacteria, and polynuclear phagocytosis seems subsidiary.

Antitoxin formation probably plays little or no part in acquired immunity, or even in recovery from infection. In diphtheria, for instance, antitoxin is not found until the disease has subsided. Possibly, in

[1] "Croonian Lectures," *Lancet*, June 25th *et seq.*, 1910.

chronic infections, antitoxin formation does play a subsidiary *rôle* in recovery.

To sum up, natural immunity is probably due to a number of factors, some or all of which may be operative in particular instances, and it is impossible to state with certainty any general law. In most cases phagocytosis is the principal means of defence, the germicidal, inhibitory, or bacteriolytic actions of the body-fluids aiding, though of subsidiary importance; in others the cells and tissues are unaffected by the bacterial toxins, sometimes because the cells are lacking in the particular side-chains or receptors which fix the toxin; sometimes because, for some unknown reason, the cells are unaffected by the toxophore group of the toxin.

As regards the immunity acquired after an attack of disease, this may be due to the "education" of the leucocytes, whereby they are attracted, whereas formerly repelled, by the products of bacterial development, or to substances which stimulate the action of the leucocytes. The germicidal, inhibitory, and bacteriolytic actions of the body-fluids may also be enhanced. It seems probable also in certain instances that the side-chains or receptors having an affinity for the toxin become in some way destroyed or used up, so that further fixation of the particular toxin cannot take place.

It is to be noted, as Metchnikoff has pointed out, that immunity is much more rapidly acquired against micro-organisms than against their toxins. In Nature, it is principally against micro-organisms that the body requires protection.

Adaptability seems to be one of the innate properties of protoplasm, and immunity is but an instance of adaptability. It might be expected, therefore, that immunity towards infection will become established,

more or less completely, when the need for it arises; and we find that this is the case, however difficult it may be to explain the mechanism by which it is attained.

The Rôle of the Serum in Phagocytosis.[1]

The fact that in an immunised animal, no sooner does the virulent organism gain access than the leucocytes migrate to the site of infection, surround the invaders, ingest and so destroy them, was at one time ascribed by Metchnikoff to "education, *i. e.* modification, of the leucocytes; but since the serum of the immunised animal injected into a non-immunised one causes the leucocytes in the latter to behave in the same manner as they do in the immunised animal, the effect must be due to something in the plasma or serum, and Metchnikoff ascribed the action to substances, "stimulins," which heighten the activity of the leucocytes. Later work has not confirmed this view, and no certain proof of the existence of stimulins is forthcoming, although Leishman attributed a stimulin action to thermostable substances in the serum in typhoid and Malta fevers. Subsequently Metchnikoff conceived the serum as acting, not on the leucocytes, but on the microbe, causing it to become positively chemotactic and no longer to repel, but to attract the phagocytes. Considerable support was given to this view by the work of Wright and Douglas, who, by a modification of Leishman's ingenious method for quantitatively estimating phagocytosis, emphasised the importance of the serum in the mechanism of phagocytosis.

Neufeld and Rimpau also concluded that substances, "bacteriotropines," are produced in the course of

[1] See Dean, *Brit. Med. Journ.*, 1907, vol. ii, p. 1409. (Bibliog.)

immunisation which promote the phagocytosis of bacteria.

Leishman's method for estimating phagocytosis.[1]—A thin suspension of some micro-organism, *e.g. M. pyogenes*, is mixed with an equal volume of blood from the finger; a droplet of this mixture is placed on a clean slide, and covered with a cover-glass, and the preparation is at once placed in a moist chamber in the incubator at 37° C. for half an hour. At the end of this time it is taken out, the cover-glass slipped off, and the films on slide and cover-glass are dried, fixed, stained and examined microscopically, and the number of microbes ingested by the polymorphonuclear leucocytes is counted.

Wright and Douglas[2] found that washed leucocytes without serum are non-phagocytic, but become so on the addition of normal serum. If, however, the serum be first heated to 60°–65° C. before being added to the mixture of leucocytes and microbes, phagocytosis does not take place; but if the unheated serum is mixed with the bacteria, the mixture kept at 37° C. for fifteen minutes and then heated to 60° C. for fifteen minutes, phagocytosis can still take place, thus demonstrating that the serum acts in some way on the bacteria, rendering them suitable prey for the phagocytes. This thermolabile serum feast preparer is called by Wright and Douglas "opsonin" (from a Greek word meaning "to cater for").

They have also shown that during the process of active immunisation the opsonic value of the serum is increased, and they have succeeded in demonstrating this opsonic immunity for a number of infections, such

[1] *Brit. Med. Journ.*, 1902, vol. i, p. 73.

[2] *Proc. Roy. Soc. Lond.*, B. lxxii, 1903, p. 357; B. lxxiii, 1904, p. 128; B. lxxiv, 1905, pp. 147, 159; B. lxxvii, 1907, p. 211. Also in *Practitioner*, May, 1908; various papers in *Lancet* and *Brit. Med. Journ.*; Wright, *Studies in Immunity*, 1909.

as the staphylococcic, Malta fever, pneumococcic, and tuberculous. If it be desired to measure the quantity of opsonins present, say in a case of furunculosis, which is almost always caused by the *M. pyogenes*, the following are required: (1) a drop or so of the patient's serum; (2) a drop of serum from a normal person; (3) a suspension in salt solution, of a culture of *M. pyogenes* preferably derived from the furuncle; (4) leucocytes washed free from the plasma. Equal volumes of the patient's serum, leucocytes, and suspension are mixed, drawn up in a capillary tube, incubated for fifteen minutes at 37° C., and films are then prepared and stained. As a control a similar mixture is prepared and treated in the same way, but using the *normal* serum instead of that of the patient. The films are then examined, and the number of cocci taken up by, say, fifty leucocytes is counted in the two specimens, and a ratio obtained. Taking the figure for the normal serum as 1, that for the patient's serum will probably be 0·5 or 0·6, and this is termed the "opsonic index" (see below, p. 229).

In subacute and chronic local infections the opsonic value of the serum is usually diminished, occasionally increased. In acute infections the index will, as a rule, be low; in chronic infections which are not strictly localised, *e. g.* tuberculosis, the index will sometimes be low, sometimes high. A low index generally indicates an infection, or a low power of resistance to the particular organism, or that a chronic but quiescent infection exists; a high index may indicate that the person has had an infection but has overcome it, or has a quiescent infection. The normal index for healthy persons varies only within narrow limits, from about 0·8 to 1·2 as extremes; an index above or below these values is therefore probably pathological.

By injecting small quantities of a vaccine consisting of a killed culture, tuberculin, etc., the opsonic index can be raised, and the infection thereby tends to be cured. The first effect of the injection is to cause a fall in the opsonic index, the "negative phase" of Wright, which is usually afterwards followed by a rise, and by properly spacing the injections a considerable rise in the opsonic value may ultimately result. If too much vaccine be given the effect may be to permanently depress the index and cause harm instead of good, hence the desirability of controlling all injections by determinations of the opsonic index. This, however, renders the treatment very laborious, and generally by employing small doses and allowing at least a week to elapse between the doses, determinations of the opsonic index are unnecessary (for dosage, etc., see p. 232). By movement, massage, etc., applied at or about the seat of a local infection, bacterial products are disseminated which may alter the index; a process of auto-inoculation may thus result.

The opsonic index may be used for diagnostic purposes; a low or high opsonic value towards a particular organism suggests that an infection by this organism exists or has recently existed.

Bulloch came to the conclusion that the blood contains a number of specific opsonins, one for tubercle another for *M. pyogenes*, and so on. Simon, Lamar, and Bispham,[1] however, from a number of carefully devised experiments, conclude that specificity of opsonins does not exist, and suggest that opsonins may be a constant quantity, and that the number of organisms taken up by the leucocytes is influenced by a second unknown and variable factor.

[1] *Journ. Exper. Med.*, vol. viii, 1906, p. 651.

Russell[1] also concludes that in *normal* serum the opsonins are "common" and not specific, and can be removed by a number of bodies. In immune serum, on the other hand, both "common" and "immune" opsonins are present, the latter being quite specific. That is to say, in the process of immunisation specific opsonins are formed, and the increase of opsonins following injection of a vaccine is probably due to the formation of immune opsonins which react specifically.

Muir and Martin[2] believe that in immune serum a specific, immune, thermostable opsonin is present, and also a normal, thermolabile opsonin.

Wright considers the opsonins to be substances distinct from all others, but Metchnikoff, Dean, and other observers suggest that they are identical with the "substance sensibilisatrice."

It is doubtful if opsonins are present in more than traces in the unaltered blood *plasma*: like alexins, they seem to develop as a result of coagulation. The *rôle* of opsonins in immunity and in recovery from infection is therefore a complex problem.

The opsonic method has been criticised of late. Thus Moss[3] says: "None of the present methods of estimating the opsonic content of the blood seems sufficiently accurate to be of practical value"; Fitzgerald, Whiteman, and Strangeways,[4] in an elaborate investigation, concluded that the method is unreliable. Whereas Wright takes into account the serum only, Shattock and Dudgeon[5] state that "the cells (*i. e.* the phagocytes) vary in value like the serum." It may be granted

[1] *Johns Hopkins Hosp. Bull.*, vol. xviii, 1907, p. 252.

[2] *Proc. Roy. Soc. Lond.*, B. lxxix, 1903, p. 187.

[3] *Johns Hopkins Hosp. Bull.*, vol. xviii, 1907, p. 237.

[4] *Bull. Committee for the Study of Special Diseases* (Cambridge), vol. i, 1907, No. 8.

[5] *Proc. Roy. Soc. Med.*, vol. i, 1908, "Medical Section," p. 169.

that the whole truth respecting the opsonic reaction and method is not yet fully known, but many of the criticisms have been based on an imperfect technique. On the whole, it may be said that Wright's method, with careful technique and in practised hands, gives information previously impossible to obtain, and the treatment by vaccines is of considerable value in particular cases.

Method of Determining the Opsonic Index.[1]

The requisites are:

1. The serum of the patient to be tested.
2. The serum of a healthy person for a control.
3. A suspension of the organism for which the determination is to be made.
4. A suspension of living leucocytes.
5. Several Wright's pipettes with india-rubber teats or nipples.

1 and 2. *The sera.*—These two specimens should be taken at about the same time, and the determination should be made as soon as possible.

The blood is preferably collected in a Wright's capsule (Fig. 35, *d*). Both ends of the pipette are broken off, and the blood is collected by immersing the *bent* end in the blood as it runs from a prick in the ear or finger. The capsule should be at least one third filled. For pricking, a flat-pointed needle of the Hagedorn type is preferable; a prick with an ordinary needle does not yield sufficient blood. After filling, the capsule is sealed in the flame, the dry or straight end being sealed first. After coagulation the capsule is centrifugalised to obtain clear serum; for this purpose the capsule is hung by the curved end in the centrifuge.

[1] This section is largely taken from the excellent account given by Emery in his *Clinical Pathology and Hæmatology* (Lewis, 1908).

Little change in the serum ensues for two to three days if the capsules are kept sealed.

3. *Suspension of the organism.*—In the case of tubercle, suitable dead cultures can be purchased. To prepare the emulsion from these, take a small portion (about as big as a grain of rice) and place it in a small agate mortar and grind it up with the pestle; then add 1·5 per cent. salt solution drop by drop until about 2 c.c. have been added, continuing to grind meanwhile. This gives an emulsion which contains isolated bacilli as well as clumps. These latter must be got rid of,

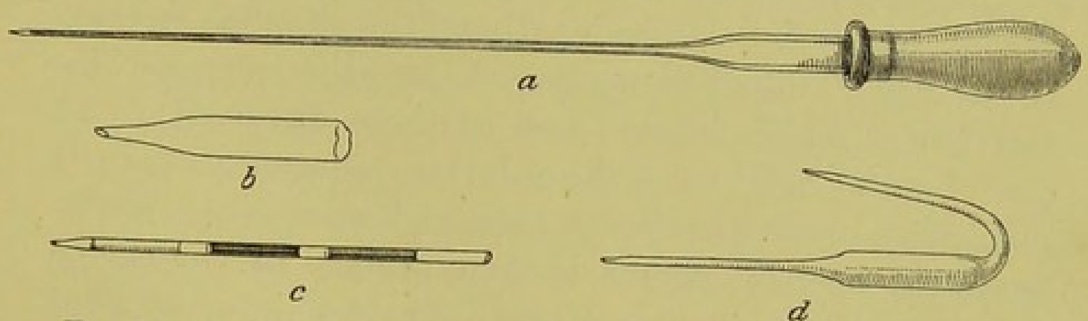


FIG. 35.—*a*. Glass pipette, with india-rubber teat for opsonic determinations, etc; *b* shows (enlarged) the contracted extremity of the pipette; *c* shows the stem of the pipette, containing the equal volumes of serum, leucocytic suspension, and bacterial suspension, before mixing; *d* is the Wright's capsule for collecting blood.

and to do this it is necessary to centrifugalise for three or four minutes. With the tubercle bacillus and gonococcus spontaneous phagocytosis is apt to occur if ordinary (0·8 per cent.) salt solution is used.

A staphylococcic emulsion is prepared by taking an agar culture not more than twenty-four hours old, adding salt solution (0·8 per cent.), and shaking gently so as to wash off the growth. When the emulsion is made it must be pipetted off into a small tube and centrifugalised for a few minutes. The emulsion must not be too thick, otherwise the leucocytes will take up an

uncountable number of cocci; the proper density can be judged by experience alone, but the emulsion should be only faintly opalescent. Emulsions of pneumococci and other organisms are made in the same way. Variations in the number of bacteria ingested may occur according as recently isolated or old strains are employed.

Instead of centrifugalising, the suspensions may be filtered through a double thickness of filter-paper.

4. *Suspension of living leucocytes.*—To prepare this, take about 10 c.c. of physiological salt solution containing ½ per cent. of sodium citrate, to prevent the coagulation of the blood. This must be freshly prepared (or kept sterile, which is inconvenient), and the simplest method is to use "soloids" prepared for the purpose by Burroughs & Wellcome; one of these dissolved in 10 c.c. of water will yield the solution required. This is put into a centrifugalising tube and warmed to blood-heat. A healthy person is then pricked in the ear or finger, and his blood is allowed to drop into the fluid until 1 c.c. or more has been collected. The tube is then put into the centrifuge, very exactly counter-balanced, and centrifugalised until all the corpuscles have come to the bottom and the supernatant fluid is left clear. If the deposit is closely examined the red corpuscles will be seen to be at the bottom, whilst above them there is a thin whitish layer of leucocytes. Then, with a capillary pipette armed with an india-rubber nipple, or with a syringe, the whole of the clear fluid is pipetted off, as close as possible to the leucocyte layer, but without disturbing the latter. The tube is then filled with saline solution, the blood and fluid are mixed, the mixture is centrifugalised, and the clear fluid again pipetted off, and this process of washing is repeated. Next, the leucocyte layer with the upper layer of red corpuscles (which also contains leucocytes) is pipetted

off into a small tube, and the whole is thoroughly mixed by repeatedly sucking into, and expelling from, the pipette. The result is a suspension of living leucocytes mixed with red corpuscles.

5. *Wright's pipettes with india-rubber teats.*—These are made of glass tubing drawn out in the blowpipe flame into the form shown in *a*, Fig. 35, which is two thirds full size. The end of the fine extremity should be contracted as shown in *b*. Glass tubing must be chosen which properly fits the teats.

The process.—(1) Prepare a pipette by placing an india-rubber teat on the thick end. Then, with a grease pencil or with pen and ink, make a transverse line about an inch from the pointed end. The volume of fluid contained in the tube between the point and this mark is spoken of as the unit.

(2) Having the patient's serum and the suspensions of leucocytes and of bacteria ready to hand, take the pipette between the index finger and thumb of the right hand and compress the nipple. Immerse the point beneath the surface of the suspension of bacilli, and relax the pressure on the nipple until the emulsion has risen exactly to the mark so that one unit has been drawn up; then remove the point from the fluid and relax the pressure again so that a *small* volume of air is sucked up. This will be quite easy if the point is a good one, otherwise it will be difficult or impossible, as the column of fluid will either refuse to stir or will oscillate violently. Next immerse the point in the suspension of leucocytes and draw up one unit. This will be separated from the emulsion of bacteria by the bubble of air. Remove the point from the emulsion and draw up a second volume of air.

Lastly, draw up one unit of the serum. There will now be in the pipette (counting from the nipple

towards the point) one unit of bacterial emulsion, a bubble of air, a unit of leucocytes, a bubble of air, and lastly a unit of serum (*c*, Fig. 35).

(3) Put the point of the pipette on to a clean hollow-ground slide or an artist's porcelain sunk palette, and express the whole of its contents, and mix well together, aspirating them repeatedly into the pipette and expelling without causing bubbles. If bubbles form, a hot wire brought near will quickly dispel them. When thoroughly mixed, aspirate the mixture into the pipette, suck up a short volume of air, and seal the tip in the flame.

Then place the pipette point downwards in the incubator, or better, in a water-bath at 35° to 37 C°., noting the time exactly, and proceed to prepare a second pipette in precisely the same way, using the same suspensions of bacteria and leucocytes, but the control serum instead of the patient's. Place this in the incubator or water-bath, by the side of the other, noting the time at which this is done. When each pipette has been incubated for a quarter of an hour it is removed from the incubator or water-bath, the end broken off and the nipple fitted to the thick end; then the contents are expelled on to a hollow slide or porcelain palette and mixed thoroughly together. Films are then prepared. This may be done by depositing a drop in the middle of a large cover-glass (1-inch squares, No. 2), dropping on to it another cover-glass and drawing the two apart. Or the films may be made on slides, for which Wright recommends roughing the slides with emery paper and spreading the film with the sharp edge of a broken slide (see next page). The films then have to be stained. For staphylococci, streptococci, pneumococci, *B. coli*, etc., the films may be fixed with formalin and stained with

carbol-thionine or borax-methylene blue, or they may be stained without previous fixing with the Leishman stain. For tubercle, the films may be fixed in a saturated solution of mercuric chloride (one or two minutes), stained in warm carbol fuchsin, decolorised with 2½ per cent. sulphuric acid in methylated spirit, and counter-stained with methylene blue.

Wright now uses the whole blood instead of the leucocyte layer only. After the blood has been drawn into the citrated salt solution it is centrifugalised, washed twice with salt solution, the fluid is pipetted off, and finally the corpuscles are well mixed. The various mixtures—washed corpuscles, bacterial suspension, and serum—are made and incubated as previously described. In order to make the film for staining and counting, the contents of the pipette are discharged on to one end of a slide roughed with fine emery paper and the mixture is spread by means of a slide which has been broken across after notching with a file or glass cutter. The object is to obtain a broken edge having a very slight concavity, and many slides may have to be sacrificed to attain this. The film is spread by drawing (not pushing) along, the leucocytes adhere to the edge of the spreader, and finally are deposited mostly at the end of the preparation, the red corpuscles being left behind.

Lastly, the films are examined with the oil-immersion lens, preferably with the aid of a mechanical stage, and the number of organisms contained in not less than fifty polymorphonuclear leucocytes is counted. Parts of the film in which the cells are broken down or not well stained, or cells containing obvious clumps of organisms, should be avoided. The ratio between the number in the control and the number in the specimen prepared with the patient's serum gives the *opsonic*

index. Thus, if in the control there are 125, while in the patient's specimen there are 75, the index would be $\frac{75}{125} = 0{\cdot}6$, *i. e.* not much more than half the normal.

Preparation of vaccines for treatment, etc.—The vaccine used for treatment is a sterilised, standardised suspension of the infecting organism, except in the case of tuberculosis, for which tuberculin (TR or BE) or an analogous preparation is employed. In certain instances a mixture of organisms is used—*e. g. M. pyogenes*, var. *aureus* and var. *albus*, with or without the acne bacillus in some cases of acne—and the strain of organism isolated from the lesion is generally to be preferred.

The vaccine is prepared by growing the organism under appropriate conditions, the staphylococcus on agar, the streptococcus, pneumococcus, and gonococcus on blood-agar, etc. The growth is then emulsified by adding a few drops of sterile 0·1 per cent. sodium chloride solution and well rubbing up with a sterile glass or aluminium rod. Two or three tubes are treated in this way; the emulsion is poured into a small sterile Erlenmeyer flask of stout glass, the tubes are rinsed out with a little more of the salt solution, and the washings added to the contents of the flask, two or three sterile glass beads are added, and the flask is shaken vigorously for some minutes thoroughly to break up the masses of organisms. The contents of the flask, which should measure 5 c.c. or thereabouts, are then centrifugalised for some minutes, and the emulsion is poured off from the deposit into a second sterile flask and is now ready for standardisation.

Standardisation is carried out by Wright's method. Two or three volumes of citrate solution are sucked up into a pipette such as that used for opsonic determinations, the finger is pricked and one volume of blood is taken up in the pipette, separated from the citrate solution by an air-bubble, and finally one volume of the bacterial emulsion, also separated from the blood by an air-bubble, is taken up. The whole contents of the pipette are then well mixed by expelling on to a clean slide and sucking up three or four times. About one third of the mixture is then transferred to each of three clean

slides, and the drops are spread with the edge of a slide so as to obtain thin uniform smears. These are allowed to dry, stained with Leishman's stain, and the number of red corpuscles and bacteria is counted in a number of microscopical fields. Assuming that there are 5,000,000 red cells in a cubic millimetre of blood, it is easy to calculate approximately the number of bacteria contained in the emulsion. Suppose that 500 red cells have been counted, and with these 1500 bacteria are admixed. Since equal volumes of blood and emulsion have been taken, one cubic millimetre of bacterial emulsion will contain $\frac{5,000,000 \times 1500}{500} = 15,000,000$ bacteria. But one cubic centimetre contains 1000 cubic millimetres, therefore the emulsion contains $15,000,000 \times 1000 = 15,000,000,000$ bacteria per cubic centimetre, and by appropriate dilution any bacterial content of the emulsion may be obtained. Thus, if 1,000,000,000 organisms per cubic centimetre is desired, 1 c.c. of the emulsion must be diluted with 14 c.c. of salt solution. To the prepared dilution of the bacterial emulsion 0·5 per cent. of carbolic acid, or 0·2 per cent. of trikresol, is added, and the flask is placed in a water-bath at 56° to 60° C. for one or one and a half hours, according to the resistance of the organism. The stock solution may subsequently be introduced into small sterile glass serum bulbs of 1–2 c.c. capacity, and the bulbs, after sealing and standing for twenty-four hours, may again be sterilised for an hour at 60° C. to ensure the destruction of the organisms; cultures may be made from the sterilised vaccine to ascertain that this is the case. The lower the temperature and the less the heating, consistent with sterilisation, the more active will be the vaccine.

Killed

The table[1] on the next page gives an idea of the doses of vaccines, their toxicity, and frequency of inoculation.

The smaller doses are given at the commencement of the treatment, and the doses are gradually increased.

The writer has employed endotoxin solutions as vaccines and believes they are very efficient.

[1] See Harris, *Practitioner*, May, 1908, p. 647.

Vaccine.	Relative toxicity.	Doses.	Frequency of inoculation.
Tuberculin	Very toxic	$\frac{1}{100000}$–$\frac{1}{10000}$–$\frac{1}{2000}$ mgrm	Every 10–14 days.
B. coli	Very toxic	5–15 millions	Every 2, 5, or 10 days.
Pneumococcic	Less toxic than *B. coli*	10–50 millions	Every 36–48 hours in pneumonia; every 10 days in chronic infections.
Streptococcic	More toxic than pneumococcic	20–60 millions	Every 7–14 days.
Staphylococcic	Less toxic than streptococcic	100–1000 millions	Every 10 days.
M. melitensis	—	$\frac{1}{10}$ sq. cm. of surface agar culture (because very difficult to count)	Every 7–14 days.
Gonococcic	Slightly toxic	100–500 millions	Every 7–14 days.

Prophylactic vaccines.—In addition to the therapeutic vaccines for the *treatment* of the declared disease, vaccines are also employed for *prevention* of disease. The preventive or prophylactic vaccines may be:

(1) Living, but attenuated, cultures, *e. g.* anthrax and cholera. This method has also been proposed for plague, and vaccinia must be regarded as of this nature (this is the "Pasteurian method").

(2) Killed cultures, autolysed cultures, and endo-toxins. The first and second are used for typhoid, plague and dysentery, and Hewlett has suggested endo-toxins for typhoid, cholera, plague and diphtheria.

(3) Immune sera give protection for a limited time.

(4) Besredka has suggested "sensitised vaccines," *i. e.* killed cultures saturated with the homologous immune body derived from an immune serum. He claims that these give rise to much less reaction than the killed cultures.

(For further particulars, see Hewlett's *Serum Therapy*, Ed. 2, J. & A. Churchill, 1910.)

CHAPTER VI.

SUPPURATION AND SEPTIC CONDITIONS.

THE subjects of septic infection and of suppuration are of great practical importance, and a knowledge of their ætiology is one of the main factors which have conduced to the great advances that were made during the Victorian era in the treatment of wounds, whether accidental or made by the surgeon's knife.

Ogston in 1881 and Rosenbach in 1884 demonstrated that micro-organisms are almost invariably present in the pus of acute abscesses, and these observations were repeatedly confirmed by subsequent investigators. A number of experiments were then initiated in order to ascertain whether these organisms bear a causal relation to the phenomena of suppuration or are merely accidentally present. These experiments showed that a large number of organisms can produce suppuration, and render it certain that in ninety-nine cases out of a hundred the suppurative and septic conditions met with spontaneously, or occurring after surgical interference, are due to the action of micro-organisms. The chief of these are several micrococci (commonly known as *staphylococci*, and the infections which they produce, as *staphylococcic infections*) and streptococci.

Under the terms "suppuration" and "septic diseases" are included such varied conditions as abscesses, boils and carbuncles, cellulitis, osteomyelitis, erysipelas, gonor-

rhœa, infective endocarditis, pyæmia, septicæmia and sapræmia, puerperal fever, and hospital gangrene.

As will be gathered from the descriptions of the individual organisms, suppuration may be set up by inoculation with several species, and a number of experiments by various observers, carried out by inunction, subcutaneous inoculation, and inoculation in the serous cavities and circulation, have conclusively proved that this is the case, not only in animals, but also in man.

A problem of great importance is whether micro-organisms are usually the cause of suppuration, or whether mechanical injury, chemical agents, etc , can also produce it. Mechanical injury alone does not seem to be capable of inducing pus production, but it is otherwise with regard to chemical agents. For a long time considerable difference of opinion existed and discordant results were published. These discrepancies have now been explained, and are found to depend upon the method of experiment and the particular animal and chemical agent employed. That chemical agents should produce suppuration was only to be expected, for it would be against analogy, derived from all other bacterial diseases, if the pyogenic organisms do not produce suppuration through the chemical substances formed by, or present within, their cells, and if these chemical substances act thus, why should not other chemical substances be found to act in a similar way ?

In experiments with chemical agents the greatest care has to be taken to exclude the entrance of micro-organisms. This is best done by sealing the sterilised substance in sterilised fusiform glass tubes and introducing these under the skin or into the tissues with strict aseptic precautions. When the wounds have completely healed the tubes are broken by pressure

and their contents allowed to diffuse into the surrounding tissues.

Sterilised cultures (above a certain amount) of the *Micrococcus pyogenes* and a crystalline body, phlogosin, obtained by Leber from its cultures, produce abscesses on inoculation. Mercury produces suppuration in the dog, but not in the rabbit; silver nitrate (5 per cent. solution) has a similar action. Ammonia fails to produce pus; it is either absorbed without damage, or if in stronger solution produces necrosis of the tissues. Turpentine produces large sterile abscesses in carnivora, and Brieger's cadaverine is likewise stated to set up suppuration.

Buchner was also able, by warming various bacteria with 0·5 per cent. caustic potash, to obtain a solution containing protein which was powerfully pyogenic, and Nannotti found that sterilised pus had a similar property. It thus seems certain that a number of chemical substances can set up suppuration. At the same time, it must be clearly recognised that suppuration and suppurative complications, as they occur naturally, are to be regarded as due to the activity of micro-organisms in almost every instance.

Of so-called "septic" diseases, sapræmia, septicæmia, and pyæmia must be mentioned. By "sapræmia" is meant the constitutional condition arising from the absorption of the toxic products elaborated by micro-organisms, the latter being localised and absent from the general circulation. In the acute form it is not a common condition, the best example being that which occurs after parturition; by simply clearing and washing out the uterus the symptoms rapidly abate. In septicæmia not only is there usually (though not necessarily) a local site of infection, but in addition micro-organisms are present in the general circulation.

It is true they are not abundant in the latter situation, and Cheyne[1] believes that they are to a large extent arrested in the capillaries. Micrococci and streptococci are the commonest forms. Pyæmia is characterised by the presence of micro-organisms, most frequently streptococci, in the general circulation, and in addition by the formation of abscesses in various situations. These arise usually from suppurative phlebitis with the formation of septic emboli and thrombi. The sequence of events, according to Cheyne (*loc. cit.*, p. 881), is (*a*) phlebitis in direct connection with the wound; (*b*) a thrombus impregnated with micro-organisms is formed in the vein; (*c*) this softens and disintegrates, and particles or emboli are carried to distant parts; (*d*) these lodge in the capillaries, with the formation of infarctions and abscesses. Suppurative pylephlebitis is a pyæmia affecting the portal system of vessels. As regards the so-called chronic pyæmia or multiple abscesses, Cheyne considers that it differs from true pyæmia in that embolism plays no part. Organisms gain access to the blood-stream, settle in any spot where the vitality of the tissues is depressed, grow and multiply, and there produce an abscess.

The mere presence of micro-organisms does not always suffice, however, for they may be present without producing suppuration; and the same organism, for example, the *Streptococcus pyogenes*, may at one time produce a localised abscess, at another diffuse cellulitis. and at a third pyæmia; a number of factors control and modify the occurrence and the particular form of septic disease.

As already mentioned (p. 208), many micro-organisms when injected into the blood-stream are rapidly disposed of; so when moderate quantities of the *Micrococcus*

[1] *System of Medicine*, Clifford Allbutt, ed. 2, vol. i, p. 876.

pyogenes are injected into the circulation of a rabbit, abscesses, as a rule, form only in the kidney. If, however, the organisms be attached to gross particles, so that they cannot pass through the capillaries, embolism occurs and abscesses form about the embolic foci. The virulence of the infecting organism, which varies much, is another factor of great importance. The effect of inflammation and injury in making a part "susceptible" is also very marked. Inject the *M. pyogenes* into animals in which the endocardium or a bone has been damaged, and in all probability an endocarditis or an osteomyelitis will ensue. The dose and concentration of the organisms are also important factors. Watson Cheyne found that 250,000,000 cocci (*M. pyogenes*) injected into the muscles of a rabbit produced a circumscribed abscess, but 1,000,000,000 caused a general septicæmia and death. So, probably, while the cells in a healthy wound can dispose of a few organisms, if the latter are abundant or in masses they may gain the mastery.

Micrococcus pyogenes, var. aureus (Staphylococcus pyogenes aureus).

Morphology and biology.—A minute spherical organism measuring about 0·75 μ in diameter. It generally occurs in more or less irregular groups, but may be met with singly or in pairs (Plate I., *c*). It is non-motile, does not form spores, and stains well with all the anilin dyes and also by Gram's method. It is aërobic and facultatively anaërobic, will develop *in vacuo*, and grows well and rapidly on all the usual culture media at temperatures from 18° to 37° C. On agar-agar it forms a thickish, moist, shining growth, cream-coloured at first, but after a day or two developing a

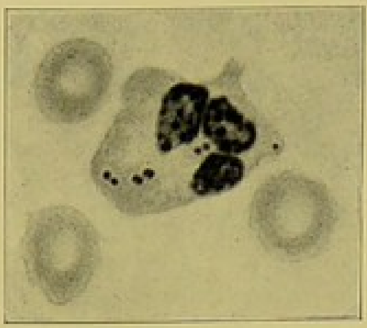
motile 0
Spores 0
Gram +
agar

characteristic orange-yellow colour. It grows in the same manner on blood-serum without liquefaction of the medium. Gelatin is rapidly liquefied, the liquefied gelatin being at first somewhat turbid from yellowish masses of organisms; these later on subside and form an orange-yellow sediment (Plate I., *d*). In gelatin plates the colonies form at first small whitish, granular points, developing in two or three days into circular areas of liquefaction with yellowish masses of the organism floating in them. On potato it forms a growth similar to that on agar. When grown in milk it produces coagulation. Acid production (lactic and butyric acids) can be demonstrated by growing on a neutral litmus glucose-agar. When grown in broth or peptone water it gives the indole reaction with the addition of a nitrite, but not without.

The rate of liquefaction of gelatin and the pigment production vary; the latter is sometimes much deeper than at others, recently isolated cultures show it better than old ones, and the presence of oxygen also seems to be necessary (see p. 240). The amount of acid production appears to vary directly with the virulence, which is likewise very variable.

Pathogenicity.—The *Micrococcus pyogenes*, var. *aureus*, is by far the commonest of all organisms met with in suppurative processes. Ogston found it alone in thirty-four, and associated with the *Streptococcus pyogenes* in sixteen, out of sixty-four cases of abscess. It occurs in acute abscesses, boils, and acne, in some cases of puerperal fever and infective endocarditis, and is almost invariably found in osteomyelitis, but only occasionally in pyæmia. The organism injected under the skin of man or animals produces an abscess, and injection into the blood-stream under certain conditions is followed by infective endocarditis or pyæmia.

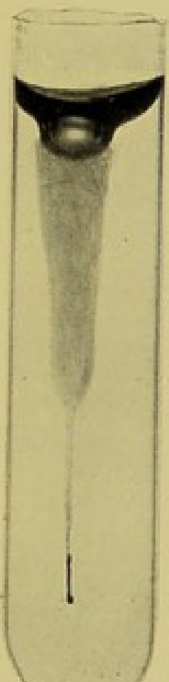
PLATE I.



a *b*

Phagocytosis by polymorphonuclear leucocytes. *a. M. pyogenes*, var. *aureus*. *b. B. tuberculosis.*

c. M. pyogenes, var. *aureus* in pus. Smear preparation. × 1000.



d. M. pyogenes, var. *aureus*. Gelatin stab-culture, four days old.

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Impetigo pustules are produced by inunction into the skin.

It may be said to be universally present on all parts of the skin, and in the mouth, and is frequently met with in the air. According to Sternberg, recent cultures in gelatin are destroyed by an exposure to a temperature of 56° to 58° C. for ten minutes; but when dried much higher temperatures, 90° to 100° C., are required, and in the dried state (on a cover-glass) it retains its vitality for more than ten days. According to different experimenters, from five to fifteen minutes are required to destroy it with a 1–1000 mercuric chloride solution; but it is evident that much depends on the state of aggregation of the organisms, and Abbott has shown that while most of the cocci in a culture are destroyed in five minutes, a few may survive much longer.

Toxins.—In a case of infective endocarditis examined by Sidney Martin, due to the *M. pyogenes*, var. *aureus*, a large amount of an albumose and of a basic body was extracted from the blood and spleen. The albumose produced fever and wasting, and retarded the coagulation of the blood.

Leber extracted a crystalline body, which he termed phlogosin, from cultures of the *M. pyogenes* var. *aureus*, and Brieger also obtained a crystalline base.

The decomposition products of the action of the *M. pyogenes*, var. *aureus*, on egg-albumen are, according to Emmerling, phenol, indole, and skatole, many volatile and non-volatile acids, betaine, and trimethylamine.

Anti-serum.—Attempts have been made to prepare an anti-serum by the injection of cultures, but the serum is of no practical value. A *vaccine*, prepared by heating a suspension of an agar culture to 65° C. for half an Vaccine

hour and standardising, has been used with much success in chronic staphylococcic infections, such as acne and boils.

Micrococcus pyogenes, var. albus, and var. citreus. Micrococcus epidermidis. Micrococcus cereus.

These organisms are of rarer occurrence than the preceding one. In morphology and cultural characteristics the first two agree with the *Micrococcus pyogenes*, var. *aureus*, except that the *albus* produces a white, shining, porcelain-like growth, and the *citreus* a lemon-yellow growth, on agar. They are said to be less pathogenic than the *aureus*, and are only occasionally found alone, being usually associated with the *aureus*. Cheyne, however, states that in his experience the *albus* is more virulent than the *aureus*, and mixed infections with the *aureus* are regarded as more severe than infection with the *aureus* alone. The *albus* has been found in some cases of panophthalmitis, and is said by Flügge to be commoner than the *aureus* in the lower animals.

Andrewes and Gordon[1] regard the *aureus*, *albus*, and *citreus* merely as variants of a single species, the *Micrococcus pyogenes*. They found that every variety of colour, from orange, through yellow to white, might be obtained by cultivation. The *Micrococcus flavescens*, met with by Babes in abscesses, may probably be placed in the same category. On the other hand, the *Micrococcus epidermidis* (*albus*), first described by Welch as occurring on the skin, in stitch abscesses, etc., and feebly pathogenic compared with the *M. aureus*, is stated by these authors to be perfectly distinct from the foregoing. Other organisms which are occasionally met with in abscesses, the *Staphylococcus cereus albus*

[1] *Rep. Med. Off. Loc. Gov. Board* for 1905–06, p. 543.

and *S. cereus flavus* of Passet, form shining waxy growths on agar, and do not liquefy gelatin, and are probably variants of another species, which may be termed the *Micrococcus cereus*. There may be many other varieties of micrococci not yet properly differentiated.[1] Well-defined micrococci occur in the saliva (*M. salivarius*), and in the scurf from the scalp. Andrewes and Gordon give the following differential table of some of these micrococci :

Chief Types of Human Micrococci.

Organism.	Broth culture.	Pigment on agar.	Clot in milk.	Liquefaction of gelatin.	Reduction of neutral red.	Reduction of nitrate.	Acid formation from				Pathogenesis.
							Maltose.	Lactose.	Glycerin.	Mannitol.	
Micrococcus pyogenes	Turbid	Orange, yellow, or white	+	+	0	+	+	+	+	+	+
Micrococcus epidermidis	Turbid	White	+	+	+	+	+	+	+	0	Feeble.
Micrococcus salivarius	Clear	White	0	0	0	+	+	0	+	0	0
Scurf micrococcus	Turbid or clear	White	0	0	0	+	0	0	0	+	0

Micrococcus zymogenes.

Isolated by MacCallum and Hastings[2] from a case of acute endocarditis. A minute micrococcus, non-motile, and staining by Gram's method. On surface agar it forms a thin, slightly elevated, moist, glistening, greyish-white growth. In gelatin stab-cultures the growth is somewhat opaque and granular, with slow liquefac-

[1] See Gordon, *Rep. Med. Off. Loc. Gov. Board* for 1903–04, p. 388.
[2] *Journ. Exp. Med.*, vol. iv, 1899, p. 521.

tion. Blood-serum is slowly liquefied. On potato a thick, moist, dirty-white growth develops, becoming dry and brownish after three days. Broth becomes slightly clouded after twenty-four hours' growth, but in three to four days the organisms settle to the bottom, leaving the medium clear. Neither indole nor gas is formed. In neutral litmus milk the litmus is decolorised after a few hours, and in twenty-four hours the milk is firmly curdled. Somewhat later liquefaction of the curd ensues from above downwards; at first the turbid fluid is reddish in the superficial layer and yellowish below; ultimately the whole curd is transformed into a turbid liquid with a reddish colour throughout. These changes in milk are characteristic of the organism. It is pathogenic to white mice, hardly so to guinea-pigs and white rats, and moderately so to rabbits; intra-venous inoculation into the latter sometimes sets up an endocarditis. Harris and Longcope[1] have reported five more instances of the occurrence of this organism (once from a cesspool, four times as secondary invasions at autopsies), and Birge[2] has isolated a similar but less virulent organism from the larynx of crows.

Micrococcus neoformans.

This organism was isolated by Doyen from malignant growths, and was supposed by him to be the causative organism of malignant disease. It is a typical Gram-positive coccus, giving a white growth on agar and liquefying gelatin in three to four days. According to Dudgeon and Dunkley,[3] it gives all Gordon's fermenta-

[1] *Centr. f. Bakt.* (1te Abt.), vol. xxx, 1901, p. 353.
[2] *Johns Hopkins Hosp. Bull.*, vol. xvi, 1905, p. 309.
[3] *Journ. of Hygiene*, vol. vii, 1907, p. 13.

tion tests for the *M. pyogenes*, var. *albus*, except that it does not acidify mannitol.

The serum of patients suffering from malignant disease does not give any marked agglutination with the *M. neoformans*, nor does it contain opsonins specific for the organism. The *M. neoformans* is non-pathogenic for rats and mice.

Pathogenic Streptococci.

Several pathogenic streptococci occur, originally described as one species, *Streptococcus pyogenes*, but of which there are several variants.

Morphology.—A non-motile, spherical coccus measuring about 1 μ in diameter. It stains well with anilin dyes and is Gram-positive.

Multiplication is by fission in one direction only, so that chains of cocci are formed. In pus these chains do not usually contain more than ten to fifteen elements (Plate II., *a*), but under cultivation, and especially in broth cultures, they may be much longer, and occasionally in a chain an irregular division occurs, so that a branch chain forms. A cell here and there in a chain is often somewhat larger than its fellows, and some authors have considered these enlarged individuals to be arthrospores (Plate II., *b*)

Cultural reactions.—It can be cultivated on the usual culture media, and grows anaërobically as well as aërobically. On agar, or better, glycerin agar, it forms in twenty-four to forty-eight hours minute whitish, semi-transparent, more or less isolated colonies (Plate II., *c*). On gelatin the growth has much the same characters, and is better seen, as this medium is clearer than agar, but it takes some days to attain the maximum. In stab-cultures it forms minute spherical colonies all down the line of the stab, but does not

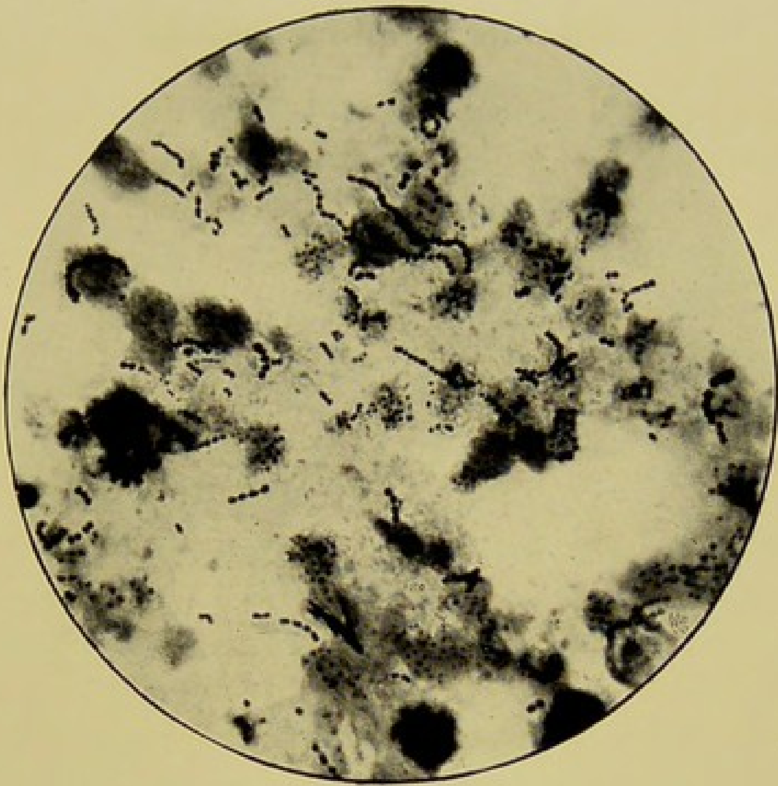
invade the surrounding medium; the gelatin is not liquefied. In broth it forms a flocculent deposit, the fluid generally remaining clear. It does not develop on potato, but in milk it grows well, usually, but not always, with coagulation. The indole reaction can be obtained in broth cultures in seven to fourteen days on the addition of a nitrite, but not without. It gives an acid reaction when grown on neutral litmus glucose-agar. It is the only organism with which the writer is acquainted that does not reduce a weak solution of methylene blue.

According to Sternberg, the thermal death-point of *Streptococcus pyogenes* is 52° to 54° C., the time of exposure being ten minutes. It is destroyed by a two hours' exposure to 1–300 carbolic acid and 1–2400 mercuric chloride solutions.

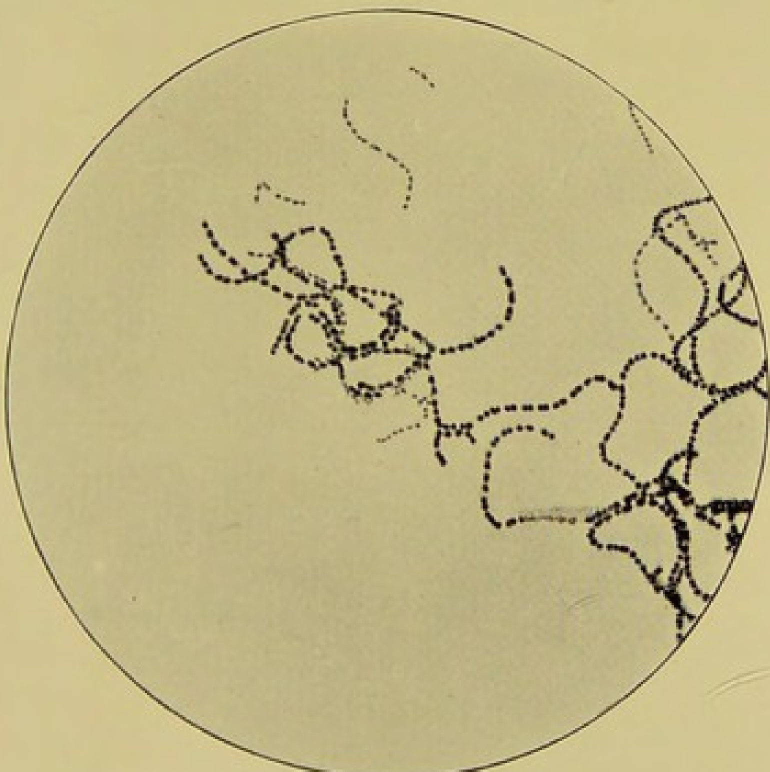
The *Streptococcus pyogenes* is sometimes found in acute circumscribed abscesses, in 16 per cent. according to Zuckermann from an analysis of the results of various observers. The streptococcus is, however, especially frequent in spreading inflammations, lymphangitis, cellulitis, and progressive gangrene, and is the usual cause of pyæmia and puerperal fever. It is met with in about one third of the cases of infective endocarditis, occasionally in acute osteomyelitis, and seems to be the cause of the septic pneumonia so often observed after operations about the mouth and throat.

In erysipelas, streptococci are present in the lymphatics at the margin of the zone of redness. These were first isolated by Fehleisen, who described the organism as the *Streptococcus erysipelatis,* and by inoculation experiments on man and animals demonstrated its causal relation to the disease. The experiments on man were made in cases of extensive and inoperable carcinoma and sarcoma, as it had been

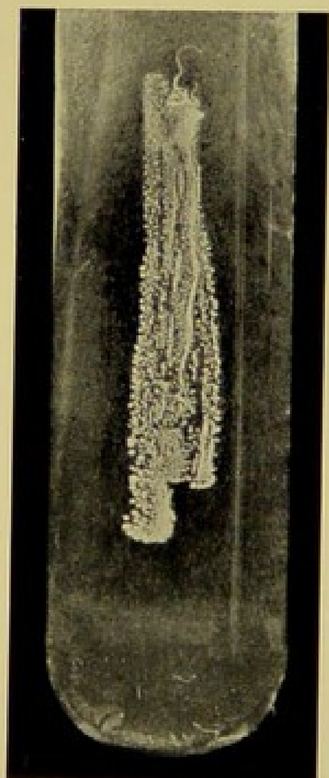
PLATE II.



a. Streptococcus pyogenes in pus. Smear preparation. × 1000.



b. Streptococcus pyogenes. Cover-glass preparation of a broth culture. × 1500.



c. Streptococcus pyogenes. Pure culture on glycerine agar.

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Gram — Cocci

	Gel	Gas	Acid
M. meningitidis	+	0	+
M. Gonococcus			
M. Catarrhalis	—		
M. Melitensis			

noticed that malignant tumours were frequently benefited after an attack of erysipelas. Several cases were inoculated, and all were successful with one exception, typical erysipelas developing (see Coley's fluid, p. 263). Jordan,[1] however, has found experimentally that typical erysipelas may be produced in a rabbit's ear not only with the streptococcus, but also with staphylococci, pneumococci, and *B. coli*, and he believes that although streptococci usually cause human erysipelas, this disease may also be produced by staphylococci, and possibly by the pneumococcus, *B. coli*, and even the *B. typhosus*.

At one time the *Streptococcus erysipelatis* was considered to be different from the *Streptococcus pyogenes*, but the two organisms are now regarded as identical, the differences in cultural characters being slight and not constant. Petruschky[2] produced a typical erysipelas in the human subject by inoculation with a pure culture of a streptococcus derived from a case of suppurative peritonitis, and Bulloch[3] has shown that an animal immunised against a streptococcus derived from a case of erysipelas is also immune against a streptococcus isolated from an abscess (but see below, p. 248).

The different effects produced by the *Streptococcus pyogenes*, abscess in one case, erysipelas in another, cellulitis or pyæmia in a third, are attributable partly to differences in virulence, partly to the seat of infection and mode of entrance into the body, partly to real differences existing between different races of streptococci (see below, p. 248). Streptococci have been described in a number of diseases about which we know little, such as variola, scarlatina (*S. scarlatinæ* or

[1] *Münch. med. Woch.*, August 27th, 1901.
[2] *Zeitsch. f. Hyg.*, xxiii, 1896, p. 142.
[3] *Tran. Brit. Inst. of Prev. Med.*, vol. i, 1897, p. 6.

conglomeratus), and vaccinia, but it is uncertain what causal relation they bear to these conditions. Strangles, a disease of horses, seems to be due to streptococci.

Anti-serum.—The important lesions due to the streptococcus and their grave nature have led to the attempt to prepare an anti-serum, but many and great experimental difficulties have to be overcome to do this. The virulence of the streptococcus has to be increased by passing it through a series of rabbits, and it is only by growing it in serum media that satisfactory cultures for the inoculation of the horses can be prepared. Human serum is the best, but is difficult to obtain; a mixture of asses' serum and peptone beef-broth comes next. The cultures are grown for about a fortnight and are then inoculated into horses, first killed and then living cultures being used, and after a time the blood acquires anti-microbic properties. It is customary now to make use of a "polyvalent" serum, *i. e.* one prepared by the injection of many strains of streptococci. The streptococcus anti-serum has been employed in erysipelas, cellulitis, puerperal fever, and pyæmia, in many cases with apparent success. Cheyne suggested its use before operations about the mouth and throat as a preventive of septic pneumonia, but a *vaccine* would probably be better for this purpose.

A *vaccine* prepared by sterilising cultures with heat may be used with benefit in streptococcic infections, which do not run too rapid a course, *e. g.* infective endocarditis.

There are slight differences in the cultural characters and morphology of the streptococci derived from different sources, and the virulence varies very considerably. Von Lingelsheim distinguished two varieties, *brevis* and *longus*, the former rendering broth turbid, growing in short chains, and

being non-pathogenic to mice and rabbits, the latter leaving the broth clear, growing in long chains, and always pathogenic to these animals. These are considered by some to be merely accidental varieties and not true species.

Gordon[1] divided the streptococci into four varieties, viz. (1) the *S. longus*, restricted to an organism forming exceptionally long chains; (2) *S. medius* including the majority of streptococci from pus, sepsis, and erysipelas, and Lingelsheim's *longus*; (3) *S. brevis*, including Lingelsheim's *brevis* and the *Diplococcus pneumoniæ*; (4) *S. scarlatinæ* or *conglomeratus*. In addition to differences between the colonies on agar and gelatin, the following table illustrates other differences:

	S. longus. Isolated from the mouth.	*S. medius*. From pus, etc.	*S. brevis** and Lingelsheim's variety; also *Dip. pneumoniæ*.	*S. scarlatinæ* or *conglomeratus*.
Morphology	Very long and comparatively straight chains	Medium-sized, curling, chains	Short chains of only a few elements	Masses of chains. Apparently bacillar (diphtheria-like) forms appear under certain conditions.
Broth . .	Remains clear. Flocculent masses of growth form at bottom of tube with no coherency	Same as *S. longus*	Becomes turbid throughout	Remains clear. Growth forms white masses with marked tendency to retain their coherency.
Gelatin at 37° C.	Similar to broth	Similar to broth	Becomes uniformly turbid	Similar to broth.
Litmus milk	Growth confined to the bottom of the tube. Acid reaction. No curdling	Acid production slight. No curdling	Slight acid production. Milk usually curdled	Much acid production. Marked curdling.

* Washbourn and Goadby state that the *S. brevis* is the commonest species in the mouth.

Recently, considerable attention has been directed to the differentiation of streptococci by Houston,[2] Andrewes,[3]

[1] *Rep. Med. Off. Loc. Gov. Board* for 1898-99, p. 482.

[2] *Rep. Med. Off. Loc. Gov. Board* for 1902-03, p. 511, and 1903-4, p. 472.

[3] *Lancet*, November 24th, 1906.

Andrewes and Horder,[1] Gordon,[2] and Besredka. Considerable differences are found in the fermentation reactions of various strains of streptococci, and Andrewes and Horder distinguish (1) *Streptococcus pyogenes* from pus, erysipelas, cellulitis, pyæmia and septicæmia, endocarditis, etc. (2) *S. salivarius*, the common type in the saliva. Also met with, probably as a "terminal" infection, in endocarditis and septicæmia. Shades into the *S. fæcalis* and *S. anginosus*. (3) *S. anginosus*, from inflamed and scarlatina throats, endocarditis, and rheumatism. (4) *S. fæcalis*, abundant in fæces, air, and dust. Met with also in endocarditis, meningitis, cystitis, and suppuration. Two strains of the *Diplococcus rheumaticus* proved to be this organism. (5) The pneumococcus. (6) *S. equinus*, present in the intestine of herbivora. They do not assert that these are absolutely defined species; at the most they seem to be species in the making, and are connected by transitional forms. Walker[3] does not consider that these reactions afford a means of distinguishing definite varieties among human streptococci.

Andrewes and Horder give the following table summarising the characters of the various streptococci:

Name.	Milk clot.	Reduction of neutral red.	Cane sugar.	Lactose.	Raffinose.	Inulin.	Salicin.	Coniferin.	Mannitol.	Growth on gelatin at 20° C.	Morphology.	Pathogenicity to mouse.	Hæmolysis.
Streptococcus pyogenes .	−	−	+	+	−	−	±	−	−	+	*longus*	+	+
Streptococcus salivarius	+	±	+	+	±	−	−	−	−	±	*brevis*	−	0
Streptococcus anginosus	+	±	+	+	−	−	−	−	−	±	*longus*	+	+
Streptococcus fæcalis .	+	+	+	+	−	−	+	+	+	+	*brevis*	−	0
Streptococcus equinus .	−	−	+	−	−	−	+	+	−	+	*brevis*	−	...
Streptococcus pneumoniæ (pneumococcus)	±	−	+	+	+	±	−	−	−	−	*brevis*	+	0

+ = Positive or acid-production. − = Negative or no acid-production.
± = Acid-production sometimes present, sometimes absent.
(These differences are not constant; with various strains one or other reaction may be lacking.)

[1] *Lancet*, 1906, vol. ii, pp. 708, 775, 852.

[2] *Ibid.*, November 11th, 1905, and *Rep. Med. Off. Loc. Gov. Board* for 1903–04, p. 388.

[3] *Proc. Roy. Soc. Lond.*, B. vol. lxxxiii, 1911, p. 541.

Bacillus pyocyaneus.

This is the organism found in green and blue pus, and it also occurs on the surface of the body. Its presence in wounds greatly retards healing, and occasionally a general toxæmia may result from it. It has been met with in otitis media and in the green pus of the pleural and pericardial cavities. It is a slender bacillus measuring 3 to 4 μ, frequently united in pairs and forming filaments. It is actively motile, does not form spores, and is aërobic and facultatively anaërobic. It does not stain by Gram's method. On gelatin it grows freely with rapid liquefaction, a greenish, fluorescent colour developing in the liquid, while whitish flocculi of growth sink to the bottom. On agar a whitish, moist layer develops, and the medium is stained a greenish colour. On potato the growth is a dirty brown or sometimes greenish.

Milk is coagulated and a greenish colour develops. Broth becomes turbid, and there is a slight film formation with a greenish colour. Oxygen is necessary for the development of the pigment, which is generally a mixture of a blue pigment, pyocyanin, and a yellow one, pyoxanthose. Pyocyanin ($C_{14}H_{14}N_2O$) is said to be an anthracine derivative; it is soluble in chloroform, and on oxidation yields pyoxanthose.[1] Various races of the organism exist, differing in their pigment production.

Subcutaneous inoculations of a small amount of a culture produce local abscesses; larger amounts cause œdema with purulent infiltration of the tissues and death. Animals can be vaccinated by means of small quantities of living cultures or by sterilised cultures. Sterilised cultures will prevent infection (experimentally)

[1] See *Centr. f. Bakt.*, xxv, p. 897; *Journ. Exp. Med.*, September–November, 1899.

by anthrax if used early—that is to say, if an animal be inoculated with anthrax, and shortly afterwards injected with a broth culture of the *Bacillus pyocyaneus*, a fatal result is averted. Emmerich and Loew[1] claim to have isolated from cultures a ferment-like body, "pyocyanase," which they state has preventive and curative properties towards anthrax and diphtheria infections. Emmerich[2] has employed the dry pyocyanase as an application in diphtheria to dissolve the false membrane.

Williams and Cameron[3] describe four cases of diarrhœa with green stools, wasting and death in infants in which the *B. pyocyaneus* was obtained, and suggest that many cases of marasmus may be due to it. A form of epidemic dysentery seems occasionally to be caused by this organism (see "Dysentery"). A few cases of general infection with this organism have also been recorded. It has also been isolated from conditions of dermatitis and bullous eruptions.[4] The *B. pyocyaneus* has been found in water, dung, soil, and in the effluent from filter beds. Lehmann and Neumann state that, with the exception of pathogenicity, there is no essential difference between this organism and the *B. fluorescens liquefaciens* so frequently met with in water.

The *B. pyocyaneus* seems to be of more frequent occurrence and of greater pathogenicity in the Tropics than in this country. A disease bearing a remarkable similarity to rabies may be caused by it (see "Rabies").

Clinical Examination.

In many cases some idea can probably be formed as to the organisms likely to be present in the pus or discharge, etc.,

[1] *Zeitschr. f. Hyg.*, 1899; *Centr. f. Bakt.*, xxxi (*Originale*), p. 1.
[2] *Münch. med. Woch.*, November 5th and 12th, 1907.
[3] *Journ. Path. and Bact.*, vol. iii, 1896, p. 344. (Refs.)
[4] See Pernet, *Brit. Med. Journ.* vol. ii, 1904, p. 992.

from the clinical characters of the disease, in which case the examination may be more particularly directed towards the isolation of the suspected organism. For example, in a urethral discharge the gonococcus will be especially looked for, in an empyema following pneumonia the *Diplococcus pneumoniæ*, and in a tropical abscess of the liver the *Amœba coli*. In all cases the pus or discharge should be collected with aseptic precautions in sterile capillary pipettes or in sterile test-tubes at the time of operation. The discharge from opened abscesses and from wounds is liable to become contaminated and the original infection to be masked. In septic wounds the infection may be a mixed one.

In all cases the examination should be commenced as early as possible.

(1) Make several smears from the pus or discharge.

(2) Stain one or two of these with Loeffler's blue and one or two by Gram's method. Mount and examine microscopically.

(*a*) If staphylococci only are detected, the presence of the ordinary pyogenic cocci may be suspected. Proceed as in 3, 4, and 5.

(*b*) If encapsuled diplococci are detected, suspect the presence of the *Diplococcus pneumoniæ*, and proceed as in 3, 5 and 7.

(*c*) If diplococci and tetracocci are present, note whether they are in groups within the pus-cells; if so, suspect the presence of either the gonococcus or *Diplococcus intra-cellularis meningitidis*, and proceed as in 6.

(*d*) If free tetracocci are detected, suspect the presence of the *Micrococcus tetragenus*, and proceed as in 3 and 4 (rare).

(*e*) If streptococci are present the *Streptococcus pyogenes* is probably the species. Proceed as in 3, 4, and 5.

(*f*) If bacilli are present they may be the colon bacillus, the *Bacillus Welchii* (*aërogenes capsulatus*), the bacillus of malignant œdema, the tetanus bacillus, the typhoid bacillus, the *Bacillus pyocyaneus*, or putrefactive bacilli of the *Proteus*

group (which see). The result of Gram-staining and the clinical history of the case will be some guide.

α. The colon bacillus, especially frequent in suppurative peritonitis and in diseases of the urinary organs. (See page 407.)

β. The *Bacillus Welchii (aërogenes capsulatus)*, especially met with in foul wounds and gangrenous conditions, with much development of gas. (See Chapter XIII.)

gas.

γ. The bacillus of malignant œdema occurs in septic wounds with septicæmic complications. (See Chapter XIII.)

δ. The tetanus bacillus is found in the wound in cases of traumatic tetanus. (See Chapter XIII.)

ε. The typhoid bacillus is rare; it may occur in suppurative conditions complicating or following typhoid fever. Proceed as in 3 and 4. (See also p. 374.)

ζ. When the *Bacillus pyocyaneus* is present the pus or discharge may be blue. Proceed as in 3 and 4.

(*g*) If yellow granules, having a rosette-like structure microscopically, are present, actinomycosis may be suspected and examined for by the methods given in Chapter XV.

(*h*) If thread forms be present, *streptothrix* or *aspergillar* infection may be suspected (see Chapters XV and XVII): if large round or ovoid cells or yeast-like forms, *Blastomycetes* or *Sporotrichon* (Chapter XVI).

(*i*) If a mixture of organisms be present, agar and gelatin plate cultivations should be prepared and further examined by subcultures from the colonies.

(*j*) If no organisms can be detected microscopically, proceed as in 3, 7, or 9. In the pus of ordinary abscesses micro-organisms can generally be detected, unless caused by the tubercle or glanders bacillus, the pneumococcus, or the *Amœba coli*. In broken-down granulomata, *e. g.* gummata, if unopened, no organisms may be present.

(3) Make several cultivations on agar and gelatin (anaërobic if required), and examine microscopically and by subcultures when the growths have developed.

(4) Make two or three sets of agar and of gelatine plate cultivations. Examine the colonies microscopically and by subcultures.

(5) Stain two or three of the cover-glass preparations by Gram's method, and counter-stain with Bismarck brown.

(6) The gonococcus and *Diplococcus intracellularis* may be identified and distinguished by the methods detailed at pp. 259 and 253.

(7) Inoculate guinea-pigs or mice subcutaneously and intra-peritoneally with the material.

(8) Organisms can rarely be detected in the blood by a microscopical examination of stained films. Therefore 2–5 c.c. of blood should be withdrawn and cultivated (p. 131).

(9) If the abscess be probably a tropical abscess of the liver, the pus or scrapings from the wall of the abscess should be examined for the presence of the *Amœba coli*. (Chapter XVIII.)

Micrococcus meningitidis.[1]

Weichselbaum in 1887 isolated from cases of epidemic cerebro-spinal meningitis (spotted fever) a coccus which he named the *Diplococcus intracellularis meningitidis*, and further research has confirmed the accuracy of Weichselbaum's discovery and the ætiological relationship of the organism to the disease.

Morphology, etc.—The meningococcus, as it may be termed, occurs as single cocci and diplococci in groups within the leucocytes (Plate III., *a*); in grouping and general appearance, in fact, it closely resembles the gonococcus, and, like the last named, is Gram-negative, though staining well with the ordinary anilin dyes and with the Leishman stain. In cultures it occurs as cocci, diplococci, and occasionally as tetrads.

Cultural characters.—The meningococcus is an

[1] See Gordon, *Rep. Loc. Gov. Board*, 1907 (Bibliog.); Arkwright, *Journ. of Hygiene*, vol. vii, 1907, p. 193; vol. ix, 1909, p. 104.

obligatory aërobe, and does not grow at a temperature below 25° C. It will occasionally grow in primary culture on glycerin agar, but frequently not, though when acclimatised it grows fairly well on agar and in broth. The organism develops best on agar smeared with blood, on ascitic-fluid agar or broth, or on the nutrose ascitic agar of Wassermann (termed by Gordon "nasgar"):

Ascitic fluid	15 c.c.
Distilled water	35 c.c.
Nutrose	1 grm.

The mixture is placed in a flask, brought to the boil with constant shaking, and filtered. It is then mixed with double the volume of ordinary nutrient agar, steamed for thirty minutes, filtered, and filled into tubes.

The colonies of the meningococcus on this medium after twenty-four hours' incubation at 37° C. appear as moist, grey, translucent, circular or oval discs with regular outline; after a further twenty hours' growth they may attain a diameter of 3 to 4 mm. The colonies never exhibit any yellowish coloration as do those of some other Gram-negative cocci. Ascitic fluid broth (ascitic fluid 1 part, broth 9 parts) is also a good culture medium, and it grows in milk without clotting or change in reaction. Arkwright found that grown in gelatin at 37° C. the meningococcus causes liquefaction, while the *M. catarrhalis* does not. The organism needs constant transplantation to maintain vitality in culture. The fermentation reactions, which are somewhat variable, are given in the table on p. 261.

Symers and Wilson[1] examined the fermentation reactions of a number of strains of the meningococcus. Glucose, maltose and dextrin were fermented with the production of acid, lævulose, galactose, lactose, mannitol,

[1] *Journ. of Hygiene*, vol. ix, 1909, p. 9.

dulcitol, and a number of glucosides were never fermented.

Pathogenesis.—In man the organism causes epidemic cerebro-spinal meningitis, and is occasionally met with in sporadic cases of cerebro-spinal meningitis. It is also capable of producing a hæmorrhagic septicæmia without meningitis. It occurs in the cerebro-spinal fluid (obtained by lumbar puncture) in the blood in 25 per cent. of the cases provided quantities of 5 to 20 c.c. be cultured, sometimes in the upper respiratory passages, particularly the nose, in the middle ear, eye and joints. Park states that the organism is usually present in the nose in the early days of the illness. The meningococcus is pathogenic to mice and guinea-pigs by intra-peritoneal or intra-pleural, but not by subcutaneous, injection. Intra-spinal injection into monkeys produces a typical meningitis.

An agglutination reaction is given in some cases, but is neither constant nor marked enough to form a sure means of diagnosis.

Symmers and Wilson[1] have found that the blood of epidemic cerebro-spinal meningitis cases may occasionally agglutinate the *B. typhosus* and *B. coli* in comparatively high dilutions.

Vaccine and anti-serum.—Cases have been reported of remarkable benefit derived by vaccinating with killed cultures.

Flexner has prepared an anti-serum with which successful results have been obtained.

Still observed in simple posterior basic meningitis of infants a diplococcus closely resembling the meningococcus but growing more freely on agar, etc. By some it is regarded as an attenuated form of the latter. According to Arkwright it

[1] *Journ of Hygiene*, vol. viii, 1908, p. 314

does not liquefy gelatin, and grows on this medium at 22° C. fails to produce acid from glucose, maltose, and galactose, and is not agglutinated by a meningococcus serum. It is in these respects very like the *M. cinereus* of Lingelsheim. Wollstein[1] failed to find any reliable criteria of difference between strains of the *D. intracellularis* and several cultures obtained from cases of posterior basic meningitis. Houston and Rankin[2] found that ten Gram-negative cocci isolated from cases of sporadic cerebro-spinal meningitis differed from the *D. intracellularis* in respect of their opsonins and agglutinins, though eight of them were identical with the meningococcus in fermentive power. *Diplococcus crassus* (Gram-positive), *D. mucosus* (grows on gelatin), *D. flavus* (produces yellow pigment), and *M. catarrhalis*, the three latter Gram-negative, may occur in the naso-pharynx. (See Arkwright, *loc. cit.*, also p. 260.)

Gram-positive cocci and other organisms may occasionally cause a sporadic cerebro-spinal meningitis, *e. g.* the pneumococcus, typhoid and Gärtner bacilli, and streptococci (*S. fæcalis* and *S. salivarius*, Symmers and Wilson, *loc. cit.*, 1909).

Micrococcus gonorrhœæ.

The *Micrococcus gonorrhœæ* was discovered by Neisser in 1879 in cases of gonorrhœal urethritis. In gonorrhœal pus it occurs usually in pairs, occasionally in tetrads, the elements of which are somewhat ovoid in shape, their opposed surfaces being flattened. The organism has a characteristic arrangement: it occurs in groups *within* the pus-cells (Plate III., *b*). The individual cocci vary somewhat in size, the average being about 0·7 μ in the long and 0·5 μ in the short diameter.

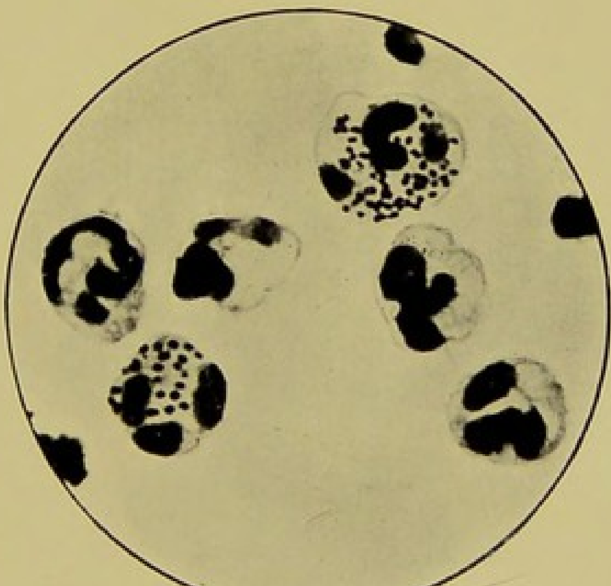
Pairs

in pus cells

[1] *Studies from the Rockefeller Inst.*, vol. x, 1910, No. 13.

[2] *Brit. Med. Journ.*, 1907, vol. ii, p. 1414.

a. The meningococcus. Smear of cerebro-spinal fluid. × 1000.



b. The gonococcus. Smear of gonorrhœal pus. × 1500.

To face page 256.

It stains readily with the ordinary anilin dyes, Löffler's blue being perhaps the best, but is decolorised by Gram's method—an important practical distinction from many other cocci.

Cultural characters.—The gonococcus is difficult to cultivate, and usually soon dies out under cultivation—within a week, unless transferred to fresh soil—but it does not seem to lose its virulence. Growth takes place between 25° and 38° C., but the optimum temperature is between 35° and 37° C. It is aërobic, and possibly facultatively anaërobic, and will develop on a feebly alkaline or acid soil. The ordinary agar and gelatin media are useless for the cultivation of the gonococcus; it will grow only on a medium containing "native" protein. Blood-serum agar gives fair results, but the ordinary Löffler's blood-serum is of no use. The best medium is agar smeared with blood. Ordinary sloping agar tubes or small agar plates may be employed. Blood obtained by pricking the finger, with antiseptic precautions, is taken up in a sterile capillary tube and deposited on the agar. A trace of gonorrhœal pus, collected with aseptic precautions, is taken up on a small sterile camel's-hair brush, and is rubbed up with the drop of blood and smeared over the surface of the agar. The cultures are incubated at 37° C., and in twenty-four hours the colonies of the gonococci appear as transparent greyish specks, which increase in size up to the end of three days. At this stage the colony measures 1 to 2 mm. in diameter, is raised, brownish, and finely granular in appearance, and roundish with a crinkled margin. The cocci from cultures resemble those in the pus, but tetrads are more frequently met with. The fermentation reactions and comparison with other Gram-negative cocci will be found in the table, p. 261. The specific virulence of gonorrhœal pus is

destroyed by exposure to a temperature of 60° C. for ten minutes.

?

Pathogenicity.—The gonococcus is a strictly parasitic organism, and seems exclusively to attack man. From inoculation experiments on the human subjects it appears to be the specific organism of gonorrhœal urethritis and vulvitis. In the female it is most frequent in the urethral or vulvar discharge, less so in that from the cervical canal, and is rarely or never seen in a purely vaginal one. It is generally, even at an early stage, associated with other organisms, particularly other diplococci (see table, p. 261) which have to be distinguished from the gonococcus. The features which serve to identify the latter are its shape and size, its non-staining by Gram's method, its arrangement in *groups within* the pus-cells, absence of growth on ordinary media, the characters of the colonies, and the fermentation reactions.

*

The gonococcus is associated with a variety of lesions besides those already mentioned, viz. epididymitis, ovaritis, salpingitis, cystitis, peritonitis, arthritis, and conjunctivitis. It has been met with in the blood, and occasionally produces endocarditis, pericarditis, and meningitis. The gonococcus is fatal to guinea-pigs and mice by intra-peritoneal inoculation.

Toxin, anti-serum, and vaccine.—Christmas[1] found that the blood-serum of the rabbit, fluid or coagulated, is an excellent culture medium for the gonococcus. By cultivating the gonococcus for ten days in an ascitic bouillon mixture he succeeded in obtaining a toxin which, when injected intra-venously into rabbits in large doses, caused death, in smaller doses fever and loss of weight, while precipitated with alcohol and injected into the anterior chamber of the eye it produced severe

[1] *Ann. de l'Inst. Pasteur*, xi, 1897, p. 609.

* Intra cellular in acute cases
Extra cellular in gleet

inflammation. By injecting rabbits with small doses of the toxin immunisation was produced, and the blood acquired antitoxic properties. A vaccine may be prepared by sterilising cultures with heat, and has proved of service in chronic gonorrhœal infections.

Clinical Diagnosis.

The diagnosis of gonorrhœa is very important, not only in clinical but also in medico-legal cases. For this purpose microscopical examination and culture methods are made use of. In a chronic gleet the material must be examined carefully and repeatedly.

(1) *Microscopical examination.*—Several thin smear specimens of the pus or discharge should be prepared. If the best results are desired the films should be air dried, and then fixed by placing in a mixture of equal parts of alcohol and ether for fifteen minutes. After fixing, a couple of the films are stained in Löffler's blue for five to ten minutes, washed in water, dried and mounted. Leishman's stain also gives good results, the films being merely air-dried and not fixed. The preparations are then examined with a $\frac{1}{12}$-inch oil-immersion; a lower power lens is useless. The ovoid cocci in pairs, and occasionally in tetrads, occurring within the pus-cells in groups of not less than four pairs are very characteristic. Diplococci situated outside the pus-cells should be neglected (it is to be noted that the nuclei of the pus-cells are deeply, the cytoplasm only faintly, stained with methylene blue). The next step is to ascertain the staining reaction by Gram's method. Stain two more films for fifteen minutes in anilin gentian violet, dip in water, place in Gram's iodine solution for two minutes, decolorise in absolute alcohol until the drainings fail to stain white filter paper, and counter stain for forty-five seconds in a saturated aqueous solution of Bismarck brown diluted with three times its volume of distilled water. The gonococci are decolorised,

and take up the brown stain. In chronic urethritis the urine may be centrifugalised, and preparations are made from the deposit and threads and stained.

(2) *Culture methods.*—Whenever a diagnosis is of great importance an attempt should be made to cultivate the organism. Plate cultures of agar smeared with blood as described (p. 257) and another set with agar only should be prepared and incubated at 37° C. In forty-eight hours colonies of the gonococcus should be recognisable on the blood-agar, but not on the plain agar.

If cultures are obtained, the fermentation tests (p. 261) may be applied.

N.B.—*The greatest caution must be exercised in declaring a case free from infection on the ground of* NEGATIVE *results of the examination.*

Micrococcus catarrhalis.[1]

This organism occurs in the nose and throat in cases of catarrh, and particularly in the "influenzal cold" (see "Influenza"), in bronchial catarrh, and occasionally in other conditions and in well people. Morphologically it occurs in pairs and tetrads, often within the polymorphonuclear leucocytes. It is Gram-negative. The primary generation develops feebly on agar, but subsequent generations grow fairly well, forming whitish translucent colonies. Blood or ascitic media should be used for isolation. Some of the fermentation reactions and a comparison with other Gram-negative cocci are given in the table on the next page.

Gram – gel grows gel?

Micrococcus tetragenus.

This organism is frequently met with in phthisical cavities and may be expectorated in the sputum, and has also been found in the pus of acute abscesses. The cells occur singly

[1] See Gordon, *Brit. Med. Journ.*, 1905, vol. ii, p. 423; Arkwright, *Journ. of Hygiene*, vol. vii, 1907, p. 145.

Glucose Galactose Maltose Saccharose

The Characters of the Chief Gram-negative Cocci (Gordon).

Organism or source.	Growth on nutrose ascitic agar at 37° C.	Growth on gelatin at 20° C.	Pathogenicity.	Glucose.	Galactose.	Maltose.	Saccharose.
M. catarrhalis. Nasal and pharyngeal discharge	Opaque, granular	Positive (grows on ordinary agar at 37° C.)	Mice and guinea-pigs by intra-peritoneal inoculation only	0	0	0	0
M. intracellularis (meningococcus). Cerebro-spinal meningitis	Clear, smooth	Negative	In some cases: mice and guinea-pigs by intra-peritoneal inoculation only	+	+	+	0
M. gonorrhœæ (gonococcus). Urethral discharge	No growth unless blood added	Negative	*Ib.*	+	+	0	0
From nasal discharge from Hertford case of influenza-like epidemic (see "Influenza")	Clear, smooth, later becomes yellowish	Negative at first, positive later (grows on ordinary agar at 37° C.)	Mice and guinea pigs by intra-peritoneal inoculation	+	0	+	0
Ib.	Opaque, granular	Negative	*Ib.*	+	+	+	+
From urethra . .	Opaque, somewhat granular, smooth edges	Positive	—	+	+	+	+
M. melitensis. Malta fever	Creamy and slightly yellowish	Positive	Monkeys. Also rabbits and guinea-pigs by intra-cerebral inoculation	—	0	0	0

+ = acid. — = alkali. 0 = no action.

(diameter 1 μ), in pairs, or in fours, and are enclosed within a capsule. It stains with the ordinary anilin dyes and also by Gram's method. On gelatin it develops slowly, with the formation of a thick, white, shining growth without liquefaction. On agar the growth has much the same characters, and on potato is white and viscous. Inoculated into animals, particularly mice, a local abscess may form, but usually it produces a fatal general infection, and the organism is found in the blood and organs. Gel 0.

A few cases of general infection in man have been described.

Sarcina ventriculi.

An organism occurring in the contents of the stomach, especially in cases of dilated stomach. Originally described by Goodsir in 1842.

It occurs as a large ovoid cell, several of which are grouped together quadrilaterally so as to form more or less cubical masses, the so-called "woolpacks." According to Falkenheim, it forms on gelatin in thirty-six to forty-eight hours roundish, prominent colonies of a yellowish colour, and in neutral hay infusion a brownish film and flocculi. It produces an acid reaction.

Other sarcinæ also occur in the stomach.

Clinical examination.—1. The organism can be detected in the vomit, etc., most readily by examination in the fresh state, a little of the material being placed on a slide, diluted with water if necessary, irrigated or not with iodine solution, covered with a cover-glass, and examined.

2 Cover-glass preparations may be stained with weak carbol fuchsin, or by Gram's method.

Other Organisms met with in Suppurative and Septic Conditions.

Many other organisms may be met with in various suppurative and septic processes, *e. g.* :

a. The *B. coli* in cystitis and pyelitis, ischio-rectal abscess, peritonitis associated with perforation and intestinal obstruction, and puerperal fever (see Chapter X).

b. The *Diplococcus pneumoniæ* in abscesses, empyema, arthritis, meningitis, pericarditis, peritonitis, etc. (see Chapter XII).

c. The *B. typhosus* in abscesses, cholecystitis, empyema, and osteomyelitis (see Chapter X).

d. The *B. œdematis* and *B. Welchii* in foul, gangrenous wounds (see Chapter XIII).

e. The *B. tuberculosis* and *B. mallei* (see Chapter IX).

f. The *actinomyces* and *streptothrix* forms (see Chapter XV).

g. Blastomycetes, Sporotrichon (see Chapter XVI) and *Hyphomycetes* (see Chapter XVII).

h. The *Amœba coli* (see Chapter XVIII).

i. Capsulated bacilli (see note, p. 271).

Coley's Fluid.

This preparation consists of the toxins of the streptococcus of erysipelas and the *B. prodigiosus.* It was devised by W. B. Coley, of New York, as a cure for inoperable malignant tumours, particularly sarcoma. The treatment is based on the undoubted fact that malignant growths may decrease or even disappear completely after an attack of erysipelas (p. 244). Originally prepared by growing a virulent streptococcus obtained from a fatal case of erysipelas in bouillon for about ten days; the culture is then inoculated with the *B. prodigiosus* and the two are allowed to grow together for another week or ten days. The culture is finally heated to from 58° to 60° C. for one hour, and a piece of thymol added to preserve it The fluid is now prepared by growing the organisms separately and then mixing the two sterilised cultures in proper proportions.

The fluid is injected subcutaneously in the vicinity of the tumour. The primary dose recommended is ¼ minim of the fluid. The dose is gradually increased each day until there is a temperature reaction of 103° to 104° F.

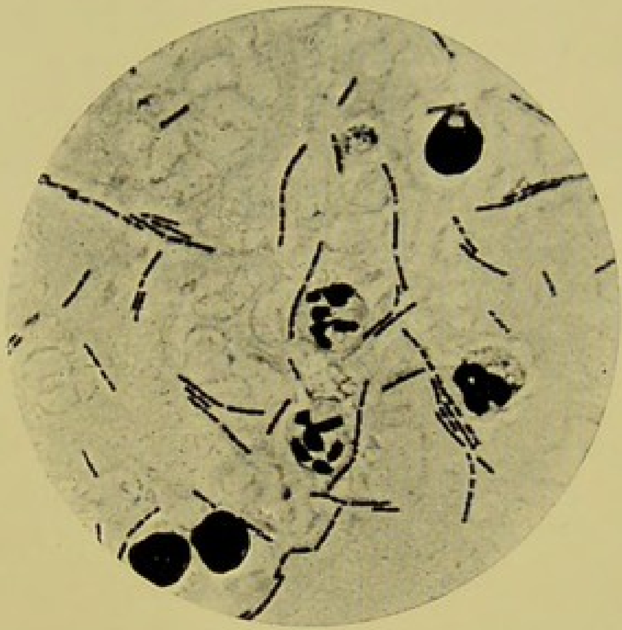
Full particulars will be found in Coley's paper (*Proc. Roy. Soc. Med.*, vol. iii, 1909–10, Surg. Sect., p. 1).

CHAPTER VII.

ANTHRAX.

Anthrax is essentially a disease of cattle known as splenic fever, and though occurring in England only sporadically, or in small outbreaks, in some parts of the world it assumes serious proportions—as in Siberia, where it has been termed the Siberian plague. In France also at one time it ravaged the sheep to such an extent as to threaten them with extinction. Man is also occasionally attacked.

Anthrax was the first disease to be definitely associated with a specific micro-parasite, for the organism was observed as glassy homogeneous rods and filaments in the blood of infected animal so long ago as 1849 by Pollender and 1850 by Davaine, and the latter also claimed in 1863 to have demonstrated by inoculation experiments the causal relation of the organism to the disease. Davaine's experiments were made by inoculating an animal directly with the blood from an infected animal, and were, therefore, hardly conclusive, as they did not comply with the second and third of Koch's postulates, which declare that the micro-organism must be cultivated outside the body, and the cultivated organism must produce the disease on inoculation, and the objection was raised that infection was due, not to the bacillus, but to something else in the blood. This objection was subsequently removed by the work of Pasteur and of Koch, who obtained pure cultures of the organism, the *Bacillus anthracis*, and with these



a. Bacillus anthracis. Smear of blood of inoculated guinea-pig. × 750

b. Anthrax. Section of kidney through glomerulus. × 500.

To face page 264.

produced results the same as had previously been obtained by inoculation with the blood of an infected animal.

Morphology.—*The Bacillus anthracis* is a rod-shaped organism varying slightly in size in different animals and under cultivation; in the blood it measures from 5 to 20 μ in length and 1 to 1·25 μ breadth (Plate IV., *a*), but in cultures long filaments develop. Examined in the fresh and living condition in a hanging-drop preparation, these rods and filaments appear homogeneous or slightly granular; in stained preparations, however, they are seen to be made up of a series of segments with unstained interspaces, each segment measuring about 4 to 5 μ in length, and the ends of the segments appear cut off square, provided care has been taken not to overheat in fixing and to stain with an aqueous solution. In the blood the filaments never exceed about five or six segments in length, except perhaps in swine, in which animals they may be somewhat longer. In cultures, however, the filaments may be of almost unlimited length, and lie parallel to one another or in more or less tangled masses. In the animal body during life, and for some hours after death, spores never occur; but in cultures more than a day or so old, and from which oxygen has not been excluded, they are always present, almost every segment containing one. The spores are ellipsoidal, measuring about 1 μ by 1·25 μ, and are centrally placed in each segment, the long axis corresponding with the long axis of the segment.

Cultural reactions.—The anthrax bacillus is aërobic and facultatively anaërobic; it is non-motile, and stains well with the ordinary anilin dyes, and especially so by Gram's method. It grows readily on all culture media at from 20° to 37° C., the latter being the optimum. Development ceases at temperatures below about 15°

and above 45° C. Small, cream-coloured, granular colonies develop in a gelatin plate in about thirty hours, and in two to three days appear as small, roundish, cream-coloured pasty masses in little pits in the gelatin, due to its liquefaction. Microscopically the colonies are somewhat characteristic; each consists of a mass

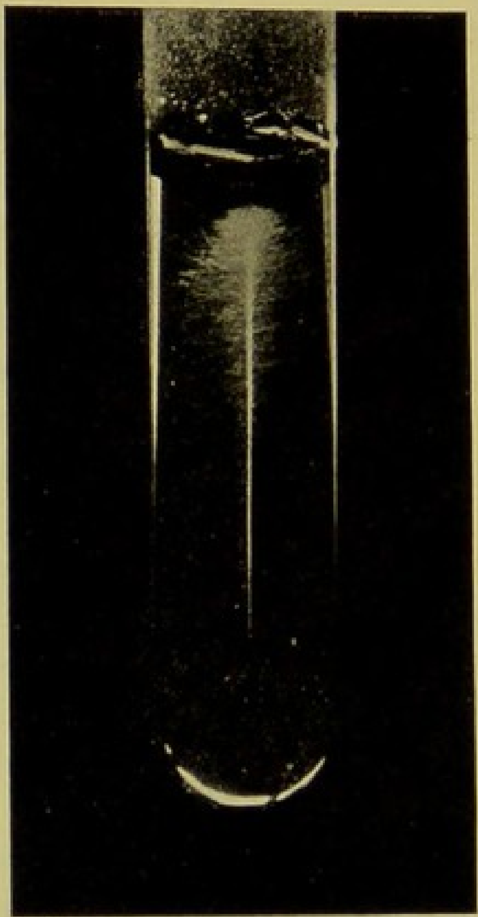


FIG. 36.—Anthrax. Gelatin stab-culture. Seven days old.

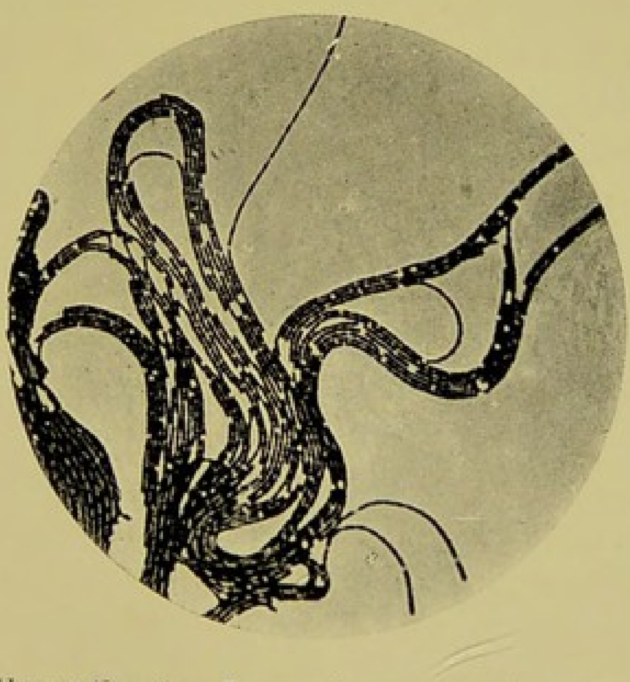
of wavy, tangled filaments like a tiny wad of cotton-wool. In gelatin streak-cultures development is slow, and in four or five days a creamy, pasty growth forms in a trough of liquefaction. In a gelatin stab-culture (preferably 5 per cent. gelatin) lateral branches spread from the central growth, longer in the upper layers, shorter below, so that at the end of a week the culture

is like an inverted fir tree (Fig. 36), and the gelatin becomes gradually liquefied from above downwards. The colonies on an agar plate develop in twenty hours at 37° C. as cream-coloured points, which microscopically consist of little masses of wavy, tangled filaments (Plate V., *a* and *b*). On an agar surface culture at 37° C. there is a copious development in eighteen hours of a thick, cream-coloured, slimy growth, which at this early stage has a finely granular, ground-glass appearance. On blood-serum a thick creamy layer forms, with slow liquefaction of the medium. On potato the organism grows freely as a dry greyish layer, with an abundant formation of spores. In broth it forms a somewhat scanty flocculent deposit, the broth remaining clear and giving the indole reaction.

In old cultures various involution forms are met with; the rods lose their regular shape and become swollen, producing the so-called torula forms, while the homogeneous appearance of the protoplasm changes and becomes granular. Spores are found in all culture media when there has been free access of oxygen, as in surface cultures on potato and agar; but in a deep broth culture, where the supply is limited, spore-formation is absent or very scanty. Spores are never met with in the living animal; they only appear some hours after death, or when matter containing the bacilli comes in contact with air, as in the bloody discharge from the nostrils. It has therefore been supposed that oxygen is necessary for spore-formation to take place, but this does not seem to be the whole explanation, for spores form in an atmosphere of nitrogen, though they do not do so in one of hydrogen. The life-history of the organism and the development of spores can be well watched in a hanging-drop specimen prepared by inoculating a droplet of broth with the blood of an infected

animal. The preparation can be observed on a warm stage, or examined at stated times, being kept in the intervals in the blood-heat incubator. At the end of twenty-four hours the short filaments, which alone are present in the blood, will have grown so long that they stretch across the field, while the protoplasm has become granular, and minute shining points are visible here and there. In another twenty-four hours the filaments extend, the protoplasm becomes still more granular, and the shining spots are now well-marked ovoid, highly refractile bodies—the mature spores. In old cultures the rods and filaments almost disappear, numbers of spores alone remaining. These spores, when placed under favourable conditions of moisture, warmth, and nutriment, again produce rods and filaments; a little bud appears at the extremity of the long diameter, which grows in length and ultimately becomes a mature rod, often with the empty spore capsule embracing one end. Sporeless varieties of the anthrax bacillus have been obtained by cultivating under unfavourable conditions, as at a high temperature (44° C.) or in the presence of minute quantities of antiseptics (1 : 1000 carbolic acid). The spores are of considerable practical importance, for they are highly resistant forms, requiring at least some minutes' boiling and three hours in dry air at 140° C. for their destruction, whereas the bacilli without spores are destroyed in ten minutes in the moist condition by a temperature of 54° C. The same resistance occurs towards various germicidal substances. While 1 per cent. carbolic acid and 1 : 10,000 corrosive sublimate solutions quickly destroy bacilli without spores, it requires at least 5 per cent. carbolic acid, acting for not less than twenty-four hours, and 1 : 500 sublimate solutions acting for not less than an hour, both at 20° C., to kill the spores. It is to be noted that

a. Bacillus anthracis. Impression preparation of a colony. × 40.



b. Bacillus anthracis. Impression preparation of a colony. × 750.

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the resistance of the spores increases with their age ; the youngest spores may be killed by 5 per cent. carbolic in a few hours, but fourteen to twenty-eight days old spores will resist 10 per cent. carbolic, cyllin, kerol, etc., for days. Anthrax spores retain their vitality and pathogenic power unimpaired for years in a dried condition.

Pathogenicity.—The anthrax bacillus is pathogenic for man, cattle, sheep, goats, rabbits, guinea-pigs, and mice. The horse and the pig are also susceptible ; but adult white rats, dogs, cats, and Algerian sheep are immune.

Young white rats, or rats fatigued by muscular work, can be infected, and frogs and fish, though immune under ordinary conditions, can be rendered susceptible by raising the temperature of their environment. Birds, such as fowls and pigeons, are also almost insusceptible, but may be rendered susceptible by lowering their temperature ; smaller birds, such as sparrows, are more susceptible. The virulence varies considerably and may be artificially modified in many ways: by passing through a series of susceptible animals it is heightened, by growing in the body of an insusceptible animal it is lowered, and the latter result is also obtained by cultivating for two or three weeks at a temperature of 42° to 45° C., or by the addition of certain chemical substances to the culture medium—for example, 0·01 per cent. of potassium bichromate. These methods of "attenuation," as it is termed, are practically applied in the preparation of the anthrax vaccine.

Symptoms of the disease in cattle are not very marked. A beast may appear a little out of sorts and the next day be found dead, or after suffering for a day or two with general malaise, fever, and rigors, and with a sanguineous discharge from the nostrils and bowel, it dies suddenly. Post mortem, the chief feature that

attracts attention is enlargement of the spleen; the organ may be two or three times larger than normal, is highly congested, and very soft and friable. Microscopically, the bacillus is found in enormous numbers in the spleen, somewhat less numerously in the blood, and still less so in the liver, kidney, and other organs.

Swine do not often suffer from this disease, unless fed with the offal of an infected animal, in which case the chief clinical sign is great enlargement about the throat; this is almost pathognomonic, while the chains of bacilli tend to be somewhat longer than in other animals.

Mice inoculated subcutaneously usually die in about twenty-four hours, and enlargement and congestion of the spleen are very noticeable. An infected guinea-pig dies in from thirty-six to forty-eight hours and usually shows no symptoms until the last, when it may suffer from rigors, with high temperature, convulsions, and staring coat. Post mortem, the muscular tissue is found to be pale and œdematous, the spleen is enlarged to two or three times its normal size and is highly congested and very soft, and minute hæmorrhages may occur in the serous membranes. Microscopically, bacilli are found throughout the spleen, and are often so numerous that in a stained preparation there appear to be more bacilli than tissue. Large numbers are also present in the blood and lungs, fewer in the liver and kidney; in the latter organ they are almost confined to the glomeruli (Plate IV., *b*). Immediately after death, however, comparatively few bacilli may be met with in the blood, the heart, and great vessels.

The spread of the disease in nature seems to result from the ingestion of spores while the animals are feeding. Although the bacilli without spores would be destroyed by the acid gastric juice, this is not the case with the

spores, which are probably generally developed from the organisms present in the bloody discharges of a stricken animal, and are distributed by wind and flood, and in this way may infect large tracts of pasture. Pasteur suggested that earthworms might bring the spores to the surface in their casts from the buried carcases of infected animals, but some experiments by Koch negatived this. The non-sporing bacilli rapidly degenerate and die in a buried carcase.

Man seems to be relatively insusceptible to anthrax. The disease is generally met with among butchers, veterinary surgeons, shepherds, etc., and among those who sort wool or hair or work with, or carry, hides, *e. g.* glove-makers, tanners, porters, etc. The disease occurs in two forms: the so-called "malignant pustule," a cutaneous infection, not unlike an angry carbuncle, occurring at the seat of inoculation, on exposed parts of the body, such as the back of the neck, the face, wrists, and hands; and "wool-sorters' disease," a general infection, severe and fortunately rare, through the lungs or stomach. Rag-sorters are likewise sometimes attacked by anthrax but there is also a distinct "rag-sorters' disease" which is stated to be due to a non-motile, non-sporing, non-liquefying, capsulated bacillus, the *Proteus capsulatus hominis*[1] of Bordoni Uffreduzzi.

Under the Factories and Workshops Act, 1895, all cases of anthrax contracted in connection with various industries have now to be reported to the Home Office.

[1] Capsulated bacilli have been met with in many septic processes. This group includes Friedländer's pneumo-bacillus, *P. capsulatus hominis*, *B. mucosus capsulatus* of Fricke, and the *B. coli immobilis*. They are met with in conditions associated with sepsis, pus production, broncho-pneumonia, ulcerating stomatitis, etc. They are shortish, non-motile, non-sporing rods, usually Gram-negative, easily cultivated and not liquefying gelatin, and in the tissues surrounded with a capsule.

In 1909, 56 cases, in 1910, 51 cases were thus reported, with mortalities of 21·5 and 17·6 per cent. respectively. In addition, in 1910 there were 31 other cases in England and Wales. Industrial anthrax has also been exhaustively dealt with by Legge.[1] It is particularly Persian wool, Chinese hides, and Russian hair which are dangerous, while Argentine, Australian, and New Zealand wools are almost innocuous. The sorting and exclusion of wool derived from infected animals seem to be impracticable, and the efficient sterilisation of the thousands of bales that are imported an impossibility. As regards hides and skins, Legge points out that it is doubtful if there is any way in which hides to be afterwards tanned can be effectively disinfected, and to be of real benefit it would have to be done before the material is opened in the warehouse; but to secure this would be impossible. Recently a method introduced by Seymour Jones has been favourably reported on[2]; it consists in soaking the skins for twenty-four hours in a mixture consisting of 1 per cent. formic acid and 1 in 5000 mercuric chloride. After this treatment the skins are soaked in a strong brine solution. As regards horse-hair, Webb and Duncan[3] carried out a number of experiments on its disinfection, from which it would seem that, leaving out of consideration white or grey hair, which is liable to change colour, no injurious effect is produced on hair by steam disinfection provided the temperature does not exceed 218° F.; but this is a comparatively low temperature for efficient disinfection, and success can then be obtained only with minute care in the construction and regulation of the

[1] *Brit. Med. Journ.*, 1905, vol. i, pp. 529, 589, and 641.

[2] Ponder, *Report to the Worshipful Company of Leathersellers*, 1911.

[3] *Ann. Rep. of Chief Inspector of Factories*, 1900, p. 472, and 1902, p. 278.

apparatus. Legge concludes that to secure certain destruction of all anthrax spores in horsehair absolute reliance cannot be placed on either steam disinfection (within the limits in which it can be applied) or simple boiling. Adoption of one or the other is a very material safeguard, but risk must always be run by those who prepare the hair for disinfection. Disinfection is now being attempted by subjecting the material to the action of certain disinfectants, but from experiments by Hall and the writer, Seymour Jones, the method seems the only one efficient.

A number of cases of anthrax, resulting in many deaths, have been reported in various parts of the United States from tanneries dealing with hides imported from China. Also a number of cattle have been infected as the result of drinking water from rivers and creeks receiving the waste liquors from these works.

Houston[1] detected the anthrax bacillus in a catch-pit in a hide factory at Yeovil, and in sewage and effluents and in the mud of the Yeo. It has also been met with in linseed cake and oats.

Toxins.—From pure cultures of the *Bacillus anthracis* Hoffa obtained small quantities of a ptomine, which produced fall of temperature and hæmorrhages, and Hankin isolated a proteose which in large amounts was fatal, but in small amounts conferred immunity to subsequent inoculation with living bacilli. Brieger and Fränkel obtained a tox-albumin from animals dead of anthrax. Marmier,[2] by growing the anthrax bacillus in a solution of peptone, glycerin, and salts, and subsequently precipitating with ammonium sulphate, obtained a toxin which he states is neither protein nor basic, and is contained within the bacterial cells.

[1] *Second Rep. Commis. on Sewage Disposal*, 1902, p. 31.
[2] *Ann. de l'Inst. Pasteur*, ix, 1895, p. 533.

Sidney Martin,[1] by growing the anthrax bacillus in alkali albumen for ten days, obtained from the culture albumoses and an alkaloidal substance. From the bodies of animals which had died of the disease, chiefly from the spleen and blood, he obtained similar substances, the amount of alkaloid being more than double that of albumose. The mixed products produced fever in animals followed by coma and death. The albumose was proved to be the fever, and the alkaloid the coma, producer; the latter also caused a spreading œdema at the seat of inoculation.

Anti-serum.—An anti-serum for anthrax was prepared by Marchoux by immunising sheep by vaccination and then inoculating with progressively increasing doses of virulent anthrax cultures. Sclavo has prepared an anti-serum by first immunising asses with a vaccine and then inoculating them with increasing doses of virulent cultures over a prolonged period. This serum has been used successfully in many cases of anthrax in man.

Vaccine.—An attenuated virus has been extensively employed for the *prophylactic* vaccination of cattle and sheep. Cultures are attenuated by growing at 42°–43° C. (Pasteur, Chamberland, and Roux). A weak vaccine is first injected, followed after ten to twelve days by an injection of a stronger vaccine. The mortality as a result of the vaccination is small and the animals are subsequently protected for some months against the virulent disease. Sobernheim has applied a combined method, 5–15 c.c. of anti-anthrax serum being inoculated on one side of the animal, and the vaccine on the other. This practically eliminates all danger from the vaccine.

[1] *Brit. Med. Journ.*, 1892, vol. i, p. 641.

Clinical Examination.

(1) *In veterinary practice.*—If an animal is suspected to have died from splenic fever, an extensive post-mortem is inadvisable because of the risk of distribution of material containing bacilli with subsequent development and dissemination of spores, with infection of pasture, etc. The abdomen should be opened and the spleen examined. If this is found to be much enlarged, and so soft that it can hardly be handled without rupture, there is a high probability of splenic fever, which the history of sudden death, with or without symptoms, coupled with a sanguineous discharge, increases. To confirm the diagnosis, some smear preparations should be made from the spleen and blood, and can be stained and examined on arriving home. If slides or cover-glasses are not available, the ear or a small piece of the spleen may be removed and taken home, where the specimen may be examined. When material is sent from a distance for examination the ear should be forwarded.

The smears may be stained with Löffler's blue and by Gram's method with eosin. Methylene-blue staining gives the most characteristic appearances, which are numbers of large bacilli forming chains of five or six segments with a pale halo round them resembling a capsule, and if the post-mortem has been made shortly after death no spores are visible. *Unless the material be quite fresh large saprophytic bacteria somewhat resembling anthrax are always present and must not be mistaken for that organism.* If a hanging-drop preparation can be made, a characteristic is the non-motility of the bacilli.

The stained preparations can be kept and produced in a court of law if necessary. Cultivations can also be made from the spleen, but the necessary culture media are not of course usually forthcoming. Finally, a guinea-pig or mouse may be inoculated subcutaneously in the abdomen with a particle of the spleen, and after death examined microscopically and by culture methods.

As regards the disposal of the carcase of an animal dead from anthrax, this should be burned if possible, but, failing

this, it may be buried in a deep pit, preferably with plenty of lime. All traces of blood and discharge must be carefully mopped up with a strong lime-wash or solution of chloride of lime, or other reliable disinfectant.

(2) *In man.*—In malignant pustule, smear specimens should be prepared from the fluid of the vesicles or with the scrapings from the incised pustule, or sections of the excised pustule may be made, and stained, some with Löffler's blue, others by Gram's method with eosin. The bacilli are not often met with in the blood, except shortly before death. At the same time cultivations on agar and gelatin should be prepared, and may yield positive results when the microscopical examination has been negative. In the later stages of the disease the bacilli may be difficult to find, even in sections.

In all cases of doubt a guinea-pig or mouse should be inoculated subcutaneously with the material, and if the animal dies the diagnosis of anthrax may be confirmed by the characteristic appearances, by a microscopical examination, and by cultivation. The animal experiment is by far the most certain method of diagnosis, a negative result being nearly as valuable as a positive one.

N.B.—It must be noted that both cultivation and inoculation experiments may fail to give positive results if the material be old or putrid.

4 negatives

CHAPTER VIII.

DIPHTHERIA.[1]

Diphtheria in England—The Diphtheria Bacillus—The Pseudo-Diphtheria Bacillus—Clinical Diagnosis—The Xerosis Bacillus—Diphtheritic Affections of Birds and Animals.

DIPHTHERIA seems to have been known from the earliest ages, being recognised by the classical (medical) writers, and it was epidemic in England and on the Continent during the Middle Ages. Bretonneau[2] experienced an outbreak at Tours, 1818–1821, and gave to the disease the name "Diphtérite" (afterwards changed to "Diphtérie") from the formation of membranes which is so marked a feature in it. In England the diphtheria deaths have only been separately scheduled since 1855. Since 1881 until recently there has been a steady increase in the prevalence of diphtheria, particularly in the large towns, but latterly the prevalence seems to be decreasing.

As regards croup, it it universally admitted that the vast majority of cases of membranous croup are cases of diphtheria.

Diphtheria is distinctly a disease of the young, especially at the ages from two to ten, and this holds good both for London and for England and Wales.

That diphtheria is an infective disease is amply

[1] See *The Bacteriology of Diphtheria*, Cambridge University Press, 1908.

[2] See *Memoirs on Diphtheria*, New Sydenham Soc., 1859.

proved by the history of epidemics, and by the recorded cases where the disease has been conveyed from one individual to another.

The disease occurs in all grades of severity, from the classical ones with wash-leather-like membrane and great prostration, to those which present a mild tonsillitis or angina.

The bacteriological study of diphtheria was commenced as long ago as 1882 by two German investigators, Klebs and Löffler. Klebs especially investigated the pathological histology, and ascribed the disease to small rod-shaped organisms, which he observed in the membrane. It was reserved for Löffler to place this observation of Klebs on a firmer basis by the isolation and cultivation of the bacillus from the membrane, and by the production of certain phases of the disease by inoculation with the isolated organism. The cause of diphtheria is, therefore, this diphtheria bacillus, which, from its discoverers, is frequently known as the Klebs-Löffler bacillus.

The isolation of the specific organism was by no means an easy matter, as a number of other species of bacteria is frequently associated with it in the membrane, but was accomplished by Löffler by the use of a special culture medium now known as Löffler's blood-serum, which consists of a mixture of blood-serum (ox serum was that originally used) 3 parts and glucose bouillon 1 part, the whole being coagulated (see p. 61). On this medium the diphtheria bacillus grows and multiplies exceedingly well, while the other organisms associated with it in the membrane are to a large extent inhibited in their growth. By rubbing a small piece of membrane from a case of diphtheria over the surface of two or three tubes, or of a plate of Löffler's serum, and incubating at 37° C. for twenty to twenty-four hours, colonies of the

diphtheria bacillus will be found more or less isolated according to the number of organisms present in the membrane, and by subculturing from these pure cultures may be obtained.

Characters of the Diphtheria Bacillus.

Morphology.—The *B. diphtheriæ* is a small, delicate bacillus, with rounded ends, measuring 3 μ or 4 μ in length. It is non-motile, does not form spores, and is aërobic and facultatively anaërobic. The size varies somewhat even on the same medium, and three varieties of the bacillus have been described, viz. long, medium, and short, according to the length. These varieties tend to be constant and to breed true. Some of the rods both in cultures and in the membrane have a swollen end, the so-called clubbing, and parallel grouping, both in the membrane and in cultures, is almost universal, the bacilli lying parallel side by side (Plate VI., *a*). This parallel arrangement arises from the peculiar mode of division of the bacillus. If a cell be observed upon a warm stage it first elongates, then becomes constricted at about its middle, and then suddenly *one* side of the cell-membrane seems to rupture and one half of the cell bends over to the other, so that the two halves form a V. This mode of division, occurring in contiguous cells and being repeated, and the cells thus becoming more and more crowded together, leads to the arrangement in parallel series. The bacilli are generally joined end to end in pairs, and distinct thread and branching forms, though of rare occurrence, may be met with. On different media the same strain exhibits considerable variation in size. On blood-serum and on gelatin the bacilli are of medium length and on the whole fairly regular in

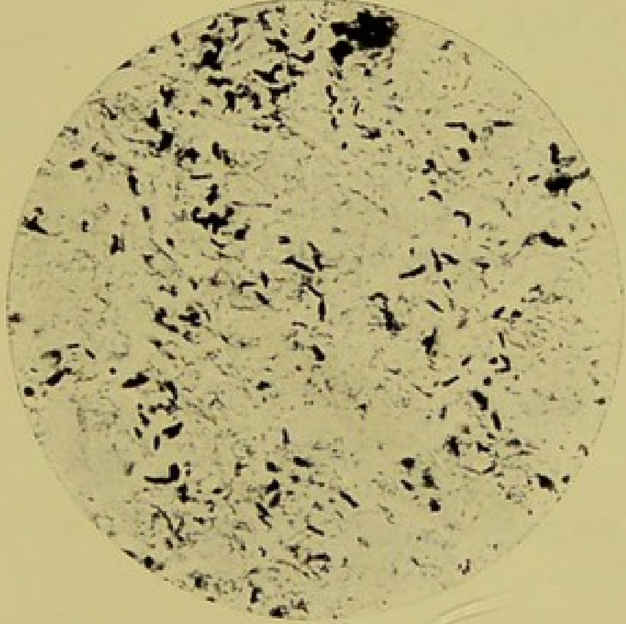
Spores O
Motile O

shape; in broth they tend to be short and stunted; while on agar, especially glycerine agar, they are much larger than on the former media, and long club-shaped, spindle-shaped and barred or segmented involution forms are abundant; on blood-serum club-shaped involution forms also occur, but sparsely in a young, eighteen to twenty hours' culture, in a forty-eight hours' culture more numerously.

Staining reactions.—The *B. diphtheriæ* stains well with the ordinary anilin dyes and is Gram-positive. With Löffler's methylene blue the coloration is usually somewhat irregular, more deeply stained portions alternating with paler intervals, the so-called segmentation, and especially marked with agar cultures. The ends of the organisms are also frequently deeply stained, the so-called polar staining, while the phenomenon known as "metachromatism" is often marked both at the poles and also in the rod, appearing as granules of a purplish tint and contrasting with the blue of the methylene blue. With Neisser's stain (p. 308) deep inky coloured dots, appearing somewhat larger in diameter than the rods, occur at the poles of the organism and occasionally at the centre.

Cultural reactions.—The diphtheria bacillus is an aërobic and also a facultatively anaërobic organism, and grows well on all the ordinary culture media, forming cream-coloured growths or colonies, the latter on serum tending to be somewhat flattened, with regular margins. It grows slowly on gelatin, forming a raised whitish growth without liquefaction of the medium, and flourishes in milk, with the production of an acid reaction, but without curdling. In broth some strains give a granular growth on the sides and at the bottom of the tube, the broth remaining clear, sometimes with a thin surface pellicle; other strains may render the

a. The Klebs-Löffler or diphtheria bacillus. Cover-glass preparation of a serum culture. × 1500.



b. Section of diphtheritic membrane with Klebs-Löffler bacilli. Gram and eosin. × 750.

To face page 280.

Gram +

4 negatives.

Spores 0
Motile 0
Gas 0
— Gelatine 0.

5 forms. on culture.
·1. Oval
·2. Long irreg. beaded
3 " reg. " (Stiefler form)
4 Segmented
·5. Uniformly stained.

Gordon's 5 chief characteristics:-
diagnostic {
α. Macroscopic & Microscop appearance on Löffler's agar
·β. Behaviour to :- { Gram + Löffler's blue Neisser's stain +
·γ. reaction to litmus in an alkaline broth.
δ Pathogenic test. (animals)
ε Virulence neutralised by antitoxin

broth turbid throughout. On potato the growth is slight and invisible.

The indole reaction can be obtained in peptone-water cultures either with or without a nitrite, but the writer has shown that this reaction is due, not to indole, but to skatolecarboxylic acid (see below, p. 301).

The diphtheria bacillus attacks glucose and lactose with the formation of acid only, no gas (see Table, p. 306). As regards the production of acid, Neisser found that during the first nine hours there is little or none; at the end of twenty-four hours a considerable quantity has been formed, and the amount increases until the end of the second day, after which the production ceases.

The *B. diphtheriæ* is agglutinated by the serum of patients and by a diphtheria serum, but the test is difficult to apply on account of the coherence of the growth, is somewhat erratic with different strains, and is of no practical value in the diagnosis of the disease. For the same reasons, the agglutination reaction is of little use for the recognition of the organism and for distinguishing it from the so-called "pseudo-diphtheria" bacilli.

The Klebs-Löffler bacillus retains its vitality in cultures for a month, and when dried for three or four weeks. According to Welch and Abbot, it is destroyed in ten minutes by a temperature of 58° C. It is readily destroyed by antiseptics when in culture, but in the membrane it is difficult to find an agent which will penetrate and kill the bacilli beneath the surface.

The diphtheria bacillus and its characters under cultivation have been described somewhat fully, because of the importance of the identification of the organism as a means of clinical diagnosis. As mentioned at the commencement of this chapter, the clinical dia-

gnosis of diphtheria presents many difficulties, and considerable assistance may be derived from a bacteriological examination. The diagnosis is based on the presence or absence of the Klebs-Löffler bacillus, either in smears, or in cultivations, made from the membrane or secretion (see p. 306). This method is of very real assistance in doubtful, and especially in mild, cases, which clinically it may be very difficult to decide whether they be diphtheritic or no. The mild cases are those which it is of the greatest importance to identify, especially in schools, for if not recognised the patients may go about and prove a source of infection to all around. The method also affords valuable evidence as to when a case can be considered free from infection; so long as bacilli are present in the throat infection must be possible, and the length of time for which they may occasionally persist is remarkable. In half the cases the bacilli disappear within three days of the disappearance of the membrane, in a few cases they linger for as long as three weeks, but occasionally they persist much longer. The writer isolated them for so long as five months (and virulent to the last); and a case is recorded in which they persisted for no less than fifteen months after the attack. In all cases two or three examinations should be made at short intervals with negative results before the bacilli can be pronounced to be absent, and no case should be discharged from hospital until the absence of bacilli has thus been proved. When bacilli persist, treatment with antiseptic sprays or gargles, combined with syringing the nose, may be tried. Syringing the nose is important, for the bacilli probably extend to the post-nasal space, where they are untouched by a throat spray or gargle. Another mode of treatment has also been adopted. A polyvalent *anti-microbic* agglutinating anti-diphtheria

serum has been prepared, dried, and compressed into tablets, one of which is dissolved in the mouth every two hours, and fifteen minutes after solution the naso-pharynx is flushed with physiological salt solution. While this treatment sometimes succeeds, it often fails. The writer has tried the use of subcutaneous inoculations of diphtheria endotoxin (0·2–1·0–2·0 mgrm.) at intervals of seven to ten days. About half the cases seem to clear after one to three injections.

With regard to the value to be attached to the bacteriological examination for diphtheria, while the finding of the bacilli is proof positive of the diphtheritic nature of the affection and its infective nature, their apparent absence is not of so much value, as various circumstances modify the result. For example, an unskilled person may not happen to touch the right spot with the swab, or from struggling, etc , on the part of the patient even a skilled operator may fail to reach any but a small portion of the mucous membrane, instead of obtaining a good mop from all over, especially when there are no definite patches of membrane. The use of antiseptic gargles or paints shortly before the swabbing is taken will likewise prevent the growth of the bacilli. It sometimes happens that a very mixed growth is obtained in the cultures, and in such cases the Klebs-Löffler bacillus may be missed. Bearing such sources of fallacy in mind, and making due allowances for them, the negative result of a bacteriological examination may have considerable value in those cases which clinically are doubtful. *In no case where there is a reasonable suspicion of diphtheria should treatment with antitoxin be delayed until the bacteriological report is obtained.*

The bacilli from the throat are frequently associated with other organisms, especially micrococci and torulæ;

and those cases in which the temperature tends to be high and the throat fetid are usually a mixed infection of diphtheria bacilli with the *Streptococcus pyogenes* or *Micrococcus pyogenes*, var. *aureus*. The fact of such mixed infection cannot, however, be definitely decided from the cultures, as these organisms may be present in the mouth or throat without necessarily taking part in the infective process. Nor can the severity of the disease be gauged from the characters or numbers of the diphtheria bacilli and other organisms present, though perhaps in a number of cases those which yield practically pure cultures will probably be more severe than the cases which yield cultures with few bacilli. It has been stated that the long form of the diphtheria bacillus is the most, and the short form the least, virulent, the medium being intermediate, but this is by no means a universal rule. Westbrook[1] has divided all forms of the diphtheria bacillus into three groups, distinguished by their staining reactions with methylene blue. Those with deeply staining granules he calls "*granular forms*," those with transverse bands "*barred forms*," and those staining evenly "*solid forms*." Each group is further divided into seven types according to shape and size, the types being designated by the letters A to G and being progressively smaller from A to G.

It is sometimes stated that a microscopical examination, unless controlled by inoculation of the isolated bacteria, is unreliable. Such a statement is extremely misleading. If the bacilli which have been cultivated from a suspicious throat possess all the characters of diphtheria bacilli, inoculation experiments are not needed, and if they were performed with a negative result (*i.e.* the bacteria are not virulent) would prove little, for the bacilli from different parts of a culture

[1] *Rep. Minnesota State Board of Health*, 1899–1900.

from a throat often possess different degrees of virulence. Occasionally, it is true, even the expert may be in doubt about a particular bacillus, but such cases are the exception. Here an inoculation experiment may help, but would be of no value if a negative result were obtained. It is absolutely essential in the microscopical examination for diphtheria to use a good lens, proper illumination, and sufficient amplification, not less than 800–1000 diameters.

Pathogenicity.—The diphtheria bacillus is pathogenic for man, the horse, ox, rabbit, guinea-pig, cat, chicken, pigeon, and finches, all of which are more or less susceptible, while mice and rats are immune. In man the respiratory tract is usually affected, though the conjunctiva and other mucous membranes, as of the vagina and stomach, and wounds may be attacked. A pseudo-membrane usually forms, consisting of laminæ of fibrin entangling a few leucocytes and other cells, and here and there small effusions of blood, together with coagulative necrosis of the underlying mucous membrane, and the bacilli are for the most part located in the superficial layers of this pseudo-membrane (Plate VI., *b*), though in all cases in which the disease has lasted for any time they are found in the lungs, spleen, and kidneys, and may occur even in the blood. If the patient recovers from the diphtheritic attack, paralytic sequelæ are not uncommon and are due to a peripheral neuritis. Pseudo-membranes may be formed by other organisms, *e. g.* by the streptococcus and pneumococcus also by the pneumobacillus, and occur in Vincent's angina (p. 311), but it is doubtful whether paralytic sequelæ follow any but a diphtheritic infection.

Some remarkable skin affections of an eczematous or ichthyomatous nature have been found by Hare[1] and others to be due to the diphtheria bacillus.

[1] *Lancet*, 1908, vol. i, p. 282.

Another affection which seems to be generally diphtheritic is membranous rhinitis. Whereas true nasal diphtheria is a serious condition, membranous rhinitis is seldom, if ever, attended with any risk to life, sequelæ do not occur, and it is rare to obtain a history of infection from cases of it. This is extraordinary and very difficult to explain, for virulent diphtheria bacilli are abundant in the nose and nasal secretion.

Diphtheroid organs can occasionally be isolated from well people and those not known to have been in contact with diphtheria cases. The Klebs-Löffler bacillus can be isolated from the throats of nearly 7 per cent. of the presumably healthy population[1]; in the throats of contacts the percentage rises to 33 or more. Murray and the writer[2] found diphtheria-like bacilli in 58 out of 385 children (15 per cent.) admitted into the Victoria Hospital, Chelsea.

Ford Robertson believes that diphtheroid organisms —possibly the Klebs-Löffler bacillus itself—may play an important part in the production of general paralysis of the insane. His views have not gained general acceptance, and Eyre (*loc. cit.*) found that the percentage incidence of all diphtheroid organisms and of the Klebs-Löffler bacillus in the throats of the insane was not greater than in well persons, and was unable to isolate the *B. diphtheriæ* post mortem from cases of general paralysis.

Traces of antitoxin can be detected in the blood after an attack of diphtheria, usually at the end of the first week of convalescence: the antitoxin has probably little to do with the actual recovery from the disease (see

[1] See Eyre, *Brit. Med. Journ.*, 1905, vol. ii, p. 1104.

[2] *Brit. Med. Journ.*, 1901, vol. i, p. 1474. See also Graham-Smith, *Journ. of Hygiene*, vol. iii, 1903, p. 216.

p. 217). A small amount of antitoxin has also been occasionally found in well people and in untreated horses. It has been suggested that in such cases there has been a latent infection with the *B. diphtheriæ*, but on Ehrlich's side-chain hypothesis it seems more likely that in such cases there happens to be an excess of the receptors which constitute antitoxin naturally free in the blood.

Guinea-pigs are the animals generally employed for experimental work on diphtheroid organisms. In order to compare the effects and virulence of various bacilli it is customary to make the inoculation with a measured volume of a forty-eight hours' broth culture. From 0·1 c.c. to 2 c.c. of such a culture, according to the virulence, inoculated subcutaneously, is usually required to kill a 250-grm. guinea-pig within three days. At the seat of inoculation hæmorrhagic œdema forms, hæmorrhages occur in the serous membranes, and especially in the adrenals, while the renal epithelium and the liver-cells undergo cloudy degeneration.

Inoculated into the trachea of the guinea-pig, rabbit, and chicken, pseudo-membranes form, and the same occurs with the superficially injured conjunctiva and vagina. It is stated by some that the diphtheria bacillus does not develop on a normal mucous membrane —this must first be injured, and the staphylococcus and streptococcus, so often associated with the diphtheria bacillus in the human subject, may play a part in preparing the way for infection by damaging the cells and tissues. Rabbits usually live somewhat longer than the guinea-pig after inoculation and paralysis frequently develops if life is prolonged, simulating the post-diphtheritic paralysis of man.

The question of the occurrence of the Klebs-Löffler bacillus in the lower animals is of considerable importance with regard to the spread of the disease and the

conveyance of infection. The so-called diphtheritic affections of pigeons, poultry, and calves (referred to more in detail below, p. 313) are as a rule diseases quite distinct from human diphtheria, and are not communicable to man. A number of observers assert, however, that cats may suffer from the disease, which in these animals runs a chronic course, and is associated with bronchitis, lobular pneumonia, nephritis, and wasting. Klein[1] points out that not only are cats liable to the disease in houses where diphtheria has occurred, but that a similar infectious disease exists naturally among cats, and symptoms similar to this natural disease may be produced by inoculating healthy cats with the Klebs-Löffler bacillus. The diphtheria bacillus has also been isolated from the horse.[2]

Several epidemics of diphtheria have been traced to an infected milk supply. In some instances the infection has undoubtedly been derived from contamination from a human source, but in others this mode of infection has not been demonstrated, and it has been suggested that certain eruptive conditions on the teats and udder of the cow may be caused by the Klebs-Löffler bacillus and the milk become infected therefrom. Klein[3] made experiments with a view of determining this point. He inoculated healthy cows in the shoulder with a bouillon culture of the diphtheria bacillus. This caused fever and local swelling, and in about a week a papular and vesicular eruption appeared on the udders and teats. The *B. diphtheriæ* was isolated from the contents of the vesicles and also from the milk on the fifth day, but not subsequently. The cows died in two to four weeks, and the *B. diphtheriæ* was obtained from the local

[1] *Rep. Med. Officer. Loc. Gov. Board* for 1889, p. 162.

[2] Cobbett, *Centr. f. Bakt.*, xxviii, No. 19, p. 631.

[3] *Rep. Med. Officer Loc. Gov. Board* for 1889 and 1890.

Cat
horse
milk

lesions. Abbott[1] obtained somewhat different results, but Klein[2] points out that these experiments were not performed under exactly the same conditions as his own.

Klein, Eyre, Dean and Marshall[3] have isolated the diphtheria bacillus from milk. It is to be noted that diphtheria-like, but non-pathogenic, bacilli are often to be found in milk and cheese (see section on "Milk").

Toxins.—Diphtheria toxin has not been obtained in a state of purity and its exact chemical nature is unknown. Löffler first investigated the chemical products formed by the diphtheria bacillus, and by precipitating bouillon cultures with alcohol obtained a white toxic substance which he classed among the enzymes.

Roux and Yersin precipitated the toxin from filtered broth cultures by means of absolute alcohol, and also by the addition of calcium chloride. They found that 0·4 mgrm. was sufficient to kill eight guinea-pigs or two rabbits, and considered it to be an enzyme.

From the blood and spleen of cases of diphtheria Sydney Martin[4] isolated albumoses (chiefly deutero-albumose) and an organic acid, but no basic body. Injected subcutaneously the albumose produces much œdema and irregularity of temperature ; in larger doses depression of temperature with paralysis and coma. Small multiple doses, not sufficient to destroy life, may give rise to some fever, and in two or three days to paralysis of the hind legs in rabbits, with general weakness and loss of weight. Post-mortem, the nerves are found to have undergone degeneration—breaking

[1] *Journ. Path. and Bact.*, vol. ii, 1894, p. 35.
[2] *Ibid.*, p. 428.
[3] *Journ. of Hygiene*, vol. vii, 1907, p. 32 (Refs.).
[4] *Brit. Med. Journ.*, 1892, vol. i, p. 641.

up and disappearance of the myelin and interruption of the axis cylinder, while the heart is fatty. The organic acid is also a nerve poison, but is not so toxic as the albumose. From diphtheritic membrane, extracted with a 10 per cent. salt solution, only traces of albumose and organic acid were obtained, but the extract was highly toxic, producing fever and paralysis. Sidney Martin suggests that a substance of the nature of a ferment may be present, and that the ferment in the membrane on absorption may perhaps form the albumose in the body. From cultures of the diphtheria bacillus in alkali-albumin, albumose and organic acid, with similar actions to those isolated from the body, were obtained.

Brieger and Fränkel (1890) were unable to find any basic substance in cultures, and concluded that the toxic substance was a protein body, which they designated a "tox-albumin." It was destroyed by a temperature of 60° C. but not by one of 50° C., even in the presence of an excess of hydrochloric acid, and hence is probably not an enzyme. The tox-albumin is non-dialysable, is precipitated by saturation with ammonium sulphate but not with magnesium sulphate, and hence is neither a peptone nor a globulin, contains a large amount of sulphur, and gives the biuret and Millon's tests. A curious property of this substance is that small quantities (2·5 mgrm. per kilogramme of the body-weight) do not produce their effects until the lapse of weeks. Brieger and Boer in a later research prepared the diphtheria tox-albumin by precipitating a bouillon culture with a 1 per cent. solution of zinc sulphate or chloride. The precipitate of the zinc double salt was washed with slightly alkaline water and decomposed with a stream of carbonic acid gas. The purified tox-albumin gives the xanthoproteic, biuret,

and Adamkiewicz's reactions, and the red coloration on heating with Millon's reagent.

According to Ehrlich the toxin broth is a complex mixture of toxic constituents belonging to the proteins, but this is denied by Madsen and Arrhenius (see p. 172). Its poisonous property gradually diminishes on keeping, and is destroyed by boiling in five minutes, at lower temperatures more slowly, and also by light.

Diphtheria antitoxin.—By the injection of sub-lethal and increasing doses of the toxin into an animal an antitoxin is generated. For the preparation of a potent antitoxin for therapeutic use the first essential is a highly toxic toxin, and for obtaining this a diphtheria bacillus of high virulence is required, and but few strains possess the necessary virulence. The virulent bacillus is grown in an alkaline broth (rendered alkaline to the extent of about 5·7 c.c. of normal caustic soda solution per litre beyond the neutral point to litmus) in Erlenmeyer flasks containing half to one litre for eight to twelve days at 37° C. Various small details have to be attended to in order to obtain toxin of maximum toxicity; it is important that growth should occur upon the *surface* of the broth. The use of meat some days old has been advocated, or of acid beef-broth in which *B. coli* has been grown for twenty-four hours, in order to eliminate the glucose (p. 27). L. Martin makes use of "peptone" prepared by the auto-digestion of a pig's stomach with dilute hydrochloric acid. The cultures are then filtered through a Berkefeld or Pasteur-Chamberland filter to remove the bacilli. The filtrate is germ-free and very toxic, and a little carbolic acid may be added to preserve it. In New York 10 per cent. of a 5 per cent. solution of carbolic acid is added to the culture, the bacilli are allowed to deposit by standing for forty-eight hours, and the culture is filtered

through paper; in this way filtration through a filter-candle is dispensed with. Less than 0·01 c.c. of the toxin should kill a 250-grm. guinea-pig in three to four days. Selected horses which have been tested with mallein and tuberculin, and kept under observation for some time to ensure that they are healthy, are then inoculated with this filtrate, commencing with a dose of 0·01 to 0·1 c.c., according to the toxicity of the toxin, or 20 c.c. of the toxin together with 10,000 units of antitoxin may be given for the first three doses. Individual horses vary very much in their susceptibility to the toxin, so that care has to be exercised with the first injections. The injections are given subcutaneously over the shoulder, and produced a local swelling and some rise of temperature and general disturbance, lasting two or three days. When this has passed away the inoculation is repeated, a larger dose being administered provided the reaction due to the former one was not too severe. The treatment is continued for five to six months, the dose of toxin administered being gradually increased until it may attain 500 c.c. or more. Cartwright-Wood found that by growing virulent diphtheria bacilli for three or four weeks in ordinary peptone broth, with the addition of 10 or 20 per cent. of blood-serum or plasma, subjecting the culture to a temperature of 65° C. for an hour and filtering before injection, much larger initial doses can be given and some degree of immunisation attained, and subsequently the ordinary broth cultures may be injected in large doses. Individual horses vary much in their capacity to yield antitoxin: on the whole those that are moderately sensitive to the toxin seem to produce most antitoxin; a horse to be of value should after three months' treatment yield an antitoxic serum containing not less than 300 units per c.c. The required potency having

been attained, as shown by the test described below, the horse is bled with aseptic precautions, the blood is allowed to coagulate, and the serum is drawn off and filled into sterile bottles each containing a dose of the antitoxic serum. A small amount of antiseptic, such as tri-kresol, is generally added as a precautionary measure to prevent the multiplication of any stray germs that may have gained access during the various manipulations.

Standardisation of antitoxin.—The potency of diphtheria antitoxin is always described in "units" and is estimated by ascertaining the quantity of antitoxin required just to neutralise a certain amount of a standardised toxin when both are injected into a 250-grm. guinea-pig. Formerly, by Roux's method, the minimal lethal dose of the toxin is first ascertained, and then the number of grammes of guinea-pig which 1 c.c. of antitoxin will protect against this minimal lethal dose is determined. If 0·01 c.c. of antitoxin protects a 300-grm. guinea-pig against the minimal lethal dose, 1 c.c. will protect $300 \times 100 = 30{,}000$ grm. of guinea-pig, and the immunising value of the antitoxin would be described as 30,000. This method is open to the fallacy that if only a portion of the lethal dose be neutralised the guinea-pig may survive, and a fictitious value be given for the potency of the antitoxin. Behring later adopted ten minimal lethal doses as the test dose of toxin, and he termed ten times the amount of antitoxin which protects a guinea-pig against the ten minimal lethal doses *a unit* (the Behring unit, which therefore = 100 minimal lethal doses of toxin), from which the Ehrlich unit, now universally adopted, is derived. Though this method eliminates to a large extent the objections to the Roux method, Ehrlich found that by it the same antitoxin tested with different

toxin broths yielded different values. This he explained by assuming that diphtheria toxin broth contains not only toxin but also other substances which combine with antitoxin. These substances, though non-toxic, or comparatively so, vary in amount in different toxin broths, and variable results, therefore, may be obtained by the simple method of testing. These substances, having an affinity for antitoxin, are toxoids and toxone. There are several varieties of toxoids, viz. (1) those having a greater affinity for antitoxin than toxin itself, *protoxoids*; (2) those having the same affinity, *syntoxoids*; (3) and those having a less affinity, *epitoxoids*.[1] Toxoids are probably derivatives of toxin; they increase in quantity in old toxin broth which has been kept, and which at the same time decreases in toxicity. The toxones also combine with antitoxin, having a less affinity for it than toxin, are *primary* secretory products of the diphtheria bacillus, and while not acutely lethal, induce induration, necrosis and paralysis. The toxoids are comparatively scanty in a fresh toxin broth and are negligible, but it is otherwise with the toxone, which is always present in appreciable quantity. Owing to the fact that toxone has less affinity for antitoxin than toxin has, if an exactly neutral mixture of toxin broth and antitoxin be prepared, considerably more than the minimal lethal dose of the toxin broth must be added to render the mixture acutely toxic, because the first portion of the added toxin simply displaces the toxone from its combination with the antitoxin, and is neutralised by the antitoxin so set free.

Thus, suppose a certain amount of a toxin broth contains 90 units of toxin and 10 units of toxone, and to this amount 100 units of antitoxin are added so as

[1] See pp. 172–175 for other views on the constitution of diphtheria toxin.

to form a physiologically neutral mixture, the combination which occurs is shown by the following "equation": 90 toxin-antitoxin + 10 toxone-antitoxin = L_0 (*i. e.* neutrality). If an amount of the toxin broth be now added, corresponding to 11 units of toxin, the effect will be as though only *one* unit of toxin has been added, as is shown by the following "equation": 90 toxin-antitoxin + 10 toxone-antitoxin + 11 toxin = 100 toxin-antitoxin + 10 toxone (free) + 1 toxin (free) = L_+ (*i. e.* just acutely lethal). Thus although the equivalent of eleven minimal lethal doses of toxin has been added to the physiologically neutral mixture of toxin broth and antitoxin, only *one* minimal lethal dose of toxin remains free and active, because ten toxin units displace the ten toxone units from the toxone-antitoxin complex and are neutralised by the antitoxin thus set free. Ehrlich, therefore, devised a method of standardisation which eliminates irregularities due to the variable proportions of toxone and toxin in the toxin broth by adopting antitoxin and not toxin as the standard. In order to standardise an antitoxin, a virulent toxin broth is employed and its minimal lethal dose is approximately ascertained—*i. e.* that amount which is just sufficient to kill a 250-grm. guinea-pig on the fourth or fifth day. A solution of accurately standardised antitoxin, which can be obtained from the Serumsprüfung Institut, Frankfort-on-Maine, is then prepared, containing one "unit" of the antitoxin in 1 c.c., and the toxin is standardised with this by mixing with one unit various quantities above and below one hundred minimal lethal doses. It is required to ascertain the amount of the toxin broth, which, when mixed with one unit of antitoxin, just suffices to kill a 250-grm. guinea-pig on the fourth or fifth day after the injection of the mixture; this amount of toxin is

known as the L_+ dose. The L_+ dose may be defined as that amount of a given diphtheria toxin broth which is not completely neutralised by one "unit" of standard antitoxin to the extent that *exactly* one simple lethal dose of toxin remains unneutralised; it corresponds usually to 105–120 minimal lethal doses. For example, suppose 0·003 c.c. of the toxin was found to be the minimal lethal dose, with separate "units" of standard antitoxin, 0·2, 0·3, 0·4 and 0·5 c.c. respectively of the toxin might be mixed, and each mixture injected into a guinea-pig; probably the guinea-pigs receiving the "unit" of antitoxin *plus* 0·2 and 0·3 c.c. of toxin would remain alive, while the animal receiving the 0·4 c.c of toxin would die in twenty-four to forty-eight hours. The death in the last case is too rapid; more than a simple lethal dose has remained unneutralised, and therefore the L_+ dose of toxin lies between 0·3 and 0·4 c.c., and further experiments would have to be performed with amounts of toxin between these limits in order to ascertain the exact dose. Death of the guinea-pig on the fourth or fifth day has been chosen because it has been found that if the dose of toxin be diminished ever so little below that producing this result, death does not ensue under nine or ten days. That is to say, an *acute* intoxication is fatal at the latest on the fourth or fifth day, a fatal result after then being due to a *chronic* intoxication. The amount of toxin which is *exactly* neutralised by one "unit" of the standard antitoxin is known as the L_0 dose. By exact neutralisation is meant absence of any reaction, general or local, at the seat of inoculation, in the inoculated guinea-pig. If toxin broth were a single substance, containing only toxin, then $L_+ - L_0 = D$, the simple lethal dose, would be equal to the minimal lethal dose. But because of the presence of toxone, which

also has an affinity for antitoxin, D, the difference between the L_0 and the L_+ doses, is usually a multiple (8–12) of the minimal lethal dose.

From these considerations we are now in a position to define the unit of antitoxin : a " unit " is that amount of antitoxin which will neutralise about 100 minimal lethal doses for the guinea-pig of diphtheria toxin. From certain considerations Ehrlich considers that the unit would exactly neutralise 200 minimal lethal doses of a theoretical toxin, containing only toxin and neither toxoid nor toxone, but, inasmuch as such a toxin is unknown practically, the unit corresponds usually to 105–120 minimal lethal doses of a toxin broth, the extremes which Ehrlich has found being 16 and 136 lethal doses. Having standardised a specimen of toxin by means of standard antitoxin, this standardised toxin is in its turn used to standardise the antitoxic serum which has been prepared for therapeutic use. The toxin is preserved by the addition of toluol, and is kept in a cool, dark place; it needs to be re-standardised every few weeks.

In standardising antitoxin, the L_+ dose of the standardised toxin is mixed with varying amounts of the antitoxin, the mixtures are injected into guinea-pigs, and the amount of the antitoxic serum which neutralises the L_+ dose of toxin is thus ascertained. If, for example, it were found that 0·05, 0·04 and 0·03 c.c. of the antitoxic serum neutralised the L_+ dose of toxin, but that the guinea-pig receiving 0·025 c.c. suffered from some local necrosis, wasted, and died in a few days, and the animal receiving 0·02 c.c. died in two or three days, 0·03 c.c. of this antitoxin would be about equivalent to one unit of standard antitoxin, and the antitoxic serum therefore contains 33 units per c.c. For all the experiments the conditions must be kept as

constant as possible, guinea-pigs weighing 250 grm. or thereabouts employed, and to eliminate irregularities a number of animals must be used. The antitoxic constituent of diphtheria antitoxin is globulin in nature, or is intimately associated with the globulin content of the serum. Thus Atkinson found that if the serum is precipitated by saturation with magnesium sulphate, the whole of the antitoxin is carried down with the precipitate, and also that the globulin content of the blood-serum of antitoxin horses is increased. His results were confirmed by Ledingham.[1]

There can now be no doubt as to the value of the antitoxin treatment of diphtheria. Since the introduction of antitoxin treatment, which was commenced about the middle of 1894, there has been a steady decline in the case mortality from diphtheria, especially in London, where probably the majority of the cases are injected with antitoxin. From 1891 to 1894 the case mortality from diphtheria in the hospitals of the Metropolitan Asylums Board averaged about 30 per cent., in 1895 it was 22·8 per cent., and afterwards steadily fell, until during the last eight years it has ranged between 8·3 and 10 per cent.

Not less than 2000 units should be injected for a dose, and early treatment is of paramount importance. As soon as there is a reasonable probability that the case is one of diphtheria the antitoxin should be used, and treatment should not be delayed for the result of the bacteriological examination. The statistics show that in cases treated on the first day of the disease the case mortality is 3·0, on the second day it is 6·5, on the third day 10·6, on the fourth day 12·9, and on the fifth day and afterwards 14·8 per cent.

In bad cases, and in those coming under treatment at a late stage of the disease, the dose may be increased to 10,000 20,000, or even 30,000 units with advantage, and to bring the patient under the influence of the antitoxin as rapidly as

[1] *Journ. of Hygiene*, vol. vii, 1907, p. 65.

possible the first dose may be administered intravenously. The dose may have to be repeated once or twice in mild cases, in bad cases perhaps every six or twelve hours until several doses have been given, the guide being the general condition of the patient and the rapidity of the separation of the membrane.

In addition to antitoxin, the recumbent posture and general and local treatment should be pursued as usual.

In cases of mixed infection, in which the diphtheria bacilli are associated with streptococci or staphylococci, diphtheria antitoxin may prove of less value, as it has no influence on the streptococcic or staphylococcic infection, and injections of anti-streptococcic serum may be given in addition.

Diphtheritic paralysis seems to be rather more frequent after the use of antitoxin than in the cases not treated with it, probably because a greater number of cases survive.

The antitoxin has also been employed as a prophylactic in schools or other places where susceptible individuals are congregated together, and where cases of diphtheria have occurred, with excellent results.

The procedure in such circumstances should consist of a bacteriological examination of the throats of *all* the inmates in the institution, isolation of those in whom the *B. diphtheriæ* is found, and the injection of everyone, or at least of all children, with a prophylactic dose, repeated if considered desirable, ten days later.[1] For this purpose a dose of about 500 units should be given. The immunity so produced does not last for more than three weeks.

The objection to the use of antitoxin for prophylaxis is that should the patient subsequently develop diphtheria, treatment with antitoxin may induce serious symptoms due to super-sensitation or anaphylaxis. The writer beleives that all the advantages of antitoxin without its disadvantages may be obtained by the use of a vaccine consisting of diphtheria endotoxin.

Some clinicians assert that antitoxin exerts its effect when

[1] On the prophylactic use of antitoxin see Norton, *Lancet*, 1907, vol. ii, p. 85.

administered by the mouth or the rectum. Hewlett was unable to detect any absorption of tetanus antitoxin from the stomach or rectum, nor Sternberg of diphtheria antitoxin from the rectum, of rabbits.

Pseudo-diphtheria and Diphtheria-like Bacilli.

Diphtheria-like bacilli are not uncommon in wounds and in pathological exudates, etc., and in connection with diphtheria an important question must be discussed, viz. the occurrence and nature of the so-called pseudo-diphtheria bacilli. The term was originally used by Löffler, and by the rule of priority should be reserved for the organism described by him under this name. The pseudo-diphtheria bacillus of all authors is an organism occurring in the throat in various anginal conditions, scarlet fever, etc., and occasionally in the throats and noses of well persons, and is non-pathogenic to guinea-pigs. Park and Beebe met with it in twenty-seven out of 330 healthy throats examined by them. Roux and Yersin, Abbott and Fränkel describe it as morphologically resembling the Klebs-Löffler bacillus, while Löffler, von Hofmann, Koplick, Park and Beebe, Peters, and Hewlett and Miss Knight,[1] consider that an organism differing somewhat from the Klebs-Löffler bacillus should alone be termed the pseudo-diphtheria bacillus; to avoid confusion it is best to designate it the Hofmann bacillus.

Morphology.—Typically, the Hofmann bacillus is a shortish rod tapering towards the ends, which are rounded, the average length being from 1·5 μ to 2 μ, and it occurs in pairs, resembling two suppositories placed base to base. It is non-motile, does not form spores, is arranged in a parallel grouping like the

[1] *Trans. Brit. Inst. of Prev. Med.*, vol. i, 1897.

Klebs-Löffler bacillus (due to the same mode of division), and involution forms are, as a rule, not met with (Plate VII., *a*). It is Gram-positive, and stains deeply and regularly with Löffler's methylene blue, segmentation and polar staining usually being absent. With Neisser's stain no inky granules are perceptible, as is the case with the diphtheria bacillus.

Cultural reactions.—The Hofmann bacillus is almost a strict aërobe; there is no growth anaërobically in hydrogen. On serum, agar, and gelatin it forms cream-coloured colonies or growths, barely distinguishable from those of the Klebs-Löffler bacillus. On ordinary potato it hardly grows at all, what growth there is being quite invisible. On alkaline potato,[1] however, it forms distinct cream-coloured colonies, usually visible by the second day. In stab-cultures in gelatin and glucose-agar no gas is formed, and the growth is confined to the upper part of the stab. In broth it forms sometimes a granular deposit, sometimes a general turbidity. On neutral litmus glucose-agar a blue colour is developed, indicating the production of alkalinity. Cultivated in peptone water an indole-like reaction with sulphuric acid alone can be obtained after a variable time, three to four weeks, while the diphtheria bacillus gives it in about a week; with a nitrite and sulphuric acid the indole-like reaction can be obtained with both the pseudo- and diphtheria bacilli in about a week. The substance giving this indole-like reaction is not indole, but skatole-carboxylic acid.[2] A broth culture reduces a weak solution of methylene blue. The bacillus does not curdle milk or liquefy

[1] Ordinary potato rendered alkaline with a 10 per cent. solution of sodium carbonate before sterilisation.

[2] Hewlett, *Trans. Path. Soc. Lond.*, vol. li, 1900, p. 187; vol. lii, 1901, p. 113.

gelatin, can be cultivated at from 22° to 37° C., and is non-pathogenic to guinea-pigs in doses of 5 c.c. or more of a forty-eight hours' broth culture. Some of the differences between the Hofmann bacillus and the Klebs-Löffler bacillus are shown in the table on the next page. Mandelbaum and Heinemann[1] state that if a glycerin-agar plate be smeared with human blood and inoculated, the diphtheria bacillus produces colonies surrounded by a yellow zone, while the colonies of the Hofmann and xerosis bacilli do not change the red colour of the blood. In addition, the Hofmann bacillus does not ferment any sugar, etc. (see Table, p. 306).

The histories of several cases investigated by Miss Knight and Hewlett seemed to show that the Hofmann bacillus is associated with mild anginal conditions, which are free from complications, end in recovery, and are not followed by sequelæ. In many of the cases the anginal condition was associated with distinct patches of membrane, and in two symptoms were present suggestive of the toxæmia which is met with in diphtheria.

In a long series of experiments Hewlett and Miss Knight believed that some evidence was obtained of the conversion of the Hofmann into the Klebs-Löffler bacillus and *vice versâ*. Moreover, the Hofmann bacillus seemed in many instances to replace the Klebs-Löffler bacillus in the throat during convalescence, and it is possible in a large series of cultures to obtain connecting links between the Klebs-Löffler bacillus on the one hand and the Hofmann bacillus on the other. Cobbett,[2] however, suggests that these facts are capable of another explanation, viz. that during the acute

[1] *Centr. f. Bakt.* (Orig.), liii, 1910, p. 356.

[2] *Journ. of Hygiene*, vol. i, 1901.

Differences between the Klebs-Löffler and the Hofmann Bacillus.

	Hofmann Bacillus.	Klebs-Löffler Bacillus.
Morphology	Rods 1·5 μ to 2 μ in length, tending to be slightly thicker at the centre than at the ends. Is "plumper," shorter, and less variable than the Klebs-Löffler bacillus. Involution forms rare	Rods averaging 3 μ to 4 μ in length. Slender and (excluding involution forms) of more uniform diameter than the pseudo. Considerable variation in size. Involution forms usually present.
Staining with Löffler's blue	Stains more deeply and regularly than the Klebs-Löffler bacillus. Polar staining rare	Staining generally more or less irregular, and polar staining common.
Neisser's stain	Negative	Positive.
Alkaline potato	Distinct cream-coloured colonies or growth visible in two days	Grows well, but growth is almost invisible.
Neutral litmus agar	Alkaline reaction	Acid reaction.
Litmus milk	Alkaline reaction	Acid reaction.
Stab-cultures in glucose agar and gelatin	Growth only at upper part of stab	Growth along whole length of stab.
Anaërobic cultures in hydrogen	No growth.	Grows well.
Indole-like reaction. (peptone-water cultures, with sulphuric acid alone)	Only after three weeks' growth. (Due to skatole-carboxylic acid)	After one week's growth. (Due to skatole-carboxylic acid).
Fermentation reactions	See table	on page 306.

stage, diphtheria bacilli being readily found, the Hofmann bacillus is likely to be overlooked, whereas at a later stage a more careful search may be necessary to detect the diphtheria bacillus, and in the course of

that search the Hofmann bacillus is therefore more frequently seen.

Miss Knight and Hewlett came to the conclusion that in some cases, at least, the Hofmann bacillus is a modified Klebs-Löffler bacillus, and the view taken of its relation to the Klebs-Löffler bacillus was, that it is a very attenuated Klebs-Löffler bacillus, *i. e.* one far removed from virulence. It would therefore seem wise to treat anginal cases in which the pseudo-diphtheria bacillus is found as possibly infective, though it would probably be inexpedient to admit to a general diphtheria ward (unless a prophylactic dose of antitoxin or of an endotoxic vaccine be given), nor would antitoxin be needed in the majority.

Most authorities have been unable to convert the pseudo-bacillus into a virulent Klebs-Löffler bacillus, or *vice versâ*, and many are of opinion that it has probably nothing to do with diphtheria (Park and Beebe, Peters, Washbourn, Cobbett, Clark). A few fatal cases have been recorded (*e. g.* by Stanley Kent) in which a careful search has failed to reveal any but Hofmann bacilli. Boycott[1] found that the seasonal prevalence of the Klebs-Löffler and Hofmann bacilli does not correspond, the former prevailing during September, October, and November; the latter is more frequent from May to August.

Priestley records an outbreak of what he terms "pseudo-diphtheria," in which the Hofmann bacillus seemed to be the causative organism, and expresses the opinion that this bacillus is not related to the Klebs-Löffler bacillus.[2]

Salter[3] claimed to have found that the Hofmann

[1] *Journ. of Hygiene*, 1905, vol. v, p. 223.

[2] *Public Health*, July, 1903.

[3] *Trans. Jenner Inst. Prev. Med.*, vol. ii, p. 113. (Bibliog.)

bacillus is virulent to many small birds (goldfinch, chaffinch, canary, etc.), and that by successive passages it becomes converted morphologically into a Klebs-Löffler form with feeble virulence for the guinea-pig. He also found the filtered broth culture of the Hofmann bacillus, though harmless to guinea-pigs, to be toxic to small birds, and that it contains a non-toxic substance (toxoid) which has the power of combining with, and neutralising, diphtheria antitoxin. Salter concluded, therefore, that diphtheritic organisms are to be met with of every grade of virulence, the weakest, known as Hofmann's or the pseudo-diphtheria bacillus, representing the most attenuated form of the Klebs-Löffler bacillus. The writer,[1] Cobbett,[2] Petrie,[3] Williams,[4] and Clark[5] have, however, quite failed to confirm Salter's results.

To sum up: the Klebs-Löffler-like avirulent bacilli met with in the thoat, the pseudo-diphtheria bacilli of Roux and Yersin, are probably modified and avirulent diphtheria bacilli. As regards the Hofmann bacillus, the general trend of opinion at present is to consider it as quite distinct from the Klebs-Löffler bacillus. Another view is to regard it as in reality including several species, of which one may be a modified Klebs-Löffler bacillus, the others having no relation with this organism. The Klebs-Löffler-like avirulent bacilli might, therefore, be regarded as true diphtheria bacilli *slightly* removed from virulence, the Hofmann bacillus, if derived from the Klebs-Löffler, as a diphtheria bacillus *far* removed from virulence.

In determining the fermentation reactions of the diphtheria-

[1] *Brit. Med. Journ.*, Sup., July 9th, 1904.
[2] *Journ. of State Med.*, vol. xi, p. 609.
[3] *Journ. of Hygiene*, vol. v, p. 134.
[4] *Journ. Med. Research*, 1902, p. 83.
[5] *Journ. Infect. Diseases*, vol. vii, 1910, p. 335.

like bacilli, the organisms should first be grown in broth until they become acclimatised to this medium, or should be grown in a medium which suits them, *e. g.* broth with the addition of serum or of ascitic fluid. Hiss's serum-water medium is satisfactory—serum 1 part, water 3 parts, with 1 per cent. of carbohydrate or other substance, tinged with litmus and sterilised in the steamer on three consecutive days. Graham-Smith[1] gives the following table of fermentation tests:

Organism.	Hiss's medium (10 days' growth).								
	Glucose.	Lactose.	Sucrose.	Galactose.	Maltose.	Lævulose.	Mannitol.	Dextrin.	Glycerin.
B. diphtheriæ, virulent and avirulent	C A	C A	0	C A	C A	C A	0	C A	C A
Hofmann bacillus* .	0	0	0	0	0	0	0	0	0
Xerosis bacillus* .	C A	0	0	0	0	C A	0	0	C A
*B. coryzæ** . . .	C A	0	0	C A	0	C A	0	0	0
Diphtheria-like bacilli: From the ear* . .	0	0	0	0	0	0	0	0	0
From the urethra* .	— A	0	0	— A	— A	— A	0	0	0
From the throat* .	C A	0	0	C A	— A	C A	0	0	0
From the fowl* . .	— A	0	0	— A	— A	...	0	0	0
(* Avirulent to the guinea-pig)									

C = coagulation; — = no coagulation; A = acid; 0 = no reaction. Slight variations were occasionally noted: for example, four out of twenty diphtheria bacilli gave no acid with lactose, and the amount of acid production and of coagulation was somewhat variable.

Clinical Diagnosis.

(A) *In man and animals*:—I. In some cases the diphtheria bacillus can be identified in the membrane or discharge, and the diagnosis established thereby.

[1] *Journ. of Hygiene*, vol. vi, 1906, p. 286.

Films are made with the exudation, or with a fragment of the membrane teased up as finely as possible on a slide, a droplet of water being added if necessary. One of these films should be stained with Löffler's methylene blue, another by Gram's method. The bacilli will be found lying parallel to one another in larger or smaller groups, together with involution forms. Films stained with Neisser's or Pugh's stain (see below) may also be of assistance. Another method is to stain the films for five seconds in dilute carbol-methylene blue (seven drops to 10 c.c. water), rinsing and drying, and counter-staining in dilute carbon-fuschin (ten drops to 10 c.c. water) for one minute, rinsing and drying (Higley).

II. Frequently the membrane is so crowded with different forms of organisms that it is extremely difficult to recognise the diphtheria bacilli with any degree of certainty. Recourse must then be had to cultivation.

For this purpose sloping blood-serum tubes, or tubes of serum-agar, must be employed; simple agar is unsuitable.

A piece of membrane or a swabbing from the throat is rubbed over the surface of one or two serum tubes, care being taken not to break up the medium. The tubes are incubated at 37° C. for eighteen to twenty hours, and are then examined microscopically whether there is any visible growth or not. If there be no visible growth a scraping is taken by means of a sterilised platinum needle from the whole surface and a film is prepared. If there is a visible growth the film should be prepared from the most likely colonies, or, if the growth be confluent, from the upper half inch or so. A microscopical examination must always be made, for some colonies—certain staphylococci and torulæ, for example—simulate those of the diphtheria bacillus very closely. The films may be stained with Löffler's methylene blue for five to ten minutes, or by Pugh's method, then washed and dried. If the films are made on a slide, after staining, washing, and drying, a drop of cedar oil may be put on the stained patch, which is then examined directly without a cover-glass. If, however, there is very little growth, it is better to make a cover-glass specimen,

as the position of the material is so much more easily located. The preparations are examined with a $\frac{1}{12}$-in. oil immersion lens magnifying not less than 800–1000 diameters, and the Klebs-Löffler bacillus is identified from the description given in the text.

Prausnitz considers that if negative results are obtained after eighteen to twenty-four hours' incubation the tubes should be incubated for a further twenty to twenty-four hours and re-examined, and undoubtedly occasionally a positive result may be obtained by this longer incubation.

Löffler's methylene blue gives much more characteristic preparations than Gram's method.

Although eighteen to twenty hours is recommended for incubating the cultures, a microscopical examination will sometimes reveal the bacilli at a much earlier period. The writer has found them in as short a time as six hours, but if bacilli are then *not* found the tubes must be incubated for the longer period.

Neisser's method of staining is as follows:

(*a*) One gramme of methylene blue (Grübler's) is dissolved in 20 c.c. of 96 per cent. alcohol, which is then mixed with 950 c.c. of distilled water and 50 c.c. of glacial acetic acid.

(*b*) Two grammes of Bismarck brown are dissolved in one litre of boiling distilled water and the solution is filtered.

The preparations are stained in (*a*) for one to three seconds, rinsed in water, and stained in (*b*) for three to five seconds, washed in water, dried, and mounted. The bacilli are stained brown, and contain two, rarely three, inky-blue dots. This is a valuable confirmatory stain for the diphtheria bacillus, but staining for a longer time than that recommended by Neisser is advisable, viz. half a minute in the blue and one minute in the brown. Tanner treats with Gram's iodine solution for half a minute after the blue. The staining solutions seem to keep well but occasionally fail to act, so should be controlled on an undoubted diphtheria culture.

Pugh's stain is also a very good one. It is a mixture

containing 1 grm. of toluidine blue dissolved in 20 c.c. of absolute alcohol and added to 1000 c.c. of distilled water and 20 c.c. of glacial acetic acid. The mixture is applied for two minutes. The protoplasm of the bacilli is stained a pale blue and the polar bodies are deeply stained and stand out in marked contrast; by artificial light they appear a reddish purple.

In the majority of cases, after a little experience, the Klebs-Löffler bacillus will be readily recognised if present. Occasionally, however, bacilli may be present which resemble the Klebs-Löffler very closely, and of which it is difficult to be certain. In such a case the following points should be noted in attempting to arrive at a decision:

1. The character of the growth on the medium.

2. The depth of staining with Löffler's blue, and the presence or absence of segmentation or polar staining: The Klebs-Löffler bacillus usually stains somewhat deeply, while the bacilli resembling it stain but feebly.

3. The presence or absence of involution forms, clubbing, etc.

4. The presence or absence of thread forms: The Klebs-Löffler bacillus does not form threads.[1]

5. The presence or absence of spores: The Klebs-Löffler bacillus does not form spores.

6. Motility in a hanging drop: The Klebs-Löffler bacillus is non-motile.

7. Gram's method of staining: The Klebs-Löffler bacillus stains well.

8. The grouping of the organism: The parallel grouping of the Klebs-Löffler bacillus is somewhat characteristic. The bacilli when lying side by side do not seem quite to touch, while the bacilli which resemble the Klebs-Löffler and show a parallel grouping frequently lie much closer together than the Klebs-Löffler bacillus ever does.

9. The reaction with Neisser's or Pugh's stain (*the culture*

[1] Klein and others have described thread and branched forms in cultures of the Klebs-Löffler bacillus in certain circumstances, but these are not likely to be observed under the conditions mentioned.

must be a young serum one): The pseudo-bacillus and other bacilli do not give the diphtheritic reaction (polar staining).

10. The final test of virulence may be applied. For this purpose the organism must be isolated in pure culture by plate cultivations. Two guinea-pigs, of 250–300 grm. weight, are each inoculated with 2 c.c. of a forty-eight hours' broth culture, one receiving at the same time 1 c.c. of diphtheria antitoxin. If the guinea-pig inoculated with culture only dies, while the one receiving culture and antitoxin lives, this is complete proof that the organism is the diphtheria bacillus; if both live no inference can be made except that the organism is non-virulent; if both die, it shows that the organism is virulent, but that it is not neutralised by antitoxin, and therefore is not the diphtheria bacillus. In cases in which bacilli persist, the test of virulence is frequently applied. If the organism proves to be non-virulent, presumably the patient is non-infective. Such a presumption, in the writer's opinion, however, is not necessarily true.

11. Agglutination tests are unsatisfactory and not of service.

It occasionally happens that a conclusion cannot be arrived at without an extended investigation.

If serum tubes are not available an egg may be used. It is boiled hard, the shell chipped away from one end with a knife sterilised by heating, and the inoculation made on the exposed white; the egg is then placed, inoculated end down, in a wine glass of such a size that it rests on the rim and does not touch the bottom. A few drops of water may with advantage be put at the bottom of the glass to keep the egg-white moist. The preparation is kept in a warm place for twenty-four to forty-eight hours and then examined. Antitoxin itself may be used as a culture medium, *provided it contains no antiseptic* (this is now rarely the case.) A test-tube is sterilised by heating, or with boiling water or steam from a kettle, antitoxin to the depth of about an inch is poured in, and is coagulated by holding the tube very obliquely in boiling water or steam. After coagulation and

a. The pseudo-diphtheria or Hofmann bacillus. Cover-glass preparation of a serum culture. × 1500.

b. Vincent's angina. Smear from exudation showing fusiform bacilli (dark) and spirilla (light). × 2000.

To face page 310.

cooling the medium is inoculated. If no incubator is available, the culture may be kept in a warm place, or in an inside pocket.

Many laboratories will now undertake the examination of material. Culture outfits are supplied by some, consisting of a sterilised tube containing a sterilised swab. Failing this, a piece of membrane may be forwarded in a tube or bottle which has been sterilised by heating, or with boiling water or steam. If there be no membrane, a swab can be readily extemporised by wrapping a little wool round the end of a piece of wire, knitting needle, hair-pin, penholder, or splinter of wood. The wool may be sterilised by moistening with water and then holding in a flame. Membrane or secretion may also be forwarded on pledgets of wool, pieces of lint or calico, and even on paper, but these are not so suitable.

(B) *In milk.*—See section on "Milk."

Vincent's Angina.

An infective malady characterised by sore throat, fetor, dysphagia, and ulceration and membrane simulating diphtheria. The diphtheria bacillus, however, is not present, and the affection is caused by an apparent association of a bacillus and a spirochaete. The bacillus (*B. fusiformis*) measures 6–8 μ to 10–12 μ in length, has *pointed* ends and is usually somewhat bent, not straight, often appears feebly motile, and does not stain by Gram. It can be cultivated anaërobically on the ordinary media to which human blood-serum, ascitic or hydrocele fluid has been added. The spirochaete is long and sinuous and very motile, but cannot be cultivated, and is stated to be developed from the fusiform bacillus. Smears may be stained with methylene blue or dilute carbol-fuchsin, and the appearance of the associated organisms is so characteristic that a diagnosis is easily effected (Plate VII., *b*).

Fusiform bacilli have been met with in various necrotic processes, *e. g.* noma (see Chap. XX).

The Xerosis Bacillus.

The xerosis bacillus was isolated by Neisser from cases of xerosis conjunctivæ, and is met with in follicular con-

junctivitis. Lawson and also Griffith isolated it from nearly 50 per cent. of all normal conjunctival sacs. In morphology and staining reactions it resembles the Klebs-Löffler bacillus very closely. It differs from the Klebs-Löffler bacillus in the following particulars: (1) Usually, but not always, in the *primary* cultivations from the eye on blood-serum, colonies do not appear under about thirty hours, while those of the Klebs-Löffler bacillus are visible in sixteen to twenty hours. This does not apply to the *secondary* cultivations, in which the colonies appear as soon as those of the Klebs-Löffler bacillus. (2) Upon agar it will seldom or never grow in primary culture, and in secondary cultures it forms only a thin, translucent, *dry* film. (3) Upon gelatin it will never grow in primary culture and seldom in secondary culture. (4) It does not give rise to acid production in milk or glucose broth. (5) It is non-pathogenic to guinea-pigs. (6) The Neisser stain is negative. The fermentation reactions will be found in the table on p. 306.

In all probability the organism is not causative of xerosis conjunctivæ.

To isolate the organism, blood-serum tubes are inoculated with a looped platinum needle from cases of follicular conjunctivitis or xerosis, and incubated at 37° C. for forty to forty-eight hours. Half the tubes will usually show a growth. Preparations may be stained with Löffler's blue and by Gram's method.

Bacillus coryzæ (segmentosus).

An organism first described by Cautley, of frequent occurrence in the nasal secretion in cases of "influenza" cold. It bears a striking resemblance morphologically to the *B. diphtheriæ* when stained with methylene blue, and is Gram-positive, but does not show granules either with Löffler blue or with Neisser's stain. On agar it grows more slowly than *B. diphtheriæ*, and in glucose broth and litmus milk acid production is slow and feeble. It is non-pathogenic to guinea-pigs. The fermentation reactions will be found in the table on p. 306.

Other Diphtheria-like Bacilli.

As already mentioned, diphtheria-like bacilli are not infrequent in wounds, pathological discharges and secretions. Some of them may be positive with Neisser's stain. They are always non-virulent. The fermentation reactions of some of these organisms will be found in the table on p. 306.

Bacillus diphtheriæ columbarum.

Pigeon diphtheria is an infectious disease of pigeons, characterised by the formation of diphtheritic-like membranes on the tongue, fauces, and corners of the mouth; occurs in extensive epizootics from time to time. Löffler isolated a bacillus to which he gave this name. It is short, with rounded ends, non-motile, does not form spores, and does not stain by Gram's method. On gelatin it forms a whitish growth without liquefaction, on agar a creamy growth, and on potato a thin grey film. Milk is not curdled and is unchanged in reaction. It is pathogenic for the mouse and pigeon, but only slightly so for the fowl and guinea-pig. It is possible to prepare a vaccine, and an anti-serum for the disease.[1] Recent research has, however, suggested that the disease may be due to a filter-passer.[2]

Diphtheritic roup of poultry is a different disease, and is stated to be due to a protozoan parasite.[3] Macfadyen and the writer[4] found Klebs-Löffler-like organisms to be present in the mouths and throats of healthy pigeons and fowls. These organisms resembled the true Klebs-Löffler bacillus in their cultural reactions, but were quite non-virulent to guinea-pigs (see table, p. 306).

The so-called diphtheria of calves is produced by an anaërobic streptothrix.

[1] See *Ann. de l'Inst. Pasteur*, xv, 1901, p. 952.

[2] Dean and Marshall, *Journ. of Path. and Bact.*, vol. xiii, 1908, p. 29.

[3] See also Gordon Sharp, *Lancet*, 1900, vol. ii, p. 18.

[4] *Trans. Path. Soc. Lond.*, vol. li, 1900, p. 13, and *Brit. Med. Journ.*, 1900, vol. i, p. 994.

CHAPTER IX.

"ACID-FAST" BACILLI.

TUBERCULOSIS—LEPROSY—THE SMEGMA BACILLUS. GLANDERS.

Gram +

"Acid-fast" Bacilli.

An important characteristic of the tubercle, leprosy, and smegma bacilli is the property they possess when stained with fuchsin of retaining the red colour after treatment with a strong mineral acid (25 per cent. sulphuric or 30 per cent. nitric). They are therefore termed "acid-fast." Most other organisms are rapidly decolorised even by 1 or 2 per cent. sulphuric acid, but it must be recognised that several apparently saprophytic bacilli are also "acid-fast." The retention of the fuchsin colour in spite of treatment with the acid seems to be due to the presence of substances of a fatty or waxy nature within the organisms with which the fuchsin either combines or is protected from the action of the acid.

Moreover, by cultivating many saprophytic bacilli in media containing butter, Bienstock and Gottstein converted them into "acid-fast" forms.

"Acid-fast" bacilli are also present in butter (Petri, Rabinowitsch, Rubner), on certain Graminaceæ (the "Timothy-grass bacillus" of Moeller), and in dung (the "Mist bacillus"). It has been suggested that these

saprophytic acid-fast bacilli may be derived from the tubercle bacillus, but Panisset's work gives no confirmation of this.

The Streptotricheæ occasionally exhibit "acid-fast" properties. Dean has found acid-fast leprosy-like bacilli in rats (see p. 355). All the acid-fast bacilli seem to be Gram-positive.

Tuberculosis.

Tuberculosis is, unfortunately, only too common in the human subject, and most of the domestic animals and wild animals in a state of captivity may be attacked by it.

The conception of tuberculosis was originally a purely anatomical one, the name being given to a condition in which the organs were studded with little yellowish points or nodules, which were termed tubercles. Laennec was the first to indicate the characters of these nodules or tubercles, and traced with considerable accuracy their development from minute lesions, the miliary tubercles, up to the large cheesy masses which may be met with in the glands and lungs.

Microscopically, the structure of a young and typical tubercle is characteristic. At the centre one or more giant-cells are found—large protoplasmic masses, each containing ten to twenty nuclei arranged round the periphery (Plate IX., *b*). They are of the nature of plasmodia, similar to the masses of fused cells which surround a foreign body in the lower animals (Adami). Around the giant-cells are well-defined epithelial-like cells with large and distinct nuclei, which are known as epithelioid, or more properly endothelioid, cells. A zone of smaller cells with scanty protoplasm and small nuclei

surrounds the endothelioid cells; they are known as lymphoid cells from their likeness to the cells of lymphoid tissue. This is the structure of a typical tubercle, but one or other of the components may be wanting, and none can be said to be absolutely characteristic of the tubercle. The nodule possesses no blood-vessels, and as its size increases by growth at the periphery the central parts undergo degenerative changes, and may become either structureless or hyaline, or be converted into a soft yellowish material somewhat like cheese and termed caseous. More or less extensive inflammatory reaction ensues in the tissues surrounding the tubercle, and the cellular elements so produced often become spindle-shaped and ultimately fibrous, so that the tuberculous nodule becomes enclosed by a capsule of fibrous tissue which may contract and convert it into a fibrous nodule. After caseation has occurred calcification may ensue—that is, lime-salts are deposited and the nodule is converted into a calcareous mass.

So far back as 1865 Villemin showed that inoculation of rabbits with human caseous material was followed by a development of nodules similar in all respects to the miliary tubercles in man. Cohnheim, Burdon Sanderson, and Wilson Fox confirmed this observation, but they also showed that the development of tubercles apparently followed the introduction, not only of tuberculous material, but also of setons, pieces of putrid muscle, and gutta-percha. It was pointed out, however, that in all probability these results were due to accidental contamination or inoculation with tuberculous matter, and, by adopting suitable precautions in order to prevent such sources of error, it was conclusively shown that non-tuberculous matter is unable to set up tuberculosis. Tuberculosis is there-

fore inoculable, and is an infective disease, and as such must be due to a specific infective agent, to the discovery of which observers then directed their attention. In 1882 Koch announced that he had discovered a special bacillus, the tubercle bacillus, in tuberculous tissues, which could be isolated and cultivated, and which reproduced the disease on inoculation.

The Tubercle Bacillus.

Morphology.—The tubercle bacillus (*B. tuberculosis*) is a slender rod with rounded ends, often slightly curved, and averaging 2–3 μ in length, though the length varies in the tissues from 1·25 μ to 6·5 μ; in cultures it tends to be short, on serum being about 1 μ. In stained preparations one or more unstained intervals are often seen in the rods (Plate VIII., *a*); these have been considered by some observers to be spores, but there are many objections to this view. Spores are usually single and not multiple, and are regular spherical or ovoid bodies, whereas the unstained spaces in the tubercle rods are irregular. Moreover, in the same specimen of sputum a varying amount of "beading," as it is termed, may be brought out by different staining methods (Plate VIII., *b*); in a preparation stained by Gram's method it is usually more pronounced than in one stained with carbol-fuchsin. In class work also it will be found that one student's specimen will show beading much more markedly than another's. These considerations render it probable that the beading is partly due to segmentation of the protoplasm, and partly, perhaps, is an artifact due to the staining process, and is not a spore formation. The tubercle bacillus, however, probably does form spores, though this is a debated point. Some observers have

described clear, regular, unstained spaces in bacilli from old cultivations, and consider these to be true spores.

The tubercle bacillus is a non-motile, strictly parasitic organism (it has been described as being both motile and flagellated). It usually occurs singly, occasionally linked in twos or threes so as to form short chains, and under certain conditions, especially in old cultures, filamentous forms develop, and Foulerton[1] and others include it among the *Streptotricheæ*. The bacillus is agglutinated by the blood-serum of a tuberculous animal (see p. 347).

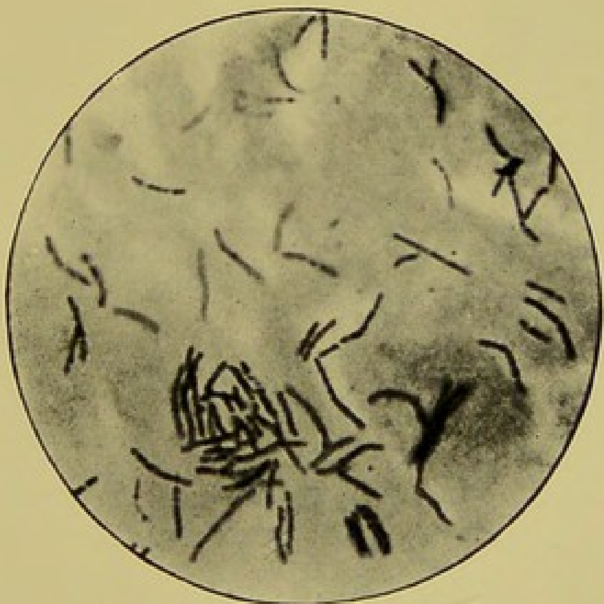
Staining reactions.—The tubercle bacillus stains indifferently with the ordinary watery solutions of dyes, prolonged treatment with, or warming, the solution being required. It stains well by Gram's method. It also stains well and deeply with carbol-fuchsin, particularly on warming, and when so stained is markedly resistant to the decolorising action of 25–30 per cent. mineral acid; that is to say, it is strongly "acid-fast," and this property is made use of for demonstrating its presence in tissues, etc., and for diagnostic purposes.

Koch states that the peculiar staining reaction of the tubercle bacillus is due to a coating of two fatty acids, which take up the stain, and are not decolorised by the mineral acid. De Schweinitz and Dorset (*loc. cit.* p. 324) have found the fatty substances to be principally a glyceride of palmitic acid, together with small amounts of lauric acid and of two other undetermined acids. Bulloch and Macleod (*loc. cit.*, p. 325) found that the fat is not acid-fast, and by saponification yields oleic, isocetinic, and myristinic acids. The acid-fast substance, according to these observers, is an alcohol.

[1] "Milroy Lectures," *Lancet*, 1910, vol. i, p. 551, *et seq.*

PLATE VIII.

a. The tubercle bacillus. Cover-glass preparation of a pure culture. × 1000.



b. Tubercle bacilli in sputum. × 1500.

To face page 318.

Cultural characters.—The tubercle bacillus is aërobic and facultatively anaërobic, and thrives best at a temperature of 37° C. or thereabouts, and development even then is slow, six weeks at least being required for an appreciable growth. The simplest method of isolating the bacillus from the tissues is to make use of Roux's potato tube (Fig. 9), the bulb being filled with 5 per cent. glycerin. The potato is inoculated with an emulsion of the tuberculous material, and incubated at 37° C. In six or eight weeks cultures will be obtained in, perhaps, 50 per cent. of the tubes. Twort[1] has successfully isolated the bacillus from sputum by direct cultures in an ericolin medium. Other media that can be employed for cultivating the organism from the tissues are glycerinated serum (preferably dog's), and glycerin brain agar. The latter is prepared by making a 3 per cent. nutrient agar of + 20 reaction, adding an equal volume of pounded ox-brain, and sufficient glycerin to make 5 per cent. in the mixture, and sterilising.

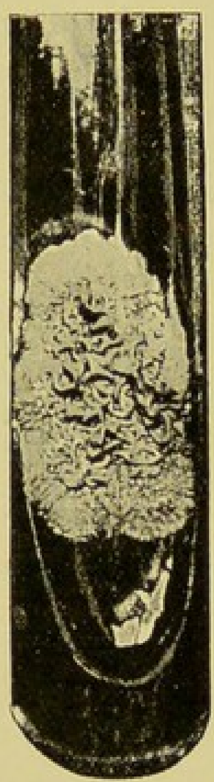


FIG. 37.—Tubercle bacillus. Glycerin - agar culture three months old.

After culture on these media for some generations, the tubercle bacillus will develop on 5 per cent. glycerin agar (reaction + 15 or 20), and in 5 per cent. glycerin broth (veal is best); it will also grow, though very slowly, on glycerin gelatine at 22° C. Gelatin and blood-serum are not liquefied. On glycerin agar the

[1] *Proc. Roy. Soc. Lond.*, B. vol. lxxxi, 1909.

growth forms a dry, crinkled and wrinkled, cream-coloured or brownish-yellow film, which has been well described as resembling the patches of lichen met with on trees (Fig. 37). The growth, however, varies considerably, both in colour and in the amount of wrinkling, though retaining more or less the characteristics just mentioned. In broth it forms soft cream-coloured, flaky masses, which increase slowly both in size and number, the broth remaining perfectly bright and clear. Sometimes a dry crinkled film forms on the surface of the broth, and may spread all over it, and tends to

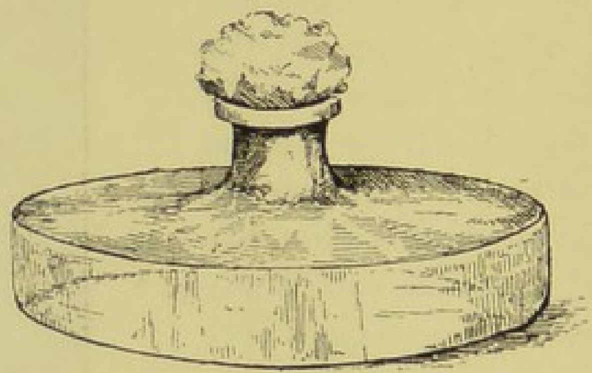


FIG. 38.—Flask for growing tuberculin.

creep up the sides of the vessel. This film formation seems to be essential for the preparation of a satisfactory old tuberculin, but it is necessary in order to start it that some of the inoculated particles should float and form nuclei from which the film spreads. The virulent organism from the primary cultivations is difficult to grow on anything but glycerinated potato or serum, or brain agar.

Tuberculins.—Extracts of, and suspensions of triturated, tubercle bacilli are employed in treatment and in the diagnosis of tuberculosis. The preparations are known as tuberculins.

Old tuberculin.—This is prepared by growing the tubercle bacillus in glycerin veal broth in a shallow layer

in flat flasks (Fig. 38), so that there is a free supply of oxygen. After some weeks an abundant growth with copious film formation develops; the latter seems to be essential, but it does not appear to matter whether the bacilli be virulent or non-virulent, or whether they be of human or of mammalian origin. The cultures, bacilli and all, are heated at 115° C. in the autoclave for half an hour, then concentrated over a water-bath to about one tenth of their volume, and finally are filtered through porous porcelain; the resulting fluid is thick, owing to the concentration of the glycerin by the evaporation, is of a dark amber colour, and possesses a curious characteristic smell. The large proportion of glycerin preserves the fluid, which keeps indefinitely in a cool dark place.

This old tuberculin possesses remarkable properties. Relatively large amounts (0·1–0·5 c.c.) may be injected into a healthy animal or individual without effect, but in a tuberculous one a minute dose, 0·001 c.c., gives rise to a marked reaction—elevation of temperature with constitutional disturbance more or less severe, and swelling and tumefaction of tuberculous lesions (glands, ulcers, etc.), and this reaction is made use of for diagnostic purposes (see p. 348). By cautiously increasing the amount a toleration is gradually induced, so that considerable doses cause little or no disturbance. Injections of tuberculin tend to produce marked changes in the tuberculous parts, leading to necrosis and exfoliation, with subsequent healthy reaction and repair. This is especially seen in cases of lupus; by continued injections a marvellous improvement results, so much so that a cure is apparently effected; but, unfortunately, when the treatment is discontinued the scar usually breaks down and the disease returns. Nevertheless, a few cases have remained permanently healed.

For treatment, the dose to commence with should not be more than 0·001–0·002 c.c., dilutions being made with 0·5 per cent. carbolic solution, and the dose is repeated when all reaction has passed away and is gradually increased. Tuberculin R, or tuberculin BE, is now generally employed (see below).

Healthy guinea-pigs bear considerable injections of tuberculin without harm; but if they be tuberculous, if the disease is advanced (eight to ten weeks after inoculation), doses of 0·01 c.c. produce death; if less advanced (four to five weeks after inoculation) a larger dose, 0·2 to 0·3 c.c., is required; but 0·5 c.c. always proves fatal. The post-mortem appearances are congestion of the lymphatics and viscera, and dark red spots, from mere points to the size of a hemp-seed, on the liver and spleen. These are due to enormous engorgement of the capillaries in the immediate neighbourhood of tuberculous deposits, actual extravasations of blood being rarely found. The hæmorrhagic-like spots on the liver are almost pathognomonic of death from tuberculin.

Absolute alcohol precipitates the active principle of tuberculin in the form of a whitish flocculent precipitate which chemically consists of proteoses. This precipitate, re-dissolved, is made use of in the *ophthalmic* reaction (p. 349). Tuberculin applied to the scarified skin also gives a *cutaneous* reaction in tuberculosis (p. 348).

Tuberculin R, or TR, *new* tuberculin, is prepared from young and virulent cultures of the tubercle bacillus. The growth is collected, dried *in vacuo* and triturated by machinery.[1] Of the triturated material, 1 grm. is treated with 100 c.c. of distilled water, and centrifugalised. The supernatant liquid is rejected, and the

[1] *Deutsch. med. Wochenschr.*, 1897, April 1st (translations or abstracts in most of the medical journals of about this date).

residue is collected, dried, again triturated and centrifugalised. The supernatant liquid is carefully pipetted off and kept, while the residue is again submitted to the same treatment, and the process is repeated until no solid residue is left. The fluids are then mixed, the solid content is estimated gravimetrically, some glycerin is added, and the liquid is diluted to the correct volume, so as to contain 2 mgrm. of solid matter per cubic centimetre (not 10 mgrm. as formerly stated), and for use is diluted with 20 per cent. sterile glycerin solution.

Tuberculin R, according to Koch, possesses distinct immunising properties, and causes neither reaction nor suppuration.

For treatment of tuberculosis in man the initial dose is equivalent to not more than $\frac{1}{100000} - \frac{1}{10000} - \frac{1}{5000}$ mgrm. of solid matter, according to the nature of the case. The doses are given subcutaneously at intervals of ten to fourteen days, and the treatment may be controlled in the earlier stages by opsonic determinations. According to Latham, tuberculin may also be given by the mouth. Cases of cutaneous or localised tuberculosis, and those in which the opsonic index to tubercle is moderately reduced, react best. In phthisis and visceral tuberculosis no striking results have been obtained.

Tuberculin, bacillary emulsion (BE), is an emulsion of the powdered bodies of tubercle bacilli in 50 per cent. aqueous glycerin. The mixture is allowed to sediment until all heavy particles have deposited, the milky supernatant fluid is pipetted off, and standardised so as to contain 5 mgrm. of solid matter per c.c. The dosage is similar to that of tuberculin R.

Behring has prepared another tuberculin, tulase or TC, by treating tubercle bacilli with chloral, which he states has a marked curative action, and is better

administered by the mouth than by subcutaneous inoculation. By giving tulase to cows, the milk is said to acquire immunising and curative properties which are transmitted to those consuming it. Other tuberculins are also on the market, and any tuberculin may be prepared with a human or with a bovine strain of bacillus.

Chemical products.—The tubercle bacillus produces no extra-cellular toxin. Crookshank and Herroun obtained from glycerin broth cultures of the tubercle bacillus a proteose and an alkaloidal body. The proteose was also obtained from "perlsucht." Both the alkaloid and the proteose (from both sources) produced a rise of temperature in tuberculous guinea-pigs, while in healthy animals the former caused a slight, and the latter a marked, fall in temperature.

De Schweinitz and Dorset[1] described chemical products isolated from the tubercle bacillus grown in a special glycerin-asparagin mixture. From the bacilli themselves an acid body was isolated, probably teraconic acid, an unsaturated acid of the fatty series. A certain amount of the same body was also obtained from the special culture medium, but only a trace from glycerin broth, in which the bacilli had been cultivated, in the latter case not because it was not formed, but because of the difficulty of isolation. This acid seemed to produce on injection depression of temperature and necrosis of the tissues locally, possessed some immunising power, and may be the substance producing caseation in the tuberculous nodules. The bacilli extracted with hot water yielded an albuminoid, which gave the tuberculin reaction. This they regard as the fever-producing substance.

[1] *Med. Journ. N. Y.*, 1897, July 24th, p. 195. Also *Fifteenth Annual Rep. Bureau of Animal Industry, U.S.A.*, 1898.

Bulloch and Macleod[1] state that the acid-fast substance of the tubercle bacillus is an alcohol. Hot xylol will remove this substance from the tubercle bacillus, and ether or 5 per cent. caustic soda that from the smegma bacillus; the organisms after this treatment are no longer "acid-fast."

Maragliano states that toxic bodies are present in the blood and urine of tuberculous individuals. Cellulose also seems to be present in small amount in the bacilli (it has also been found in tuberculous nodules).

Tubercle bacilli, living or dead, are with great difficulty absorbed when in any quantity. The dead bacilli when injected under the skin invariably cause suppuration, and several months later it is still possible to detect in the pus numerous bacilli which stain well; introduced into the circulation of rabbits they give rise to nodules in the lungs similar to the tuberculous nodules produced by living bacilli (Koch).

Action of heat and antiseptics on the tubercle bacillus.—The thermal death-point of the bacillus has been the subject of some controversy. Sternberg found that tuberculous sputum exposed for ten minutes to a temperature of 90°, 80°, and 66° C. failed to infect guinea-pigs in inoculation, while another specimen of the same sputum heated for ten minutes to a temperature of 50° C. produced tuberculosis in a guinea-pig, so that from these experiments the thermal death-point lies between 50° and 66° C.

Yersin in 1888, by culture methods, failed to obtain any growth from bacilli which had been heated to 70° C. for ten minutes, while those heated to 55° C. and 60° C. gave growths in glycerin broth in ten days and twenty-two days respectively. Macfadyen and the writer, in the course of some experiments on the

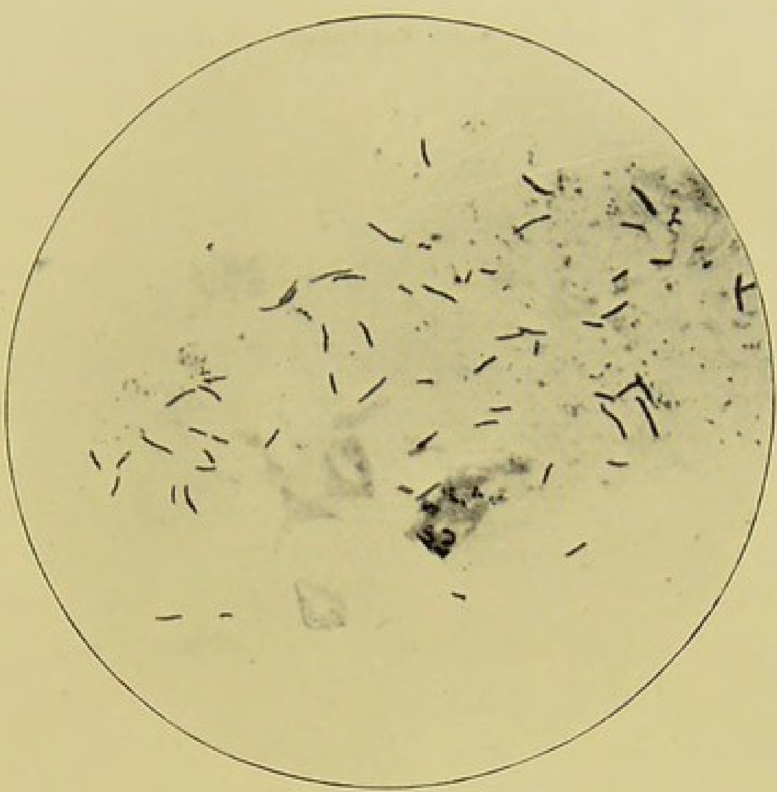
[1] *Journ. of Hygiene*, vol. iv, 1904, p. 1.

sterilisation of milk, found that milk to which powdered dried sputum had been added was rendered innocuous by a momentary heating to 67°–68° C. These experiments indicate that a temperature of 65° C. and over is probably rapidly fatal to the tubercle bacillus, so that milk which has been pasteurised (*i. e.* heated to 68°–70° C. for twenty to thirty minutes) may be regarded as quite safe. Experiments by the Royal Commission on Tuberculosis with virulent tuberculous milk gave somewhat irregular results; in one instance heating to 65° C. for two and a half minutes rendered the milk innocuous, in another instance after five minutes at 70° C. it was slightly virulent, but twelve minutes at the same temperature rendered it inert (see also section on "Milk"). Foulerton found that emulsified tuberculous material from tuberculous guinea-pigs did not lose its power of infecting unless heated to 70° C. or over for ten minutes.

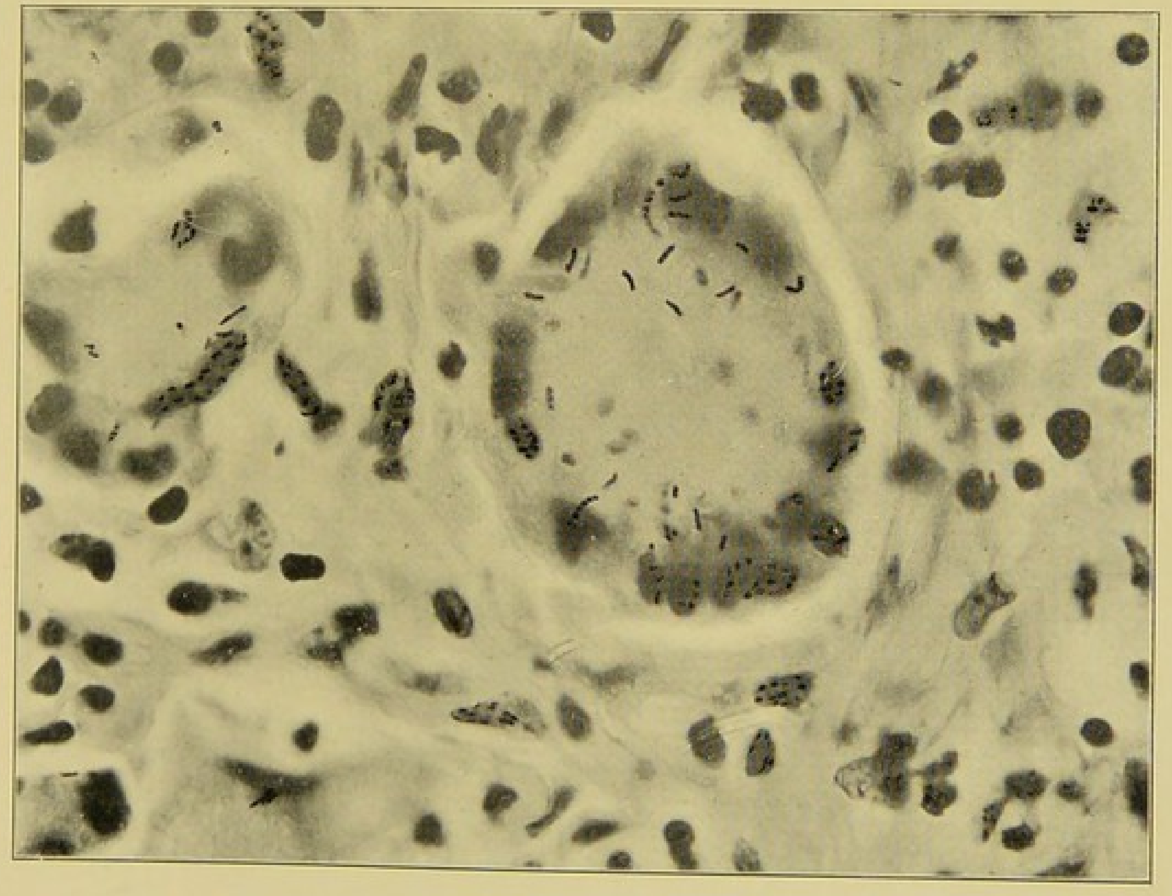
The tubercle bacillus offers considerable resistance to the action of antiseptics and germicides. Yersin found that it was killed by 5 per cent. carbolic acid in thirty seconds, by 1 per cent. in one minute, by absolute alcohol in five minutes, and by mercuric chloride, 1–1000, in ten minutes. Crookshank found that tuberculous sputum mixed with an equal volume of 5 per cent. carbolic was rendered innocuous in a few minutes, and this without any special precautions as to breaking up the masses. For disinfecting sputum mercuric chloride is unsuitable. (See also Chap. XXI.)

Pathogensis, etc.—Man is, unfortunately, only too frequently attacked with tuberculosis, the manifestations of which tend to differ somewhat at different age periods. Thus, in the very young, general miliary tuberculosis, tuberculous meningitis, and tuberculous disease of the peritoneum, intestine, and mesenteric

PLATE IX.



a. Tubercle bacilli in sputum. × 1000.



b. Giant-cell in tubercle containing tubercle bacilli. × 1000.

To face page 326.

In very young { intestinal / brain } { ! milk

Puberty —— Bones / joints

adult. Lungs

glands (tabes mesenterica) are the commonest ; in older children, up to the age of puberty, the lymphatic glands, especially in the neck, joints and bones, and the skin (lupus) are mostly attacked ; young adults suffer from disease of the lung (consumption, phthisis), and older people from chronic disease of the lung and tuberculous disease of the urinary organs and testes, and of the suprarenal capsules (Addison's disease). Scrofula and struma were terms formerly much employed; both denote a swollen neck, and were applied to cases suffering from chronic tuberculous inflammation with enlargement of lymphatic glands, especially of the cervical glands, with which other conditions, such as inflammation of the ear, throat and eye, and implication of bones and joints, are frequently associated.

The distribution of the bacillus in the tissues varies considerably. In young and active tubercles the bacilli are more plentiful and more easily demonstrated than in older and more chronic ones. They tend to be more numerous in some animals than in others—in the ox and horse than in man, for example. In man the bacillus is difficult to demonstrate (by staining) in enlarged and caseating glands, in pus, in synovial membranes, and in lupus. In some animals, especially the ox and horse, bacilli can usually be readily demonstrated, and may be present in large numbers, and frequently have the typical distribution, viz. within and at the periphery of the giant-cells, though they are by no means confined to this locality (Plate IX *b*).

It has been asserted, particularly by Rosenberger and Forsyth, that tubercle bacilli can be detected in the blood in the majority of cases of pulmonary tuberculosis. Hewat and Sutherland,[1] however, made twenty-

[1] *Brit. Med. Journ.*, 1909, vol. ii, p. 1119 (References).

two blood examinations on twenty patients in all stages of the disease and in only one detected two acid-fast bacilli. Schroeder and Cotton tested the blood of forty-two cattle in all stages of tuberculosis by inoculation into guinea-pigs with negative results.

Tuberculosis in animals.—The majority of the domestic animals are subject to tuberculosis. It is most common in the ox, pig, and horse, much less so in the sheep and goat, cat and dog. Wild animals, both mammals and birds, in a state of captivity are also specially prone to be attacked, and a large number of the deaths in Zoological Gardens, particularly among the apes, are due to this disease.

Fish

In carp, tubercle-like nodules are occasionally met with in which a bacillus resembling the tubercle bacillus in morphology and staining reactions is present. It grows, however, much more freely than the true tubercle bacillus, and though inoculable into fish and frogs, is non-inoculable into warm-blooded animals. But it yields a tuberculin which reacts with mammalian tuberculosis, and by feeding carp on the mammalian tubercle bacillus this can apparently be transformed into the piscian variety.

Avian

Bird or avian tuberculosis undoubtedly differs in many respects from mammalian tuberculosis. The tuberculous new formations may be very large, but do not show nearly such a disposition to caseation or suppuration as the human lesions. Epithelioid cells form the major part of the growth, and giant-cells are very infrequent. One remarkable feature is the enormous numbers of bacilli which may be present in the tissues; in places they may be so numerous and closely packed as to form distinct masses or nodules. The bacilli of avian have the same staining reaction as those of mammalian tuberculosis, but on cultivation and inoculation

various differences between the two races become evident.

The mammalian bacilli flourish best at about 37° C., and growth ceases at 41° C., whereas the avian bacilli thrive luxuriantly at 43° C, and the growth of the latter on glycerin agar is much moister and more wrinkled, and often more pigmented, than that of the former. Fowls and dogs are with difficulty infected with human bacilli, but dogs are susceptible to infection with avian bacilli. By cultivation on boric-acid agar and on eggs, etc., the mammalian bacilli are stated to assume the characters of the avian.

Avian tuberculosis is of practical importance not only as attacking poultry, but also in human pathology, as several cases have been recorded in which the bacilli cultivated from human cases seemed to be of the avian type, and were therefore probably derived from an avian source of infection. Two types of tuberculosis also occur in the horse—one in which the lesions are chiefly abdominal, in the other the lungs and bronchial glands are most affected. Nocard states that generally the bacillus obtained from the pulmonary variety is of the ordinary mammalian type, while that of the abdominal one belongs to the avian.

Relation of human and mammalian tuberculosis.—It has long been noticed that there are certain differences between the bacilli of human and of bovine tuberculosis, the latter tending to be shorter and thicker and less readily cultivated than the former; also, while human tuberculous material injected into a rabbit generally produces small discrete lesions in the organs which tend to retrogress, bovine material induces a progressive disease with large caseating masses.[1] These

[1] The bacilli derived from tuberculosis of the sheep, pig and horse are also of the bovine type.

distinctions were regarded as being due to variations in the bacilli as a result of growing upon a different soil and not to any fundamental difference between the two strains of bacilli. In 1901, however, Koch stated[1] that young cattle and swine cannot be infected with human tuberculous material, and he therefore concluded that human and mammalian tubercle bacilli are essentially different. As a result of his experiments he made the statement that "though the important question whether man is susceptible to bovine tuberculosis at all is not yet absolutely decided, if such a susceptibility really exists, the infection of human beings is but a very rare occurrence."

This view met with considerable opposition, and a second Royal Commission was appointed to investigate the question, and the following summarises the results obtained up to the present, from which it will be gathered that while there is no justification for assuming that man is infected from human sources alone, infection from human sources is probably vastly more frequent than from any other. Thirty different viruses isolated from cases of tuberculosis occurring spontaneously in bovines have been studied, and the results of introducing them into a number of different animals by feeding and inoculation are recorded. In calves, inoculation usually results in generalised progressive tuberculosis, but the effect is somewhat dependent on the dose, *i.e.* the number of bacilli, administered. Thus whereas 50 mgrm. of culture always induced a fatal generalised progressive tuberculosis, in two instances much smaller doses—0·01–0·02 mgrm.—produced only limited retrogressive tuberculosis. Feeding, on the other hand, usually produced lesions limited to the neighbourhood of the digestive tract, which

[1] See *Brit. Med. Journ.*, 1901, vol. ii, p. 189.

generally retrogress and become calcareous. The bovine bacillus, when introduced into *rhesus* monkeys or chimpanzees, either by inoculation (even in so small a dose as 0·001 mgrm.) or by feeding, induces rapid generalised tuberculosis, and, considering the close relation that exists between the anthropoid apes and man, these results are of the highest importance. In pigs, generalised progressive tuberculosis is readily set up both by feeding with, and by the inoculation of, bovine bacilli. Goats, dogs, and cats are relatively less susceptible, but more or less tuberculous infection can similarly be produced in them. On this part of the investigation the Commissioners remark that the bacillus of bovine tuberculosis is not so constituted as to act on bovine tissues only, and the fact that it can readily infect the anthropoid apes, and, indeed, seems to produce this result more readily than in the bovine body itself, has an importance so obvious that it need not be dwelt on. The viruses isolated from sixty cases of the disease in man were also studied, and the results obtained show that they may be divided into two groups, subsequently referred to as Group I. and Group II. The bacilli of Group I. comprised fourteen viruses, one obtained from sputum, three from tuberculous cervical glands, and ten from mesenteric glands of primary abdominal tuberculosis in children. The results produced by introducing these viruses into animals are identical with those produced by the bovine bacillus. The bacilli of Group II. comprised forty viruses obtained from various forms of human tuberculosis—cervical glands, mesenteric glands (8), lungs and bronchial glands (10), joint and bone disease (9), testis, kidney, etc.—grow more luxuriantly in culture than those of Group I., and inoculated into calves and rabbits do not produce the generalised and

fatal disease caused by the bovine bacillus, but in *rhesus* monkeys and in the chimpanzee set up a general tuberculosis. Certain human viruses, differing in certain respects from those of Groups I. and II., were also met with and are classed as Group III., but an opinion on their significance is reserved for a future report.

The Commissioners conclude that the tubercle bacillus in its nutritive and reproductive powers resembles other simple organisms, and that the essential difference between one strain and another depends on variations in these factors, and they classify those bacilli that grow with difficulty on artificial media as *dysgonic*, and those that grow readily on media as *eugonic*.

The bearings of the results obtained are thus summarised :

" There can be no doubt that in a certain number of cases the tuberculosis occurring in the human subject, especially in children, is the direct result of the introduction into the human body of the bacillus of bovine tuberculosis, and that in the majority of these cases the disease is introduced through cow's milk. Our results clearly point to the necessity of measures more stringent than those at present enforced being taken to prevent the sale or the consumption of tuberculous milk."

As regards the histological appearances of the tuberculous process in different animals, Dr. Eastwood states that there is an underlying unity of the morbid processes produced experimentally by infection with every variety of bovine and human tubercle bacillus.

Eber,[1] in an extended investigation, succeeded in infecting calves from three cases of human pulmonary tuberculosis. The bacilli isolated from the human

[1] *Centr. f. Bakt.*, Abt. I (Orig.) lix, 1911, p. 193.

material were of the human type, but after passage through the calf became transformed into the bovine type. He affirms, therefore, the essential identity of the human and bovine types of tubercle bacilli.

With regard to the channel of infection in human tuberculosis opinions differ. Koch insisted that inhalation of air-borne bacilli derived from dried human sputum is the principal source of infection; Von Behring, on the other hand, expressed the opinion that tuberculous milk fed to children is the main source of infection both of children and of adults; in the latter case he suggests that bacilli are ingested in childhood and lie dormant for years before becoming active.

Calmette similarly believes that in the young, infection by the digestive tract, especially by tuberculous milk, is the more frequent, and attaches little or no importance to dry dust containing tubercle bacilli as a source of infection. Ravenel considers that the alimentary tract, particularly in children, is a frequent portal of entry for the tubercle bacillus, which he believes is able to pass through an intact mucous membrane. Of sixty cases of human tuberculosis investigated by the Royal Commission on Tuberculosis, twenty-eight possessed clinical histories indicating that in them the bacillus was introduced by the alimentary canal.

Flügge, on the other hand, states that his experiments show that tuberculosis can be communicated to animals by inhalation, and that the dose of bacilli required to infect by the respiratory tract is far less than that required to infect by the alimentary canal. The mode of infection in man doubtless varies, and he believes that children may be infected by the digestive tract, by tuberculous food, particularly milk, but the

Koch

Behring

Calmette

most extensive source of infection is the number of droplets of tuberculous expectoration coughed up by consumptives; these float in the air and serve as sources of infection to others. Ribbert and Schrötter, also, from the evidence of autopsies, considered inhalation as the chief mode of infection in man.

Pulmonary Human

Bulloch,[1] from a careful survey of the literature, concludes that pulmonary tuberculosis is invariably caused by bacilli of the human type, and, therefore, is presumably due to inhalation of human bacilli.

McFadyean,[2] also, from a critical survey of the experimental evidence, concludes that (1) inhalation of tubercle bacilli suspended in the air is a very certain method of infecting susceptible animals; (2) experimental infection by the digestive tract is comparatively difficult to realise; (3) inhalation is probably the commonest natural method of infection, both in man and in animals; (4) infection by the digestive tract can be inferred only when the lesions are confined to the abdomen. He finally states that "the whole of the experimental evidence on which the theory of the intestinal origin of pulmonary tuberculosis in man was built up has been swept away."

Thus there has been a reversion to Koch's original view, and, inasmuch as the death-rate per 1000 living from all forms of tuberculosis is about 1·64, that from phthisis is 1·14, so that by far the greater part of the mortality from tuberculosis must be ascribed to infection from human sources.

Final R.C.

While this book was in the Press, the final report of the Royal Commission was issued. The Commissioners conclude that a considerable amount of human tuberculosis is caused by bacilli of the bovine type, and that tuberculosis may be

1 *"Horace Dobell Lecture,"* 1910.

2 *Journ. Roy. Inst. Public Health*, vol. xviii, 1910, p. 705.

communicated to man from infected cow's milk, and from tuberculous meat, either beef or pork.

So far, therefore, from any relaxation of the existing supervision of milk-production and meat-preparation being possible, the Commissioners press upon the Government the enforcement of food regulations, "planned to afford better security against the infection of human beings through the medium of articles of diet derived from tuberculous animals." More particularly they urge such action "in order to avert or minimise the present danger arising from the consumption of infected milk."

Of young children who died of wasting disease of the intestine, the bovine bacillus was present in nearly half the cases. Further, a large proportion of cases of tuberculous cervical glands in both children and adults was due to the same bacillus. The wording of the report is: "Whatever, therefore, may be the animal source of tuberculosis in adolescents and in adult man, there can be no doubt that a considerable proportion of the tuberculosis affecting children is of bovine origin, more particularly that which affects primarily the abdominal organs and the cervical glands. And further, there can be no doubt that primary abdominal tuberculosis, as well as tuberculosis of the cervical glands, is commonly due to ingestion of tuberculous infective material. The evidence which we have accumulated goes to demonstrate that a considerable amount of the tuberculosis of childhood is to be ascribed to infection with bacilli of the bovine type transmitted to children in meals consisting largely of the milk of the cow.

"We are convinced that measures for securing the prevention of ingestion of living bovine tubercle bacilli with milk would greatly reduce the number of cases of abdominal and cervical gland tuberculosis in children, and that such measures should include the exclusion from the food supply of the milk of the recognisably tuberculous cow, irrespective of the site of the disease, whether in the udder or in the internal organs."

The occurrence of tuberculosis in the domestic animals raises points of practical importance, especially the occurrence of infection from the consumption of meat and milk from diseased animals. In the ox the tuberculous lesions are most frequently met with in the lymphatic glands and serous membranes, particularly the pleura, and in the lungs and liver, while the fat and muscular tissues, which constitute the major part of "meat," are very rarely affected. On the pleura the growths take the form of nodular masses, which from their arrangement are popularly termed "grapes" or "angle berries." There can be no doubt that the carcase of an animal extensively affected with tuberculosis, especially if wasting has occurred, should be condemned as unfit for food, and likewise all parts in which there are tuberculous deposits. But it becomes an important question for the community, financially as well as from a hygienic point of view, as to the method of procedure with the meat from a beast comparatively slightly affected with tuberculosis—an enlarged gland or two, and a few nodules on the pleura. No doubt the ideal method in such a case is the condemnation and destruction of the whole carcase, be the amount of tubercle ever so little; but from financial considerations this procedure is hardly practicable on account of the large amount that would have to be paid in compensation. Experiment has demonstrated that the tubercle bacilli are practically confined to the tuberculous areas and are extremely rarely met with in the muscular tissue, and these portions, therefore, it might seem, could be eaten with impunity, especially as they would be cooked before consumption. As regards swine, however, it is generally held that tuberculosis anywhere condemns the whole carcase.

The report of the first Royal Commission on Tuber-

culosis, however, indicated two dangers. Firstly, in cutting up a carcase the butcher will most likely use the same knife throughout, and in this way may infect the meat with tuberculous matter by smearing with the knife. Secondly, cooking cannot be depended upon to destroy the bacilli unless the joints are under 6 lb. in weight; when the weight is above this the temperature in the interior may not rise sufficiently high. Evidently one of the first measures to be taken is the abolition of private slaughterhouses and the establishment of municipal abattoirs where the meat would have to be passed by competent inspectors. In this way all badly affected carcases would be condemned, and those only slightly affected could be separately dealt with and special precautions taken to eliminate tuberculous pieces, etc.

Tuberculous milk also raises many important points. Probably some 10–15 per cent. of *all* samples are infective to guinea-pigs, but this does not necessarily indicate that this proportion would be dangerous to man, for the material is introduced into the guinea-pigs by inoculation after concentration by centrifuging (see also section on "Milk"). Tubercle bacilli are present in milk not only when the udder is tuberculous, but also when the cows are suffering from tuberculosis elsewhere which is *clinically recognisable*. Thus, when the lungs are affected, bacilli are disseminated from the air-passages and also by the fæces. It is noteworthy that the incidence of abdominal tuberculosis in young children occurs just when cow's milk in the staple article of their diet. At the same time this incidence does not seem to fall on those who consume most milk.

Much might be done by the registration of all dairy premises, the use of selected cows, the elimination of all tuberculous animals, and by enforcing the inspection

of dairy cattle by competent inspectors at intervals of not longer than a fortnight, making the notification of any disease of the udder compulsory, and the sale of milk from such a diseased udder illegal under a heavy penalty (Roy. Com. on Tuberculosis). In the absence of inspection and the use of selected cows, treatment of milk intended for the food of infants and young children by pasteurisation or sterilisation has been recommended, but has disadvantages (see section on "Milk"). The ideal method, and one which commends itself at first sight as being the most satisfactory, is the elimination by slaughter of all animals which are tuberculous. This was adopted in the State of Massachusetts; under an order of the Board of Cattle Commissioners all beasts in the State were tested with tuberculin, and every animal that reacted was slaughtered, and strict quarantine combined with the tuberculin test imposed on all imported cattle. Even in this small state such a plan was found to be unworkable, the expense being so heavy. A middle course seems to be the only practicable one, viz. all manifestly tuberculous animals, especially where wasting or tuberculous udder is present, to be slaughtered; other animals to be tested with tuberculin, and those which react to be separated from the healthy and to be disposed of as soon as convenient, and in the meanwhile kept as much as possible in pasture.

Measures should be adopted by local authorities and others to prevent the spread of tuberculosis, and, thanks to the attention directed to the subject, tuberculosis is diminishing. It can hardly be doubted that the disease, or at least phthisis, should be made notifiable, though there are many difficulties in carrying this out. Patients should be warned of the danger of disseminating their expectoration, and should use pocket-spittoons containing an antiseptic, or handkerchiefs (such as the Japanese

paper ones) which can be destroyed. Rooms which have been inhabited by tuberculous patients should be disinfected, for which purpose Delépine recommended spraying with a 1–100 solution of chloride of lime. Although the occurrence of direct infection can rarely be proved, the possibility of this cannot be ignored. Not only should the dissemination of infection be prevented, but the resistance of the individual should be raised by providing a healthy environment and by inculcating the importance of fresh air.

Serum therapeutics and vaccine.—Maragliano's serum is prepared by injecting cows with watery tuberculin and with a bacillary pulp made by grinding up tubercle bacilli, emulsifying in water and filtering through a porcelain filter. Subcutaneous injections of the two preparations are given in increasing doses, commencing with 5 c.c. of each, until 20 c.c. is reached, the frequency of the injection being determined by the temperature reaction and general symptoms produced.

Marmorek's serum is prepared by growing tubercle bacilli in a medium consisting of a leucotoxic calf-serum (prepared by injecting calves with leucocytes) and glycerine liver bouillon.[1] The filtered culture is injected into horses, and their serum, after several injections, becomes antitoxic to a slight degree. It cannot be said that even encouraging results have been obtained with these or other sera.

For *vaccine treatment*, tuberculins R and BE are usually employed (p. 322). Latham has found that tuberculin given *per os* produces its characteristic effects.

Immunity.—Attempts have been made from time to time to produce immunity against the *B. tuberculosis*, particularly in cattle. Thus McFadyean[2] found that

[1] *Bull de l'Inst. Pasteur*, i, 1903, p. 851.
[2] *Trans. Path. Soc. Lond.*, vol. liii, 1902, p. 20.

heifers which had previously been subjected to repeated doses of tuberculin (old) in some cases resisted infection with virulent bacilli. Behring[1] also employed human tubercle bacilli for the vaccination of cattle with satisfactory results. His tulase likewise confers immunity when given either by the mouth or by the stomach.

Theobald Smith[2] also concludes that vaccination of calves with the human type of bacillus is harmless, and that the procedure leads to a relatively high resistance to fatal doses of the bovine bacillus.

Clinical Examination.

I. The "*complement-fixation*" *test* was first used in tuberculosis by Wassermann and Brück. The method has been further elaborated by Emery.[3] He makes use of a standard emulsion of tubercle bacilli in salt solution, containing about 4 per cent. by volume of solid bacillary substance. This is sterilised by intermittent sterilisation and keeps for four to six weeks. Bacilli from various sources vary somewhat, so that the emulsion should be standardised so as to give an absorption-time with normal sera of about 20 minutes, *i.e.* the complement of normal serum should be just completely absorbed in about 20 minutes. A water-bath kept at a constant temperature of 38° C. is used to warm all the constituents and mixtures. One part of the serum to be tested is mixed with four parts of the bacillary emulsion in a small tube (*e.g.* a Durham's tube) in the water-bath, the time of mixing being accurately noted. After 2½ minutes' incubation, 4 volumes of the mixture are removed by means of a capillary pipette with teat (Fig. 35, p. 225), into which also a single volume of sensitised corpuscles (*i.e.* a hæmolytic system, p. 191) is taken up and the whole is expelled into a small tube already standing in the water-bath. The process is repeated after 5, 10, 15 and 20 minutes, and longer if

1 *Brit. Med. Journ.*, 1906, vol. ii, p. 577.

2 *Journ. Med. Research*, vol. xviii, 1908, p. 451.

3 *Lancet*, 1911, vol. i, p. 485.

necessary. By the occurrence or absence of hæmolysis in the various tubes, the time taken for the absorption of complement is ascertained, the complement used being that contained in the serum itself, which therefore should be fresh. A control with normal serum should always be performed at the same time. With normal serum complete absorption should take place in about 20 minutes; with tuberculous sera it is often complete in $2\frac{1}{2}$ minutes. If, then, absorption of complement is complete in much less than the time necessary for absorption with a normal serum, presumably the serum is derived from a tuberculous individual. (But see Emery's paper for limitations.)

II. The *examination of sputum, etc.*, for the tubercle bacillus is a routine procedure of the greatest value in forming a diagnosis. Fortunately, owing to the peculiar staining reaction of the tubercle bacillus, discovered by Koch, the method is comparatively simple.

1. *Sputum.*—Film specimens are prepared by smearing with a platinum needle a little of the sputum on a slide so as to form a thin film covering two thirds of the surface, or by placing a particle of the sputum on one slide, applying another slide, pressing together, and then drawing apart so that a thin film is left on each slide. The thick portion of the sputum should be used, the thin mucoid portion being rejected. If there are any small yellow caseous particles present these should be chosen, and sufficient material should be used so as to form a distinct but not too thick film; a little experience will soon decide the right amount. Preparations may also be made by smearing the sputum on a cover-glass or between two cover-glasses instead of using slides. Whichever plan is adopted, the film is dried and fixed in the usual manner (generally by heat), and then stained by one of the following methods:

(*a*) *Ziehl-Neelsen method.*—Film specimens on slides are most conveniently stained by flooding with filtered, undiluted carbol-fuchsin and warming for 2 to 5 minutes on a piece of asbestos cardboard supported on a tripod, or on a heated penny (p. 114), or slides or cover-glasses flooded with the stain

may be held in the forceps and carefully warmed over a flame, or the preparations may be immersed in a watch-glass or dish of the stain, covered, and placed in the warm incubator for half an hour. In no case must the stain be allowed to boil; it should only be warmed sufficiently to steam (50°–60° C.), and with slides or cover-glasses as evaporation takes place more stain (always filtered), or better, 5 per cent. carbolic, should be added. After staining, the preparations are rinsed in water and are then decolorised by treating with 25 per cent. sulphuric or 30 per cent. nitric acid. The preparation may be flooded with the acid, but a better method is to have a pot (fig. 20, p. 113) containing the acid in which it is immersed. In the acid the colour changes after a few seconds to a yellowish brown, the preparation is then rinsed in water, and some of the pink colour returns. The treatment with acid and with water alternately is repeated until the preparation is nearly colourless when rinsed in water. With sputum this is usually the case after three or four rinses in the acid, but it varies with the thickness of the film and with the number of tubercle bacilli present; when these are absent the film often decolorises more readily than when there are many. The presence of blood renders the decolorisation difficult. After decolorising and washing, the preparations are stained for one minute in Löffler's methylene blue, washed in water, and mounted in water, or, better, dried and mounted in Canada-balsam or cedar oil. When the preparation is made on the slide, after washing and drying, it can be examined directly without a cover-glass with the oil-immersion after applying a drop of cedar oil, unless a permanent specimen is desired, in which case it should be mounted in Canada-balsam.

The tubercle bacilli appear as delicate red rods, often beaded or segmented, on a blue background composed of cells, mucus, and putrefactive or other bacteria. Occasionally here and there a little red colour may be present in addition to the tubercle bacilli. Hair and keratinised material generally, such as horny epithelium, and red blood-corpuscles, retain the red colour after the foregoing treatment, and the spores of bacteria are also liable to retain the red somewhat persistently.

These exceptions are not, however, likely to prove a source of error, for the tubercle bacilli should be recognised not only by their red colour, but also by their characteristic size, shape, and general appearance. It is conceivable that acid-fast bacilli not tubercle might be present in sputum, but such an event is a very unlikely one. For the microscopical examination, a $\frac{1}{6}$-inch with good illumination is sufficient when the tubercle bacilli are present in any number. When they are scanty it is necessary to use a $\frac{1}{12}$-inch oil-immersion, and this is the better lens in any case. (See Plate IX., *b*, and Plate X., *a*.)

If tubercle bacilli are not found, other specimens should be prepared and examined. *It is only by repeated examinations on different occasions that the negative evidence*, the absence of tubercle bacilli, *becomes of any value.*

The tubercle bacillus is occasionally not acid-fast[1]; probably the bacilli in such cases are degenerate, and, like all degenerate bacteria, fail to stain well. Dold has examined a number of methods that have recently been introduced for staining and differentiating the tubercle bacillus, but does not find that any presents a marked advantage over those usually employed. In material which has been preserved a long time, *e. g.* sputum with carbolic, or tissue in spirit, the bacilli may be much less acid-fast than in fresh material.

Various methods have been recommended for the solution of the sputum and the examination of the sediment of the bacilli. In one method 5 c.c. of sputum are mixed with 50 c.c. of normal KOH solution; the mixture is warmed in a water-bath to 60°–65° C. until the sputum is dissolved (about 3 hours); 50 c.c. of cold water are next added, the whole is well shaken, and again warmed for ½ hour. Petroleum ether, 2 c.c., is next added, the whole is well shaken, and is then kept at 60° C. until the ether has separated. The bacilli will be concentrated in the fluffy layer at the junction of the ether and water; this is pipetted off and films are made with it and stained. Antiformin (a mixture of sodium hypochlorite and sodium hydrate) has also been recommended. Into a boiling-tube or small flask of 50 c.c. capacity, 5 c.c. of

[1] See *Lancet*, 1908, vol. i, p. 1222.

the sputum are introduced. To this are added 25 c.c. of antiformin solution (10–20 per cent. aqueous solution) diluted with 10–20 c.c. of water according to the density of the sputum. The mixture is well shaken until homogeneous (about 15 minutes), then centrifuged, the deposit is washed three times with salt solution by centrifuging, and films are made with the washed deposit and stained by the Ziehl-Neelsen method.

If it is inconvenient to examine the sputum for a day or two a little 1–20 carbolic should be added. This preserves the sputum and the tubercle bacilli retain their staining power for some time.

If the tubercle bacillus cannot be detected microscopically after repeated examinations, and a certain diagnosis is important, the inoculation method may be employed. A couple of guinea-pigs are inoculated subcutaneously in the thigh or abdomen with 0·5 to 1 c.c. of the sputum. If tubercle bacilli are present the animals will show signs of tuberculosis in three to six weeks (see below, "Urine").

(*b*) *Gabbet's method.*—Prepare and stain the specimens in carbol-fuchsin as in method (*a*). Then wash and treat with the following solution for two to three minutes; wash, dry, and mount:

Alcohol	50 parts
Water	30 ,,
Nitric acid	20 ,,

Saturate with methylene blue and filter.

The solution both decolorises and counter-stains.

2. *Tissues.*—The histological appearance of the tubercle is usually sufficient for diagnostic purposes without the demonstration of the tubercle bacilli, which in many instances may be difficult in human material, as the bacilli may be very scanty, or practically impossible to find, *e g.* in lupus. Sections should be prepared either by the freezing or the paraffin method, stained with hæmatoxylin, and counter-stained with eosin, or orange-rubin, or with the Ehrlich-Biondi mixture.

In order to demonstrate the tubercle bacillus in fresh tissue smears may be made and stained like sputum, or

sections prepared and stained in warm carbol-fuchsin for about ten minutes. For frozen sections the stain may be contained in a watch-glass or small glass capsule, and is warmed until it steams, but not boiled, on a piece of asbestos cardboard or a sand-bath. Paraffin sections should be fixed to the slides with glycerin albumin, and may be stained by flooding with the carbol-fuchsin and warming on asbestos cardboard, or a heated penny, for ten minutes. After staining, the sections are washed in water and are then decolorised in 25 per cent. sulphuric acid. This is a longer process than with sputum, and the sections after being in the acid for a few seconds are washed in water and then returned to the acid, and this alternate rinsing in acid and in water is repeated until they are nearly colourless when placed in water. It is not necessary to remove the colour absolutely; a faint pink remaining does not matter. After rinsing in fresh water to remove all the acid, the sections are counter-stained in Löffler's methylene blue for two minutes, rinsed in methylated spirit, passed through absolute alcohol somewhat rapidly to avoid removing too much of the blue, cleared in cedar oil or xylol, and mounted in balsam. The sections may also be counter-stained with hæmatoxylin or Bismarck brown.

Instead of using the strong acid solution for decolorising, an acid alcohol solution may be used with advantage.

Gram's method may also be used, but is, of course, not distinctive for the tubercle bacillus.

The following is a good method for staining tubercle in sections (Kühne, modified by Delépine[1]):

(*a*) The tissues should be fixed in corrosive sublimate, acidulated or not, hardened in alcohol, embedded in paraffin, and the sections fixed on slides with glycerin albumin.

(*b*) The sections are stained with hæmatin solution for ten to twenty seconds to obtain a pure nuclear stain (not too deep), then washed thoroughly in water.

(*c*) They are then stained with carbol fuchsin, kept at a temperature of about 47° C. for twenty to thirty minutes.

[1] See *Med. Chronicle*, vol. v, 1896, p. 17.

The slides are during that time kept in a moist chamber to prevent the stain from drying on the specimen.

(*d*) The stain is rinsed off with water, and the sections are treated with 2 per cent. watery solution of hydrochloride of anilin for a few seconds.

(*e*) The sections are then decolorised in 75 per cent. alcohol till they are apparently free from stain; this will take from fifteen to thirty minutes.

(*f*) They are then counter-stained with a solution of orange (one part of saturated watery solution of orange to twenty to forty parts of 50 per cent. alcohol), dehydrated, cleared, and mounted.

Where a positive diagnosis is important, a small piece of the tissue may be inserted under the skin of the thigh or abdomen of a guinea-pig. If tuberculous, the animal will show signs of tuberculosis in two to three weeks (see below, "Urine").

Cover-glass specimens of pure cultivations of the tubercle bacillus may be stained in warm carbol-fuchsin for two to five minutes, rinsed in the sulphuric or nitric acid solution, washed, dried, and mounted. They can also be stained by Gram's method, which usually brings out the beaded appearance very markedly. Differentiation from the leprosy bacillus will be found at p. 356, and from the smegma bacillus and other acid-fast organisms at p. 357.

3. *Urine.*—The tubercle bacillus is often very difficult to demonstrate in urine. The urine must be allowed to stand in a conical glass for twenty-four hours or centrifugalised, and film specimens are prepared with the sediment and treated by one of the methods for sputum given above. Several specimens should be made and must be very carefully examined. It is important to exclude the smegma bacillus, and the urine is preferably drawn off by a catheter. Staining may be carried out by Housell's method, by which the smegma bacillus is decolorised, viz. after staining in warm carbol-fuchsin the specimen is washed and dried. It is then immersed in acid alcohol (alcohol + 3 per cent. hydrochloric) for ten minutes, washed in water, counter-stained for a few seconds in a saturated alcoholic solution of methylene blue, washed, dried,

and mounted (see also p. 357). An electrolytic method for the concentration of the tubercle bacilli has been devised by Russ.[1]

If a diagnosis is of importance inoculation should be resorted to. Two guinea-pigs are inoculated subcutaneously in the thigh or abdomen with 0·5 to 1 c.c. of the deposit from the sedimented or centrifugalised urine, or one may be inoculated subcutaneously, the other intra-peritoneally. If tubercle bacilli are present the animals may show signs of tuberculosis as early as two to three weeks after inoculation. Delépine[2] recommends the inoculations to be made on the inner aspect of the leg about the level of the knee. The order of infection after inoculation is as follows: the popliteal, superficial and deep inguinal, and sub-lumbar glands, the retro-hepatic, mediastinal and bronchial, deep cervical, and subscapular glands, the spleen, liver, and lungs. The inoculated animals are killed in two to three weeks, dissected, and the lesions examined microscopically. Others inoculate two guinea-pigs, one subcutaneously in the abdomen, the other intra-peritoneally. Negative results are nearly as valuable as positive ones.

In *fæces*, if definite yellow caseous particles can be found, these should be picked out, and films made and stained. Antiformin may also be used. About a cubic ½ in. of fæces is mixed with 20 c.c. of 15 per cent. aqueous antiformin in a conical glass, will agitated and broken up, and an equal volume of the dilute antiformin is then added. The mixture is allowed to stand for an hour, and films are prepared from the white curdy layer which forms, stained, and examined.

4. *Milk*.—See section on milk (Chapter XXI).

III. *Agglutination reaction*.—The method of agglutination was proposed by Arloing and Courmont for the diagnosis of tuberculosis, but is difficult to carry out and is not much employed. A special method has to be employed to obtain homogeneous cultures of the tubercle bacillus or a powder of

[1] *Proc. Roy. Soc. Lond.*, B. 1909.

[2] *Brit. Med. Journ.*, 1893, vol. ii, p. 664. The results only apply to ordinary forms of tuberculosis, and not to certain modified forms such as lupus and the avian variety.

pulverised or ground-up bacilli may be used: this powder may be purchased. The reaction may be carried out either microscopically or macroscopically; for the latter small sterile test-tubes may be employed. For each test three dilutions of the serum are made, a 1 in 5, a 1 in 10, and a 1 in 20, and the tubes filled with these dilutions are allowed to stand in an inclined position (45°) for five to ten hours. In man the serum of normal individuals may agglutinate up to a dilution of 1 in 5, while in animals this is variable—imperceptible in the guinea-pig, rabbit, and calf; feeble in the goat; in the adult ox up to 1 in 5, but in the dog it may be up to 1 in 10 or even 1 in 20.

A positive serum reaction in a suspected subject is a sign of great value in establishing the diagnosis; a negative serum reaction is of less value.

IV. *The opsonic method.*—The general mode of carrying this out is described at pp. 224–230, the tubercle bacilli being suspended in 1·5 per cent. salt solution.

V. *Tuberculin reactions.*—The *old* tuberculin is used for diagnostic purposes; it is not perhaps very safe. A dose of 0·002 c.c. is injected subcutaneously, and the temperature taken four-hourly during the succeeding thirty-six hours. A rise of 2°–3° F. or more ensues a few hours after injection in tuberculous subjects. If no reaction occurs another dose of 0·005 c.c. may be given after the lapse of some days.

This method has now almost completely been superseded by the cutaneous or by the ophthalmo reaction.

The cutaneous tuberculin reaction.—Von Pirquet[1] discovered that when tuberculin is introduced into the superficial layers of the skin of tuberculous individuals, as in vaccination, a reaction occurs consisting of the formation of a papule with redness, slight swelling and exudation, and sometimes small vesicles. This reaction is usually at its height twenty-four to forty-eight hours after inoculation. In healthy individuals no reaction follows the inoculation. The method is to scarify a small spot on the forearm through a drop of a dilution of the old tuberculin, and protect the patch with a simple dry

[1] *Wien. med. Woch.*, July 6th, 1907.

dressing. Moro has modified the method by applying the tuberculin to the skin in the form of ointment.

The ophthalmo-tuberculin reaction.—Calmette transferred the site of inoculation from the skin to the conjunctiva. He makes use of material prepared by precipitating the old tuberculin with alcohol, of which a 1–100 solution is prepared in distilled water. One drop of this is instilled into the inner half of the conjunctiva of one eye. In tuberculous individuals a reaction follows, usually in six to sixteen hours after medication, consisting of a conjunctivitis, ranging in intensity from a local redness to a redness extending over the whole eye and having the appearance of an acute conjunctivitis. The reaction soon passes off, generally without leaving ill effect. On the whole the reaction appears to be fairly constant in tuberculous individuals, but absence of reaction is not certain proof that the case is not tuberculous.[1]

VI. *Tuberculin for veterinary use.*—The dose of the various preparations in the market varies according to their strength; it corresponds to 0·1 c.c. or 0·2 c.c. of Koch's original tuberculin.

(*a*) The dose is injected subcutaneously in the neck.

(*b*) The temperature should be taken immediately previous to inoculation, and, if possible, morning and evening for two or three days previous to inoculation.

(*c*) The temperature should be taken at the twentieth hour after injection, or, if it can be done, at frequent intervals from the twelfth to the twentieth hour.

(*d*) The reaction consists of a rise of temperature of 1·5° to 6° F. above the average normal, occurring eight to twelve hours after injection, and lasting twelve to fourteen hours, accompanied by some systemic disturbance.

(*e*) A healthy animal is unaffected by the injection, and if an animal be very extensively affected with tuberculosis the reaction may not be given, or may be masked by the febrile condition present.

An ophthalmo-reaction may also be employed in cattle.

[1] See articles in *Brit. Med. Journ.* and *Lancet*, 1907, vol. ii, and 1908, vol. i.

Calmette

Johne's Disease.

Johne's disease,[1] a bovine enteritis, is due to an acid-fast bacillus closely resembling the tubercle bacillus in morphology. It occurs in scrapings of the affected mucous membrane of the bowel, and also in sections of the intestinal wall. The Johne bacillus is not inoculable into the guinea-pig or rabbit, and does not grow on any of the ordinary laboratory media. Twort states that it can be cultivated on the medium employed by him for growing the leprosy bacillus (p. 354).

Pseudo-Tuberculosis.

The term "pseudo-tuberculosis" (which is not a good one, and should be discarded) has been applied to a number of different conditions which have as a common character the presence of tubercle-like nodules, but which are not caused by the tubercle bacillus. Such are produced by certain parasitic worms, by *Blastomycetes*, *Streptothrix* and *Aspergillus*, Protozoa, and by several bacteria.

Pfeiffer's *Bacillus pseudo-tuberculosis* produces nodular deposits in the organ, accompanied by wasting, very like true tuberculosis. The disease, however, runs a more rapid course, death ensuing in the guinea-pigs two to three weeks after inoculation. Guinea-pigs, rabbits, mice and monkeys can be readily infected. The nodules consist of masses of round cells which undergo necrosis and caseation. The bacillus in the tissues is not readily stained, carbol-methylene blue being the best solution, as it is not acid-fast, nor does it stain by Gram's method. Morphologically it is a small rod 1–2 μ in length, usually non-motile, although, according to Klein, it possesses a single flagellum or two flagella at one end. On gelatin it forms a whitish

[1] See MacFadyean, *Journ. Comp. Path. and Therap.*, vol. xx, 1907, p. 48.

growth without liquefaction, like that of the colon bacillus, but confined to the needle-track. It produces alkali, forms no gas, and does not curdle milk. Broth remains clear, with a whitish stringy flocculent deposit. The bacillus grows readily and rapidly.

MacConkey has found that the fermentation reactions of this organism and of the plague bacillus are practically identical (see "Plague," p. 416), and sterilised cultures of either will protect against the other.

Ovine caseous lymphadenitis, a disease of sheep simulating tuberculosis, is due to a short plump bacillus with rounded ends which stains well by Gram's method, and grows best on blood-serum, on which it forms greyish colonies.[1]

Leprosy.

Leprosy, the elephantiasis Græcorum or true elephantiasis, is a disease which has existed from the earliest times, and was recognised by the ancients. It was undoubtedly somewhat prevalent in the British Isles from the twelfth to the fifteenth centuries, as the many leper houses and enactments against lepers testify. At the same time, no doubt a number of other skin diseases, syphilides, psoriasis, lupus, etc., were at that early period of medical diagnosis confounded with it. In the present day leprosy, although extinct in the British Isles, may be said to have a world-wide distribution, for it is met with in Iceland and Scandinavia, Russia and the Mediterranean coasts; in Persia, India, China, Siberia, and Japan; in Africa from north to south, in the American continent in many districts, and also in the Pacific Islands. Three varieties of leprosy are described—the tuberculated or nodular, the anæsthetic, and the mixed.

[1] *Sixteenth Ann. Rep. Bureau of Animal Indust. U.S.A.*, p. 638.

The mode of spread is probably by personal contact (though possibly insects play some part), and throughout ancient and mediæval times leprosy was considered to be a contagious and communicable disease, as witness the stringent regulations in the Mosaic and other laws for the segregation of lepers. J. Hutchinson supposes that fish in the diet, particularly if stale, decomposed, or badly cured, in some way is a causative factor; but he is practically alone in this view.

A bacillus, the *Bacillus lepræ*, is abundant in the tissues, and was discovered by Hansen in 1879. In form it resembles the tubercle bacillus, but is slightly more slender; it probably does not form spores, though in stained preparations the same irregularity in staining—namely, the occurrence of unstained intervals, the so-called "beading"—is met with as in the tubercle bacillus, and is assumed by some to be due to the presence of spores. The organism as obtained from the tissues is non-motile, stains readily with the ordinary anilin dyes, and by Gram's method, which brings out the beaded appearance very well, and is markedly acid-fast, thus closely resembling the tubercle bacillus, and the methods used to demonstrate it are the same as for the latter organism.

The *Bacillus lepræ* is found in enormous numbers, usually crowded together in bundles or masses, in the leprous nodules in the skin (Plate X., *a*), liver, spleen, and testicles, and in the affected nerves in the anæsthetic form; it has also been found in the blood, but only in the febrile paroxysms which set in when the disease is approaching a fatal termination. The exact situation of the leprosy bacilli in the tissues has been a matter of controversy. By some it has been held that they are contained within certain round cells, the so-called leprous cells, and this may be the case, but to

PLATE X.

a. Leprosy. Section of skin × 1500.

b. The smegma bacillus. Smear preparation of smegma. × 1500.

To face page 352.

Gram +

Gel
agar } usual grow
milk } hence. O.
Indol O.

Not so acid fast as T.B.

Stains more easily than T.B.
Eg. with cold Carbol Fuchsin

an inconsiderable extent. Unna has always regarded these leprous cells as really being transverse sections of lymphatic vessels containing bacillary thrombi, and this seems to be usually the case. Giant-cells are occasionally present in the leprous nodules. One of the most constant and earliest situations in which the *B. lepræ* is found is the nasal mucous membrane.

Although the organism is present in such enormous numbers and is so readily demonstrable, it is very difficult to cultivate on artificial media and to inoculate into animals. Babes, Bordoni-Uffreduzzi, Czaplewski, are some of those who in the past believe that they have cultivated the leprosy bacillus. Van Houten[1] claimed to have succeeded by cultivating in glycerin fish broth. The bacillus cultivated was acid-fast, and agglutinated with, and was sensitised by, lepers' serum.

Deycke,[2] by taking fragments of leprosy tissue and incubating for several weeks in physiological salt solution at 37° C., obtained a growth of a semi-acid-fast streptothrix, *S. leproides*. He is uncertain if this is a true growth of the leprosy bacillus. Injected into leprosy patients it seemed to produce a beneficial effect. The acid-fast property resides in a fatty substance which can be extracted with solvents, particularly benzoyl chloride. The fatty substance Deycke terms "nastin"; it is a neutral fat, the glycerin ester of a fatty acid of high molecular weight. Injected into leprosy patients it sometimes produces marked reaction, sometimes not. In solution in benzoyl chloride it is much more active, and Deycke hopes that it will act as a curative vaccine in leprosy. On the whole, the results obtained with nastin have been disappointing.

[1] *Journ. Path. and Bact.*, vol. viii, 1903, p. 260.
[2] *Brit. Med. Journ.*, 1908, vol. i, p. 802.

Twort[1] claims recently to have cultivated the *B. lepræ* on a medium consisting of eggs, glycerin and ground-up tubercle bacilli. Clegg states that the leprosy bacillus will grow in symbiosis with amœbæ, and Duval that it grows in 1 per cent. human serum in symbiosis with some bacteria.

In 1904 Rost announced that he had obtained cultures of the leprosy bacillus in a chlorine-free medium, but this was not confirmed. In 1909 he again claimed success by cultivating in a medium consisting of the fluid obtained by the steam distillation of rotten fish to which is added a little Lemco broth and milk, and Bannerman believes that he is correct.[2] Williams has grown a non-acid-fast streptothrix in ordinary broth, and has also cultivated acid-fast bacilli in a modified Rost medium (substituting distilled water for the fish distillate). The writer has also grown a non-acid-fast streptothrix from a case of leprosy on brain agar containing the juice from disintegrated *B. megaterium*. The fact seems to be that the *B. lepræ* is really a streptothrix, that it is acid-fast only under certain conditions, viz. in the body or in media containing fat, and that the streptothrix may break up into non-acid-fast diphtheroid bacilli or into acid-fast leprosy bacilli in cultures.

A certain number of positive results of the inoculation of leprous material into the lower animals have been reported by Ortmann and others. Nicolle[3] has reported the successful inoculation of a macaque monkey, but most of the attempts have ended in failure; positive results are open to criticism and may be fallacious, for lepers not infrequently suffer from coincident tuber-

[1] *Proc. Roy. Soc. Lond., B.*, 1911.
[2] See *Sc. Mem. Gov. of India*, No. 42, 1911.
[3] *Comp. Rend. Acad. Sc.*, 1905.

culosis, and the animals therefore may have been infected with tuberculosis. Japanese dancing mice are also stated to be slightly susceptible. The local lesion induced in animals may be simply inflammatory, produced by the leprous material acting as a foreign body, and the bacilli may be diffused without proliferating. Human beings have also been inoculated, but the positive results obtained are all open to objection.

The differentiation of leprosy from tuberculosis, although the bacilli are so similar, does not in the majority of cases present much difficulty. The large number of bacilli present in the skin and in the leprous lesions elsewhere forms a marked distinction from tuberculosis, while the *Bacillus lepræ* stains more readily and with watery solutions in a shorter time than does the *Bacillus tuberculosis*, though this distinction is hardly marked enough for diagnostic purposes.

Cases of leprosy, both of the nodular and anæsthetic varieties, have been treated with injections of Koch's tuberculin, which has been found to produce a certain amount of reaction followed by some amelioration in their condition. Rost and Williams with their cultures have prepared vaccines with which treatment is being pursued.

Dean[1] and others have met with a leprosy-like disease in the rat. Nodules are found in the tissues which contain large numbers of an acid-fast bacillus closely resembling the *B. lepræ*. The organism cannot be cultivated. Inoculated into rats it reproduced the disease after some months, but had no effects on guinea-pigs.

Clinical Examination.

(1) If cutaneous nodules be present, one is clamped, pricked, and film specimens are prepared with the juice that exudes and stained as for tubercle. The occurrence of large numbers of

[1] *Journ. of Hyg.*, vol. v, 1905, p. 99.

bacilli, having the same staining reactions as the tubercle bacillus and obtained from the cutaneous structures, is diagnostic of leprosy (the smegma bacillus may be present *on*, but not *in*, the skin).

(2) In the tissues the diagnosis must be based on the presence of the bacilli in large numbers in the so-called leprosy-cells.

Tissue sections are stained in the same manner as tuberculous material.

(3) Leprosy is not inoculable in guinea-pigs.

N.B.—It must be remembered that lepers not infrequently suffer from coincident tuberculosis.

(4) The differentiation of the leprosy from the tubercle bacillus by staining methods cannot be said to be satisfactory. By staining in a saturated aqueous solution of fuschin in the cold for five to seven minntes, and subsequently decolorising with acid alcohol (nitric acid 1 part, alcohol 10 parts), it is stated that the leprosy bacillus is stained, the tubercle bacillus not.

The Smegma Bacillus.[1]

The smegma bacillus is an organism found in the smegma præputii, between the scrotum and thigh, and between the labia. It also occurs in the cerumen, occasionally on the skin, and possibly in the sputum.

It is a small bacillus resembling the tubercle bacillus in size and appearance, and, like the latter, is difficult to stain, but when stained with carbol-fuchsin, retains the colour after treatment with a 25 per cent. mineral acid (Plate X., *b*); it is also Gram-positive. It has, therefore, to be distinguished from the tubercle bacillus in certain localities, viz. in urine and about the external genitals. It is non-inoculable on animals, and does not

[1] See Czaplewski, *Münch. med. Woch.*, 1897, No. 43; Grünbaum, *Lancet*, 1897, vol. i; Neufeld, *Arch. f. Hygiene*, xxxix, p. 184; *Zeitschr. f. Hyg.*, xxxix, 1901; and Moeller, *Centr. f. Bakt.*, xxxi, 1902 (Originale), p. 278.

usually grow in primary cultures on ordinary media, but can be isolated by the use of blood-serum or nutrose-agar, on which it forms delicate, ropy colonies. After isolation it grows freely on agar as a thin, slightly brownish, creamy layer, in which the bacilli may be very short but retain their acid-fast properties; on potato it forms minute (0·5–1 mm.) greyish colonies. It has been suggested that the syphilis bacillus of Lustgarten is identical with the smegma bacillus; neither is decolorised by Lustgarten's permanganate method, but while the smegma bacillus after staining is with difficulty decolorised by acid, and is easily decolorised by alcohol, the reverse is the case with Lustgarten's bacillus.

Staining and Differentiation.

Cover-glass specimens of smegma may be stained in exactly the same manner as for tubercle, after treating the preparations with ether to get rid of fatty material.

The urine should be drawn off with a catheter when it is to be examined for the tubercle bacillus; this will generally exclude the smegma bacillus. Young and Churchman[1] conclude that the smegma bacillus is a scant invader of the male urethra, and that by washing the glans and irrigation of the urethra it may be eliminated from the urine.

If there is reason to suspect the presence of the smegma bacillus when staining for tubercle, Bunge and Tranteroth[2] recommend that the cover-glass specimens should be treated as follows:

(1) Immerse in absolute alcohol for three hours.
(2) Immerse in 5 per cent. chromic acid for fifteen minutes.
(3) Stain in warm carbol-fuschin.

[1] *Johns Hopkins Hospital Rep.*, vol. xiii, 1906, p. 15.

[2] *Fortschrit. der Med.*, xiv, 1896, Nos. 23 and 24. See also *ibid.*, No. 9.

(4) Decolorise in 25 per cent. sulphuric acid for two to three minutes.

(5) Counter-stain in a concentrated alcoholic solution of methylene-blue for five minutes.

The smegma bacillus will be decolorised by this method (see also p. 346).

Coles recommends (*Journal of State Medicine*, vol. xii, 1904, p. 225) the following staining method:

(1) Spread thin and even films on slides, and fix by heat, in the ordinary way.

(2) While still warm from the heat fixation flood with filtered carbol-fuschin, and allow the preparation to remain for half a minute. Again warm for a a few seconds over the flame without actual boiling. Allow it to stand and stain for seven minutes.

(3) Wash thoroughly in running water, and then decolorise in either of the following solutions:

(*a*) *In Pappenheim's solution.*[1] Place the preparation in a wide-mouthed bottle containing the solution for not less than four, and not longer than twelve, hours. Wash, dry, and mount. Tubercle bacilli are the only organisms stained red.

(*b*) *In Pappenheim's solution without methylene-blue.* Proceed as in (*a*); wash in water and counter-stain for a minute in weak aqueous methylene-blue solution. The tubercle bacilli are brilliantly red.

(*c*) *In* 25 *per cent. sulphuric acid.* Pour on a few drops of the acid and allow it to act for half a minute. Pour off, and then place the preparation in a wide-mouthed bottle containing the acid for not less than sixteen hours and not more than twenty-four hours. Wash thoroughly, counter-stain with weak aqueous methylene-blue. Tubercle bacilli are the only bacilli which retain the red.

Acid-fast bacilli in milk and butter.—Numerous acid-fast

[1] *Pappenheim's solution* consists of one part of corallin (rosolic acid) in 100 parts of absolute alcohol, to which methylene-blue is added to saturation; 20 parts of glycerin are then added.

bacilli have been obtained from milk and butter. They usually grow freely and quickly on agar and on gelatin without liquefaction, sometimes as a creamy layer, sometimes as a dry, crinkled film, which may be pigmented (yellow, orange, pale brown or brick red). Some are pathogenic to guinea-pigs by massive intra-peritoneal inoculation only, producing a plastic peritonitis, but not nodules in the organs. In culture, the bacilli are acid-fast and occasionally resemble *B. tuberculosis*, but are genenerally thicker. (See Petri, *Arb. a. d. Kais. Gesundheitsamte*, xiv, 1897; Rabinowitsch, *Zeitschr. f. Hyg.*, xxvi, 1897; Grassberger, *Münch. med. Woch.*, 1899, Nos. 11 and 12; Tobler, *ibid.*, xxxvi; Swithinbank and Newman, *Bacteriology of Milk* [Murray, 1903].)

Grass bacilli and mist bacillus.—Moeller isolated from a grass (*Phleum arvense*) an acid-fast bacillus which he termed the Timothy-grass bacillus; other grasses also yield acid-fast bacilli (Grass Bacillus II.). They grow readily on culture media, and are not so acid-fast as the tubercle bacillus. The Mist bacillus was isolated from dung, and is considered by Pettersson to be identical with the Timothy-grass bacillus. (See Moeller, *Deutsch. med. Woch.*, 1898, p. 376; Herr, *Zeitschr. f. Hyg.*, xxxviii, 1901; Pettersson, *Berl. klin. Woch.*, 1899, p. 562.)

Glanders.[1]

Glanders is a disease which has been known from the earliest times, being recognised by the Greek and Roman writers, by whom it was termed μᾶλις and *malleus* respectively. It is distinctly a disease of the horse, mule, and ass, but is also communicable to man and to certain other animals. It is caused by a small bacillus discovered by Löffler and Schütz in 1882.

In the horse the lungs are always affected, and frequently the nasal mucous membrane (Fig. 39).

[1] See McFadyean, *Journ. of State Med.*, vol. xiii, 1905, pp. 1, 65 and 125.

Nodules form which afterwards break down and ulcerate, and a muco-purulent discharge appears; in the older writings the name "glanders" covered only these advanced cases of the disease. In "farcy" the lymphatic vessels and glands are affected, the enlarged glands being known as "farcy buds" (Fig. 40).

In man the disease is rare, an average of four deaths per annum being caused by it in this country. It occurs in two forms—the acute and the chronic. The

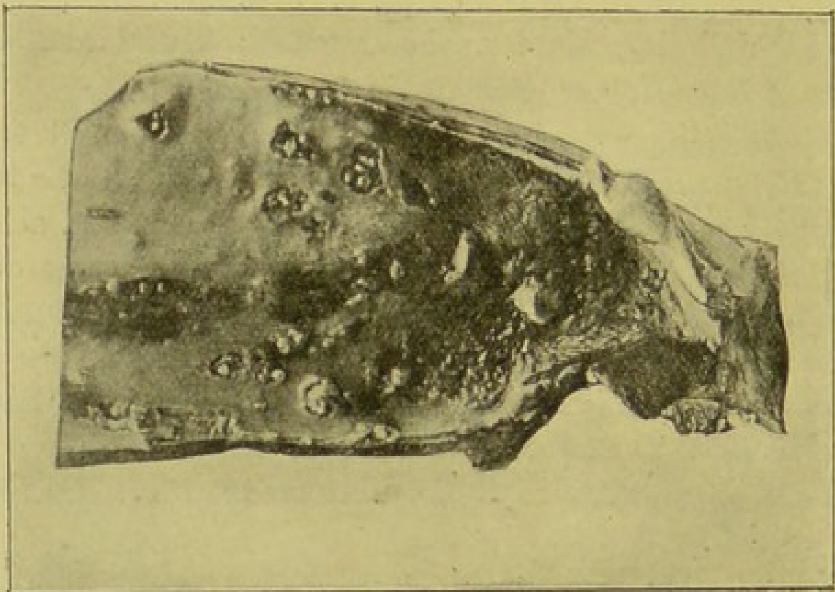


Fig. 39.—Nasal septum of glandered horse, showing ulceration of Schneiderian membrane (McFadyean).

former is a very serious affection, accompanied by high fever, prostration, and delirium, and almost invariably fatal in from two to three weeks. The seat of infection is usually the hand or arm, the nasal mucous membrane being sometimes subsequently involved, and deposits may form in the lymphatic glands, internal organs, and muscles. In the chronic form intra-muscular abscesses are frequent, from the breaking down of which indolent ulcers may result; the disease runs a prolonged course of weeks or even months, and about half the cases end

in recovery. In the early stage an eruption may develop on the forehead and face simulating very closely that of smallpox.

The Glanders Bacillus.

The glanders bacillus (*B. mallei*) is an obligatory parasite with the equine species for its normal host. It hardly grows on artificial media below about 20° C., and

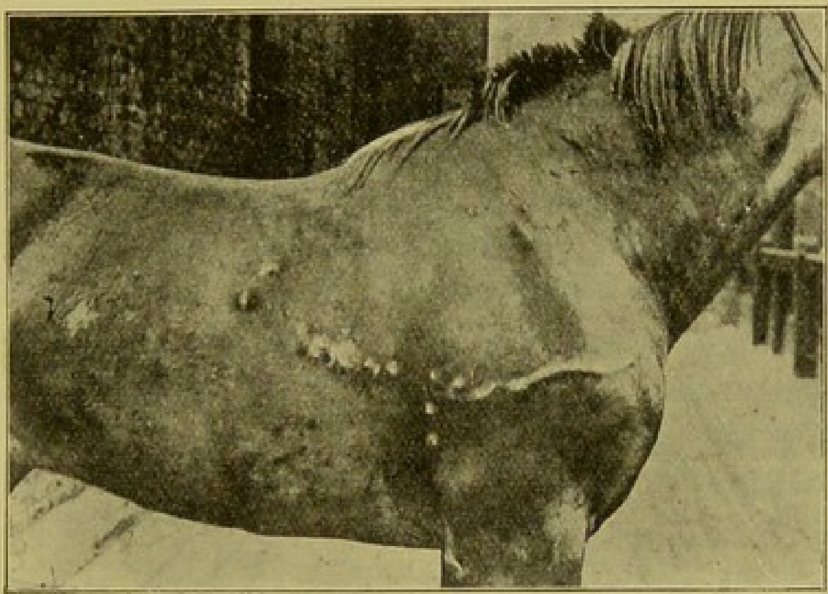


FIG. 40.—Horse affected with farcy (McFadyean).

probably cannot maintain a saprophytic existence outside the animal body.

Morphology.—The glanders bacillus occurs in the tissues as a cylindrical rod with rounded ends, varying between 2 μ and 5 μ in length, and generally straight, though sometimes slightly curved. The bacilli are usually irregularly scattered, and do not tend to form colonies. In stained preparations they often appear more or less beaded, or may exhibit bipolar staining, but some stain uniformly. The bacilli from young cultures not more than twenty-four hours old are almost always short rods, a little thicker than those found in

the lesions (Plate XI., *a*). In old broth cultures the surface growth is largely composed of filaments, which do not show any regular segmentation, but may exhibit lateral branching, and may have club-shaped extremities. From these features some have inferred that the glanders organism belongs to the *Streptothricæ*. The bacillus does not form spores, and is probably non-motile, though in a hanging-drop preparation a very active Brownian movement is present.

Staining reactions.—The bacillus is Gram-negative, *and is not acid-fast*, but from young cultures stains readily with the ordinary anilin dyes. In smears of glanders or farcy material, a simple staining with any of the basic anilin dyes, with subsequent decolorisation with dilute acetic acid, suffices to demonstrate it if it is present in any number, a difficulty in recognising the organism being the presence of deeply staining nuclear detritus. In sections, methylene-blue staining with decolorisation in dilute acetic and mordanting with tannin gives the best results (p. 369). The bacillus shows dark staining dots when treated with osmic acid, suggesting fat-globules (Shattock).

Cultural characters.—The *Bacillus mallei* is an aërobic and facultatively anaërobic organism. The growth on gelatin at 22° C. is scanty and pale brownish in colour without liquefaction. On glycerin agar it forms a thick cream- or slightly brown-coloured growth, and on blood-serum a somewhat amber-coloured growth, which afterwards becomes brownish. The growth on potato at 37° C. is most characteristic, and practically diagnostic. If the surface of the potato is inoculated with a loopful of farcy pus or material from the centre of a glanders nodule, the resulting growth is usually not distinctly visible until the third day, when raised, translucent, viscid, amber-yellow coloured growth or colonies

appear. With continued incubation the colonies coalesce, the growth becomes thicker and fawn-coloured, then reddish-brown, and finally generally chocolate-brown. The growth is also odourless, limited to the site of implantation, and does not stain the potato. Broth or glycerin broth becomes uniformly turbid, and after a week or so patches of a whitish surface scum form, and after three weeks the broth is nearly covered with this surface growth, which is slimy and easily broken up on shaking. Broth cultures give the indole reaction. Litmus glucose agar becomes pink. Milk is not coagulated.

Resistance to Germicides, etc.—The glanders bacillus is but little resistant, and cultures frequently die out in a month or so. Complete desiccation at 37° C. of nasal discharge, farcy pus, or bacilli from cultures, is frequently fatal in twenty-four to forty-eight hours. Young broth cultures are soon destroyed by bright sunlight, and an exposure of ten minutes to a temperature of 55° C. is fatal to the cultivated bacilli. A 3 per cent. solution of carbolic acid, a 1 per cent. solution of potassium permanganate, and a 1 in 5000 solution of corrosive sublimate are fatal in two to five minutes.

Pathogenicity, etc.—The glanders bacillus varies considerably in virulence, and under continued cultivation may become almost non-pathogenic.

Glanders is met with exclusively among horses, asses, and mules, and man is infected from these animals, nearly all cases of human glanders being among ostlers, grooms, and coachmen, and the usual mode of infection is by farcy pus or nasal discharge coming into contact with a cutaneous wound or abrasion. A remarkable immunity, however, is enjoyed by the slaughterers, who have to deal with the carcases of glandered animals, and who might be supposed to run the greatest risk.

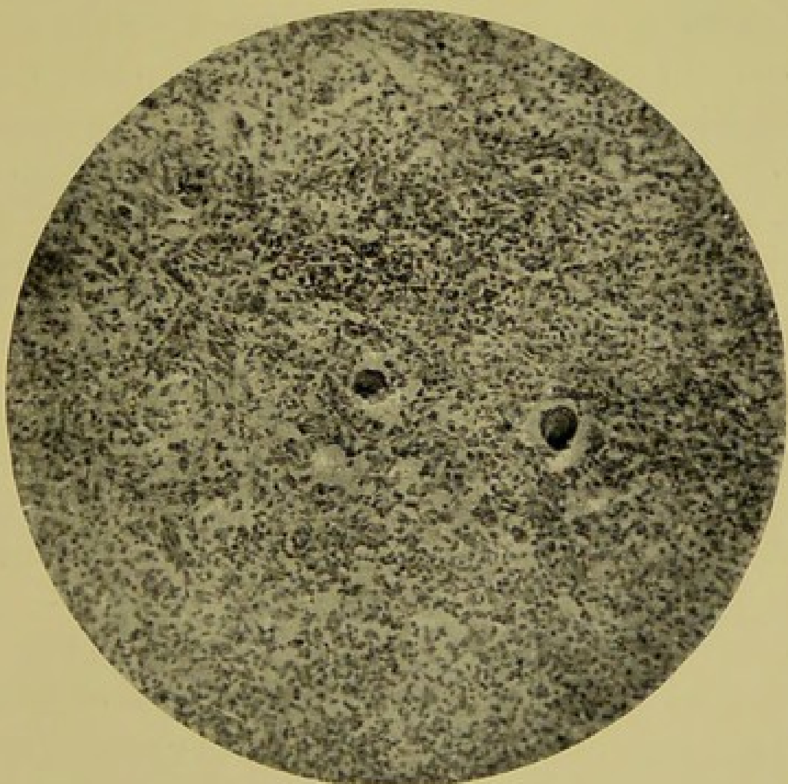
But it must be remembered that Babes frequently found at the post-mortem on persons who had to do with horses, and who died from diseases other than glanders, encapsuled glanders nodules in the lungs and internal organs, suggesting that the disease may often be latent in man, who appears to be relatively insusceptible, and that infection may be possible by inhalation. In the horse glanders is readily transmissible experimentally both by ingestion and by inoculation, and ingestion is probably the common mode of infection naturally, infection by inhalation occasionally occurring. Even when glanders bacilli are administered experimentally by the mouth in the horse, the lesions may be most prominent in, or even be confined to, the lungs. In the horse, the disease has periods of epidemic prevalence, and is particularly frequent in London. In 1892 there were 3000 equine cases in Great Britain, in 1903 there were 2499 cases, and nearly 90 per cent. of all cases occur in the Metropolitan area. These, it is to be noted, were cases in which the disease was well-developed and manifest, but there are also numerous others in which it is latent. Guinea-pigs and field mice are highly susceptible to the disease, which may also be contracted by some of the Carnivora, such as the cat, lion, and tiger, by inoculation or by feeding on diseased carcases. The rabbit, sheep, and dog are but slightly susceptible, while cattle, swine, and house-mice are stated to be immune. Shattock[1] found that the white mouse is somewhat susceptible, and suggests that in all probability the house-mouse is similarly so.

In the horse the most constant seat of glanders lesions is the lung, and McFadyean states that no case of glanders with lesions elsewhere than in the lungs,

[1] *Trans. Path. Soc. Lond.*, vol. lix, 1898, p. 333.

PLATE XI.

a. The glanders bacillus. Cover-glass preparation of a pure culture. × 1000.



b. Section of a glanders nodule, showing giant-cells (after McFadyean).

To face page 364.

and with these organs unaffected, has ever been recorded. In nearly every case of farcy, also, nodules are present in the lungs. The lung lesions have the form of rounded, firm, or shotty nodules. The number present is variable, rarely less than a dozen ; exceptionally there are hundreds, fairly evenly distributed throughout the lung tissue. The nodule commences as a collection of polymorphonuclear leucocytes, around which a zone of congestion is present. Later, the alveolar walls undergo necrosis, and the leucocytes necrose and disintegrate, but their chromatin persists as rounded fragments which retain their affininity for nuclear stains (chromatotexis). The nodule may become surrounded with a layer of thin fibrous tissue, between which and the necrotic central area a zone of endothelioid cells with giant-cells may be present (Plate XI., *b*).

The lesions of farcy are at the onset histologically identical with the glanders nodule, but by the progressive liquefaction of the tissues actual abscesses form.

The lesions set up in an inoculated guinea-pig are very characteristic, and can be used for diagnostic purposes. With a very virulent culture, such as can be obtained by several passages through a susceptible animal, a guinea-pig may die in four or five days, and the post-mortem lesions are slight, consisting of some caseation at the seat of inoculation and slightly enlarged spleen, which contains a few small yellowish nodules resembling miliary tubercles. The material from human cases as a rule seems more virulent than that from the horse, and death of the guinea-pig often ensues a few days after inoculation.

The culture or material from a glandered horse does not usually produce death of a guinea-pig until a lapse of two or three weeks. A male guinea-pig being chosen, the changes observed are caseation

followed by ulceration at the seat of inoculation, when this is done subcutaneously, and great enlargement of the testicles; on cutting into these they are found to be partially or almost entirely converted into a pasty caseous material, while the skin covering them is so adherent that it can only be detached by cutting, and the spleen is very much enlarged and studded with small yellowish nodules. In a female guinea-pig the ovaries are attacked. These appearances are of importance in the diagnosis of the disease. The difficulty of finding the bacillus in the discharges by microscopical and staining methods is so great that these cannot be employed with any certainty. Löffler and Straus therefore recommend the inoculation of a male guinea-pig intra-peritoneally with the discharge or other material. If the glanders bacillus is present the lesions thus described rapidly ensue, and the diagnosis is established in four or five days (Straus's test[1]). At the present time the inoculation method has been almost entirely superseded by the introduction of mallein, the former being reserved for clinical diagnosis in man.

McFadyean found that the blood of a glandered animal produces agglutination or clumping of the glanders bacillus similar to that obtained in the agglutination (Widal) test for typhoid, and has suggested this reaction as a means of diagnosis. As an aid to the clinical diagnosis of the disease in man it is doubtful if the method of serum diagnosis can be applied, for Foulerton found that typhoid and diphtheria sera also produce agglutination of the glanders bacillus.

Toxins.—Mallein, a preparation analogous to tuberculin, is prepared by growing a virulent glanders bacillus for a month or six weeks in glycerin veal-

[1] See also Nicolle, *Ann. de l'Inst. Pasteur*, xx, 1906.

broth in flat flasks such as are employed for tuberculin (Fig. 38), so that there is free access of oxygen. The culture is then autoclaved for fifteen minutes at 115° C., filtered through a Berkefeld filter, concentrated to one fourth of its volume, and mixed with an equal volume of a $\frac{1}{2}$ per cent. solution of carbolic acid. This yields an active mallein, 1 c.c. of which is a dose, and gives a good reaction. Like tuberculin, it possesses feeble curative properties, though a few cases of cure by prolonged use have been reported by Babes and others, but is used for diagnostic purposes; the veterinary authorities are unanimously agreed that it is one of the most certain means we possess for diagnosing glanders in the horse. Injected into an unglandered horse little or no effect is produced, but in a glandered animal, about twelve hours after injection, the temperature rises 1·5° to 3° C. above the normal, a large and painful swelling forms at the seat of inoculation (it may be as large or even larger than half a cocoanut), while any affected lymphatic vessels or farcy buds become swollen. Reaction may, however, be produced in the absence of glanders if the horse is being treated with bacterial products, toxins, etc.[1]

Epizootic lymphangitis has a superficial resemblance to farcy in the horse, and must not be mistaken for the latter (see "Sporotrichosis", Chapter XVI).

The greatest care should be exercised when working with glanders material or cultures, several fatal laboratory accidents having unfortunately happened.

Clinical Examination.

(1) Prepare and stain film preparations of the pus or discharge in Löffler's blue, with subsequent partial decolorisation in 4 per cent. acetic. The ordinary pyogenic cocci will

[1] See Südmersen and Glenny, *Journ. of Hygiene*, vol. viii, 1908, p 14.

not be found unless a secondary infection has occurred, and the material may appear sterile, for the glanders bacilli may be very scanty.

(2) Several tubes of glycerin-agar and potato should be inoculated and incubated at 37° C. for seventy-two hours. On the agar, colonies of the glanders bacillus will develop in twenty-four to thirty-six hours, but the potato will not show the characteristic amber-yellow growth under forty-eight to seventy-two hours.

(3) It will usually be necessary (in man, at any rate) to confirm the diagnosis by an inoculation experiment. A fully developed male guinea-pig is chosen, and a little of the discharge, or an emulsion of the material (0·5 to 1 c.c.) is injected intra-peritoneally, if the material be fairly sterile, but if not, subcutaneously. In three to five days the animal should show the characteristic swelling of the testicles if the material be glandered.

(4) An ophthalmo-reaction is stated to be reliable both in man and in animals.

(5) In animals the mallein test may be applied :

(*a*) The dose is injected subcutaneously in the neck over the vertebræ, and midway between the jaw and the shoulder.

(*b*) If possible the temperature of the animal should be taken morning and evening for two or three days previous to inoculation.

(*c*) The temperature of the animal should be taken at the twentieth hour after inoculation, or, if possible, at frequent intervals from the twelfth to the twentieth hour.

(*d*) A complete reaction comprises (i) a rise of temperature of more than 2·5° F., (ii) an extensive hot and painful swelling at the seat of inoculation.

(*e*) Systemic disturbance, such as prostration, loss of appetite, shivering, etc., may occur.

(*f*) The temperature reaction is unreliable in all cases in which the temperature at the time of inoculation is 2·5° F. above the normal. In such cases, if there be any suspicious clinical signs to assist, reliance may be placed upon the local swelling.

(6) In animals the agglutination reaction is stated by Moore and Taylor[1] to give accurate results. In man this test might give an inconclusive result (see *ante*).

(7) In the tissues the glanders bacillus is difficult to demonstrate. Sections may be stained for half an hour with carbol methylene-blue, treated with 4 per cent. acetic for a few seconds, washed, and rapidly dehydrated with alcohol, cleared and mounted. McFadyean recommends, after treating with the acetic and washing, flooding with a saturated solution of tannic acid in water for fifteen minutes, washing, counter-staining in a 1 per cent. aqueous solution of acid fuchsin for fifteen to thirty seconds, washing, dehydrating, and clearing in cedar oil.

Twort's method may also be employed (see section on *Amœba coli*, "Clinical Diagnosis").

[1] *Journ. of Infect. Diseases*, Sup. No. 3, May, 1907, p. 85.

CHAPTER X.

TYPHOID FEVER—PARA-TYPHOID FEVER—BACILLUS ENTERITIDIS AND THE GÄRTNER GROUP—SWINE FEVER—BACILLUS DYSENTERIÆ—BACILLUS COLI.

The organisms considered in this chapter form a natural group or family, and pass as it were by gradations in cultural characters from the typhoid bacillus to the colon bacillus. Löffler classes them together in a family, the Typhaceæ, which is divided into sub-families by the reactions of the organisms included in it on certain culture media. These culture media are: (1) the *typhoid solution*, an aqueous solution containing 2 per cent. peptone, 1 per cent. nutrose, 1 per cent. grape sugar, 5 per cent. lactose, and 1·5 c.c. per cent. normal potash; (2) the *para-typhoid solution*, having the same composition with the exception that the glucose is omitted. To 100 c.c. of the solution, in each case, 1 c.c. of a 0·2 per cent. solution of chemically pure malachite-green crystals (Hoechst, No. 120) is added.

Löffler's classification is as follows:

Family.—Typhaceæ.

Sub-family (1)—Typheæ. This group does not ferment the typhoid solution, but either leaves it unchanged or precipitates it. The para-typhoid solution is either not fermented, is unchanged, or becomes milky. The following organisms are contained in it: (*a*) *B. typhosus*, actively motile, precipitates the typhoid solution, has no action on the para-typhoid solution. (*b*) *B. dysenteriæ* (Flexner), motile,[1] does not precipitate the typhoid solution, and the para-typhoid solution is unchanged. (*c*) *B. dysenteriæ* (Shiga-Kruse), non-

[1] It is usually stated to be non-motile.

motile, has no action on either solution, but precipitates the typhoid solution if the green be omitted. (*d*) *B. typhosimilis*, motile, does not precipitate the typhoid solution, but renders the para-typhoid solution milky; occurs in impure water and fæces. (*e*) *B. pseudo-dysenteriæ*, does not alter either solution, is not agglutinated by either Flexner or Shiga-Kruse serum.

Sub-family (2)—Josarceæ.[1] This group ferments the typhoid solution with gas-formation and frothing, and alters the para-typhoid solution. It includes the following organisms: (*a*) *B. paratyphosus* A, motile, ferments the typhoid solution, renders the para-typhoid solution a darker green; pathogenic for mice *per os*. (*b*) *B. paratyphosus* B, motile, ferments the typhoid solution, decolorises the para-typhoid solution; occurs in man, ox, sheep, swine, horse. (*c*) *B. typhimurium*, resembles (*b*); pathogenic *per os* for mice and field-mice only. (*d*) *B. Danysz*, resembles (*b*), is agglutinated by *paratyphosus* B serum; pathogenic for rats and mice. (*e*) *B. psittacosis*, resembles (*b*); pathogenic for parrots and man. (*f*) *B. enteritidis-isosarcinus* (Gärtner), resembles (*b*), but not agglutinated by *paratyphosus* B serum; sometimes forms thermostable poisons. (*g*) *B. isosarcinus*, n. sp., from cases of food-poisoning; resembles (*b*), not agglutinated by Gärtner or *paratyphosus* B serum. (*h*) *B. suipestifer*, resembles (*b*); pathogenic.

Sub-family (3)—Coleæ. This group ferments both solutions with gas-formation and frothing. Includes (*a*) *B. coli*, slightly motile or non-motile; ferments both solutions. (*b*) *B. paracoli*.

In addition, all the organisms are agglutinated by their homologous sera, and none liquefies gelatin.

Typhoid Fever.

The specific organism of typhoid fever is a bacillus originally isolated by Eberth in 1880, and more closely studied by Gaffky in 1884.

The Eberth-Gaffky bacillus, or *Bacillus typhosus*, is

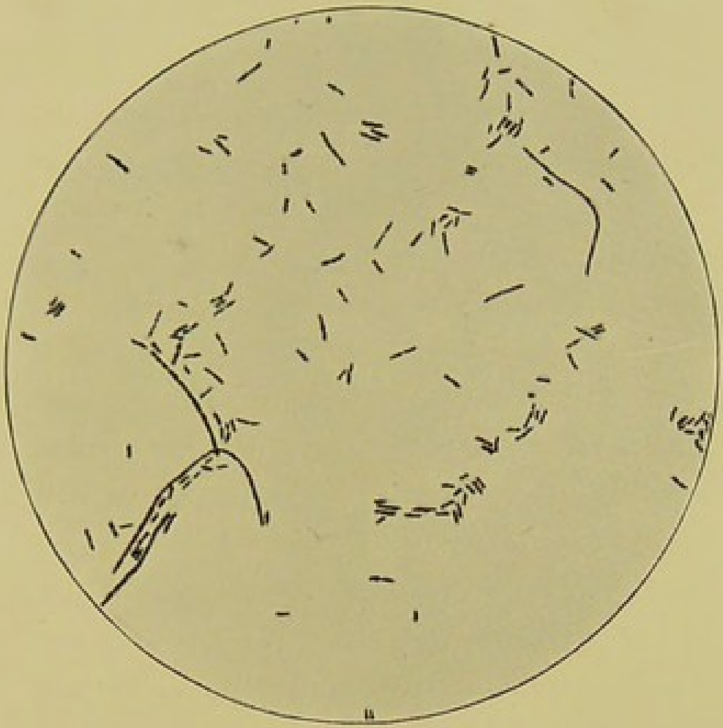
[1] From ἰός, poison, and σάρξ, flesh.

best observed in sections of the spleen, in which it occurs in groups or colonies consisting of short rods with rounded ends, each measuring about 3 μ in length. It has also been demonstrated in the mesenteric glands and liver, in the swollen Peyer's patches before ulceration, and in other situations.

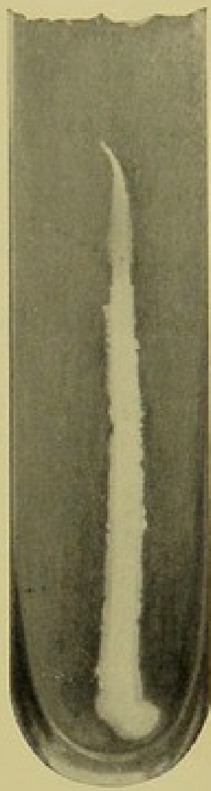
In order to obtain pure cultivations, it is preferable to make use of the spleen. The organ is washed, and then cauterised lineally by means of a red-hot iron, in order to destroy the saprophytic bacteria on and near the surface. An incision is made with a sterilised knife through this cauterised area, and a little of the splenic pulp is taken with a sterilised platinum needle and inoculated on to tubes or plates, preferably of litmus lactose, Conradi-Drigalski, or malachite-green, agar. These are incubated at 37° C. for twenty-four to forty-eight hours, and the growths which develop are examined and tested by microscopical and culture methods. The organism may also be obtained by cultivation from the blood of a patient (p. 389). The following are the characters of the *Bacillus typhosus*.

Morphology.—Bacilli with rounded ends 2 to 3 μ in length, and 0·6 μ broad. It is, however, in cultivation a markedly pleomorphic organism, and very short rods, long rods, and thick filaments 10 to 30 μ in length occur; the latter are known as involution forms (Plate XII., *a*). It does not form spores, but granulation and vacuolation may be observed in the protoplasm.

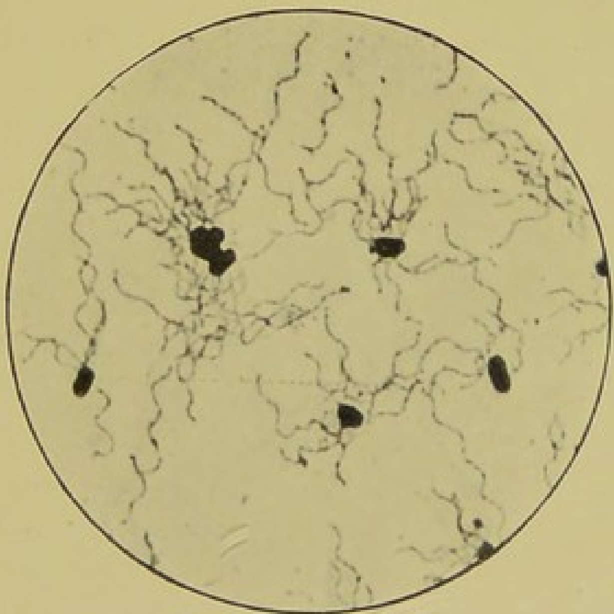
It is actively motile, and possesses a number of flagella, arranged peritrichically both at the poles and sides (Plate XII., *c*). The flagella are long and wavy, and average eight to twelve in number, which is an important point of differentiation from the *Bacillus coli*, which usually has only three or four. It stains by the ordinary anilin dyes, but not by Gram's method. In



a. Bacillus typhosus. Cover-glass preparation of a pure culture. × 1500.



b. Gelatine culture of *B. typhosus*, six days old.



c. Bacillus typhosus. Cover-glass preparation showing flagella. × 1500.

To face page 372.

stained preparations unstained spaces or vacuoles may frequently be seen.

Cultural characters.—The *B. typhosus* is aërobic and facultatively anaërobic, and grows well on the ordinary culture media. On agar it forms a thick, moist, greyish layer. On gelatin it grows slowly, and the growth, which is usually scanty and confined to the needle-track, is white and shining, and somewhat irregular (Plate XII., *b*). The colonies in gelatin are visible in about forty-eight hours, and form small roundish-white points, which are granular and brownish in colour by transmitted light. In broth it produces a general turbidity, without film formation. The growth on potato acid in reaction is somewhat characteristic; it forms a moist, grey, shining layer, which is almost invisible. If, however, the reaction of the potato is neutral or alkaline, the growth may be yellowish. The *B. typhosus* grows well in milk, with slight permanent acidity, but without coagulation.

Acid is formed in small quantity during its growth in many media (volatile fatty acids, and lactic acid), which can be demonstrated by cultivating in litmus milk, or in litmus glucose media, and the organism will grow in slightly acid media. Neither gas nor indole[1] is formed in cultures; acid is produced from glucose, but no gas; lactose is unacted upon. The fermentation reactions on various media are given in the table on p. 400, and are there contrasted with those of the *B. coli* and other organisms (see also p. 405). Chatterjee[2] finds that agar on which the typhoid bacillus has been grown contains substances which inhibit further

[1] Occasionally a feeble indole reaction may be obtained by careful testing.

[2] *Trans. Fourteenth Internat. Cong. of Hygiene* (Berlin, 1907), Bd. iv, p. 34.

development of the organism if it be inoculated on to an agar culture which has been scraped so as to remove all growth.

Pathogenicity.—In cases of typhoid fever in man the *Bacillus typhosus* is widely distributed in the body, in the various tissues, and in the blood, from which it may be obtained by cultivations made from at least 0·5 c.c. (see "Clinical Diagnosis," p. 389). The bacillus is constantly present in the blood from the commencement of the disease, though not in large numbers, and cultures from the blood in competent hands result in the recovery of the organism in approximately 100 per cent. of the cases; in the later stages of the disease it is less frequently recovered.[1] In addition to being present in the Peyer's patches, mesenteric glands, and spleen, the *B. typhosus* has been found in the rose-spots of the eruption, in the sweat, in the sputum and lungs in the pulmonary complications, and in the urine. In the urine it is so frequently present that special disinfection should be practised, more particularly during convalescence, and in some cases it may be so abundant as to produce a turbidity (typhoid bacilluria) and cystitis. It is also pyogenic, and occurs (usually in pure culture) in concurrent or post-typhoidal complications, *e.g.* empyema, abscesses, osteomyelitis, suppurating ovarian cysts, etc. Clumps of bacilli in the gall-bladder have been suggested as the nuclei of gall-stones, and the bacilli may be so numerous in the gall-bladder and bile-ducts as to cause cholecystitis and cholangitis. The organism is by no means easy to isolate from the stools, simple plate cultivations usually fail, and the best medium to employ is the Conradi-Drigalski or malachite-green agar (see "Water").

Injected intra-peritoneally into mice and guinea-pigs,

[1] Coleman and Buxton, *Amer. Journ. Med. Sci.*, June, 1907.

the *B. typhosus* usually produces death, and the same result follows from intra-venous injections in rabbits, but the pathogenic effects so obtained are not specific. By continuous cultivation it loses its pathogenic properties. Given by the mouth no result follows, and the same is the experience of most observers who have fed animals on typhoid stools; a disease process analogous to typhoid fever in man has rarely been induced experimentally. Remlinger[1] states that by feeding young rabbits on vegetables, cabbage, etc., soaked in water, to which had been added some culture of the typhoid bacillus, he has succeeded in inducing a condition resembling typhoid fever in man. The charts which accompany the paper show a typical rise of temperature, a period of pyrexia with morning remission, followed by a typical fall of temperature. The animals suffered from diarrhœa, and their blood gave the agglutination reaction. Post mortem, the intestine was congested and filled with yellow diarrhœic matter, the Peyer's patches were swollen and in some places commencing to ulcerate. The spleen was increased to two or three times its normal size, and cultures of the typhoid bacillus were obtained from it. Metchnikoff[2] has infected the chimpanzee *per os* with typhoid fæces.

The proof of the causal relation of the *Bacillus typhosus* to enteric fever is based on the following facts. It is met with in the tissues in cases of enteric fever, can be obtained from the spleen during life by puncturing with a hollow needle, and may be isolated from the urine and blood during the course of the disease, and is not met with in other diseases. The writer has had under his care three cases, and knows of several others, in which the disease was almost

[1] *Ann. de l'Inst. Pasteur*, xi, 1897, p. 829.

[2] See *Ann. de l'Inst. Pasteur*, xxv, 1911, p. 193.

certainly contracted in the laboratory from working with pure cultures. The blood and blood-serum of an animal immunised against the *B. typhosus* are found to bring about cessation of movement and agglutination or aggregation of the bacilli in a broth culture of the organism. A similar result occurs when the serum of a patient, in the second week of an attack of typhoid fever, acts on the *B. typhosus*, the reaction not occurring with healthy individuals or in other diseases (Plate XIII., *a*). This indicates that in the body of an individual suffering from typhoid fever the same substances are formed as in an animal artificially immunised by cultures of the *B. typhosus*. This reaction is now recognised as a valuable clinical test in doubtful cases of enteric fever (the "Widal" or agglutination reaction[1]).

The agglutination reaction.—For the method of carrying out the agglutination reaction see p. 198. *Normal* serum will generally agglutinate the typhoid bacillus in a dilution up to 1 in 3 or 4, but occasionally is more active. Dead bacilli may be used. The reaction is not obtained before the sixth or seventh day of fever, occasionally not until much later. Very rarely the reaction seems to be intermittent. The blood may retain its agglutinating power for years after an attack, and inoculation with Wright's vaccine also confers agglutinative properties. Cases do occur in which agglutination is absent throughout, but they are rare and often tend to be severe and to terminate fatally. If the blood during the course of an attack fails to give a reaction when tested on three occasions at intervals of three to four days, it is improbable that the case is one of typhoid fever. Moreover, cases

[1] Some controversy has arisen as to the discoverer of this reaction, Grünbaum claims to have first observed it.

occur, simulating typhoid closely, due to infection with the so-called para-colon or para-typhoid bacilli. These "para" bacilli belong properly to the *B. enteritidis* group of organisms (see p. 391). If a positive reaction be obtained, yet the case does not seem to be one of typoid, a previous attack or inoculation with Wright's vaccine must be excluded. The previous injection of a typhoid anti-serum into the patient might induce a non-typhoid infection to give the reaction.

Gwyn[1] found that out of 265 cases diagnosed as typhoid and accurately studied, only one persistently failed to give the reaction. The blood of this case, however, reacted typically with a Gärtner-like organism obtained from the blood (a case, therefore, of para-typhoid infection with all the symptoms of typhoid fever).

Johnson and McTaggart[2] found that typhoid blood dried for sixty days still gives a typical agglutination reaction. An incomplete reaction was occasionally obtained as early as the end of the second day, and the complete reaction was rarely delayed beyond the fifth day. They also noticed that the blood of the horse often produced clumping, etc., of typhoid bacilli, indistinguishable from an agglutination reaction with typhoid blood; but the same agglutinating effect was also produced on the colon bacillus. Many chemical substances also produce agglutination of typhoid bacilli, so that it is necessary to exclude them in making a diagnosis. For example, corrosive sublimate (0·7 : 1000), alcohol, salicylic acid, vesuvin, and safranin (1 : 1000) agglutinate, while carbolic and lactic acids, chloroform, caustic soda, and ammonia do not, the two last only provided the test typhoid emulsion be made

[1] *Johns Hopkins Hosp. Bull.*, vol. viii, 1900, p. 387.
[2] *Brit Med. Journ.*, 1896, vol. ii, p. 629.

with distilled water. Safranin has a powerful agglutinating action on the typhoid bacillus, but not on the colon bacillus.[1]

While there is no constant connection between the activity of agglutination and the severity of the disease, active agglutination tends to go with cases which recover, and cases in which agglutination is feeble or absent tend to be severe.

Toxins.—From cultures of the typhoid bacillus Brieger isolated a base which he termed typhotoxin, and which is isomeric with gadinine. In animals it produced salivation, profuse diarrhœa, paralysis, and death. Brieger and Fränkel isolated from cultures a toxic protein body. Fenwick and Bokenham[2] extracted from spleens of typhoid fever patients a proteose, an alkaloid, and a fatty residue. The proteose produced fever, anorexia, and loss of weight in guinea-pigs and rabbits, but the alkaloid and fatty matter were without effect.

The toxins of the typhoid bacillus, however, seem to be largely intra-cellular, and filtered broth cultures are usually almost non-toxic. Sidney Martin[3] by cultivating in a protein medium was able sometimes to obtain a toxic filtrate, a few c.c. of which produced lowered temperature, diarrhœa and death. Macfadyen and Rowland,[4] by disintegrating large quantities of typhoid bacilli, filtering, and so obtaining the intra-cellular constituents in the filtrate, found that small doses of the latter produced a transient rise of temperature in guinea-pigs and a loss of weight which was soon recovered from. Animals so treated were pro-

[1] Malvoz, *Ann. de l'Inst. Pasteur*, xi, 1897, p. 582.
[2] *Brit. Med. Journ.*, 1895, vol. i, p. 801.
[3] *Ibid.*, 1898, vol. ii, pp. 11 and 73.
[4] *Centr. f. Bakt.*, xxx, p. 753.

tected against a certain lethal dose of typhoid bacilli, and their blood exhibited agglutinative and bacteriolytic properties towards the typhoid bacillus. Macfadyen[1] later obtained the intra-cellular juice of typhoid bacilli by disintegration after freezing with liquid air, and found it to be very toxic to guinea-pigs by intra-peritoneal, and to rabbits by intra-venous inoculation. The writer found that cultures of the *Bacillus typhosus* do not give the "diazo" reaction.

Survival of the typhoid bacillus in the body.—Bacilli may persist in the spleen for weeks, in the gall-bladder for years, and in suppurative lesions for six years or more. Foster and Kayser obtained pure cultures from the gall-bladders of seven out of eight cases, and in 2 per cent. of the cases this "cholecystitis typhosa" becomes a chronic process, and typhoid bacilli may be discharged into the bowel for long periods. Dean[2] found this to be the case in a patient who had had enteric fever twenty-nine years previously. Such "typhoid carriers" have been the subject of much investigation recently.[3] A. and J. Ledingham record three instances met with in an asylum in which mysterious cases of typhoid had occurred—31 cases during fourteen years. Davies and Walker Hall[4] relate similar outbreaks, the carrier in this case being a woman who had suffered from enteric fever in 1901, milk serving as the vehicle of transmission, and a number of other instances have been recorded. Three fourths of the cases are women (and three fourths of the cases of gall-stones occur in women), and usually

[1] *Proc. Roy. Soc. Lond.*, B. lxxi, 1902, p. 77.

[2] *Brit. Med. Journ.*, 1908, vol. i, p. 562.

[3] See Ledingham, *Rep. Med. Off. Loc. Gov. Board* for 1909–10 (Bibliog).

[4] *Proc. Roy. Soc. Med.*, vol. i, 1908, Epidemiolog. Sect., p. 175.

the serum of the carriers gives a marked agglutination reaction, and their stools frequently contain such large numbers of typhoid bacilli that these largely replace the natural bacterial flora of the intestine and may often be recovered from the stools by simple plating. Obviously the typhoid carrier is a source of serious risk to the community, and mysterious outbreaks of enteric fever, ascribed by some in the past to a "*de novo*" origin of the specific organism, become explicable. The typhoid bacillus may occur in the contents of ovarian cysts, usually causing suppuration, and may survive for months—twelve in a case recorded by Taylor[1]—after the attack of typhoid.

Survival of the typhoid bacillus outside the body.—The *Bacillus typhosus* has been isolated in a few instances from WATER SUPPLIES which have become infected, and have given rise to epidemics, as in the case of the Lincoln epidemic in 1905.[2] This is the exception, however, and the isolation of the typhoid bacillus from an infected water is a very difficult matter on account of the fact that the bacillus may have died out before the investigation is commenced, that it is generally in a small minority and admixed with numbers of *coli*-form organisms, and that until recently no medium was available which inhibited the growth of the *coli*-form organisms without at the same time inhibiting the growth of the *B. typhosus*. By the use of malachite or brilliant green media, the last-named difficulty seems to have been overcome (see section on "Water").

In sterilised waters, including distilled water, the *Bacillus typhosus* maintains its vitality for upwards of a month, and in some cases for much longer. The

[1] *Journ. Obstet. and Gynæcol. Brit. Empire*, November, 1907.

[2] *Rep. Med. Off. Loc. Gov. Board* for 1905–06.

survival is not necessarily longer in an organically polluted water than in a pure water. Infecting sterilised Thames water (from the Temple Embankment) and sterilised tap-water of the Chelsea Waterworks with typhoid cultures, the writer found that, examining small quantities (1 c.c.) of the water, the bacillus appeared to die out in the former in two to three weeks, in the latter in four to five weeks.

The survival of the typhoid bacillus in natural waters must be influenced by many circumstances—temperature, chemical composition, struggle for existence with the natural bacterial flora, etc., of the water. Experiments by Russell and Fuller,[1] in which the organism, suspended in collodion sacs, was subjected to the action of lake water, indicated that the maximum was eight to ten days. Houston,[2] using raw Thames, Lee, and New River waters artificially infected with varying quantities of ordinary laboratory typhoid cultures, and examining quantities of 100 c.c. of the water, found that in none of eighteen experiments was a negative result obtained in four weeks, and it was only after nine weeks that the typhoid bacillus could not be isolated from this quantity in all the experiments. But in subsequent experiments,[3] in which typhoid bacilli, obtained directly from the urine of a carrier case by centrifugalising and without culturing, were added to the water, the number of bacilli was reduced by 99·99 per cent. after a week, and after ten days the organism could not be isolated from 100 c.c. of the infected water, indicating that the uncultured bacillus rapidly dies in a natural water, and that even a week's storage of water affords enormous protection

Houston

[1] *Journ. Infect. Diseases*, Sup. No. 2, February, 1902, p. 40.

[2] *First Rep. on Research Work*, Metropolitan Water Board, 1908.

[3] *Sixth Research Report*, Metropolitan Water Board, 1911.

against water-borne typhoid. In aërated (CO_2) waters the *B. typhosus* does not survive a fortnight. The methods of isolation from water are given in Chapter XXI.

The *Bacillus typhosus* may gain access to shell-fish,[1] oysters, mussels, cockles, etc., particularly if obtained from sewage-polluted laying. Such polluted shell-fish may give rise to typhoid epidemics—as at Winchester and Southampton in the case of oysters, and in the case of cockles, derived from the Thames Estuary and imperfectly cooked, to typhoid cases. Buchan found that out of 855 primary cases of typhoid fever occurring in households in Birmingham, 124, or 14·5 per cent., had a history of mussel eating, and in seventeen instances the histories were conclusive of mussel infection. Mussels, under certain conditions (which are not well understood), are liable to develop mytilotoxin, etc. (p. 39), which gives rise to gastro-enteritis. Shell-fish from sewage-polluted layings contain *B. coli* in varying numbers, but from uncontaminated layings are free from this organism, which may therefore serve as an index of pollution (see "Examination of Shell-Fish," Chapter XXI). Contaminated shell-fish, removed to pure water, gradually cleanse themselves—probably after two to three weeks' sojourn. Klein obtained the typhoid bacillus from artificially infected oysters, kept in tanks of sea-water, after nine, sixteen, and even eighteen days from the commencement of the experiment, the oysters showing no abnormal condition.

As regards the vitality of the *Bacillus typhosus* in

[1] On pathogenic organisms in shell-fish see Reports by Bulstrode to the Local Government Board, 1894 and 1911; *Rep. Med. Off. Loc. Gov. Board* for 1899–1900, p. 574; Houston, *Fourth Report of the Sewage Commission*, vol. iii, 1904; McWeeney, *Loc. Gov. Board, Ireland*, 1904; Buchan, *Journ. of Hygiene*, vol. x, 1910, p. 569.

sewage we have little certain information ; probably it tends to die out within a few days. In sterilised sewage inoculated with it the *B. typhosus* hardly multiplies at all, and at the end of ten days has died out. Certain organisms in sewage seemed to have a deleterious action on the *B. typhosus*, hastening its extinction, viz. the *B. fluorescens liquefaciens* and *B. fluorescens stercoralis*. Russell and Fuller, subjecting the bacillus to the direct action of sewage, found the survival to range from three to five days.

In *dry* garden earth, according to Dempster,[1] the *Bacillus typhosus* does not live longer than eighteen days (Firth and Horrocks recovered it up to twenty-five days), and in peat it dies within twenty-four hours. In *moist* soil, however, the bacillus still survived on the forty-second day. In an *artificially* dried soil it was not found alive after the seventh day.

Sidney Martin found that in moist *sterilised* soil kept at temperatures from 3° to 37° C., the *B. typhosus* maintains its vitality for upwards of fifteen months, but that in unsterilised soil it rapidly dies.[2]

Mair[3] concludes that the typhoid bacillus can survive in natural soil in large numbers for about twenty days, and is still present in a living condition after seventy to eighty days, but that there is no evidence that it is capable of multiplying and leading a saprophytic existence in ordinary soil. He suggests that Martin's result (the rapid extinction of the bacillus in unsterilised soil) may be explained by the use of *broth* cultures for infection, the broth added causing a multiplication of the saprophytes. Firth and Horrocks[4]

[1] *Med.-Chir. Trans.*, vol. lxxvii, 1894, p. 263.

[2] *Reps. Med. Off. Loc. Gov. Board* for 1896–1901.

[3] *Journ. of Hygiene*, vol. viii, 1908, p. 37.

[4] *Brit. Med. Journ.*, 1902, vol. ii, p. 936.

similarly conclude that the typhoid bacillus displays no tendency to increase in numbers, nor to grow upwards or downwards in soil, though it may be washed by water through a thickness of 18 inches. Neither virgin nor sewage-polluted soils differed much in these respects.

Vitality of B. typhosus in dust, fomites, etc.—Firth and Horrocks found the *B. typhosus* to be alive in soil dry enough to form dust for as long as twenty-five days, and consider that infective material can be readily transmitted from dried soil and sand by means of winds and air-currents. Doubtless much depends on the degree of dryness of the substratum. From khaki drill and serge inoculated with cultures the bacillus was recoverable for from ten to twelve weeks, and for from ten to seventeen days from the same materials fouled with enteric fæces.

Semple and Greig,[1] with cloth and blanket infected with typhoid urine, failed to obtain the bacillus after seventeen days. This, however, was in India, and the survival of the typhoid bacillus on fomites probably greatly depends on the degree of drying of the material. A striking instance of the conveyance of infection by fomites was that of the blankets used in the South African war and brought to this country, which gave rise to many cases of typhoid fever.

Firth and Horrock demonstrated that house-flies can convey enteric infective material from specific excreta or other polluted material to objects on which they settle or feed, and the Commission which investigated the prevalence of enteric fever in the Spanish-American war ascribed to flies the principal part in the dissemination of the disease (see also p. 411).

There has always been considerable discussion on the

[1] *Sc. Mem. Gov. of India*, No. 32, 1908.

exact relation of "sewer-gas" to disease. It is generally held that sewer-gas is at least a predisposing cause to enteric fever, diphtheria and tonsillitis. Some have considered that the specific organisms are present in the emanations from sewers, and this may occasionally be the case. Thus Horrocks,[1] in some experiments performed at Gibraltar, by pouring sewage artificially infected with typhoid culture down drains, showed that specific bacteria present in sewage may be ejected into the air of ventilation pipes, inspection chambers, drains and sewers by (*a*) the bursting of bubbles at the surface of the sewage, (*b*) the separation of dried particles from the walls of pipes, chambers and sewers, and probably by (*c*) the ejection of minute droplets from flowing sewage. "Sewer-gas" may also lower vitality and increase susceptibility. Thus Alessi found that animals exposed to drain emanations are at first more susceptible to infection, but after a month or so acquire tolerance and are no more susceptible than animals kept under ordinary conditions. There is no evidence that sewer-men or those employed at sewage-works suffer from ill-health.

Action of heat, germicides, etc.—The *B. typhosus* in broth culture is killed by a temperature of 53°–54° C. in half an hour, and of 56°–60° C. in ten minutes. It is readily destroyed by antiseptics. (*See* table, Chap. XXII.

Semple and Greig (*loc. cit.*) found bright sunlight to be germicidal in from two to six hours.

Wines and spirits have some germicidal action on the typhoid bacillus. Champagne destroys the bacillus in ten minutes, white wines in fifteen to twenty minutes, red wines in thirty minutes or thereabouts. If diluted with water the germicidal action takes much longer to accomplish, and the acidity, not the alcohol content,

[1] *Journ. Roy. San. Inst.*, May, 1907, p. 176.

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seems to be the active factor.[1] Spirits, such as whisky or brandy, if diluted with not more than one to two times the volume of water, kill in ten to twenty minutes.

Anti-typhoid serum.—Attempts have been made to prepare an anti-typhoid serum by inoculating horses with increasing doses of typhoid bacilli, first killed (by heat, chloroform, etc.) and then living, but such sera have proved quite useless.

Macfadyen[2] prepared an endotoxic serum by treating horses with the endotoxin obtained by triturating the bacilli in the presence of liquid air. The writer continued the work, and obtained a serum which gave promising results.[3]

Chantemesse,[4] by cultivating a virulent strain of the typhoid bacillus in a special broth made with ox spleen, heating the culture to 55° C., centrifugalising and injecting horses with the fluid, obtains a serum which he claims has marked curative properties, the mortality being 4·3 per cent., as against 17 per cent. for those subjected to ordinary treatment. The patients receive very small doses of the serum—five or six drops—and the dose is repeated only two or three times. This dosage is quite different from that of an ordinary anti-toxic or antimicrobic serum, and Wright suggested that *toxins* (and not anti-bodies) in the serum may be the active agents. Chantemesse has accepted this view, and the treatment, therefore, seems to be a vaccine one.

The disease has also been treated with a *vaccine* (consisting of a killed culture) with promising results

1 Sabrazès and Marcandier, *Ann. de l'Inst. Pasteur*, 1907.

2 *Proc. Roy. Soc. Lond.*, B, vol. lxxi, 1903, pp. 76 and 351; *Brit. Med. Journ.*, 1906, vol. i, p. 905.

3 See Hewlett, Goodall and Bruce, *Proc. Roy. Soc. Med.*, vol. ii, 1907–08 (Medical Section), p. 245 *et seq.*; and Hewlett's *Serum Therapy*, p. 220.

4 *Trans. Fourteenth Internat. Cong. Hygiene and Demography*, 1907.

by Semple, Smallman, Leishman, and others. The initial dose is 40–100 millions, and the amount is cautiously increased up to 300–400 millions.

Anti-typhoid vaccine.—Wright first prepared an anti-typhoid vaccine by the following method.[1] A typhoid culture of moderate virulence (the virulence being kept up by intra-peritoneal passage through guinea-pigs) is grown in peptone beef broth in flasks at 37° C. for from fourteen to twenty-one days. The flasks are then so heated that their contents attain, and remain at for a few minutes, a temperature of 60° C. To obtain uniform toxicity, the contents of several flasks should be mixed, and to safeguard the vaccine from contamination one twentieth of its volume of 10 per cent. lysol is added. Various ingenious devices have been adopted by Wright and Leishman to prevent contamination and for standardisation.

The strength of a typhoid vaccine depends upon the number of bacilli it contains, and on the particular strain of bacillus used. The vaccine is standardised by counting the number of bacilli it contains by Wright's method (p. 230). Leishman[2] now cultivates for about forty-two hours, and the bacteria are killed by heating to 53° C. for one hour, the higher temperature having proved to be deleterious, and 0·4 per cent. of lysol is then added; it is not necessary to employ a virulent bacillus. In the early days the symptoms produced by the inoculation were often severe, but with the use of improved methods are now hardly appreciable. Two doses of the vaccine should be given, with an interval of about ten days between the two, the doses being 500 and 1000 millions respectively.

Inoculation is now being extensively practised, and

[1] Wright and Semple, *Brit. Med. Journ.*, 1897, vol. i, p. 256.

[2] See *Journ. Roy. Inst. Pub. Health*, vol. xviii, 1910, pp. 385, 449, 513.

Leishman (*loc. cit.*) gives the following statistics of its value: total number under observation, 18,483–19,314; average period under observation, twenty months; number inoculated, 10,378; number uninoculated, 8936; case-incidence of enteric per 1000, inoculated 5·39 $\pm$ 0·48, uninoculated 30·4 $\pm$ 1·23; case-mortality per 100, inoculated 8·9, uninoculated 16·9. Other forms of vaccine have also been devised.

Variation of the B. typhosus.—Allusion has already been made to Twort's work on the "education" of *B. typhosus* to ferment lactose, and on the apparent conversion of *B. typhosus* into *B. alkaligines* by Horrocks (p. 6). Penfold also records variations in the fermentive powers of *B. typhosus* (*Journal of Hygiene*, vol. xi, 1911, p. 30).

Relapses.

Various hypotheses have been advanced to account for the relapses which occur in typhoid and other diseases (*e. g.* Malta and relapsing fevers). Chantemesse and Widal[1] showed that if the *B. typhosus* is injected into an animal together with toxins of the streptococcus, *B. coli*, or *Proteus*, its virulence is enhanced, or the animal's resistance may be lowered. If, then, immunising and bactericidal properties of the blood and tissues are but slightly acquired during the attack, an absorption of toxic substances from the alimentary tract may be sufficient to give the typhoid bacilli still present a fresh start, and so produce a relapse. This Sanarelli[2] was able to do experimentally. Wright and Lamb formulated another hypothesis.[3] The organisms in typhoid, Malta, and relapsing fevers, are deposited in the spleen and internal organs, multiply and form colonies there, which become protected

[1] *Ann. de l'Inst. Pasteur*, vi, 1892, p. 755.

[2] *Ibid.*, vi, 1892, p. 721; and *ibid.*, viii, 1894, p. 193.

[3] *Lancet*, 1899, vol. ii, p. 1727; *Sc. Mem. Med. Officers of Ind. Army*, pt. xii.

from the bactericidal substances by the formation of a non-anti-bacterial envelope. When the anti-bacterial substances in the blood and lymph have increased to such an extent as to penetrate and abolish the non-anti-bacterial envelopes which surround these colonies, the production of toxins will be so diminished that the temperature will fall. If, however, for some reason or other, even a single colony escapes the full anti-bacterial power of the lymph, owing, it may be, to being shut off in a capillary which has become blocked, or in some other part not freely infiltrated by the blood- or lymph-streams, the bacteria of this colony will go on multiplying until the blood has become modified in such a manner as to bring about a diminution of the anti-bacterial substances, and thus render a relapse possible.

A third theory has been suggested by Durham.[1] He regards a given infection as due to the "result of the action of a sum of a number of infecting agents, each of which is similar but not identical in its nature," the apparently simple infection being "in reality a complex phenomenon brought about by a number of varieties and sub-varieties of the given microbe." He suggests, therefore, that in a typhoid infection a particular race of typhoid bacilli is in excess, and when the anti-bodies for this particular race have been formed in sufficient quantity, the disease process comes to an end. There may, however, be present at the same time other races which have produced little of their specific anti-bodies; these then begin to grow and multiply, and a relapse ensues.

In the case of relapsing fever the organism is probably a protozoon, and in this and other protozoan diseases relapses coincide with developmental cycles of the parasite, *e. g.* in malaria.

Clinical Diagnosis.

(1) *Blood cultures.*—Three to 5 c.c. of blood are withdrawn from a superficial vein with a syringe with aseptic precautions, and 0·5 c.c. of the blood so obtained is sown into each of

[1] *Journ. Path. and Bact.*, vol. vii, 1901, No. 2, p. 240.

several tubes containing 15 to 20 c.c. of sterile broth. The tubes are incubated at 37° C., and if organisms develop these are isolated and examined culturally for the typhoid bacillus. Coleman and Buxton recommend the following culture medium: Ox-bile 90 c.c., glycerin 10 c.c., and peptone 2 grm. Distribute in small flasks, 20 c.c. in each, and sterilise. Each flask is inoculated with 2 to 3 c.c. of blood, incubated for eighteen to twenty-four hours, then streaks from each are made on to litmus lactose agar plates, which are incubated for a few hours. If the growth does *not* redden the medium and a typhoid-like bacillus is present, it is tested for agglutination with typhoid-immune serum.

(2) *Agglutination reaction.*—This is carried out by the microscopic or the macroscopic (sedimentation) method described at p. 198. Dilutions of 1 : 30 and 1 : 50 should be made, and some make a 1 : 100 in addition. The microscopic method is the more rapid. Various apparatus (agglutinometers) can be obtained, consisting of measuring devices and a supply of dead culture, with which the sedimentation test can be carried out by anyone, and seem to be satisfactory.

(3) *Ophthalmo-diagnosis.*—Chantemesse (*loc. cit.*) has devised a method analogous to the ophthalmo-diagnosis for tuberculosis (p. 349). The material is prepared from agar cultures of typhoid which are emulsified, dried, triturated, and extracted, and the extract is precipitated with absolute alcohol and dried (for details see Hewlett's *Serum Therapy*, p. 382). The dry substance is powdered in an agate mortar, and for use 8 to 10 mgrm. are dissolved in 1 c.c. of sterile water. Of this solution a drop is instilled into the conjunctival sac; in a case of typhoid, after a lapse of two to three hours the conjunctiva becomes red and there is a sensation of heat, after six to ten hours there is a marked conjunctivitis, which may persist for one to three days and then passes off. In healthy persons and in other diseases no conjunctivitis ensues. A cutaneous reaction has also been devised.

(4) *Puncture of the spleen with a sterilised hypodermic needle and syringe.*—A little of the blood and pulp is withdrawn with the syringe, and cultivations are made as in (1). This

method seems hardly justifiable, and now that the blood-culture method and agglutination reaction have been introduced should be discarded.

(5) *Examination of pus.*—Cultivations may be made as in (1) if the bacillus is present, apparently in pure culture. If not, plate cultivations, preferably on litmus lactose agar, Conradi-Drigalski, malachite- or brilliant-green, agar, may be prepared (see "Water").

(6) *Examination of the stools.*—This is hardly practicable for clinical diagnosis; it takes too long, is tedious and uncertain. Plate cultivations from the diluted stools are made on Conradi-Drigalski, malachite- or brilliant-green, agar (see "Water").

The Gärtner or Enteritidis Group of Bacilli.

The Gärtner group of bacilli, of which the type is the *B. enteritidis* of Gärtner, are bacilli morphologically resembling the *B. typhosus*, *i. e.* they are pleomorphic, actively motile, multi-flagellate, non-sporing, and non-Gram-staining, but culturally are intermediate between *B. typhosus* and *B. coli*. Thus, like *B. coli*, they ferment glucose with the production of gas and acid and change neutral red; like *B. typhosus* they do not attack lactose and do not curdle milk. In litmus milk they usually first produce slight acidity, followed after three to four days by a change to alkalinity, and the milk ultimately becomes limpid. The fermentation reactions of some members of the Gärtner group are given in the table on p. 400. The organisms of the Gärtner group may be divided into four sub-groups:

1. *Enteritidis group.*—Produce acute gastro-intestinal disturbance in man. The cause of epidemic meat-poisoning, *e.g.* the *B. enteritidis* of Gärtner.

2. *Pneumonic group.*—Produce pneumonic symptoms in man. The cause of some outbreaks of epidemic pneumonia, *e.g. B. psittacosis.*

3. *Paratyphoid group.*—Produce a disease resembling

typhoid fever in man. May also produce "food-poisoning" with gastro-enteritis. Subdivisions A or α and B or β.

4. *Group non-pathogenic to man, e. g. B. typhi murium.*

The Bacillus enteritidis.

A number of outbreaks of what has been termed "epidemic meat poisoning" have been traced to infection with the *B. enteritidis*. (See also "Food Poisoning," Chap. XXI.) The disease takes the form of an acute gastro-enteritis—urticaria, abdominal pain, vomiting, diarrhœa, nervous symptoms and collapse—occuring from eight to thirty-six hours after partaking of a meat meal, usually pork (sausage, pork-pie, ham), occasionally beef and tinned meat. The principal outbreaks of this nature have been those at Jena, in 1888, investigated by Gärtner, and from which he isolated the type form of the *B. enteritidis*; Welbeck in 1880; Middlesborough in 1888; Mansfield in 1896; and Derby in 1902. A small outbreak occurred at Bedford in 1907.[1] These outbreaks are usually caused by varieties of the *B. enteritidis* having the general characters of the group, which usually do not ferment lactose, and are distinguishable by agglutination reactions and fixation tests, the organism isolated as a rule agglutinating well with the patient's serum.

The *B. enteritidis* in morphology, motility, and staining reactions resembles the *B. typhosus*, forms no, or only traces of, indole, and changes neutral red to a fluorescent yellowish colour. Litmus milk after a faint acidity becomes alkaline, and is converted into a thin watery translucent fluid, without coagulation. It does not attack either salicin or glycerin. The fermentation reactions are given in the table on p. 400. Savage[2]

[1] *Public Health*, vol. xx, 1907–8, p. 310.

[2] *Rep. Med. Off. Loc. Gov. Board* for 1909–10, p. 446.

obtained this organism from only one out of fifty-three specimens of human excreta examined. A number of variants were isolated from various materials, some fermenting salicin, some glycerin, and some both these substances (See "Meat," Chap. XXI).

Swine Fever or Hog Cholera.[1]

Swine fever, or hog cholera (to be distinguished from swine erysipelas, which see), is an infective disease of pigs, highly contagious, and causing considerable mortality. The duration of the affection is usually three to four weeks; the animals lie about, their temperature is raised, and they may suffer from cough and frequent respiration, and some lameness in the hind legs. Towards the end mucous diarrhœa is a prominent symptom. Post mortem, the large intestine is found to be ulcerated, the ulcers much resembling the typhoid ulcers of man, and according to Klein, pneumonia is commonly present, whence he termed the disease "pneumo-enteritis." McFadyean, however, from his own experience and that of the Board of Agriculture, considers pneumonia very infrequent. The ulcers occur mainly in the cæcum and colon, and are due to a well-defined circular necrosis involving the whole thickness of the mucous membrane and occasionally extending to the wall of the bowel. A diffuse diphtheroid lesion also occurs, due to a superficial necrosis with deposition of a thin layer of fibrinous exudate on the surface of the mucous membrane. All gradations are found between the well-defined circular necrosis and the diffuse diphtheroid lesion.

An organism constantly present is a member of the paratyphoid sub-group of the Gärtner group (*B. suicholeræ* or *suipestifer*, apparently identical with *B. aertryck*), but it seems to be a terminal infection and not the true ætiological agent, as the blood and tissues filtered through a porcelain filter are still infective—*i.e.* the organism is probably ultra-

[1] See Uhlenhuth, *Trans. Fourteenth Internat. Cong. of Hygiene* (Berlin, 1907), Bd. iv, p. 50; *Journ. Roy. Inst. Pub. Health*, 1911.

microscopic. Some confusion exists in the nomenclature of the disease. Swine fever is the British, and hog cholera the American, name. In addition, a disease of swine was formally described under the designation " swine plague " (" Schweine-seuche," Schütz). This clinically much resembles swine fever, but pneumonia is a prominent lesion, and a non-motile, stumpy, bi-polar staining bacillus belonging to the group of the hæmorrhagic septicæmic bacilli is present (see under " Chicken Cholera "). This is now regarded as a secondary infection and the disease as being really swine fever. The *B suicholeræ* is apparently identical with the *B. icteroïdes* of Sanarelli. (See also Chap. XIX.)

Although the lesions are very similar, swine fever has nothing to do with typhoid fever of man, nor with ulcerative colitis.

Other organisms belonging to the Gärtner group are:

1. The Danysz bacillus, used as a virus for exterminating rats (the Danysz virus).

2. The *B. icteroïdes* of Sanarelli, supposed by him to be the cause of yellow fever, but apparently identical with the *B. suicholeræ* (see " Yellow Fever," Chap. XIX).

3. The *B. typhi murium* of Löffler, used as a virus for exterminating mice.

4. The *B. psittacosis* of Nocard, causing an infective disease of parrots and transmissible to man (bird-fanciers, etc.), in whom it produces a severe and often fatal broncho-pneumonia.

5. *Summer diarrhœa.*—Morgan[1] concluded that the summer or epidemic diarrhœa of infants is not caused by the dysentery bacillus (see p. 401). In 50 per cent. of the cases he isolated a bacillus which appears to be most closely allied to the hog-cholera bacillus, differing from the latter by producing alkalinity in litmus milk (without previous acidity) and much indole, and by failing to produce acid and gas from mannitol, arabinose, maltose, and dextrin. Eyre and Minett[2]

[1] *Brit. Med. Journ.*, 1906, vol. i, pp. 908 and 1131; *ibid.*, 1907, vol. i, p. 16.

[2] *Brit. Med. Journ.*, 1909, vol. i, p. 1227.

examined the normal faces of sixty young children, and in four only isolated a bacillus allied to the Morgan bacillus. The method of isolation was by means of plates of bile-salt agar containing 1 per cent. of mannitol and coloured with neutral red. (See also Chap. XX.)

Para-typhoid Fever.[1]

The name "para-colon" bacillus was given by Gilbert in 1895 to races of bacilli intermediate in type between the typhoid bacillus and the colon bacillus, and this designation was also applied by Widal and Nobécourt to a bacillus isolated by them from an abscess in the neighbourhood of the thyroid. The name "para-typhoid" bacillus appears first to have been used by Archard and Bensaude in 1896, and was reintroduced by Schottmüller in 1901, and would seem to be the preferable designation for those micro-organisms that produce typhoidal symptoms.

Para-typhoid fever may be defined as a disease much resembling typhoid fever in its clinical aspect, which is, however, caused, not by the typhoid bacillus, but by organisms belonging to the para-typhoid sub-group of the Gärtner group of bacilli. Para-typhoid infections sometimes occur in epidemics, may be spread by drinking-water and by "carriers," and occur in all parts of the world.

Para-typhoid bacilli are also occasionally the pathogenic agents in cases of "food poisoning" with gastro-enteritis, particularly *B. suipestifer* (or *aertryck*).

The para-typhoid bacilli are morphologically like the typhoid bacillus and are actively motile, but they ferment glucose with the production both of acid and of gas. A number of races have been isolated differing

[1] See Savage, *Rep. Med. Off. Loc. Gov. Board* for 1908-9, p. 316; Bainbridge and O'Brien, *Journ. of Hygiene*, vol. xi, 1911, p. 68 (Bibliog.).

from one another in their source, rate of fermentation of glucose, action on milk, action on neutral red, and agglutination reaction, and are distinguished by the names of those who isolated them.

Two groups of the para-typhoid may be distinguished which have been termed A and B by Buxton. Group A produces less gas in glucose media than group B; with group A milk remains permanently acid; with group B it becomes alkaline after a transient acidity; and though group A changes neutral red to yellow, the red colour tend to return after three weeks or so, while with group B the yellow colour is permanent. That is to say, in its reactions group A is allied more closely to the typhoid bacillus than group B.

B. paratyphosus A or *a* is rarely found, the vast majority of cases of para-typhoid fever being associated with the presence of the B or *β* type. The fermentation reactions of some of the para-typhoid bacilli are given in the table on p. 400.

As regards the agglutination reaction, the blood of the para-typhoid fever patient either does not agglutinate the typhoid bacillus or agglutinates it only in low dilution—*e.g.* 1 in 10 to 40, while it agglutinates the para-typhoid bacilli in far higher dilution—*e.g.* 1 in 100 or 200, or even higher; thus in Cushing's case the patient's serum agglutinated the para-typhoid bacillus isolated from it up to 1 in 8000.

The diagnosis of para-typhoid fever would be based on (*a*) the agglutination reaction; (*b*) the isolation of a para-typhoid bacillus by cultures from the blood (p. 389).

Bacillus dysenteriæ.

In one type of dysentery, the so-called epidemic or bacillary form (see "Dysentery," Chap. XX), a bacillus,

B. dysenteriæ, is the causative agent. The *B. dysenteriæ* includes a group of closely allied organisms.

The dysentery bacillus was first isolated in 1897 by Shiga in Japan. Somewhat later Kruse isolated an almost identical bacillus in Germany, and this type is known as the Shiga-Kruse type. Later, Flexner and Strong isolated another type of the dysentery bacillus, and during the last few years similar organisms, but differing from the Shiga-Kruse and Flexner types in some of their fermentation and other reactions, have been isolated; these are sometimes termed "pseudo-dysentery" bacilli.

The Shiga-Kruse and other types of dysentery bacilli have been isolated by Flexner and Strong in the Philippines, Park, Duval, Bassett, Martini, Hiss, Russell and others in the United States, Castellani in Ceylon, Rogers and others in India, Ruffer and Willmore in Egypt (El Tor), and Eyre, McWeeney and others in the British Isles.

Morphology.—The *B. dysenteriæ* are small slender bacilli much resembling the colon bacillus. They are non-motile, but Brownian movement is often active,[1] Gram-negative, and non-sporing, and are readily destroyed by heat (58°–60° C.) and antiseptics.

Cultural characters.—The dysentery bacilli are aërobic and facultatively anaërobic. On agar a thinnish creamy growth develops; on gelatin a white growth nearly limited to the inoculation track, and without liquefaction. The colonies on a gelatin plate closely resemble those of the typhoid bacillus. On potato the growth is either thin, grey and slightly visible, or thicker and yellowish or brownish. The colour of neutral red media is unaltered. Litmus milk first becomes faintly acid,

[1] Flagella have been described by some observers, but cannot usually be demonstrated.

then markedly alkaline; no clotting. Indole is generally not formed; occasionally a trace may be detected. All strains ferment glucose with the formation of acid only, no gas; none ferments lactose. Some strains (the Flexner type) ferment mannitol with the formation of acid only, no gas; other strains (the Shiga-Kruse type) have no action on this alcohol. The principal fermentation and other reactions are given in the table on p. 400. These reactions are very variable with different stains, but differentiation may be accomplished by agglutination, saturation, and complement fixation, tests.

Agglutination reaction.—The agglutination reaction is given by the blood of patients suffering from the bacillary form of dysentery, but not by the amœbic form (unless a double infection be present, which occasionally is the case). The agglutination reaction is obtained in dilutions of 1 in 10 to 1 in 100, but may occur only with the particular strain causing the infection.[1] Thus by the agglutination reaction variations between different strains of the *B. dysenteriæ* may be detected.

Pathogenic action.—The organism seems limited to the bowel and its mucous membrane and does not gain access to the blood. No characteristic lesions are produced in animals by administration of the dysentery bacillus *per os*. In man, cultures given by the mouth are stated to have induced a typical dysentery. Animals such as rabbits, guinea-pigs and mice are very sensitive to injections of living and killed cultures; in fact, it is very difficult to immunise animals against the organism. Amounts of 0·1–0·2 mgrm. of an agar culture given intra-venously or intra-peritoneally are fatal to these animals.

In man the organism is abundant in the bloody

[1] See Hewlett, *Trans. Path. Soc. Lond.*, vol. lv, 1904, p. 51.

mucoid discharge from the bowel, and at an early stage is easy to isolate by means of Conradi-Drigalski agar plates, on which it forms small blue colonies; at a later stage (after two to three days) the other organisms in the bowel multiply to such an extent that isolation may become very difficult. "Carriers" occur and help to spread the disease, which may be conveyed by infected water and food and by flies.

Toxins.—The filtrate of dysentery cultures (four to six weeks old) in a somewhat highly alkaline broth (broth just alkaline to litmus + 7 c.c. normal NaOH per litre) is markedly toxic, 0·1 c.c. being a fatal dose for a large rabbit.[1]

Anti-serum and vaccine.—The serum of horses immunised with the toxin, or with dead and then with living cultures, possesses marked antitoxic properties, and the use of this antitoxic serum has been successful in cases of acute bacillary dysentery. Shiga obtained a reduction in mortality from 22 to 7 per cent. by the use of serum in a severe epidemic, and striking results were obtained by Ruffer and Willmore[2] in Egypt. It is necessary, however, to employ a serum prepared with the particular strain of the disease.

When the disease has become chronic the use of a *vaccine*, consisting of a culture sterilised by heat, is sometimes beneficial. Castellani also suggests the use of a vaccine for prophylactic purposes.

Para-dysentery bacilli.—In the dysenteries of Ceylon, Castellani[3] has sometimes isolated dysentery bacilli nearly related to the Shiga-Kruse type, but showing differences from it in agglutination, persistence of acid

[1] Todd, *Journ. of Hygiene*, vol. iv, 1904, p. 480 (Bibliog.).

[2] *Brit. Med. Journ.*, 1909, vol. ii, p. 862, and 1910, vol. ii, p. 1519.

[3] *Journ. of Hygiene*, vol. iv, 1904, p. 495.

[1] *Fermentation Reactions of Organisms belonging to the Typhoid-Coli group, with others.*

Organism.	Glucose.	Laevulose.	Maltose.	Galactose.	Arabinose.	Raffinose.	Lactose.	Cane-sugar.	Mannite.	Sorbite.	Dulcite.	Adonit.	Dextrin.	Starch.	Inulin.	Litmus Milk.	Liquefaction of Gelatin.	Indole.	Motility.	Glucose $\frac{H}{CO_2}$	Gas Percentage in Glucose.	Voges-Proskauer's Reaction.
B. typhosus	A	A	A	A	—	A	—	—	A	A	—	...	A	—	—	A	—	—	+	...	...	—
B coli	+	+	+	+	+	+	+	∓	+	+	+	—	+	—	—	AC	—	+	±	$\frac{2}{1}$	25	—
B. enteritidis	+	+	+	+	+	+	—	—	+	+	+	...	+	—	—	A Alk.	—	∓	+	$\frac{1+}{1}$	...	—
B. para-coli (Day)	+	+	+	+	+	+	—	—	+	A	A	...	+	...	...	A Alk	—	...	+	$\frac{1+}{1}$	...	—
B. para-typhosus (Schottmüller, A and B, and Brion and Kayser)	+	+	+	+	+	+	—	—	+	+	+	...	+	...	...	A Alk.	—	...	+	$\frac{1+}{1}$	...	—
B. dysenteriæ (Shiga)	A	A	A	A	—	A	—	—	—	—	—	...	—	—	—	A Alk.	—	∓	—	...	...	—
B. dysenteriæ (Flexner)	A	A	A	A	A	A	—	—	A	—	—	...	A	—	—	A Alk.	—	...	—	...	...	—
B. icteroïdes (Sanarelli)	+	+	+	+	A	+	—	—	+	+	A	...	+	—	—	A Alk.	—	...	+	$\frac{1+}{1}$	...	—
B. suicholeræ	+	+	+	+	A	A	—	—	+	+	A	...	+	...	...	A Alk.	—	...	+	$\frac{1+}{1}$	...	—
B. capsulatus (Pfeiffer)	+	+	+	+	+	+	+	+	+	+	—	+	+	±	—	...	—	+	—	$\frac{2}{1}$	25	+
B. lactis aërogenes	+	+	+	+	+	+	+	+	+	+	—	+	+	±	—	AC	—	+	—	$\frac{2}{1}$	25	+
B. acidi lactici (Hüppe)	+	+	+	+	+	+	+	—	+	+	—	+	+	—	—	AC	—	+	—	$\frac{2}{1}$	15-30	—
B. cloacæ (Jordan)	+	+	+	+	+	+	+	+	+	+	—	...	+	—	—	AC	+	—	+	$\frac{1}{1}$	75	+
B. pneumoniæ (Friedländer)	+	+	+	+	+	+	+	+	+	+	+	+	+	...	...	A ±C	—	+	—	$\frac{3}{1}$	30	—

A = acid production only. C = curdling of milk. $\frac{A}{Alk.}$ = first acid, then alkaline.
\+ = acid and gas production, or positive. — = no change in medium, or negative. ... = not tested.
± = sometimes acid and gas production, sometimes no change; or sometimes positive, sometimes negative.

See MacConkey, *Journal of Hygiene*, vol. v, 1905, p. 333, and vol. vi, 1906, p. 385.

reaction in litmus milk, and virulence; these he has termed "para-dysentery" bacilli.

Asylums dysentery and summer diarrhœa of infants.—Both in America and in England some cases of summer diarrhœa of infants are found to be associated with the *B. dysenteriæ* (see above, p. 394). The asylums or institutional dysentery, or ulcerative colitis, is also due to this organism, and the blood of patients gives the agglutination reaction.[1] In both instances the *B. dysenteriæ* present is of the Shiga-Kruse type.

Bacillus coli.

The *Bacillus coli*, or colon bacillus (*B. coli communis*), is an organism of considerable importance, both in connection with the *Bacillus typhosus*, in pathological processes, and in water supplies as an indication of pollution. As its name implies it is a constant inhabitant of the intestinal tract in man and animals (except perhaps in certain arctic animals), and is one of the most widely distributed organisms in nature. While the term "colon bacillus" is applied to a fairly well-defined organism (the "typical *B. coli*"), there are a number of allied organisms differing from the type in one or more characters—*e. g.* motility, indole production, fermentation reactions, rate and extent of milk curdling, etc.—and these varieties are said to belong to the "colon group," or are termed "coliform."

The *B. coli* may be readily isolated by inoculating litmus lactose bile-salt peptone-water tubes with a trace of a suspension of fresh fæces, growing for from twenty-four to forty-eight hours at 42° C., and plating the culture on litmus lactose agar, on gelatin, or on Conradi-Drigalski agar, or by direct plating of the

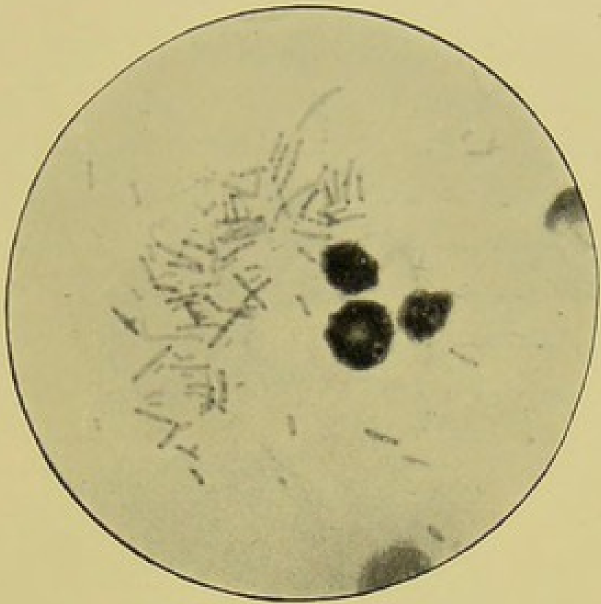
[1] Hewlett, *Trans. Path. Soc. Lond.*, vol. lv, 1904, p. 51.

fæces suspension on the last-named medium (see also "Water").

Morphology.—The *B. coli* is a short rod with rounded ends, 2 or 3 μ long and 0·4 to 0·6 μ broad, frequently linked in pairs or more. It is often so short that it is merely ovoid in shape; and, on the other hand, longer individuals and involution forms occur 10 μ or more in length (Plate XIII., *b*). It is feebly motile, and possesses lateral flagella to the number of three or four on an average, which are shorter and straighter than those of the typhoid bacillus. It is sometimes met with in a diplococcoid form, which by cultivation in ascitic fluid may become fixed. Capsulated forms have been described.

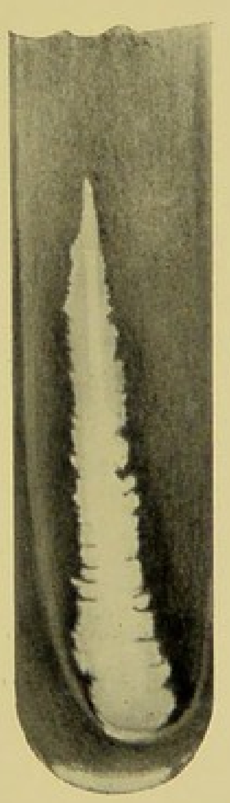
Spore-formation does not occur, but vacuolation may sometimes be observed. The organism is well stained by the ordinary anilin dyes, but is Gram-negative.

Cultural characters.—The *B. coli* is aërobic and facultatively anaërobic, and grows readily on the ordinary culture media from 20° to 37° C. In gelatin plates the colonies are visible in twenty-four to forty-eight hours. The deep colonies are spherical, granular, and of a pale brownish colour, darker at the centre than at the periphery. The superficial colonies are at first punctate, round and almost transparent, but subsequently spread on the surface and may attain a diameter of 3 mm., their margins become irregular, the surface is smooth, they are finely granular, opalescent in appearance, and are thicker at the centre than at the periphery (Fig. 41). On a gelatin streak a copious white, shining, smooth growth develops, the margins of which are irregular and crenated (Plate XIII., *c*), and in old cultures the medium becomes opalescent. In a gelatin stab-culture a white growth develops along the line of inoculation with one or more gas-bubbles. The gelatin



a. The agglutination reaction. A clump of typhoid bacilli. × 1500.

b. Bacillus coli. Cover-glass preparation of a pure culture. × 1000.



c. Gelatine culture of *B. coli*, six days old.

To face page 402.

is not liquefied. On agar and on blood-serum a thick, moist, shining, greyish layer forms. There is abundant formation of gas in a stab-culture in glucose-agar and in gelatin shake cultures (Fig. 42), provided the medium be made with meat; "lemco" gelatin, however, generally fails to give gas. On acid potato it forms a straw-yellow or brownish-yellow, moist, thick growth, but if the potato is not fresh and acid in reaction the

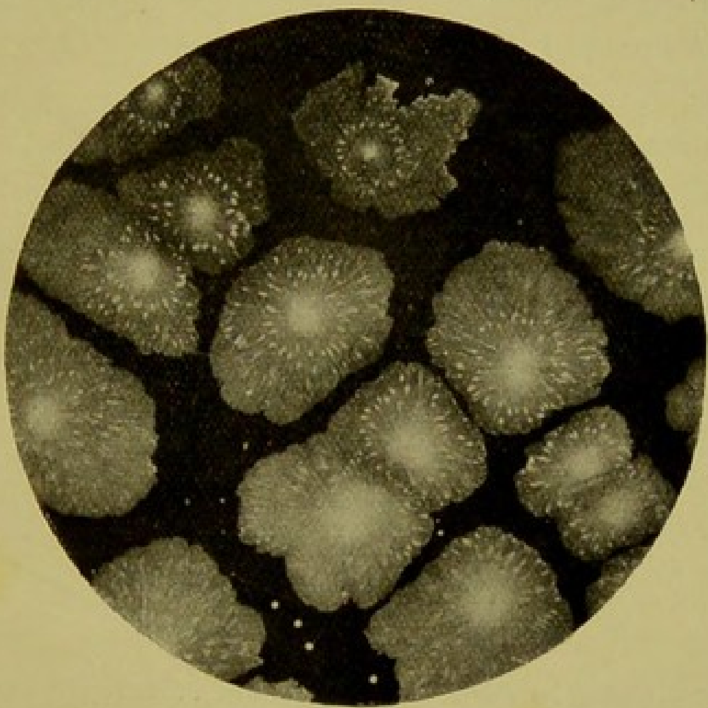


Fig. 41.—Colonies of the colon bacillus, superficial and deep.

growth may be colourless. Milk is a good culture medium, and is curdled in twenty-four to seventy-two hours. This curdling is principally due, not to an enzyme, but to the formation of a considerable amount of lactic acid, though a milk-curdling enzyme has been described by Savage[1] as being formed under certain conditions. The gas which is produced in culture media under anaërobic conditions consists of hydrogen

[1] *Journ. Pathol. and Bact.*, November, 1904.

and carbon dioxide. Under aërobic conditions marsh gas is stated to be also formed. The ratio of H to CO_2 is about 2 : 1 for dextrose and lactose. In broth it produces a general turbidity without film formation, and the culture gives the indole reaction on the addition of a nitrite in twenty-four to forty-eight hours.

The fermentation reactions are given in the table, p. 400. It will be seen that the *B. coli* is an active fermenter of many carbohydrates, alcohols, and gluco-

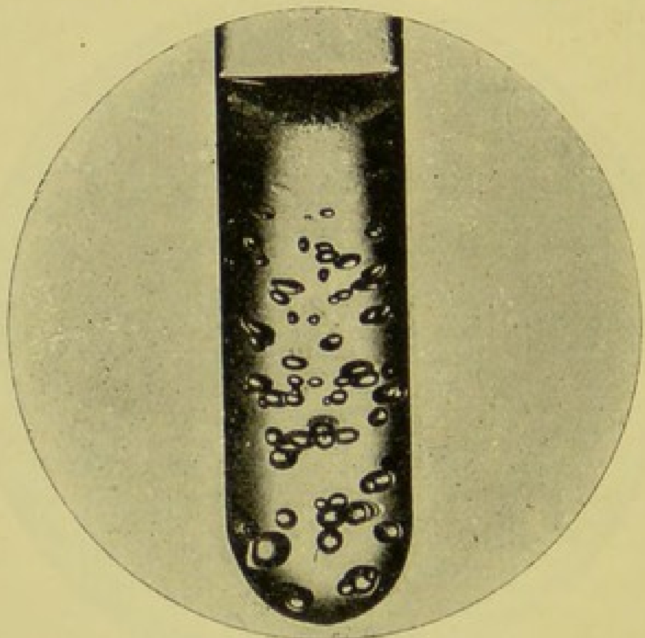


Fig. 42.—Colon bacillus. Gelatin shake culture showing gas production.

sides,[1] *e. g.* glucose, lactose, galactose, mannitol and dulcitol, but not of adonit. Cane-sugar may or may not be fermented; sometimes only acid is formed, sometimes both acid and gas are produced. To the variety producing both acid and gas from cane-sugar Durham gave the name *B. coli communior*. Prescott and Winslow consider that the term *B. coli* should be applied only to an organism that does not attack ketonic sugars. Neutral red in glucose broth is

[1] See Twort, *Proc. Roy. Soc. Lond.*, B, vol. lxxviii, p. 329.

changed to a fluorescent yellow, and Houston describes a typical *B. coli* as "flaginac," *i. e.* producing fluorescence in neutral red glucose peptone-water (fl), acid and gas from glucose (ag), indole in peptone-water (in), and acid and curd in milk (ac). The colonies on Conradi-Drigalski agar are large and red (see "Water"). The *B. coli* does not give the Voges-Proskauer reaction (p. 410).

The differentiation of the *B. coli* from the *B. typhosus* should present no difficulty if the morphology and motility of the organisms and their fermentation and agglutination reactions be compared. Bacteriologists usually make use of the following tests for the differentiation of *B. coli*: (1) Morphology, (2) motility, (3) Gram staining, (4) character of growth and colonies on gelatin, (5) non-liquefaction of gelatin, (6) action on milk, (7) indole formation, (8) fermentation of glucose, (9) fermentation of lactose and saccharose, (10) action on neutral red. MacConkey suggests that instead of tests Nos. 4, 6, 7, 8, and 10, the following should be substituted: (*a*) fermentation of dulcitol, but not of adonit and inulin; (*b*) the Voges-Proskauer reaction.

Other media which have been recommended for the differentiation of *B. coli* from *B. typhosus* are the Proskauer-Capaldi media and Petruschky's litmus whey, but are not now much used.

The Proskauer-Capaldi medium No. 1 is an asparagin-mannitol solution with certain salts; medium No. 2 is a peptone-water-mannitol solution. Both solutions are carefully neutralised and tinged with litmus.

If these media be inoculated with *B. typhosus* and *B. coli* respectively and incubated at 37° C. for twenty-four hours, the following changes will be noted:

	Medium No. 1.	*Medium No.* 2.
B. typhosus	No growth or change in reaction.	Growth with strongly acid reaction.
B. coli .	Growth with acid reaction.	Growth with neutral or faintly alkaline reaction.

Petruschky's litmus whey is prepared as follows: Fresh milk is warmed and the casein precipitated by the addition of a minimal amount of hydrochloric acid. It is filtered, and the filtrate of clear whey is carefully neutralised with dilute caustic soda solution. The fluid is then steamed for two hours and filtered; the filtrate should be clear, colourless, and neutral in reaction. Enough neutral litmus solution is then added to render it well coloured, and the mixture is distributed into test-tubes and sterilised. This medium is rendered slightly acid (represented by 6–10 c.c. N/10 caustic soda per cent.) by *B. typhosus*, very acid (40–50 c.c. ditto) by *B. coli*.

The thermal death-point of the organism, according to Weisser and Sternberg, is 60° C. with an exposure of ten minutes. The *B. coli* will grow freely in a slightly acid medium, and in media containing as much as 0.15 per cent. of carbolic acid. In this respect it is a more resistant organism than the *B. typhosus*.

Chemical products.—The acids produced are mainly lævo-lactic acid with some dextro-lactic acid from glucose, lævo-lactic acid only from mannitol; also acetic, formic and succinic acids, and alcohol. According to Harden, *B. coli* attacks glucose in a characteristic manner, each molecular proportion of sugar yielding half a molecular proportion of acetic acid and of alcohol, and one molecular proportion of lactic acid, together with a small amount of succinic acid, and gaseous carbonic acid and hydrogen.[1] Nitrates are reduced to nitrites.

[1] See also Revis, *Centr. f. Bakt.* (2te Abt.), xxvi. 1910, p. 161.

No toxin, or a trace only, is formed in cultures, but the dead bacilli are toxic and pyogenic, and a toxin is obtained by autolysis of cultures or by triturating the bacilli with liquid air (Macfadyen).

Vaughan,[1] by washing large quantities of colon and typhoid bacilli, extracting the bacterial cells first with alcohol, then with ether, and then digesting the ground residue with alcohol containing 2 per cent. NaOH, states that two constituents are obtained, one soluble in alcohol and toxic, the other insoluble in alcohol and non-toxic. The latter confers a certain degree of immunity on animals injected with it.

Pathogenicity.—The pathogenic action and pathogenicity of the *B. coli* are very varied. Introduced into the circulation or into the peritoneal cavity in guinea-pigs or rabbits it usually causes death in from one to three days with a general septicæmia. Some varieties are, however, non-virulent to animals.

In man the colon bacillus is associated with a number of important pathological processes. It is usually the organism causing the peritonitis which is due to infection from the intestine, as in hernia with obstruction or perforation, in ulceration of the bowel and enteritis, in cancerous growths, and affections of the appendix, biliary canals, and gall-bladder. The exudation in these cases is often characteristic; at first it is clear and greenish, it then becomes greenish-yellow, thin, semi-opaque and foul-smelling, and finally purulent. An important point is that the colon bacillus may pass through the intestinal wall where it has been damaged, as by strangulation, but not yet perforated.

The *B. coli* is a pyogenic organism, and has been

[1] *Trans. Fourteenth Internat. Cong. Hygiene* (Berlin, 1907), Bd. iv, p. 28.

met with in ischio-rectal abscesses (probably the *B. pyogenes fetidus* of Passet). Possibly it causes in some instances the pneumonia and pleurisy occurring after peritonitis, for it has been obtained from the lung and pleura in these conditions, but it must be recognised that the *B. coli* is a common secondary or terminal infection. Puerperal fever is another condition sometimes caused by the *B. coli*, and cystitis and infections after urinary operations are also commonly due to it.

In the Pictou cattle disease, characterised by extensive hepatic cirrhosis, Adami found a minute diplococcus or short bacillus. A similar form was afterwards isolated by him in hepatic cirrhosis in man. Miss Abbot,[1] from a study of several such cases, came to the conclusion that this organism is a variety of the *B. coli*. It has been suggested that hepatic cirrhosis is produced by poisons or toxins, *e. g.* of the *B. coli*, and that alcoholism, the usual cause assigned, is but an exciting or secondary agent.

Anti-serum and vaccine.—Attempts have been made to prepare an anti-serum for *B. coli* infections but they have met with little or no success.

A *vaccine* prepared by sterilising cultures by heat and standardising has been used successfully in the treatment of chronic *B. coli* infections, *e.g.* cholangitis, cholecystitis, pyelitis, and cystitis. The *B. coli* vaccine is more toxic than most vaccines, and small doses must therefore be given (see p. 232).

Clinical Examination.

(1) The appearance and odour of the pus are often characteristic. Smears of the pus show small bacilli, which are decolorised by Gram's method.

[1] *Journ. Path. and Bact.*, vol. vi, 1900, No. 3, p. 315 (Bibliog.).

(2) The organism may be isolated by plating on gelatin, agar, litmus lactose agar, Conradi-Drigalski agar, or by the use of neutral red or bile-salt media (see "Water"). The isolated organism must be tested as to its morphology motility, non-Gram staining, non-liquefaction of gelatin, indole production, curdling of milk, and fermentation of glucose, lactose, dulcitol, mannitol, etc.

(3) An agglutination reaction may likewise be tried, but if negative is of little value, as there are so many varieties of the colon bacillus, and one variety may not be agglutinated by the specific serum obtained with another variety. A positive reaction must also be carefully controlled, as the colon bacillus is much more readily agglutinated by normal serum than is the typhoid bacillus.

Varieties of Bacillus coli.

Organisms are frequently met with in fæces, manure, sewage and polluted water which resemble the typical *B. coli* in many of their characters, but which differ from it in certain particulars. Thus the colonies on gelatin, instead of being smooth, may be wrinkled; milk may be but slowly curdled (three to eight days); acid or gas production, or both, in sugars may be less marked than usual. These organisms are generally regarded as varieties of the *B. coli*, and are perhaps derived from typical *B. coli*. There is, however, little evidence that *B. coli* can be transformed into such varieties, or that these varieties can be reconverted into typical *B. coli*. Revis (*loc. cit.*) has obtained evidence of alterations of fermentive power, and in the characters of the colonies of certain coliform organisms.

Organisms that have been Regarded as Variants of B. coli.

A number of organisms have been regarded as being varieties of the *B. coli* (consult table of fermentation reactions, p. 400).

(1) *Bacillus cavicida* (Brieger).—This resembles *B. coli* in most of its characters, but was stated to be non-motile. MacConkey says it is motile.

(2) *Bacillus neapolitanus* (Emmerich).—Isolated from the bowel in cases of cholera. It differs from *B. coli* by not being motile, and by fermenting cane sugar.

(3) Gas-forming bacilli of Laser and Gärtner.[1]

(4) Aërobic bacillus of malignant œdema (Klein).

(5) *Bacillus lactis aërogenes* of Escherich.—Found in the intestine of nurslings. Much like *B. coli*, but is non-motile. It differs from *B. coli* by not fermenting dulcitol, by fermenting saccharose and adonit, and by giving the Voges-Proskauer reaction (see table, p. 400). According to Harden and Walpole,[2] its action on glucose differs from that of *B. coli*, more alcohol being produced and formed at the expense of that part of the molecule of the sugar which in the *B. coli* fermentation yields acetic and lactic acids.

The Voges-Proskauer reaction is obtained by growing the organism in 2 per cent. glucose broth in a fermentation tube (Fig. 17, p. 85) for three days and adding some strong caustic potash solution; on standing exposed to the air a pink colour develops. According to Harden and Walpole[3] the reaction is probably due to acetylmethyl-carbinol, which in the presence of air and potash is oxidised into diacetyl, which then reacts with some constituent of the peptone in the medium, giving the pink colour.

The *B. lactis aërogenes* (which may be classed among the capsulated bacilli, see p. 271) is occasionally pathogenic, causing peritonitis.[4] In these circumstances, it is capsulated, but the capsule is difficult to stain.

(6) *B. cloacæ* (Jordan).—Met with in sewage. In general characters it much resembles *B. coli*, but produces more gas

[1] *Centr. f. Bakt.* (1te Abt.), xiii, 1893, p. 217, and xv, 1894, pp. 1 and 276.

[2] *Journ. of Hygiene*, vol. v, 1905, p. 488; *Proc. Roy. Soc. Lond.*, B, vol. lxxvii, 1906, p. 399.

[3] *Proc. Roy. Soc. Lond.*, B, vol. lxxvii, 1906, p. 399.

[4] See Churchman, *Johns Hopkins Hosp. Bull.*, vol. xxii, 1911, p. 116.

(75 per cent.) from glucose and liquefies gelatin in four to five to thirty days. Like *B. lactis aërogenes*, saccharose is always fermented and the Voges-Proskauer reaction is positive, but neither dulcitol nor adonit is fermented. (See table, p. 400.)

Flies as Carriers of Infection.

Flies and other "insects" may convey infection (1) by infecting food, etc., (2) by direct inoculation, (3) by inoculation after a cycle of development—in which case the carrier is more or less specific; *e. g.* anopheline mosquitoes in malaria. In the first method the organisms are generally bacteria, occasionally ova of worms; in the second, bacteria or protozoa; in the third, invariably protozoa, filaria, etc., *i. e. animal* organisms.

The ordinary domestic fly, the blue-bottle and other similar flies (of which there are many) have no biting proboscis, but undoubtedly carry infection by infecting food, etc., directly by organisms upon various parts of their body, or by the organisms passing through the digestive tract and infecting the food with the fæces. In this way, typhoid, bacillary dysentery, *B. enteritidis*, summer diarrhœa, cholera, and possibly anthrax may be conveyed.

The ordinary house-fly breeds in dung and garbage containing dung, and it has a possible range of flight of about a mile. The house-fly experimentally infected remains grossly infected for at least three days, and a smaller degree of infection persists for ten days or even longer.[1]

[1] See *Reports to the Loc. Gov. Board on Flies as Carriers of Infection*, Nos. 1-4, 1910 and 1911.

CHAPTER XI.

BUBONIC PLAGUE—CHICKEN CHOLERA—MOUSE SEPTICÆMIA.

Bubonic Plague.

PLAGUE was epidemic throughout Europe during the Middle Ages; in England in the fourteenth century it appeared as the Black Death, and in the seventeenth century as the Great Plague of London, while numerous other lesser visitations have been recorded. For some years plague has been practically pandemic. The disease seems always to have been endemic in certain centres, *e.g.* in Asia Minor, on the Persian Gulf, in Yunnan, in Uganda, etc. A characteristic of plague is the manner in which it appears and remains prevalent for a time in a district and then disappears, to reappear again after a considerable interval; this has happened not only in Europe, but also in Persia, Syria, India, and China.

Three main types of the disease are recognised, the bubonic in which the femoral (rarely the inguinal), axillary and other glands become enlarged (whence the disease derives its name), the septicæmic, and the pneumonic. In India the disease has been mainly bubonic (70 per cent. of the cases). Occasionally the majority of the cases are pneumonic, as was the case in Accra, in China in 1910–11, and in the small outbreak in Suffolk in 1910. Septicæmic cases are the

exception, but any form tends to become septicæmic on the approach of death.

At the commencement and at the end of an epidemic the disease may assume an extremely mild type, the so-called "pestis minor."

Bacilli were first observed in this disease in the blood, buboes, and organs by Kitasato in 1894. In the same year (1894) Yersin investigated the outbreak

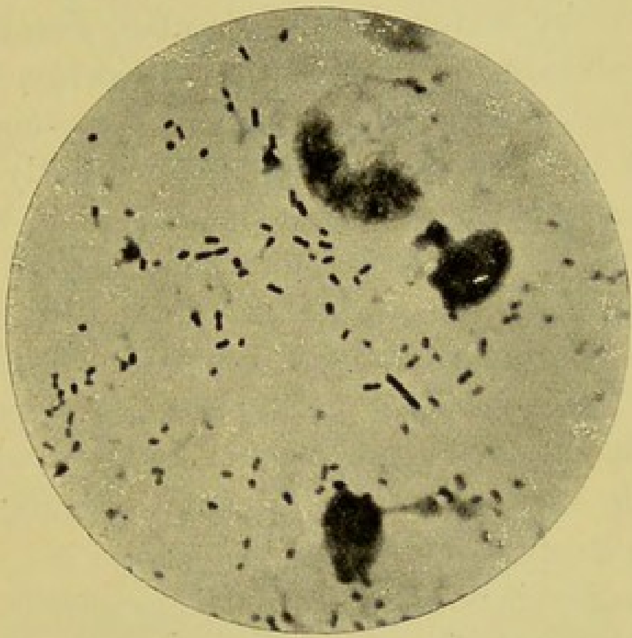


Fig. 43.—Plague. Smear preparation from spleen of inoculated guinea-pig. × 1000.

of bubonic plague at Hong Kong, and described the bacillus met with in the buboes and its cultural and pathogenic properties very fully. This organism is known as the *Bacillus pestis*.

Morphology.—The *B. pestis* belongs to the group of hæmorrhagic septicæmic bacilli (chicken cholera, rabbit and ferret septicæmia, swine plague, etc., see p. 427), and is a markedly pleomorphic organism. *In the animal body* it occurs for the most part as a short, plump, non-sporing rod, measuring 2–3 μ by 1–2 μ,

but longer forms may be seen here and there measuring as much as 5 μ (Fig. 43). Polar staining is a marked feature (Plate XIV., *a* and *b*). Occasionally swollen involution forms occur. The typical form of the organism, the bi-polar staining, short, stumpy bacillus, is met with in smears from the buboes, in the sputum in the pneumonic form, and in the blood in the septicæmic variety, but only in the earlier stages of the disease. Later the typical forms tend to disappear, their place being taken by a few large, rounded, ovoid, or pear-shaped involution forms. *Under cultivation* the bacilli in young cultures (twenty-four to forty-eight hours) are so short as to be almost coccoid or slightly ovoid; on agar their size is about the same as that in the animal body, on gelatin they are somewhat smaller, but a few well-marked rods and even threads are always present. In older cultures, rod, thread and involution forms occur more numerously; on agar containing 2–3 per cent. of salt the latter are swollen and yeast-like.

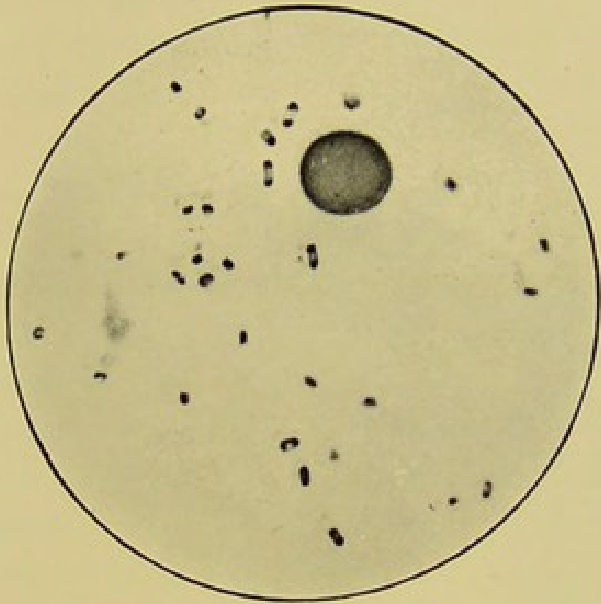
In broth chains of slightly ovoid organisms occur resembling streptococci (Plate XV., *a*).

The organism is non-sporing and non-motile, although Gordon described the presence of one or two fine spiral terminal flagella (others have not found flagella).

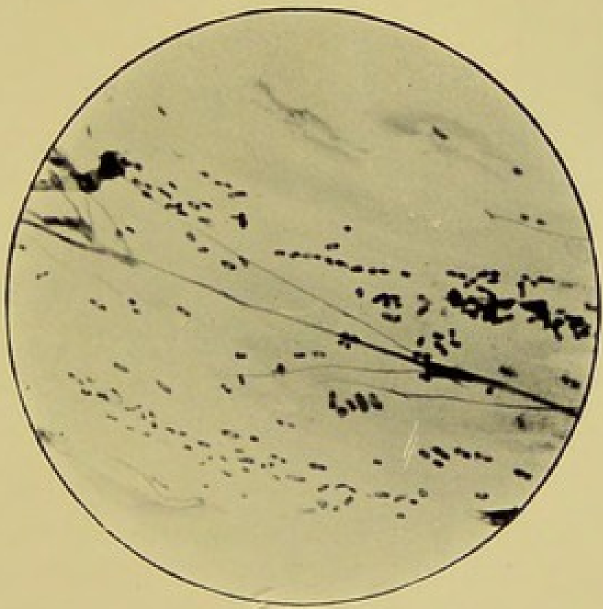
Sometimes in hanging-drop cultivations a capsule is apparently present, but the writer has failed to verify this by staining methods.

The *B. pestis* stains well with Löffler's blue and anilin-gentian violet, polar-staining being a marked feature, especially in smear preparations. It does not stain by Gram's method. With old laboratory strains polar staining may be completely absent, but in such cases may sometimes be obtained by first treating the preparations with alcohol or by the Gram

PLATE XIV.



a. Bacillus pestis. Smear preparation from a bubo. × 1000.



b. Bacillus pestis. Smear preparation of sputum. × 1000.

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method, and subsequently staining with Löffler's blue or weak gentian violet. Sections are best stained with carbol methylene or thionine blue.

Cultural characters.—The *B. pestis* is aërobic and facultatively anaërobic. On blood-serum it forms moist, smooth, shining, cream-coloured colonies or growths, slightly raised above the surrounding medium. The blood-serum is not liquefied.

On agar the colonies are raised, round and cream-coloured, finely granular, denser at the centre than at the margins, which are regular. Size 0·25 to 0·5 mm. in two days at 37° C.

On surface agar the *B. pestis* forms a thick, opaque, moist, smooth, cream-coloured growth, the margins of which are usually markedly crenated (Fig. 44); the growth is very sticky and tenacious. Haffkine states that when grown on *dry* agar (agar which has been kept in the warm incubator for two to three weeks) and viewed from behind the growth has an appearance like that given by the back of a mirror—*i. e.* a dull, silvery appearance.

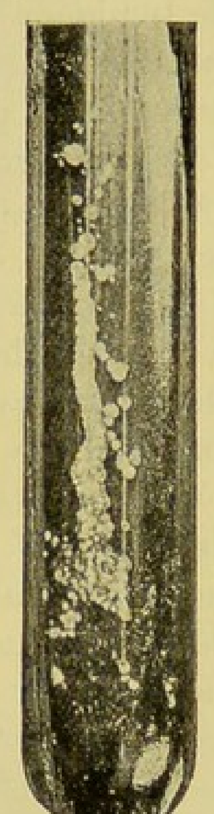


Fig. 44. — Plague, surface culture on glycerine agar, forty-eight hours old.

On a salt agar (2·5–3·5 per cent. of sodium chloride) Hankin describes the development of remarkable spherical or pear-shaped involution forms.

On gelatin the colonies are whitish, filmy, finely granular with regular margins. Size, 0·1 to 0·25 mm. in five days at 22° C.

On surface gelatin the organism forms a thin, white,

granular growth, with slightly irregular surface and margins, and nearly confined to the inoculation track. The growth does not penetrate into the medium, nor does it render it cloudy. The growth is very adherent.

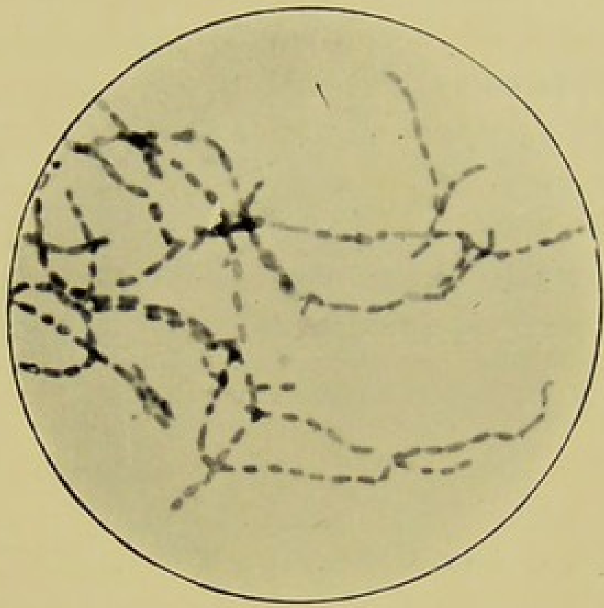
In a stab gelatin culture a delicate whitish, finely granular growth develops to the end of the stab, with little tendency to spread from the needle track. The gelatin is not liquefied. Both in agar and gelatin cultures fresh punctate growths sometimes develop in the original growth, simulating a contamination. No growth occurs on ordinary potato, and milk is not coagulated.

In broth the growth is somewhat characteristic. For two or three day the broth remains perfectly clear, but a flocculent growth forms and gradually increases in amount on the bottom and sometimes upon the sides of the tube. After some days the broth may become a little cloudy. A delicate flocculent film develops if the tube be kept absolutely at rest. In broth to which a little butter-fat or ghee has been added little islands of growth appear on the surface, and from these flocculent tapering dependent growths form in about a week, provided the tubes or flasks be kept absolutely at rest, the bulk of the broth remaining clear. This is the stalactite growth of Haffkine, and is very characteristic (*B. pseudo-tuberculosis* also gives it). Broth cultures reduce a weak solution of methylene blue.

With sulphuric acid alone a feeble indole reaction can be obtained with week-old broth cultures. With sulphuric acid and a nitrite a well-marked indole reaction can be obtained under the same conditions.

The fermentation reactions of the *B. pestis*, which MacConkey has pointed out are practically identical with those by the *B. pseudo-tuberculosis*, are as follows: Acid production, but no gas, in glucose, lævulose,

PLATE XV.



a. Bacillus pestis. Cover-glass preparation of a 48-hours' broth culture. × 1500.

b. Chicken cholera. Cover-glass preparation of blood of fowl. × 1000.

To face page 416.

Gram –
Motile 0 ? flagella
Stl. 0.

'Creaming on agar
/broth – milky at stalactite growths
like B. Pseudo tuberculosis
in other respects too

Easily killed

P.M. Spleen enlarged much

very little toxin
∴ an gang endotoxin

galactose, maltose, mannitol, and dextrin, no change in lactose, cane-sugar, and dulcitol.

Action of antiseptics, etc.—The plague bacillus is readily destroyed by antiseptics; a 1 : 1000 corrosive sublimate or 1 : 100 chloride of lime solution being efficient. An *acid* solution of corrosive sublimate is preferable, and for the practical disinfection of native houses a 1 : 250 solution of sulphuric acid may be employed. A temperature of 65° C. kills the organism in about fifteen minutes. Desiccation over sulphuric acid at 30° C. is also rapidly fatal.

Vitality and virulence of cultures.—Cultures retain their vitality for at least a month. As regards virulence, the organism varies much according to the source from which it is obtained. Under cultivation it gradually loses its virulence unless subcultured in the following manner: The cultures are made every week on surface agar, are placed in the blood-heat incubator for twenty-four hours, and are then removed and kept at room temperature. If inoculated into animals the virulence may be heightened for a particular species by successive passages, but in so doing is diminished for other species.

Pathogenic action.—In addition to man, the following animals are liable to contract plague under natural conditions—the monkey, cat, rat, mouse, squirrel, ground squirrel, ferret, bandicoot, and marmot. The guinea-pig and rabbit are also susceptible to inoculation. The horse, cattle, sheep and goat are relatively insusceptible, though Simpson[1] stated that calves and poultry may be infected by feeding, and suffer from a chronic form of the disease (this observation of Simpson's has not been confirmed by other workers). Birds are not easily susceptible, and vultures feeding

[1] *Report on the Plague in Hong Kong.*

on the corpses of the plague-stricken do not seem to contract the disease. The mouse, rat, and guinea-pig are the animals chiefly used for experimental purposes in the laboratory; the first two are highly susceptible, a simple prick in the thigh with an infected needle being sufficient to induce the disease.

A guinea-pig inoculated with plague material or with a pure cultivation usually dies in from two to seven days, the symptoms being sluggishness and loss of appetite, sometimes a discharge from the eyes, and towards the end staring coat and perhaps convulsive and paralytic attacks. The post-mortem appearances are extensive hæmorrhagic œdema at the seat of in-

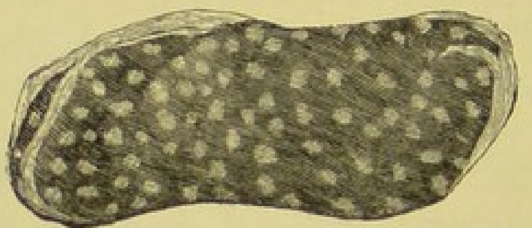


Fig. 45.—Spleen of guinea-pig inoculated with plague. (Nat. size.)

oculation, enlargement and congestion of the spleen, and enlargement of, and hæmorrhages into, the inguinal and axillary lymphatic glands. If the animal live six or seven days, the glands may be as large as small nuts (see some admirable preparations in the College of Surgeons Museum). The spleen may be enormous, six times its natural size, and studded with small yellowish nodules resembling miliary tubercles, consisting of necrotic areas with masses of bacilli (Fig. 45); the lungs also may be more or less inflamed, and contain small and large necrotic foci. The bacilli are extremely numerous at the seat of inoculation, in the glands, and in the spleen, less so in the peritoneal fluid, liver, and blood; if the death of the animal is

delayed the exudation in the bronchi may contain considerable numbers. Some bacilli may generally be found in the duodenum, trachea, and larynx. Mice usually die in from two to three days, and rats in from three to seven days after inoculation. In rats and mice the post-mortem appearances are similar to those in the guinea-pig. A very small dose of a pure culture may fail to kill an inoculated animal. Rabbits are much less susceptible to plague than guinea-pigs, and may be injected with considerable doses of living cultures without showing marked illness. Rats can be infected by feeding on the corpses or carcases of men or animals dead from the disease.

In man the bacilli are found in large numbers in the fluid in the buboes, either alone or mixed with strepto-cocci or micrococci, and in the sputum in the pneumonic form. They are not usually found in any number in the blood except in the septicæmic variety, or shortly before death, and in stained preparations appear as short plump bacilli, often in pairs, with polar staining and unstained centres (Plate XIV., *a* and *b*). If the organisms are found to be free and numerous in the buboes the prognosis tends to be grave, but if they are largely present within the phagocytic polymorphonuclear leucocytes the prognosis is better and the disease will probably remain localised.

Toxins.—The plague bacillus forms but little toxin, the minimal fatal dose of the most active filtered broth culture for a mouse being about 0·02 c.c. In order to prepare a vaccine or an anti-serum it is necessary, therefore, to employ unfiltered cultures—*i.e.* the microbes themselves.

Macfadyen obtained an endotoxin by triturating the bacilli frozen with liquid air.

Vaccines and immunity.—Of the plague vaccines,

Haffkine

that of Haffkine, the Haffkine prophylactic, is the best known, and has been extensively employed. It consists essentially of a four to six weeks old butter-fat broth culture of the plague bacillus, killed by heating to 65° C. for an hour, with a small addition of antiseptic. As to the value of Haffkine's prophylactic a mass of figures is available. By its use both the incidence of, and the mortality from, plague are markedly diminished. Wilkinson collected the following data of the efficiency of the vaccine: Among the inoculated the case incidence was 1·8 and the case mortality 23·9 per cent.; among the uninoculated the figures were 7·7 and 60·1 respectively. The immunising products seem to be mainly intra-cellular, but the broth itself is not without action.

Other vaccines have also been devised. Lustig and Galeotti prepared one by digesting the growth from agar cultures with 1 per cent. caustic soda solution, filtering through paper, and precipitating with very dilute acetic or hydrochloric acid, or by saturation with ammonium sulphate. The precipitate is dissolved in a 0·5 per cent. solution of sodium carbonate, and filtered through a Chamberland filter; this forms the vaccine fluid. Calmette prepared a vaccine by emulsifying an agar growth in water, well washing the organisms with sterile water to remove adherent toxin, emulsifying again in sterile water, heating to 70° C. for an hour, and finally drying *in vacuo*. The dry substance can be kept for a considerable time without change. For use 1–2 mgrm. are emulsified in 2–3 c.c. of sterile salt solution and injected.

Calmette

Yersin proposed vaccinating with living culture of feeble virulence, which has been done by Strong in Manilla. Though such a method might be used in a plague-stricken district, it is obviously one that can be used only with the greatest caution.

Klein[1] has prepared a prophylactic by drying the organs of a guinea-pig dead of plague for three days at 46° C.,

[1] *Rep. Med. Off. Loc. Gov. Board* for 1905–06.

rubbing the material to a powder, and further drying at 37° C. for three days. Of this dry powder 15–16 mgrm. protected a rat, and 25 mgrm. a monkey.

With reference to experimental immunity and protection in plague, Klein[1] found that a guinea-pig which had been three times injected with an amount of living culture insufficient to kill was still capable of being infected; that the blood of a guinea-pig which had twice passed through an attack of plague did not contain an appreciable amount of germicidal substances; and that the immunisation of guinea-pigs by sterilised cultures is an extremely slow and difficult process. Calmette also found that the guinea-pig was extremely difficult to immunise.

Calmette, from laboratory experiments, surmised that protection with a vaccine is not attained for some days, and that in the interval susceptibility to infection is increased. These observations are not borne out in practice, for Bannerman[2] found that so far from there being an increase in mortality among those who have been inoculated and who develop plague within ten days of inoculation the reverse is the case, and that in a small community where the population had been partly vaccinated and partly not vaccinated, the incidence of plague during the week following vaccination was less among the vaccinated than among the unvaccinated, pointing to the rapid production of protection.

Anti-plague serum.—This is prepared by growing the *B. pestis* on the surface of agar in plate bottles, washing off and emulsifying the growth, and for the earlier injections the emulsion is heated to 65° C. for one hour, and the commencing dose is $\frac{1}{24}$ part of a flask. The injections are given intra-venously at intervals of a week. At the end of three months the bactericidal power of the blood will have become very marked, and living cultures are then injected

[1] *Rep. Med. Off. Loc. Gov. Board* for 1896–97, App. B., p. 2.

[2] *Centralbl. f. Bakt.* (1te Abt.), Bd. xxix, p. 873 (Bibliog.).

Like Diphth. but living cultures used at last.

for a further period of about three months until a whole flask-culture is given at a dose. An interval of a fortnight is allowed to elapse between the last dose and the bleeding of the animal. The serum is tested upon mice.

The anti-plague serum, which is mainly anti-microbic, is not very potent, and to be of service large amounts and early treatment are essential.[1]

Epidemiology.—The mode of infection in man has been a matter of controversy. The pneumonic form arises generally from aërial infection by the respiratory tract. It is extremely fatal and infectious, while the bubonic and septicæmic varieties are hardly even contagious. Although a gastric and intestinal form of the disease has been described, and there is evidence to show that food or drink may be the vehicle of infection, this must be a rare mode of infection. Yersin claimed to have isolated the bacillus from the dust and earth of a native dwelling, and Hankin from the brackish water in a field. The observations of Hankin and others indicate, however, that contagion is likely to occur only from immediate contact with man or animals, or their excretions, infected with plague, and not from a saprophytic form of the organism.

Certain animals, especially the rat (*Mus rattus* and *Mus decumanus*), are important agents in spreading the disease. The association of sickness and of death among the rats with an epidemic of plague has been established by a number of observations, and in some instances the epizootic among the rats has been definitely shown to precede the epidemic in man. The epidemics at Sydney are perhaps the most striking instances of rat-borne plague; discussing the first

Rats

[1] See Hewlett's *Serum Therapy*, 1910.

one Tidswell says : "The one clear fact in our epidemic was that human beings were not becoming infected from one another." In the first epidemic the mode of introduction of the disease was never traced to any human source. During an epidemic the rats may be found in all stages of illness and the plague bacillus can be found in large numbers in their carcases. In the various epidemics at Sydney, cases of plague first occurred among the rats and mice, followed after an interval of days or weeks by human cases. Other animals may also occasionally be the means of disseminating the disease. The experiments of the Advisory Committee on Plague Investigation in India have conclusively shown the important part played by rats in the dissemination of the disease, though the origin of the primary infection in rats is doubtful. They may possibly become infected from the dust of earthern floors of the native houses soiled with excreta or discharges of plague patients, or from their clothing, poultices or dressings, but the readiest method is probably by feeding on the dead. Once the epizootic has started, further infection is simple; rats fight, and so may directly inoculate one another; the sick rats may soil grain or other food-stuffs, and the dead rats are eaten by their fellows. Moreover, parasitic insects, especially fleas, undoubtedly may transmit the disease from one animal to another. Thus it is found that if guinea-pigs be placed in a plague-infected house, many of the animals contract plague; but if the animals be placed in cages of wire-gauze, the mesh of which is small enough to prevent access of fleas, the animals do not contract plague. The transmission of the disease from rats to man is similarly due to transmission by fleas (except in the pneumonic forms in which infection is direct from

Fleas

the sick to the healthy). The great majority of rat fleas are *Xenopsylla cheopis*, *Ceratophyllus fasciatus*, *Cer. anisus*, *Ctenopsylla musculi*, and *Ctenophthalmus agyrtes*, of which the first is most prevalent in the tropics and subtropical regions, the second in cooler regions.[1] The manner in which the periods in the year when human plague does not occur are bridged over is unknown. In such periods rats suffering from plague have been found, but these are regarded as having a retrogressive form of the disease rather than a chronic infection. The destruction of rats, either by trapping, poisoning, or asphyxiating, or by the use of the Danysz rat virus (see p. 394), is, therefore, one of the means to be adopted in fighting the disease. The extermination of rats seems quite impossible, but by rat destruction there is a likelihood of destroying infected animals and the subsequent development of a healthy race.

On Plague, see Simpson, *Treatise on Plague* (Cambridge University Press); Klein, *Bacteriology of Oriental Plague;* "Reports on Plague Investigations in India," *Journ. of Hygiene* (extra numbers), vols. vi–xi; *Rep. of the Indian Plague Commission;* numerous reports published by the Indian Government.

Clinical Examination.

If it cannot be examined immediately, plague material may be placed in a solution containing glycerine 20 c.c., distilled water 80 c.c., calcium carbonate 2 grm. The bacilli retain their vitality and virulence in this for thirteen days (Albrecht-Ghon method).

(1) Withdraw a little of the fluid from the bubo by means of an antitoxin syringe. Make smears and stain with

[1] See Chick and Martin, *Journ. of Hygiene*, vol. xi, 1911, p. 122 (Bibliog.).

methylene or thionine blue. Search for short plump bacilli often in pairs, with polar staining and unstained centres. They are not stained by Gram's method.

N.B.—There may be a mixture of organisms in the buboes,

(2) Make agar plates and broth cultures. Incubate the cultures at 25°–27° C., *not* at 37° C. From colonies on the agar plates the organism may be isolated and its cultural and pathogenic characters ascertained. The appearance of the broth cultures, if characteristic, would be very suggestive of plague, but if uniform turbidity develops this may be due to contaminating organisms, *e. g.* micrococci.

(3) Inoculate mice, rats, or guinea-pigs subcutaneously with the fluid or with the culture. Some of the animals should be inoculated by the cutaneous method—rubbing a little of the material on the shaved abdomen, and also as in (4). Inoculation of rats serves to distinguish the *B. pseudo-tuberculosis* from the *B. pestis*. If the animals die, investigate for the *Bacillus pestis* by staining and culture methods.

(4) In the pneumonic form, dilute the sputum with a little boiled water, inoculate several agar tubes, and incubate at 25°-27° C. Examine in two to three days. Also daub the nostrils of a guinea-pig or rat with a brush or pledget of wool dipped in the diluted sputum, avoiding wounding the mucous membrane. Smears of the sputum may also be made, stained, and examined. Gram's method will distinguish the *B. pestis* from the *Diplococcus pneumoniæ*; the latter stains well by Gram.

(5) *Agglutination reaction.*—The Indian Plague Commissioners state that in their opinion no practical value attaches to the method of serum diagnosis in plague, but a modified method is considered by Dunbar[1] to be of considerable value. The method is carried out as follows:

A small quantity of peptone solution, inoculated with the tissue-juice from the suspected organ, is mixed with an equal quantity of plague-serum of such a strength that the dilution reduces it to 1 : 200 (approximately). A second dilution of 1 : 400 and a third of 1 : 800 are also prepared.

[1] *Centralbl. f. Bakt.*, xli (Originale), 1906, p. 860.

As a *control*, an equal quantity of the inoculated peptone water is mixed with normal serum (rabbit or horse serum), the dilution being 1 : 100.

In a few minutes a distinct difference is observable. The "control" shows with the oil-immersion lens a few isolated non-motile bacteria, while the plague-serum dilution 1 : 200 shows larger and smaller masses of agglutinated bacteria.

After two hours' incubation the same result is obtained with the plague-serum dilution of 1 : 400. No agglutination, however, is observed after incubation for twenty-four hours of the dilution of 1 : 800. This agglutination reaction, in conjunction with other suspicious phenomena, justifies an official notification of suspected plague.

In the examination of rats suspected to be suffering from plague infection, it is essential not only to take the naked-eye characters into account, but to make microscopical preparations and cultures, and to test the cultures by animal inoculations. *Care must be taken not to mistake hæmorrhagic septicæmic bacilli* (see pp. 413, 427), *and other organisms for the plague bacillus.* The *B. coli*, *B. proteus*, and other organisms are recorded by Klein (*loc. cit.*) as simulating the *B. pestis.*

Chicken Cholera.

Chicken cholera is a disease of poultry characterised by profuse diarrhœa; its course may be very rapid, and the bird found dead without having shown signs of illness. The organism is a very short rod, non-motile, so short that it is almost ovoid, 0·6 to 0·8 μ in length, and 0·4 to 0·5 μ in diameter. It stains by the ordinary anilin dyes, but not by Gram's method, and the staining tends to be polar, so that Pasteur, who first investigated the disease, described it as a diplococcus (Plate XV., *b*). The organism grows freely on the various culture media from 20° to 38° C., on agar forming a thick, moist, cream-coloured layer, on gelatin a shining, white, expansive growth without liquefaction. In broth a general turbidity forms, but growth on potato is indifferent. It produces acid, does not ferment glucose or lactose, is aërobic

and facultatively anaërobic, does not form spores, and is killed by a temperature of 60° C. in fifteen minutes. If dried it dies in a few days, but retains its vitality for a considerable time in damp earth or in water, and so infection is readily conveyed. Fowls die after subcutaneous, intra-muscular or intra-venous inoculation and by feeding, the organisms being found abundantly in the blood. Post mortem, the serous membranes may be inflamed and hæmorrhagic, the liver large and soft, and the intestine shows hæmorrhagic spots, and is sometimes ulcerated and contains a mucoid fluid stained with blood. Other birds, pigeons, pheasants, sparrows, wild and domestic ducks are also susceptible to the disease, and rabbits and guinea-pigs can be successfully inoculated; in the latter animal a local abscess sometimes forms instead of a general infection. By continuous cultivation with free access of oxygen the virus becomes attenuated, and Pasteur was able thus to prepare a vaccine which protected fowls.

The bacillus of chicken cholera belongs to the group of *hæmorrhagic septicæmic bacilli* (p. 413), and seems to be identical with Koch's bacillus of rabbit septicæmia, and with the bacillus of swine *plague* (see p. 394). These organisms tend to form a stalactite growth in butter broth.

Organisms have been described by Klein in fowl enteritis, grouse disease, etc., differing somewhat from the bacillus of chicken cholera.

Mouse Septicæmia.

This disease may be conveniently described here. Koch first obtained a minute bacillus by injecting putrefying material subcutaneously into mice. It seems to be identical with the bacillus found in swine erysipelas. The organisms are met with in large numbers in the blood and tissues of mice. They measure only 1 μ in length, and occur in considerable numbers in the leucocytes. The bacillus stains well by Gram's method, and is stated by some writers to be motile.

It grows readily, forming on agar extremely delicate, almost invisible colonies; in stab gelatin cultures after some time a delicate cloudiness radiates from the central puncture. From an agar culture the bacilli are somewhat larger than those found in the animal body, and form filaments. It is pathogenic for swine, rabbits, and mice.

CHAPTER XII.

PNEUMONIA, INFLUENZA, AND WHOOPING-COUGH.

Pneumonia.

PNEUMONIA is of two types, lobular, catarrhal, or broncho-pneumonia, and lobar or croupous pneumonia. The former may be primary, or may be secondary and arise in connection with many of the specific fevers, as in measles, whooping-cough, diphtheria, enteric fever, influenza, plague, etc. The broncho-pneumonia occurring in the course of other diseases may be due to the causative organism of the disease, or may be due to other organisms. Eyre[1] examined 62 cases of broncho-pneumonia occurring in the course of other diseases and 102 cases in which the broncho-pneumonia was the primary lesion. Of these 164 cases, 52·4 per cent. yielded pure cultivations of some one or other of six bacteria—pneumococcus, *Strep. longus*, *M. pyogenes* var. *aureus*, *M. catarrhalis*, *B. pneumoniæ*, and *B. influenzæ*; whilst 47·5 per cent. gave a mixed growth of one or more of these six in association with one or more of five other bacteria—*M. tetragenus*, *B. pertussis*, *B. pyocyaneus*, *B. typhosus*, *B. diphtheriæ*. The *B. coli* also occurs in broncho-pneumonia. Acute croupous or lobar pneumonia in many of its characters resembles an acute specific infection, and while frequently a primary disease, may also occur secondarily in almost any condition.

5

anaerobic

5

Friedländer in 1882–83 first described organisms in cases of pneumonia.

[1] *Journ. Path. and Bact.*, vol. xiv, 1910, p. 160.

In 1883–85 Talamon, Klein and Sternberg each described in pneumonic sputum an oval encapsuled organism, which induced pneumonia in animals; it was termed by the former the *Micrococcus lanceolatus*, and by Sternberg the *Micrococcus Pasteuri*. This and Friedländer's organisms were at first believed to be identical, but Fränkel and Weichselbaum subsequently showed that they are quite distinct, and that the former is the ætiological agent of acute croupous pneumonia.

The majority (95 per cent.) of cases of acute croupous pneumonia are caused by the *Diplococcus pneumoniæ*, and Friedländer's organism, now termed Friedländer's pneumobacillus, or *B. pneumoniæ*, is of ætiological significance in only a small minority, if at all. The latter is, however, associated with certain pathological processes which will be referred to below.

From pleuro-pneumonia of cattle, Nocard and Roux succeeded in cultivating in broth in collodion sacs in the peritoneal cavity of rabbits an organism just visible as minute granules with a magnification of 2000 diameters. Bordet[1] states that it may be grown on the medium employed by him for the cultivation of the *B. pertussis* (p. 441), and then appears as fine, straight, curved, undulating or even spirillar filaments not unlike spirochaetes.

The Diplococcus (Streptococcus) pneumoniæ.[2]

Synonyms, Fränkel's pneumococcus, *Micrococcus Pasteuri* (Sternberg), *Micrococcus lanceolatus* (Talamon), *Micrococcus pyogenes tenuis* (Rosenbach).

Morphology.—The *Diplococcus pneumoniæ* in the sputum and tissues occurs as an oval or lance-shaped coccus united in pairs, occasionally in chains of three or four elements, and then often almost spherical, and is generally surrounded by a well-marked capsule (Plate XVI., *a*). In order to isolate the organism several tubes of glycerin agar, serum or serum-agar may be inoculated with rusty sputum and incubated for

[1] *Ann. de l'Inst. Pasteur*, xxiv, 1910, March.

[2] On the pathology of pneumococcus infection see *Brit. Med. Journ.*, 1901, vol. ii, p. 760; Eyre, *Lancet*, 1908, vol. i, February 22nd.

forty-eight hours; in some a pure culture may be obtained. A more certain method is to inject a drop or two of the rusty sputum into the peritoneal cavity of a mouse or young rabbit. The animal will die in from twenty-four to thirty-six hours, and the organism will be found in considerable numbers in the lung and blood, from which cultures may be obtained. It is non-motile, stains with the ordinary anilin dyes and by Gram's method.

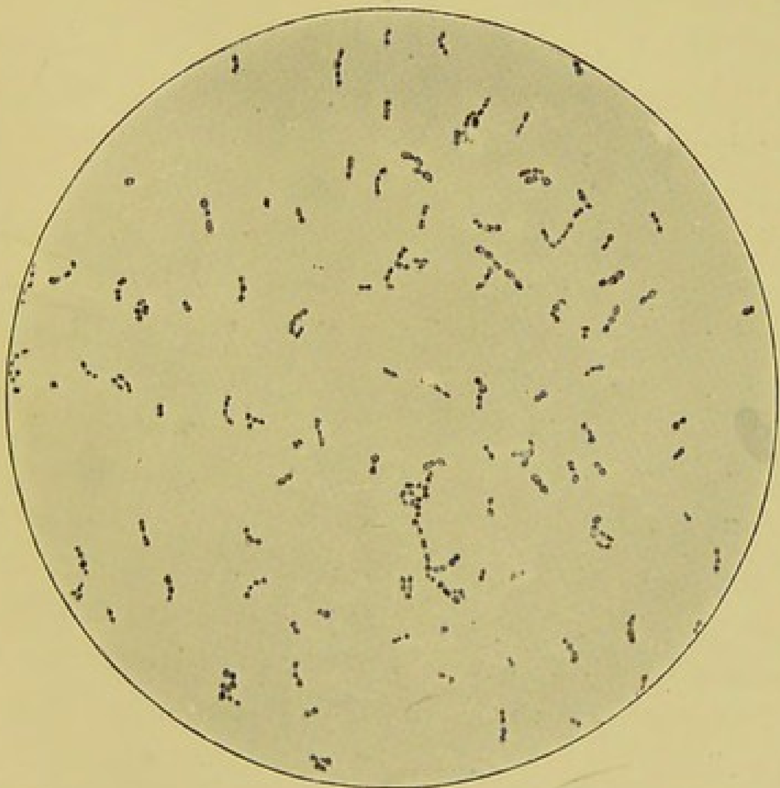
Cultural characters.—The *D. pneumoniæ* is aërobic and almost facultatively anaërobic. On glycerin agar at 37° C. it forms minute, transparent, almost invisible colonies like droplets of fluid; on serum the growth has much the same characters, but is somewhat more abundant. It hardly grows on gelatin at the ordinary temperature, but in a 20 per cent. gelatin at 25° C. minute white colonies develop without liquefaction. In broth it produces a slight cloudiness; it does not grow on potato but develops in milk, which is usually coagulated; neutral litmus glucose-agar becomes red during growth, indicating the production of acid. The fermentation reactions are given in the table on p. 248. Hiss's medium (p. 306) with inulin is fermented and coagulated; most other streptococci fail to ferment inulin. On the ordinary culture media it retains its vitality for a short time only, not more than about a week; but if a little blood be smeared over the surface of the agar the vitality may be prolonged for a month or even longer. Washbourn recommended an agar rendered alkaline to the extent of 4 c.c. of normal caustic soda per litre, after neutralisation, rosolic acid being the indicator. This medium is smeared with blood, placed in the incubator for twenty-four hours to ascertain whether it be sterile, then inoculated, capped, and kept at 37° C. Foa's method for keeping Fränkel's

pneumococcus alive and virulent is to receive the infected blood of an inoculated animal into a small glass tube 5 mm. in diameter and 20 cm. long, so that the blood completely fills the tube, which is then sealed and kept away from the light at the ordinary temperature. If inoculated on to ordinary gelatin, which is then kept in the *blood heat* (37° C.) incubator, the organism retains its vitality for a month or six weeks.

Under cultivation the *D. pneumoniæ* usually assumes the form of a short streptococcus (Plate XVI., *b*) (included by Gordon in his *S. brevis* class) and the capsule is lost, but is regained again on passage through a susceptible animal, or by growing in fluid serum. A good deal of variation occurs in the morphology of the organism obtained from different sources and under cultivation. The thermal death-point of the *D. pneumoniæ* according to Sternberg is 52° C., the time of exposure being ten minutes, and it is readily destroyed by the ordinary germicides, by light, and by desiccation; but in dried sputum it may retain its vitality and virulence unimpaired for weeks.

Pathogenic action.—The *D. pneumoniæ* is pathogenic for a number of animals, the most susceptible being mice, then in decreasing order, rabbits, rats, guinea-pigs, and dogs. Pigeons and fowls are immune. Death follows after subcutaneous, intra-venous, intra-peritoneal, or intra-thoracic injection of a virulent culture, or of rusty pneumonic sputum, into mice and rabbits in twenty-four to forty-eight hours. The virulence of the organism varies considerably; under cultivation it may be completely lost, while by a series of passages through a susceptible animal it may be much increased. The less virulent it is the longer it tends to retain its vitality under cultivation. Except when injected into the lung or into the trachea, pneumonia does not result,

a. Diplococcus pneumoniæ. Cover-glass preparation of blood of inoculated animal. × 1000.



b. Diplococcus pneumoniæ. Cover-glass preparation of a pure culture. × 1500.

To face page 432.

but the disease runs the course of a septicæmia with high temperature and dyspnœa, death being generally preceded by a subnormal temperature and often convulsions. The *post-mortem* appearances are much œdema and inflammatory infiltration at the seat of inoculation, hæmorrhages in the serous membranes, enlargement and congestion of the spleen, and congestion of the lungs. The organisms occur in large numbers in the blood, lungs, and spleen, usually in the form of oval diplococci with well-marked capsules (Plate XVI., *a*), but sometimes as short chains of streptococci. When injected into the lung or trachea a typical fibrinous or croupous pneumonia results.

The *D. pneumoniæ* is the cause of acute croupous pneumonia in man, and occurs in large numbers in the rusty sputum and hepatised lung, and in 20 per cent. of the cases can be isolated from the blood if 5–10 c.c. be cultured. The production of a typical pneumonic process experimentally and the presence of the diplococcus in a large proportion of cases of acute croupous pneumonia point to its specific relationship to the disease. With regard to the latter observation, Weichselbaum obtained it in 94 cases out of 129 examined, Wolf in 66 out of 70 cases, and Netter in 75 per cent. of the cases examined. In America the disease has of late been much on the increase, in Chicago the mortality having reached as high as 20 per 10,000 inhabitants. Acute croupous pneumonia sometimes occurs in epidemic form.

The organism is frequently present in the saliva of healthy individuals, as shown by Netter, Sternberg, and others, and the generally accepted idea of the relationship of "catching cold" to an attack of the disease is explicable on the theory that the action of cold lowers vitality, and renders the tissues vulner-

able to the attacks of the organism already in close proximity to them.

Besides acute croupous pneumonia, more than half the cases of broncho-pneumonia, both primary, and secondary in the course of other diseases, are due to the *D. pneumoniæ*, which is also associated with a number of other important pathological conditions in man. It is a pyogenic organism, producing abscesses when inoculated into a relatively insusceptible animal such as a dog, and has been met with in abscesses, empyema, suppuration in the antrum, and purulent arthritis. It is also found in about half the cases of purulent meningitis, sometimes in cerebro-spinal meningitis, in about a third of the cases of otitis media and infective endocarditis, sometimes in purulent pericarditis, and occasionally in peritonitis.

Toxins.—Auld separated a proteose and an organic acid from the blood and organs of infected animals, and from cultivations of the *Diplococcus pneumoniæ* in alkali-albumin the same products were apparently obtained, the alkaline medium soon becoming permanently acid. The proteose on subcutaneous or intravenous injection produced some fever ; on intra-thoracic injection fever and dyspnœa, and post-mortem pleurisy and consolidation of the lung were found. The organic acid produced slight rise of temperature, but no other symptom. Macfadyen[1] obtained an endotoxin by triturating cultures with liquid air.

Anti-serum.—Immunity can be conferred on susceptible animals by treating them with attenuated cultures, and also by inoculation with increasing doses of filtered broth cultures of the virulent organism. G. and F. Klemperer used recent broth cultures heated to 60° C. for one or two hours. Washbourn used filtered

[1] *Brit. Med. Journ.*, 1906, vol. ii, p. 776 (Refs.).

cultures in defibrinated blood, 20 c.c. of which injected subcutaneously into a rabbit conferred immunity against virulent cultures, an immunity persisting for fifty or sixty days. The blood-serum of such immunised animals will protect other animals when injected, and Klemperer, Issaef, and Washbourn have prepared a pneumonic anti-serum. The latter, by first immunising a horse with filtered cultures, increased the immunity by injection with gradually increasing doses of living virulent cultures, until a very high degree of immunity is obtained. This anti-serum has been used in the treatment of pneumonia and other pneumococcic infections, but the results have not been very encouraging. The protective serum seems to produce aggregation of the cocci when added to a culture of the diplococcus. Klemperer and Washbourn found that the serum of convalescent patients possesses some degree of protective power. The serum, however, taken during the pyrexial stage of the disease rather increases the susceptibility of animals to pneumococcic infection.

Vaccine.—A vaccine prepared from cultures killed by heat and standardised has been found of service in chronic pneumococcic infections, and has also been employed in acute croupous pneumonia.[1]

Friedländer's Pneumo-bacillus.

This organism, already referred to above in the general discussion of pneumonia, and originally believed by Friedländer to be the cause of the disease, has been obtained by recent observers in only a small proportion of cases of pneumonia.

Morphology.—The *B. pneumoniæ* is a very pleomorphic organism, occurring in sputum or in the blood

[1] Willcox and Morgan, *Brit. Med. Journ.*, 1909, vol. ii, p. 1050.

of an inoculated animal generally as a short rod with rounded ends surrounded by a marked capsule. It is non-motile, does not form spores, and is readily stained with the ordinary anilin dyes, but not by Gram's method—an important distinction from the *Diplococcus pneumoniæ.* In cultivations it forms short rods, long rods, chains, and even filaments, the capsule being absent, but this is regained on passage through a susceptible animal.

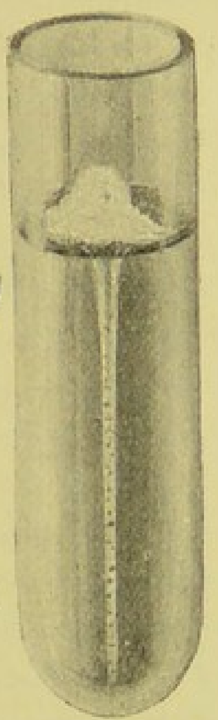


Fig. 46.—Friedländer's pneumo-bacillus. Gelatin stab-culture, seven days old.

Cultural characters.—The *B. pneumoniæ* is aërobic and facultatively anaërobic, and may produce indole. It grows readily on the various culture media from 20° to 37° C., on agar and blood-serum forming a copious, viscid, greyish growth; on gelatin, a thick, white, shining, porcelain-like growth without liquefaction; and in stab-cultures in gelatin a so-called nail-shaped growth is developed (Fig. 46), consisting of a white growth along the needle-track, tapering from above downwards, and at the surface heaped up and expanded, forming the "head" of the nail. On potato a copious whitish growth develops, while milk is curdled and gas-bubbles frequently form in stab-gelatin cultures. It is an active fermenter of carbohydrates, and the fermentation reactions are given in the table, p. 400.

Pathogenic action.—The pneumo-bacillus of Friedländer is pathogenic to mice and guinea-pigs, but rabbits are immune. Post-mortem, the spleen is enlarged, the lungs are congested and consolidated in patches, and the organism is found in large numbers

in the blood. In a small percentage of cases of croupous pneumonia Friedländer's bacillus may be associated with the *D. pneumoniæ.* Friedländer's bacillus may sometimes set up a broncho-pneumonic or bronchitic process, and is occasionally associated with anginal conditions, which are characterised by the formation of a false membrane, with an absence of any general symptoms. A microscopical examination of the membrane will show the organisms surrounded with a capsule and unstainable by Gram's method. If a culture be made on serum, the large, round, greyish colonies of the bacillus will be recognisable in fifteen to twenty hours, and should be examined microscopically. To obtain a pure culture a white mouse should be inoculated from a colony; it will die in twenty-eight to sixty hours. Friedländer's pneumo-bacillus has also been met with in water by Grimbert. According to him, it is identical with the *B. capsulatus* of Mori.

Clinical Examination (Pneumonia).

1. Make cover-glass specimens from the rusty sputum, and stain some with Löffler's blue, and others by Gram's method with eosin. By a microscopical examination the oval diplococci will be readily recognised, the *B. pneumoniæ* and *B. pestis* being distinguished from the *Diplococcus pneumoniæ* by being decolorised by Gram's method. The latter organism is the only one, moreover, which is likely to be ordinarily met with.

2. If the diplococci are found to be fairly abundant in the sputum, and other organisms nearly absent, an attempt may be made to cultivate by inoculating several glycerin-agar and serum tubes and incubating at 37° C. for forty-eight hours.

3. If the diplococci are scanty, or so mixed with other organisms that it is difficult to identify them, and probably impossible to obtain a pure culture, a drop or two of the

sputum should be injected into the peritoneal cavity of a mouse or rabbit. The animal will die in from twenty-four to thirty-six hours, and the *Diplococcus pneumoniæ* will be found plentifully in smears prepared from the blood or lung-juice, and pure cultures can be readily obtained by inoculating glycerin-agar tubes with the blood or lung-juice.

4. The culture or inoculation method, preferably both, will probably have to be adopted for the recognition and isolation of the *Diplococcus pneumoniæ* in pus from empyemata, abscesses, etc.

5. Friedländer's pneumo-bacillus can be readily isolated by making gelatin-plate cultivations, in which its colonies form white, shining, heaped-up points.

heaped up

Influenza.

A minute bacillus was first described in this disease by Pfeiffer in 1892, who found it in large numbers in the bronchial secretion. In order to isolate the organism a patient with bronchial expectoration should be chosen; he rinses his mouth and gargles his throat with hot water several times, and then, after coughing, the expectoration is obtained. A little of this expectoration is washed by shaking in a test-tube with sterile salt solution, then repeating the washing with sterile salt solution in a second and finally in a third test-tube. By means of a platinum needle a number of glycerin-agar and blood-agar tubes are inoculated with the sputum after the last washing, and incubated at 37° C.

Aerobe

Morphology.—The influenza bacillus is one of the smallest bacilli with which we are acquainted. It is a minute rod 0·5–1·5 μ in length, and is non-motile and non-sporing. It does not stain by Gram's method, and not very readily with the ordinary dyes, dilute carbol-fuchsin or prolonged staining with Löffler's blue

Gram 0

yielding the best results, the poles tending to stain more deeply than the centre. In the sputum it occurs singly, in short chains, in small groups, or in larger masses, being most numerous early in the acute stage of the disease.

Cultural characters.—The bacillus is strictly aërobic, and no growth occurs on media at 22° C. On glycerin agar and blood-serum at 37° C. it forms very small, transparent, drop-like colonies in from twenty-four to forty-eight hours, which, according to Kitasato, never become confluent. There is no growth on potato. The organism grows best on media containing blood, such as agar smeared with sterile human, rabbit's, or pigeon's blood. In broth it grows at the surface in fine white flakes which subsequently sink.

It soon dies out in cultivation, but according to Klein can be kept alive for some weeks in gelatin incubated at 37° C. The liquefied gelatin remains clear, the growth forming a delicate flocculent precipitate at the bottom. Preparations from cultures show long twisted chains and threads of bacilli, aggregated so as to form dense networks and convolutions. These chains or threads are composed of bacilli placed end to end, and united by a continuation of the cell-membrane. Involution forms occur. It is stated to grow better in association with the *M. pyogenes*, var. *aureus*, than alone. The organism does not seem to be able to live outside the body for any length of time, and is readily destroyed by desiccation, weak antiseptics, and by a temperature of 60° C. acting for five minutes.

Pathogenic action.—Canon stated that he obtained this bacillus from the blood in a number of cases, but many other investigators have failed to find it. Klein also obtained it in six cases out of forty-three examined,

According to Pfeiffer the bacillus is pathogenic only to monkeys and rabbits. Klein, however, was unable to obtain any definite effects in these animals by the injection either of sputum rich in bacilli or of pure cultures.

The influenza bacillus is met with in all uncomplicated cases of influenza in the nasal and bronchial secretions, often almost in pure culture, and in the bronchial tubes and lung in the pneumonic complications accompanying the disease. The organisms disappear with convalescence, and are not met with in other diseases. Klein[1] appears to consider that the pneumonia often complicating the disease is probably directly due to the bacillus. The typical influenza pneumonia is of the lobular type with a cellular rather than a fibrinous exudate. True lobar pneumonia, due to the *Diplococcus pneumoniæ*, may, however, often complicate the influenzal attack. The organism also occurs in bronchitis, broncho-pneumonia, and whooping-cough.

Although the typical influenza may be due to the *B. influenzæ*, many febrile conditions attended with pulmonary catarrh and frequently termed "influenza" are not due to this organism. In an epidemic simulating influenza occurring in Essex in 1905, the examination was negative as regards streptococci, *B. diphtheriæ*, and *B. influenzæ*, but the *M. catarrhalis* was present in numbers in most cases (twenty-two out of twenty-four). This organism was originally isolated by Seifert in a small epidemic of infectious bronchitis, afterwards by Pfeiffer in cases of broncho-pneumonia in young children (see p. 260). Two other Gram-negative cocci were also isolated from three other cases (see table, p. 261).

[1] "Further Report on Epidemic Influenza," 1889–92, *Loc. Gov. Board Report*, 1893, p. 85.

Clinical Examination.

In cases of influenza, accompanied with bronchitis or pneumonia, the influenza bacillus may be met with in large numbers in the sputum, and their presence may aid in confirming the diagnosis. Cover-glass preparations may be stained with carbol-methylene blue.

Whooping-cough (Pertussis).[1]

An influenza-like bacillus has been isolated by Koplik, Czaplewski and Hensel, Davis and others in this disease, but the researches of Bordet and Gengou have shown that it is distinct from the influenza bacillus.

The *B. pertussis* is a minute bacillus, very like the *B. influenzæ*, non-motile, non-sporing, and Gram-negative. It is scanty in the bulk of the expectoration, but is abundant in the viscid exudate, rich in leucocytes, coming from the depth of the bronchi, and voided at the end of a paroxỳsm of coughing.

It is best isolated on a medium consisting of defibrinated blood (human or rabbit), thoroughly mixed with an equal volume of 3 per cent. agar containing a little extract of potato made with 4 per cent. aqueous glycerin. It forms on this a fairly thick whitish streak, the subjacent blood being hæmolysed. It may also be grown in serum or blood broth in shallow layers. After acclimatisation to artificial media it will develop on the ordinary laboratory media.

The *B. pertussis* is agglutinated feebly by the blood of patients, but complement-fixation is marked.

Monkeys are stated to develop a typical whooping-cough on inoculation, but the ordinary laboratory animals are susceptible only to massive intra-peritoneal or intra-venous inoculation, death ensuing from a septicæmic process.

Attempts have been made to treat the disease with a vaccine.

[1] See Bordet, *Brit. Med. Journ.*, 1909, vol. ii, p. 1062.

CHAPTER XIII.

Anaërobic Organisms.

TETANUS—MALIGNANT ŒDEMA—BLACK QUARTER—BACILLUS WELCHII (AËROGENES CAPSULATUS, ENTERITIDIS SPOROGENES)—BACILLUS CADAVERIS SPOROGENES—CLOSTRIDIUM BUTYRICUM.

Tetanus.

The causation of tetanus was for a long time involved in mystery. No obvious or characteristic changes being met with after death, the disease was regarded by many as "functional." Others believed that a primary lesion of the central nervous system might be the cause of the affection, while a few classed it with the specific diseases.

It had long been noticed that wounds soiled with earth were specially prone to be followed by tetanus, and Sternberg in 1880, and Nicolaier in 1884, produced tetanus in rabbits by introducing a little garden earth beneath the skin. The latter observer found at the seat of inoculation and in his impure cultures—for he was unable to obtain pure ones—a distinctive bacillus, and he was able with these cultures, and with the pus from the seat of inoculation, to induce tetanus in other animals. Carle and Rattone subsequently showed that the bacillus of Nicolaier was present in the tissues of, and secretions from, the wound, in cases of traumatic tetanus in man, and that inoculation with the pus from such a wound produced tetanus in the lower animals—observations which were confirmed by Rosenbach in 1885. The bacillus was

isolated in pure culture by Kitasato in 1889 by taking the impure cultures obtained from the wound in a case of traumatic tetanus, heating to 80° C., and plating the heated cultures, the plates being incubated anaërobically in hydrogen.

The Bacillus tetani.

Morphology.—The *Bacillus tetani* is a straight, slender rod with rounded ends, but under cultivation the rods may grow into longish filaments. It is somewhat motile and possesses a large number of flagella, three or four of which are generally thicker than the rest.[1] Spores are freely formed; they are spherical and develop at one extremity of the rod, and their diameter being much greater than that of the rod, the spore-bearing organism has been likened to a "pin" or "drum-stick" (Plate XVII., *a*). It stains with the ordinary anilin dyes, and also by Gram's method. "Drum-stick" bacilli are not necessarily tetanus; other anaërobic bacilli, *e. g. B. putrificus* (*coli*), may also have large terminal spores.

Cultural characters. — The *B. tetani* is a strictly anaërobic organism, and will not grow in the presence of a trace of free oxygen, nor in an atmosphere of carbon dioxide. It can be cultivated in deep stabs in glucose agar and gelatin, or in broth by Buchner's method, or in an atmosphere of hydrogen (p. 73). In a gelatin stab-culture at 22° C. the growth radiates from the central puncture, and the gelatin is slowly liquefied. In a glucose agar stab-culture it forms feathery, radiating outgrowths from the central puncture, a small amount of gas being formed (Fig. 47). Broth becomes turbid with the formation of some gas

[1] Kanthack and Connell, *Journ. Path. and Bact.*, vol. iv, 1897, p. 452.

and the development of a foul odour; there is no film formation. The colonies have a central opaque portion surrounded by diverging rays. It grows on serum without liquefaction and in milk without curdling. The tetanus bacillus remains alive for some time, possibly indefinitely, in cultures, and the spores retain their vitality for years in the dried state, withstand a temperature of 80° C. for an hour, but are killed by boiling for five minutes. Carbolic acid (1 : 20) does not destroy the spores under about fifteen hours.

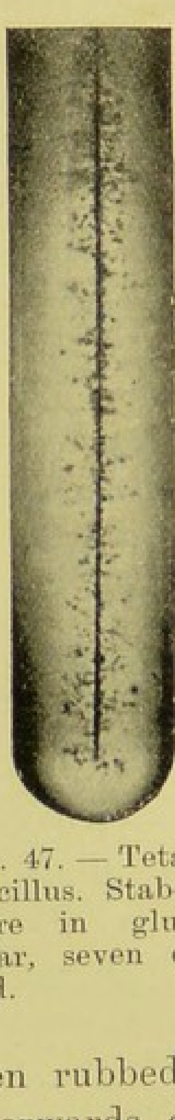


Fig. 47. — Tetanus bacillus. Stab-culture in glucose agar, seven days old.

Occurrence and pathogenic action.— Man and the horse are most subject to tetanus; cattle and sheep are rarely affected, while the fowl, frog, triton, snakes and tortoise are immune. Mice, guinea-pigs and rabbits are all very susceptible. The bacillus is present in the superficial layers of the soils in many localities, but not in all, and this accounts for the fact that tetanus is rare in some places and frequent in others. Curiously enough, some of the savage inhabitants of the Solomon Islands have made use of poisoned arrows, the poisonous nature of which is due to tetanus-bearing earth. The arrows are tipped with a viscid fluid, then rubbed in the soil from a mango swamp, and afterwards dried. Individuals wounded with these arrows often develop tetanus.

Tetanus spores are frequently present in the dejecta of cattle, horses, and other animals, and occasionally of man (p. 445).

The bacillus is confined to the seat of inoculation, or at most is met with in the nearest lymphatic glands, so that the general symptoms are due to the absorption of toxin. The researches of Ransom and Meyer have shown that the tetanus toxin is mainly absorbed by the nerve-trunks (see also p. 166). The organisms associated with the tetanus bacillus in earth are probably of considerable importance in the production of the disease, for it has been shown that if the tetanus bacilli and their spores be carefully washed so as to remove all adherent toxins, they fail to set up tetanus on inoculation, while if the same washed bacilli be injected, together with a little lactic acid, tetanus follows, the explanation being that the bacilli are unable to multiply unless the surrounding tissues are damaged and phagocytosis is prevented. The associated organisms in the wound probably effect this, and do not act by producing a condition of anaërobiosis as has been suggested. Semple[1] has recently found that tetanus spores are occasionally present in the human intestinal tract (Hamilton suggested that tetanoid organisms in the intestinal tract might be the cause of the so-called idiopathic or rheumatic tetanus). He injected guinea-pigs with washed spores, and tetanus did not ensue, but the tissue at the site of inoculation, examined five to seven months later, still contained the living spores. Semple suggests that such latent spores may in some instances be disturbed and become active by the hypodermic or intra-muscular injection of quinine, owing to the tissue necrosis and inhibition of phagocytosis produced by the drug.

Toxins.—Cultivated anaërobically in broth, the tetanus bacillus forms a most potent extra-cellular toxin, so that if the culture be filtered through a

[1] *Sc. Mem. Gov. of India*, No. 43, 1911.

porcelain filter, 0·001 c.c., 0·0001 c.c., or even 0·00001 c.c. of the filtrate is a fatal dose for a guinea-pig.

Tetanus toxin broth contains a tetanising substance, termed tetano-spasmin, and also a hæmolysin, tetano-lysin. The toxin has a special affinity for nerve-tissue (see p. 166). Injected into animals such as the mouse, guinea-pig and rabbit, the toxin broth produces tonic, not clonic, spasm, and with small doses the muscles at or near the seat of inoculation tend first to be affected, so that the spine may be curved, the leg paralysed, etc. (Fig. 48).

By treatment with carbon disulphide, tetanus toxin broth becomes practically non-toxic, though it still retains its power of immunising on inoculation and of combining with antitoxin—that is to say, bodies are formed analogous to the toxoids of diphtheria toxin.

Brieger, from impure cultures of the tetanus bacillus, obtained two basic bodies which he termed "tetanine" and "tetano-toxin," the former producing tetanic symptoms in mice, and the latter tremor, paralysis, and finally convulsions. Brieger also isolated tetanine from the amputated limb of a tetanic patient. Brieger and Fränkel obtained a tox-albumin from bouillon cultures which induced tetanus in guinea-pigs. Brieger and Cohn subsequently investigated the tetanus poison obtained by precipitating veal-broth cultures with ammonium sulphate added to saturation, and purifying by re-dissolving, precipitating the protein with basic lead acetate, and removing other soluble impurities by dialysis. The purified product forms yellow flakes, soluble in water, but not giving the Millon and xanthoproteic reactions. It is not precipitated by most metallic salts, and is not carried down by Roux and Yersin's method of precipitation

with calcium phosphate. It contains no phosphorus and only traces of sulphur. Of the most active preparation 0·00000005 grm. killed a mouse.

In a case of tetanus examined by Sidney Martin, an albumose, chiefly deutero-albumose, was extracted from the blood. Injected into an animal, it produced depression of temperature, followed by progressive wasting, but no spasm or paralysis.

Antitoxin.—If an animal is cautiously injected with

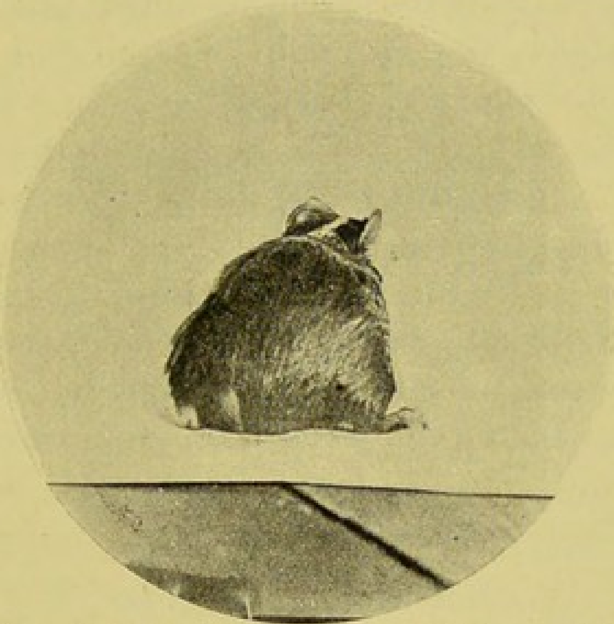


FIG. 48.—Guinea-pig inoculated with a small dose of tetanus toxin, showing paralytic condition of right hind leg due to spasm.

tetanus toxin, commencing the treatment with a weakened toxin, and increasing the dose very gradually, a high degree of immunity is ultimately obtained, and the blood-serum acquires marked antitoxic properties. The toxin is obtained by growing the tetanus bacillus in bouillon in an atmosphere of hydrogen for about three weeks, and filtering through porous porcelain. To obtain an active serum treatment has to be prolonged, a horse immunised by the writer requiring six months. The antitoxic serum so obtained is by far

the most active of any of the sera, and is now recognised as the proper remedy to use in cases of tetanus in man. The antitoxic treatment of tetanus is not nearly so successful as that of diphtheria, and for this reason: in diphtheria, in a large proportion of the cases, a local manifestation is present to aid diagnosis before any serious absorption of the toxin has taken place, whereas in tetanus the disease is only recognisable by the symptoms induced by such absorption. Nevertheless, it can hardly be doubted that it is our duty to employ the antitoxin not only in the fully developed disease, but also in certain cases as a prophylactic. As the toxin is at once fixed by the nerve-tissue, the antitoxin should be injected into the central nervous system in order to obtain immediate action.

The antitoxin may be standardised by the Roux or by the Behring method (see p. 293). Recently a method analogous to that used for standardising diphtheria antitoxin has been introduced.[1]

Clinical Examination.

The symptoms of tetanus are usually so obvious that a bacteriological examination is not needed to establish the diagnosis, and unless there is an evident wound it will be difficult, if not impossible, to detect the tetanus bacillus.

(1) Prepare several smears of the pus or discharge, and stain by Gram's method. Examine microscopically, looking for the spore-bearing rods or "drum-sticks." A "drum-stick" bacillus is, however, not necessarily the tetanus bacillus (see p. 443).

(2) If "drum-sticks" be found, an attempt may be made to isolate the bacillus by making anaërobic plate cultivations

[1] On the standardisation and therapeutic use of tetanus antitoxin, see Hewlett's *Serum Therapy*, 1910.

from the discharge, after heating it in capillary pipettes to 80° C. for half an hour.

(3) Inoculate mice and guinea-pigs with the heated discharge. If they die with tetanic symptoms, treat the pus at the seat of inoculation as in (2).

Malignant Œdema.

Malignant œdema is met with in man in connection with wounds soiled with septic matter, compound fractures, contused and lacerated wounds, etc. Usually there is a putrefactive and œdematous condition of the tissues with subcutaneous emphysema. Animals also occasionally suffer from the disease, which can be produced artificially by inoculation with dust, dust from straw, the upper layers of garden earth, and decomposing animal and vegetable matter.

If a guinea-pig be inoculated subcutaneously with a little garden earth, it will very likely die in forty-eight hours. Post mortem, the subcutaneous tissues around the seat of inoculation will be found to be œdematous and blood-stained, with more or less development of gas. The internal organs are only slightly altered, but the spleen may be somewhat enlarged. The juice from the seat of inoculation will be found to contain a mixture of organisms, but in the blood and organs few will be found. Under the capsule of the spleen, however, long slender rods may be seen; these are the bacilli of malignant œdema.

Morphology.—The bacillus of malignant œdema is a long and slender rod, several of which may be united into a thread. It is motile, possesses several flagella, and is readily stained by the ordinary anilin dyes, but not by Gram's method. It spores freely at temperatures above 20° C., the spores being large and central.

Cultural characters.—The bacillus of malignant œdema

is strictly anaërobic. In a deep stab in glucose-agar it forms a thick line of growth in the needle track, with irregular outline and greyish-white in colour. There is profuse development of gas, accompanied by a foul odour, and attended with disruption of the medium into several portions.

The bacillus of malignant œdema is an organism which has to be distinguished from anthrax, and there should be no difficulty in doing this. Post mortem, the spleen is rarely found much enlarged in malignant œdema, the organism is not very abundant, is almost entirely absent from the blood, and is only found under the capsule of the spleen, not at its centre. If, however, several hours have elapsed since death occurred, the organism may have wandered into the blood and the centre of the spleen. The bacillus of malignant œdema is motile under anaërobic conditions, the anthrax bacillus non-motile; the former occurs as a long slender filament, which on staining is seen to consist of two or three long segments; it does not stain by Gram's method (except by Claudius's modification), and is strictly anaërobic.

Bacillus botulinus.

In certain forms of meat poisoning (see Chap. XXI) van Ermengem isolated an anaërobic bacillus, the *B. botulinus*. It is chiefly met with in ham and sausage, and the symptoms are caused by the absorption of toxin, which has a special effect on the nerve centres.

The organism is a large Gram-positive anaërobic bacillus, often occurring in pairs or in short chains. In glucose gelatin it forms a whitish streak in the line of the stab, with lateral out-growths, liquefaction of the medium, and gas-formation. The cultures have a rancid odour, due to butyric acid production. The colonies in gelatin are semi-transparent spheres. The optimum growth is from 20°–30° C.

The *B. botulinus* in broth cultures forms a potent extra-cellular toxin. The toxin is also produced in the infected ham, sausage, etc. With the toxin an antitoxin can be prepared.

anaërob
gas +
liquef
gel +
Gram +
nerve symptoms
gas +
Characteristic growth
odour

Bacillus Welchii.[1]

Probable synonyms.—*B. aërogenes capsulatus* (Welch and Nuttall), *Granulo-bacillus saccharo-butyricus immobilis liquefaciens* (Grassberger and Schattenfroh), *B. enteritidis sporogenes* (Klein), *B. perfringens* (Veillon and Zuber), gas-phlegmon bacillus (Fränkel), bacillus of acute rheumatism (Achalme : see "Rheumatism").

This organism was originally described by Welch and Nuttall under the name *B. aërogenes capsulatus*, and occurs in conditions accompanied by much development of gas in the tissues, as in cases which might be described either as phlegmonous erysipelas or as emphysematous gangrene, especially after injuries. It is also met with occasionally in perforative peritonitis and in various septicæmic and pyæmic conditions, in the puerperal state,[2] complicated stricture, etc.

The *B. Welchii* is widely distributed, and has been cultivated from the soil, dust, and contents of the intestine. It has either been described under a variety of names, or a group of closely related bacilli may exist. Gas-bubbles found in the blood and internal organs ("foamy organs") at an autopsy seem generally to be due to this organism, but may occasionally perhaps be caused by other putrefactive bacteria.

Morphology.—The *B. Welchii* is a non-motile, sporing, anthrax-like bacillus, variable in size, being 3 to 6 μ in length (Plate XVII., *b*). It occurs singly, in short

[1] See Welch and Nuttall, *Bull. Johns Hopkins Hosp.*, vol. iii, 1892, p. 81; Welch, 'Shattuck Lecture,' *ibid.*, vol. xi, 1900, p. 185; Dunham, *ibid.*, vol. viii, 1897, p. 68; Welch and Flexner, *Journ. Exper. Med.*, vol. i, 1896, p. 5; Herter, *Bacterial Infections of the Digestive Tract*, 1907; Kamen, *Centr. f. Bakt.*, Orig. xxxv, 1904, pp. 554, 686; *Archiv f. Hyg.*, vol. liii, 1905, p. 128; and Blake and Lahey, *Journ. Amer. Med. Assoc.*, vol. liv, 1910, p. 1671.

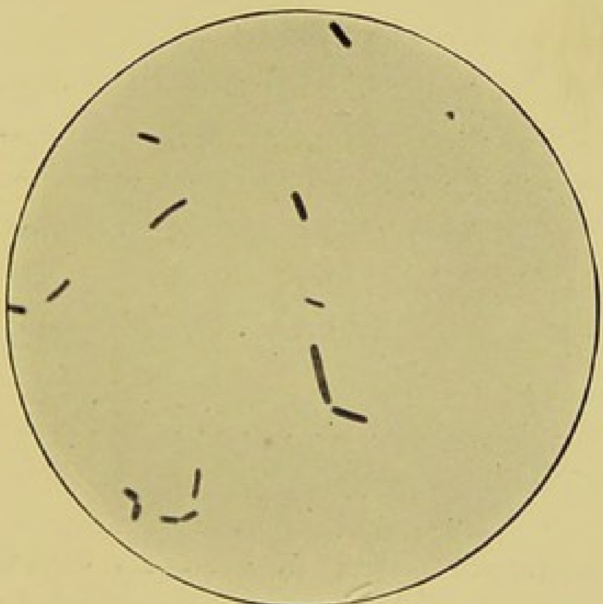
[2] See Little, *Bull. Johns Hopkins Hosp.*, vol. xvi, 1905, p. 136.

chains, or in clumps, and occasionally in long threads. It stains well with the ordinary anilin dyes and also by Gram's method. A capsule is often present, but spores are only formed in blood-serum cultures.

Cultural characters.—The *B. Welchii* grows well on all the ordinary culture media, slowly at 20° C., rapidly at blood-heat, but is strictly anaërobic. It forms greyish-white colonies on agar, and gelatin is liquefied. In glucose-broth it produces at first a diffuse cloudiness, but later the fluid becomes clear and a whitish viscid sediment settles. Milk is coagulated, the casein forming a thick, stringy, honeycombed mass on the surface of a clear watery whey. On potato the growth is almost invisible. There is abundant formation of gas in culture media, the gas both in dextrose media and in milk, according to Theobald Smith, consisting of hydrogen and carbon dioxide in the ratio 2 : 1 or 3 : 2.

Pathogenicity.—The *B. Welchii* is pathogenic for guinea-pigs and mice, but slightly so for rabbits. The whey of a milk culture in quantities of 0·5–2 c.c. per 100 grm. of body-weight produces death of a guinea-pig within forty-eight hours. Post mortem, if injected subcutaneously, the hair strips readily from the skin, which may be green and gangrenous; the subcutaneous tissue may also be green and gangrenous, or more or less digested, so that the skin hangs loose, and the sac formed contains gas and exudation, sometimes scanty, sometimes abundant, thin and sanguinolent, and containing numbers of bacilli. If the post-mortem be delayed, or if the heart-blood be taken up into tubes, and these are sealed and incubated for some hours, many of the bacilli will spore. Pigeons, by intra-muscular inoculation, are also susceptible. Injected intravenously into a rabbit, the animal killed immediately and the carcase incubated at 37° C. for twenty-four hours

a. Bacillus tetani. Cover-glass preparation of a pure culture. × 1500.



b. Bacillus Welchii. Cover-glass preparation of a milk culture. × 1000.

To face page 452.

and examined, there is an abundant formation of gas, particularly in the liver, which is riddled with gas-bubbles. This is a very characteristic test (Welch-Nuttall test).

The *B. Chauvæi* also produces this "foaming" condition of organs when similarly treated, but spores freely, whereas the *B. Welchii* does not spore under such conditions. Monkeys fed with considerable numbers of *B. Welchii* are unaffected. In the human intestine the organism is almost absent or scanty in nurslings and children, but becomes more and more abundant as age advances. It is probable that it is capable of producing necrotic changes in the intestinal mucous membrane. Different strains seem to vary much in virulence.

Products and toxins.—The gas production has already been mentioned. Butyric and allied acids are freely formed, but lactic acid is scanty. Indole may or may not be produced. Hæmolytic substances can be readily detected in blood-bouillon cultures, and the organism is abundant in the intestine in some cases of primary anæmia and possibly may have some relation to the condition. In some cases of infection the blood-serum agglutinates the organism.

Under the name *B. enteritidis sporogenes*, Klein[1] isolated a bacillus similar to the *B. Welchii* from the evacuations of, and from milk consumed by, patients suffering from an epidemic diarrhœa which occurred in St. Bartholomew's Hospital; as did Andrewes,[2] from cases of diarrhœa admitted into the same hospital. Klein believed this organism to be the cause of the diarrhœa, and stated that it could not be found in the intestinal evacuations of healthy individuals. Klein also

[1] *Rep. Med. Off. Loc. Gov. Board*, 1895-96, p. 197; *ibid.*, 1897-98, p. 225.

[2] *Ibid.* for 1896-97, p. 225.

found it in water, sewage, manure, and milk. The writer, however, showed that it could generally be found in the normal dejecta, also in road and laboratory dust, and frequently in milk, and the opinion he formed was that it was probably a ubiquitous organism and had little to do with the diarrhœa.[1] Glynn also found the organism to be very widely distributed, and fed guinea-pigs with, and himself ingested, cultures without result.[2]

The *B. enteritidis sporogenes* in its morphology, staining reaction, and cultural characters is almost, if not quite, identical with the preceding organism, the *B. Welchii* or *B. aërogenes capsulatus* of Welch. The only point of difference between them is that the former, according to Klein, is motile and flagellated, while the latter, according to Welch, is non-motile and non-flagellated. Spores are only formed in serum or gelatin, not on agar. It is abundantly present in sewage and sewage-contaminated water (see Chapter XXI). The *Clostridium butyricum* of Botkin, an energetic butyric-acid-forming anaërobic bacillus (p. 456), produces in milk changes similar to those of the *B. Welchii*, but is non-pathogenic.

Clinical Examination (Malignant Œdema and B. Welchii).

The character of the wound and discharge will probably give some indication of the existence of infection with malignant œdema or with *B. Welchii*. The tissues are softened, œdematous, and discoloured, and soaked with a foul-smelling, sanguineous fluid, which may be frothy from the development of gas. Other bacilli will probably be present.

(1) Make films from the discharge. Stain some with Löffler's blue, and others by Gram's method. Examine microscopically, and look for bacilli of the forms described. *B. Welchii* stains, malignant œdema does not stain, by Gram.

[1] *Trans. Jenner Inst. Prev. Med.*, vol. ii, 1899, p. 70.
[2] *Thomson Yates Lab. Rep.*, vol. iii, Pt. ii, 1901, p. 131.

(2) Inoculate two guinea-pigs subcutaneously with the discharge or with portions of the tissues. If the animals die, look for the characteristic organism.

(3) An attempt may be made to isolate the bacillus by anaërobic cultures and plate cultivations, prepared from unheated, and heated (80° C. for ten minutes), material.

Bacillus cadaveris sporogenes.

This is another organism isolated by Klein,[1] and has to be distinguished from the *B. Welchii*. The two organisms are morphologically very similar and both stain by Gram's method, but the *B. cadaveris sporogenes* does not produce the typical changes in milk. In a culture two or three days old the milk below the cream layer commences to clear, and later this change proceeds rapidly, so that at the end of a week three layers are apparent—an upper of unchanged cream, a middle, yellowish and watery, and a lower of precipitated casein. Its colonies on agar are also different, sending out ramifying, anastomosing threads from their margins, and it spores freely on agar in two to three days.

Black Quarter.

Syn.: Black Leg, Quarter Evil, Symptomatic Anthrax, Rauschbrand.

Black quarter is a disease affecting sheep and oxen, and is unknown in man. The names black quarter, black leg, and quarter evil are derived from the dark discoloration of the muscles of the leg and flanks or quarters of the affected animals. When the muscles are cut into a thin sanguineous fluid exudes, and in this slender bacilli are present, some of which are swollen or club-shaped from the presence of spores. The muscles are dark, slightly crepitant owing to the presence of gas, and have a rancid odour.

The organism, the *B. (Clostridium) Chauvæi*, is a slender rod never forming long threads, is strictly anaërobic and motile, but loses its motility in the presence of oxygen. Some

[1] *Centr. f. Bakt.* (1te Abt.), xxv, p. 278.

of the rods are cylindrical throughout, others form slender spindles, others are oval or lemon-shaped. It stains with the ordinary anilin dyes but not by Gram's method (except by Claudius's modification). Occasionally in the tissues it seems to stain by Gram. The organism forms endogenous spores, the spore-bearing rods being enlarged or club-shaped, and therefore should be termed a "clostridium."

It can be grown in deep stabs in gelatin and agar. Gelatin is rapidly liquefied. In glucose-agar it forms a thick, irregular, greyish growth, with much development of foul-smelling gas. The writer has found extreme difficulty in isolating and in maintaining cultures of the organism. The guinea-pig is susceptible if inoculated subcutaneously or into the muscles, the bacilli being found at the seat of inoculation, but not in the blood or internal organs. Artificial immunity can be induced in various ways: by bacilli attenuated by heat or by successive cultivations, or by heating the dried muscle to 85° to 90° C. for six hours (Kitt), also by inoculating the susceptible animal at the tip of the tail. Hanna,[1] by growing the organism in a mixture of blood-plasma and broth, obtained toxins which, by careful injection, conferred immunity on rabbits, the animals after injection yielding an antitoxic serum.

Hamilton has described specific anaërobic bacilli in braxy, louping-ill, and other diseases of sheep and deer.[2]

Clostridium butyricum.

An anaërobic organism occurring in milk, in which it produces a marked butyric acid fermentation with changes like those of the *B. Welchii*. It forms short rods, and also long ones 3 to 10 μ in length, and filaments are met with. Spore-formation takes place freely in enlarged segments. It forms a whitish growth on agar, and gelatin is rapidly liquefied, a scum forming on the surface. It is non-pathogenic (p. 454).

[1] *Journ. Path. and Bact.*, vol. iv, 1897, p. 383.

[2] *Rep. Louping-Ill and Braxy Com.*, Board of Agriculture and Fisheries, 1906.

CHAPTER XIV.

ASIATIC CHOLERA—SPIRILLUM METCHNIKOVI—SPIRILLUM OF FINKLER AND PRIOR—SPIRILLUM TYROGENUM—SPIRILLUM RUBRUM.

Asiatic Cholera.

The bacteriological study of Asiatic cholera may be said to date from the researches of Koch, who in 1884 was sent by the German Government to investigate the disease in Egypt and India. He described an organism present in the intestine and in the dejecta which he believed to be the specific contagium, and termed it the "comma bacillus" from its curved shape. This name is a misleading one, for the organism is not shaped like a printer's comma, but is a curved rod, and belongs to the group of spirilla; however, it is commonly known as "Koch's comma bacillus."

Spirillum choleræ asiaticæ.

Morphology.—Curved rods with rounded ends 1 to 2μ in length, sometimes forming half a circle, sometimes united in pairs forming an S-shaped curve (Plate XVIII., *a*). It is more or less abundant in the intestine and in the alvine discharges, especially in the rice-like flakes, but is not found in the blood, organs, or tissues. In the rice-like flakes it is frequently so numerous that in a cover-class specimen the "commas" appear in masses crowded together and lying parallel to one

another (known as the "fish-in-stream" arrangement). It stains well with the ordinary anilin dyes, especially with dilute carbol-fuchsin, but is decolorised by Gram's method. It is actively motile, and typically possesses a single terminal flagellum at one end only, but there is some variation in this respect. Spores are not formed, though in old cultures Hueppe described bodies which he believes to be arthrospores. In such cultures the bacilli lose their regular shape, and swollen and distorted involution forms are seen.

Under cultivation in bouillon or in a hanging-drop specimen the organism tends to develop into a spirilliform filament, and the commas are therefore regarded as resulting from the breaking up of a spirillum; but if the conditions of growth are very favourable multiplication may be so rapid that the curved rods or commas are alone produced, the organism dividing before it has had time to grow into a spiral. Such a curved rod is commonly known as a "vibrio."

Cultural characters and biology.—The Koch's spirillum is aërobic and facultatively anaërobic, and grows well on the ordinary culture media from 20° to 37° C.

According to Frankland, although it grows readily in an atmosphere of hydrogen, it does not develop in one of carbonic acid gas. In gelatin plates at 22° C. small cream-coloured colonies appear in about twenty-four hours, soon accompanied by liquefaction, so that in two or three days the plate becomes pitted. Microscopically, the young colonies are rounded with irregular margins, cream-coloured, and coarsely granular. In stab-cultures development occurs all along the stab as a whitish, opaque, punctate growth, thicker above than below. Liquefaction commences about the second day and progresses slowly; in the early stage it is confined to the surface, and looks like

a little bead or air-bubble (Plate XVIII., *b*), but in a fortnight or so the greater part of the gelatin may be liquefied. Liquefaction varies greatly both in rate and in extent in different cultures and stocks; in some old laboratory cultures it may be almost absent. On surface agar a thick, moist, shining, greyish growth quickly develops with more or less crenated margins, often becoming brownish when old. On blood-serum much the same growth occurs with slow liquefaction. A thin brownish layer is formed on potato at 37° C.; and broth becomes turbid, a delicate film forming on the surface. Peptone water, or Dunham's modification of it (1 per cent. NaCl), is a good cultivating medium, and becomes turbid, especially in the upper layers. In milk it multiplies rapidly without curdling; neutral litmus glucose-agar is reddened from the development of acid, but no gas is produced under cultivation. Acid, but not gas, is produced from glucose, maltose, saccharose, lactose, and starch.

An important characteristic of the cholera spirillum is the rapid formation of indole in considerable quantity, and the reduction of nitrates to nitrites, especially in peptone water. This forms the basis of the important cholera-red reaction; a few drops of pure sulphuric or hydrochloric acid added to a peptone-water culture, eight to twelve hours old, give a pink colour, and the colour is intense when the culture is two to three days old, and of a purplish-red colour, like that of potassium permanganate. Some specimens of "peptone" are unsuitable for the peptone water used for obtaining the reaction, either on account of the absence of a tryptophane nucleus, or of nitrates and nitrites. The medium should be sugar-free, and the addition of 0·01 per cent. potassium nitrate to it is an advantage. Some believe that two pigments are formed in the

reaction, a cholera-red and the nitroso-indole pigment.[1] The reducing action of the cholera spirillum can also be shown by growing in litmus broth, which becomes decolorised (Cahen's test).

Kraus and Prantschoff[2] noticed that certain vibrios dissolved red blood-corpuscles, but came to the conclusion that no true recently isolated cholera vibrio is hæmolytic (see also p. 465).

Strong,[3] in the Philippines, found that all vibrios which agglutinated well with a cholera serum were genuine cholera vibrios and that none of them was hæmolytic. On the other hand, Baerthlein[4] found that seven freshly isolated strains of the cholera vibrio were definitely hæmolytic in suspensions of sheep's corpuscles in from twenty-four to forty-eight hours. Van Loghem[5] employs goat's blood in hæmolytic tests for the cholera vibrio. He asserts that goat's blood is quickly hæmolysed by hæmolysing cholera-like (*e. g.* El Tor, p. 465) vibrios, but that recently isolated cholera strains, if they hæmolyse at all, do not do so for some time—twenty-four to forty-eight hours.

The cholera spirillum retains its vitality in cultures for a month. It can multiply in water and on the surface of moist linen, but rapidly dies on drying. Its thermal death-point, according to Sternberg, is 52° C. with an exposure of four minutes; according to Kitasato, 55° C. in about ten minutes. It is easily destroyed by the ordinary germicides.

In some experiments by Dempster[6] it was found that

[1] Wherry, Bureau of Government Laboratories, Manila, *Bulls.* 19 and 31, 1904 and 1905.

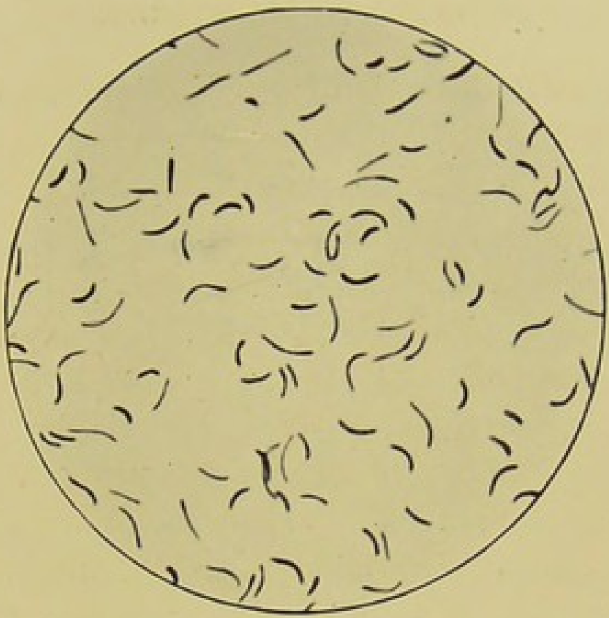
[2] *Wien. klin. Woch.*, 1906, p. 299.

[3] *Philippine Journ. of Science*, vol. v, 1910, p. 403.

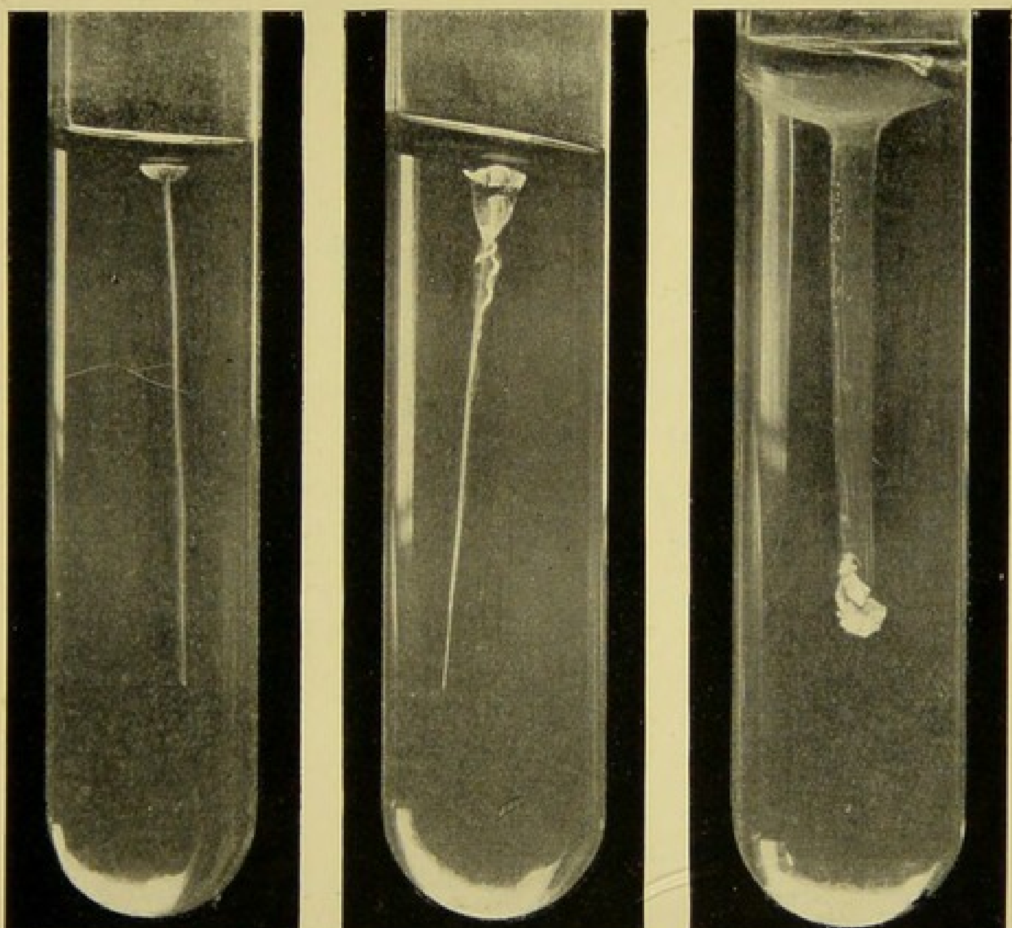
[4] *Arb. aus dem kaiserl. Gesundheitsamte*, xxxvi, 1911.

[5] *Centr. f. Bakt.*, Abt. I (Originale), lvii, 1911, p. 289.

[6] *Med.-Chir. Trans.*, vol. lxxvii, 1894, p. 263.



a. Spirillum choleræ. Cover-glass preparation of a pure culture. × 1500.



b *c* *d*

Gelatin stab-cultures, two days old, of (*b*) *Sp. choleræ*, (*c*) *Sp. Metchnikovi*, (*d*) *Sp. Finkleri.*

To face page 460.

the comma bacillus lived from three to five days in dry soil, but only one day in an artificially dried soil, while in moist soil it lived from twenty-eight to sixty-eight days. In peat, however, it was invariably dead within twenty-four hours. In sterilised salt solution (0·75 per cent.) the comma bacilli were alive on the 159th day, and in fresh urine (sterilised) they lived fourteen days at 37° C. and twenty-nine days at 22° C.

In sterilised distilled water the cholera spirillum usually rapidly dies, as a rule within twenty-four hours. The addition of sodium chloride greatly increases the length of time it may remain alive, a survival of five or six weeks having been recorded. In ordinary sterilised potable waters it may survive many months. In unsterilised potable waters its survival is greatly influenced by the presence of salts; in some cases it dies out rapidly; in others, especially in those containing a large proportion of salts, it may remain alive for some time. Houston[1] found that cholera vibrios die very rapidly in *raw* Thames, Lee, and New River waters as the result of storage in the laboratory. At least 99·9 per cent. perish within one week, and it was not possible to isolate any, even from 100 c.c. of the water, three weeks after infection. Klein[2] found that the cholera vibrio could retain its vitality for at least fourteen days in unsterilised sea-water, while from the interior of oysters, kept in water infected with the vibrios, it was obtained up to nine days after infection. In sterilised sewage the cholera spirillum multiplies and survives for months; in unsterilised sewage it may survive for two to four weeks (Houston).

Pathogenicity.—The disease is spread mainly by infected water; milk, salads, vegetables and flies are

[1] Metropolitan Water Board, *Fifth Rep. on Research work*, 1910.
[2] *Rep. Med. Off. Loc. Gov. Board* for 1896, p. 135.

also other sources of infection. The organism has been found in the dejecta of contacts not suffering from the disease, and it may sometimes persist for long periods after convalescence.

The relation of the cholera spirillum to the disease has been a very vexed question in the past, but the outcome of the voluminous researches which have been made is to confirm Koch's work. The organism is found in all cases of cholera, and several instances of laboratory infection from cultures have been recorded.

None of the lower animals suffers from or contracts a disease in any way comparable to Asiatic cholera, so that the test of animal experiments cannot be applied except in the case of young suckling rabbits (see below, "Anti-serum"). By first neutralising the acidity of the gastric juice by an injection of sodium carbonate solution into the stomach, then diminishing peristalsis by an injection of tincture of opium into the peritoneal cavity, and finally injecting a broth culture of the cholera spirillum into the stomach, Koch succeeded in inducing in guinea-pigs a condition somewhat similar to cholera in man—namely, indisposition with falling temperature, weakness of the extremities, and death in forty-eight hours. Post mortem, the small intestine was congested and filled with a watery fluid containing large numbers of the cholera spirillum. Injected into the peritoneal cavity of mice, guinea-pigs and rabbits, it usually produces death from a general septicæmia.

Metchnikoff[1] ascribes the immunity of animals to intestinal cholera as largely due to the inhibitory action of the other organisms present in the digestive tract. In man digestive disturbances are often an important predisposing cause of an attack. The acidity of the

[1] *Ann. de l'Inst. Pasteur*, vii, pp. 403, 562; vol. viii, pp. 257, 529.

gastric juice is also probably a means of defence (see "Water").

The blood-serum of an animal immunised by injections of the cholera spirillum gives a typical agglutination reaction with recent cultures of the organism. The reaction can also be obtained with the blood-serum of cholera patients sometimes as early as the first day of the disease, but it is probably of little use for diagnostic purposes, as the course of the disease is generally so rapid.

Occurrence of the vibrio.—That the cholera spirillum is associated with the disease seems to be beyond any doubt, and so constant is its presence in true cholera that all investigators, even those who at one time opposed Koch's views, rely on its detection for the bacteriological diagnosis. The matter, however, has become complicated owing to the detection in various natural waters of pathogenic spirilla which, although not identical with the cholera spirillum of Koch, resemble it so closely that it is difficult to classify them as anything but varieties of the cholera spirillum. In certain epidemics in India variations have also been noted in the cholera spirilla that have been isolated. Sanarelli[1] isolated from the Seine and Marne thirty-two spirilla, of which four were almost indistinguishable from cholera, except that they were only slightly pathogenic, but by passage through a series of animals their pathogenic power was much enhanced. Sanarelli believed that these were the descendants of true cholera spirilla that had gained access to the rivers during some previous epidemic of cholera. At the same time it is to be noted that vibrios may also be present in the normal intestinal tract of man and animals, and may therefore gain access to streams (Sanarelli). Dunbar

[1] *Ann. de l'Inst. Pasteur*, vii, p. 693, and ix, p. 129.

similarly, from the Elbe, Rhine, and other rivers, isolated a number of spirilla which could not be distinguished from the cholera spirillum (*Spirillum Elwers*). It was afterwards noticed that some of these under certain conditions of oxidation and temperature became phosphorescent,[1] but Rumpel[2] has also found that cultures of the genuine cholera spirillum may exhibit phosphorescence, so this cannot be used as a differential character for the separation of non-choleraic forms. Neisser isolated a spirillum, which he termed *Vibrio Berolinensis*, which agreed with the cholera spirillum in every particular except that the colonies in a gelatin plate were invisible to the naked eye in forty-eight hours. Heider found in the Danube a spirillum, named by him the *Vibrio Danubicus*, which resembled the cholera spirillum closely, but its colonies were somewhat different, and it was more actively pathogenic to mice. Ivanoff similarly obtained a spirillum which could only be distinguished from cholera by the finer granulation of its colonies and more distinct spiral form. Lastly, there is the *Spirillum Massowah*, isolated from an epidemic of cholera at Massowah, which differs from the Koch spirillum in having two terminal flagella at each end. Cunningham has also described several spirilla differing but slightly from the cholera spirillum.

Applying the Pfeiffer and agglutination tests to the spirilla in question, the following results were obtained. In the first place, each of the organisms gives a complete positive reaction to both tests with its own serum; this, of course, is only to be expected. Pfeiffer found that, using his reaction, the variety *Ivanoff* gave a positive reaction with cholera serum, and Durham found that *Ivanoff* and *Berolinensis* reacted completely

[1] *Centr. f. Bakt.* (1te Abt.), xviii, 1895, p. 424 (Kutscher).

[2] *Munch. med. Wochenschr.*, 1895, No. 3.

with cholera serum. Conversely, positive reactions with cholera spirilla were obtained with *Massowah*, *Danubicus*, and *Elwers* sera, while *Massowah* and *Elwers* react completely to each other. From these considerations it would therefore seem probable that some of these spirilla—*Sanarelli*, *Berolinensis*, and *Ivanoff*—may be varieties of the Koch spirillum. The Massowah spirillum is usually considered not to be a true cholera vibrio.

Ruffer[1] in 1905 at El Tor isolated vibrios, which may be distinguished as "El Tor vibrios," from the intestine of pilgrims returning from Mecca and suffering from various diseases (dysentery, diarrhœa, pneumonia, rheumatism), but among whom there had been no cholera, nor had they been in contact with cholera. These vibrios were subjected to detailed examination by the agglutination, saturation and fixation tests, and Pfeiffer's reaction with Berlin cholera-immune serum, and also by the hæmolysis test. Vibrios isolated from a previous epidemic of cholera (referred to as Group 1), and other vibrios isolated from cholera and other stool (Groups 3 and 4), were also compared with the El Tor vibrios. Ruffer's results were as follows:

Group 1 (undoubted cholera vibrios).—Those which react positively to the four principal tests with cholera serum—namely, the agglutination, saturation, and fixation tests, and Pfeiffer's reaction. They do not hæmolyse, even when remaining in contact with red corpuscles for three days at the temperature of the laboratory.

Group 2.—The second group contains the vibrios agglutinated by and giving the saturation and Pfeiffer's

[1] *Researches on the Bacteriological Diagnosis of Cholera.* Sanitary, Maritime, and Quarantine Council of Egypt, Alexandria, 1907. (Also *Brit. Med. Journ.*, 1907, vol. i, p. 735.)

reactions with cholera serum, but not fixing the cholera-immune body. These vibrios are strongly hæmolytic. This group consists of the El Tor vibrios only.

Group 3.—The third group is formed by vibrios which are not agglutinated by immune serum, do not give the saturation or Pfeiffer's reaction, but fix the cholera-immune body. These vibrios also hæmolyse, but feebly and late, often only after thirty-six to forty-eight hours.

Group 4.—The last group is formed by strongly hæmolytic vibrios not reacting at all to cholera-immune serum.

Ruffer concludes that the El Tor vibrios are not genuine cholera vibrios. He says: "The only possible classification is to group together all the vibrios reacting in the same way to all tests, separating them from those which, under the same conditions, behave in a different way. If this method be applied to the vibrios found at El Tor, there is no difficulty in distinguishing them from the true cholera vibrios, in spite of several of the reactions of both being similar. And it follows also that the agglutination, saturation and Pfeiffer's tests are not in themselves of absolute diagnostic value for cholera vibrios."

Neufield and Haendel,[1] however, after a re-examination of some of these vibrios, consider that they are true cholera vibrios. The matter therefore remains undecided.

Klein found that the cholera vibrio kept in sea-water showed marked variation from the original strain. In the East many cases of cholera are mixed "vibrionic" infections; the stools may contain several varieties of vibrios, some agglutinating with cholera serum, others not; some monociliate, others multiciliate.

[1] *Arbeit. a. d. Kais. Gesundheitsamte*, xxvi, 1907, p. 536.

It may be that, like the *B. dysenteriæ*, the cholera vibrio is not a single definite organism, but that cholera may be caused by any one of a group of closely allied vibrios.

Toxins.—Brieger in 1887 obtained cadaverin and putrescin and two other basic bodies from cholera cultures. Brieger and Fränkel isolated a tox-albumin, and Gamaleia a ferment-like body. Hueppe believes that the cholera poison is a tox-albumin formed in the culture medium, but that immunising substances are derived from the bacterial cells.

Rontaler compared the chemical products of the ordinary cholera and of the Massowah spirilla, and could find little difference between then.

Wesbrook[1] obtained albumoses and other bodies from alkali-albumin, egg, and Uschinsky medium, cultures. This observer also found aërobic cultures of the cholera spirillum to be much more toxic than anaërobic ones.

Pfeiffer found that cholera cultures killed with chloroform vapour contained a toxic substance fatal to guinea-pigs in small doses, with extreme collapse. He believed the substance to be an integral part of the bacterial cells.

Metchnikoff,[2] Roux and Salimbeni demonstrated the existence of a soluble cholera-poison in a very ingenious manner. Collodion sacs of 2 c.c. to 3 c.c. capacity were sterilised, filled with peptone solution, inoculated with the cholera spirillum, and closed. The closed sac was then introduced into the peritoneal cavity of a guinea-pig, which died in three or four days from the effects of the soluble toxins dialysing through the walls of the sac (see also next page).

[1] *Journ. of Path. and Bact.*, vol. iv, 1896, p. 1.
[2] *Ann. de l'Inst. Pasteur*, x, 1896, p. 257.

Brau and Dernier[1] obtained a toxic filtrate by cultivating the cholera vibrio in a medium consisting of horse-serum with an addition of 10 per cent. of defibrinated horse-blood.

Macfadyen obtained a highly toxic endotoxin by triturating cholera cultures with liquid air.[2]

Emmerich[3] strongly supports the view that the cholera intoxication is not a toxin intoxication, but is due to nitrite poisoning, the nitrites being produced by the reducing action of the vibrios on nitrates present.

Anti-serum.—By growing the cholera spirillum in a shallow layer with free access of oxygen in a peptone gelatin-salt medium, Metchnikoff and his co-workers obtained a toxic fluid after three or four days' growth. During incubation the fluid becomes concentrated to about one eighth by evaporation. After filtration, 0·25 c.c. killed a 300-grm. guinea-pig in eighteen hours. Goats having been inoculated with increasing doses of this toxin, commencing with 10 c.c. and reaching 200 c.c. in six months, become immunised and yield an antitoxic serum, 1 c.c. of which will neutralise four times the lethal dose of toxin. Metchnikoff had previously found that young suckling rabbits suffer from an intestinal cholera when fed with cultures, so that the effect of the cholera antitoxin in preventing intestinal cholera could be tested on these animals. Experiment showed that of the treated rabbits, 51 per cent. survived, of the untreated only 19 per cent. Salimbeni employed a serum prepared in this manner in the treatment of cases of cholera in the Russian epidemic, 1910.

Animals may be inoculated with dead and living

1 *Ann. de l'Inst. Pasteur*, xx, 1906.

2 *Lancet*, 1906, vol. ii, p. 494.

3 *Munch. med. Wochenschr.*, 1911, No. 18, p. 942.

cultures and an immune serum so prepared, but no practical value has yet attended the use of anti-sera in the treatment of cholera. Macfadyen immunised a goat with cholera-cell juice, and obtained a serum of which $\frac{1}{500}$ c.c. protected a guinea-pig against three lethal doses of cholera culture.

The writer prepared an anti-endotoxic serum in this manner, with which a few cases of cholera were treated in Russia.[1]

Vaccine.—Ferran in 1885 first prepared a vaccine by making cultures (mixed) in broth from cholera stools and injecting 0·3–0·5 c.c. subcutaneously, but the reports of the commissions sent to investigate the method were unfavourable.

Haffkine subsequently prepared a vaccine against cholera from cultures of the Koch spirillum, which seems to be efficacious in preventing the disease. For example, a number of labourers were inoculated during an epidemic, and among the inoculated the mortality was only 2·25, whereas among the uninoculated it was nearly 19 per cent. In another instance amongst 654 uninoculated there were 71 deaths, a mortality of 10·86 per cent., while among 402 inoculated there were only 12 deaths, a mortality of 2·99 per cent., and a reduction in mortality of 72·47 per cent.

In the Haffkine method two vaccines are made use of. The first or weak vaccine is prepared from cultures of the cholera vibrio attenuated by growing on the surface of agar, with free aëration, for several generations. The second or strong vaccine is prepared by enhancing the virulence of a cholera culture by a succession of passages through the peritoneal cavity of guinea-pigs. The virulence of this culture must be maintained in the same manner.

[1] *Lancet*, 1910, vol. ii, October 22nd.

For making both vaccines, "standard" agar cultures are employed. These are tubes in which the sloping surface of agar measures 15 cm. in length, and the cultures are incubated for twenty-four hours. The whole growth on such a tube is emulsified in 8 c.c. of broth or salt solution; the dose of this is 1 c.c., and the vaccines (living, or killed by heat, or carbolised) are injected into the flank, the second or strong being given seven to ten days after the first or weak.

Besredka,[1] by making a mixture of cholera culture and cholera-immune serum, allowing this to stand for twelve hours, heating to 56° C. for one hour and then injecting subcutaneously, claims that an immediate and lasting (six months) immunity may be produced.

Strong[2] prepares a vaccine from autolysed cultures. The cholera vibrio is grown on surface agar for twenty-four hours at 37° C.; the growth is then washed off with sterile water, the suspension is kept at 60° C. for twenty-four hours, and then at 37° C. for two to five days, and is finally filtered through a porcelain filter.

Clinical Diagnosis.

Some of the rice-like flakes should be picked out of the stool and well rinsed in sterile salt solution.

1. From one of the whitish, slimy, rice-like flakes in the evacuations or the intestine films are prepared, stained in Löffler's blue, washed, dried, and mounted. If on examination large numbers of curved rods lying in groups parallel to one another are observed, the diagnosis of Asiatic cholera may be made with some degree of certainty. Koch states that this is so in quite half the cases, especially the acute ones. (Single, or a few, vibrios are of no diagnostic significance; they may occur in normal and diarrhœa stools. The

[1] *Ann. de l'Inst. Pasteur*, 1902, p. 918.
[2] *Bureau of Gov. Laboratories, Manila*, Bull. No. 16, 1904. (Bibliog.)

presence of numbers of vibrios having the "fish-in-stream" arrangement is also not absolutely characteristic.)

2. Gelatin and agar plates should be prepared from an emulsion of rice-like flakes. Agar plates are best prepared by smearing the flake over the surface of the solidified agar. The plates are incubated at 22° C. and 37° C. respectively. In the gelatin plates the characteristic colonies of the cholera vibrios should be recognisable in about twenty-four hours, in the agar plates in from twelve to sixteen hours. The likely colonies should be examined microscopically and peptone-water and other cultures prepared from them.

A better medium to employ is Dieudonné's blood alkali agar. Equal parts of defibrinated ox-blood and normal caustic soda solution are mixed and sterilised in the steamer. Of this 30 c.c. are mixed with 70 c.c. of ordinary peptone-agar (neutral to litmus), previously melted. Plates are poured and the plates are kept at 60° C. for half an hour, and are then allowed to stand for twenty-four hours for ammonia to vaporise. On this little else than the cholera vibrio develops (except cholera-like vibrios, which develop equally well).

3. With other rice-like flakes several peptone-water cultures should be prepared and incubated at 37° C. This is best done in the small Erlenmeyer flasks containing a shallow layer (1–2 cm. deep) of Dunham's peptone-water, without wool plugs, but capped with a piece of sterile filter-paper. In eight to ten hours the upper layers of the fluid should be examined microscopically for the presence of commas, and gelatin, agar and Dieudonné agar plates and subcultures in peptone water are also made by inoculating from the surface layer of fluid. The peptone-water culture may then be tested for the presence of indole by carefully adding a few drops of pure concentrated sulphuric acid. In cases of Asiatic cholera the indole reaction can be obtained as early as eight hours after inoculation.

4. To vibrios that have been isolated, the agglutination, saturation, and fixation tests and Pfeiffer's reaction should be applied, a high-grade authentic cholera-immune serum

being used. The hæmolysis test should also be applied (p. 189).

Agglutination tests are, however, somewhat variable with different strains.

5. If the case has lasted any time the agglutination reaction may be applied, testing the patient's serum on a known strain of cholera vibrio, but this is of doubtful value.

Spirillum Metchnikovi.

Isolated by Gamaleia from the intestinal contents of chickens dead of an infectious gastro-enteritis which occurred in certain parts of Russia. The disease, although resembling chicken cholera in some respects, is quite distinct from the latter. This spirillum forms curved rods and spiral filaments, generally slightly shorter, thicker, and more curved than the Koch spirillum. It is decolorised by Gram's method, and is best stained with weak carbol-fuchsin. It is readily cultivated and is aërobic and facultatively anaërobic. In gelatin plates it forms small whitish colonies, visible within twenty hours, which grow more rapidly than the cholera vibrio, and in two or three days produce marked areas of liquefaction. In a stab-culture in gelatin a whitish granular growth occurs along the line of puncture with liquefaction, much like that of the Koch spirillum, but the rate of growth and the liquefaction are more rapid (Plate XVIII., *c*). Grown in eggs by Hueppe's method typical appearances are produced. After ten days the white becomes transformed into a yellowish limpid liquid, while the yolk, though retaining its form and consistence, is quite black. On surface agar a thick cream-coloured layer develops; on potato the growth is brownish, and milk is coagulated. It grows freely in broth and peptone water, the fluid becoming uniformly turbid, and a slight

film forms on the surface, and these cultures give a marked indole reaction on the addition of sulphuric acid alone, in this respect resembling the Koch spirillum. The *S. Metchnikovi* is pathogenic to chickens, pigeons and guinea-pigs, but not to rabbits or mice except in large doses. It is, however, more pathogenic to guinea-pigs than the cholera vibrio. Pigeons are killed by intra-muscular inoculation, and fowls are susceptible to feeding, whereas the cholera vibrio is not fatal to pigeons and fowls under these conditions. It is not agglutinated with cholera-immune serum. Abbott[1] isolated a pathogenic spirillum from the Schuylkill River, Philadelphia, which resembles the *S. Metchnikovi* closely, and is probably identical with it.

Spirillum Finkleri (of Finkler and Prior).

Isolated from the stools in certain cases of cholera nostras, but its ætiological significance is doubtful. It occurs as short, thickish, curved or straight rods, and sometimes as spiral filaments. It is aërobic and facultatively anaërobic, does not form spores, and does not stain by Gram's method. In a gelatin stab-culture a yellowish growth forms with rapid liquefaction (Plate XVIII., *d*). On agar a thick, slightly brownish, moist layer develops. Serum is rapidly liquefied. On potato a slimy brownish growth occurs even at room temperature. It grows in broth and peptone water, producing a general turbidity. It does not as a rule give the indole reaction with sulphuric acid alone, but the ordinary laboratory cultures after three to four days' growth occasionally give a slight reaction. It is stated to be pathogenic to guinea-pigs by intra-peritoneal inoculation.

[1] *Journ. of Exper. Med.*, vol. i, 1896, p. 419.

Spirillum tyrogenum.

Obtained by Deneke from old cheese, and frequently spoken of as Deneke's spirillum. It forms curved rods and spiral filaments somewhat closely resembling the Koch spirillum. It grows well on the ordinary culture media at room temperature, but development is usually slight or absent at 37° C. In a gelatin stab-culture a yellowish growth occurs with liquefaction, which is much more rapid than that of the Koch spirillum, but less so than than of the Finkler-Prior spirillum. On agar a thinnish, brownish, somewhat membranous and coherent layer slowly develops at room temperature. On potato a yellowish growth occurs. It is stated to be slightly pathogenic to guinea-pigs by intra-peritoneal inoculation.

Spirillum rubrum.

A chromogenic spirillum obtained by Koch from the putrefying tissues of a mouse. In a gelatin stab-culture a dark red growth slowly develops along the line of puncture without liquefaction; at the surface, however, the growth is colourless. In broth at 37° C. it grows freely, producing a general turbidity with a red deposit at the bottom of the tube; there is no film formation. In such a broth culture large numbers of typical spirillar filaments can be seen, which are thin and delicate, of varying length, and actively motile. It is non-pathogenic.

Vibrios are common in the mouth, and may be met with in the discharge of septic ulcers.

CHAPTER XV.

STREPTOTHRIX INFECTIONS — ACTINOMYCOSIS — MYCETOMA — LEPTOTHRIX BUCCALIS — CLADOTHRIX DICHOTOMA — MYCOSIS TONSILLARIS.

Streptothrix Infections (Streptothricosis).[1]

THE Streptotricheæ are a group of thread-forming organisms showing true, but not dichotomous, branching. Their exact position in the botanical scale is uncertain; by some they are considered to belong to the higher Schizomycetes, forming a connecting link between these and the Hyphomycetes; others place them among the latter, and others make them a separate and distinct group.

The Streptotricheæ form a filamentous network, or mycelium, the individual threads of which show branching, while their terminal portions undergo segmentation, with the formation of rounded bodies regarded as spores. The mycelial network, unless old, stains by Gram's method, and occasionally possesses "acid-fast" properties.[2] The leprosy bacillus apparently sometimes grows as a streptothrix, and the tubercle, glanders, and perhaps diphtheria, bacilli may belong to this group.

Pathogenic streptothrix forms are not uncommon, the best known being those causing actinomycosis of the ox and other animals and of man, the white variety of mycetoma, the *S. Eppingeri*, more or less acid-fast, originally isolated from a cerebral abscess, and also causing a variety of madura foot, *S. Nocardii* of the ox, and *S. canis* of the dog. Doubtless

[1] See Musgrave, Clegg and Polk, *Philippine Journ. of Science*, vol. iii, 1908, p. 447; Foulerton, *Lancet*, 1910, vol. i, p. 551, *et seq.*

[2] See Birt and Leishman, *Journ. of Hyg.*, vol. ii. Pt. ii, 1902.

cases of streptothrix infection in man may occasionally be missed, as the clinical characters are those of tuberculosis.

Actinomycosis.

In man, actinomycosis in its clinical history and pathological lesions closely resembles tuberculosis, with which in the past it was frequently confounded.

In cattle, actinomycosis has long been known, but its exact pathology was involved in considerable doubt until the researches of Bollinger in 1876. It forms tumours chiefly affecting the tongue, jaw, face, and throat, and was described under such varied names as wen, scrofula, scirrhus, osteo-sarcoma, cancer, wooden tongue, etc.

The tumours after a time break down and discharge, the tongue often protrudes from the mouth, the saliva drips, and the animal becomes much emaciated.

On cutting into a "wooden tongue," or wen, a grating sensation is felt, such as that experienced in cutting a turnip or unripe pear; on examining the section little rounded, yellowish, frequently almost caseating areas will be noticed, resembling old tubercles. On making sections and examining with a low power it is found that these rounded areas are composed of masses of small round-cells, with occasionally giant-cells, surrounded by a capsule of fibrous tissue. The growth may be so soft as to be practically purulent, and abscesses varying in size from a pin's head to that of an orange may be present in the affected areas. Like tubercles, the growths may become caseous, calcified, or fibrous. In the growth or in the pus from abscesses, when examined fresh with a low power, yellowish or yellowish-white granules will be found here and there, which may be very minute, or as large as a small pin's head, and are somewhat soft

a. Actinomycosis bovis. Section of tongue. Gram. × 350.

b. Mycetoma. Section of tissue, white variety. Gram. × 350.

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Note Clinically &
Pathogenically
liber Tuberculosis

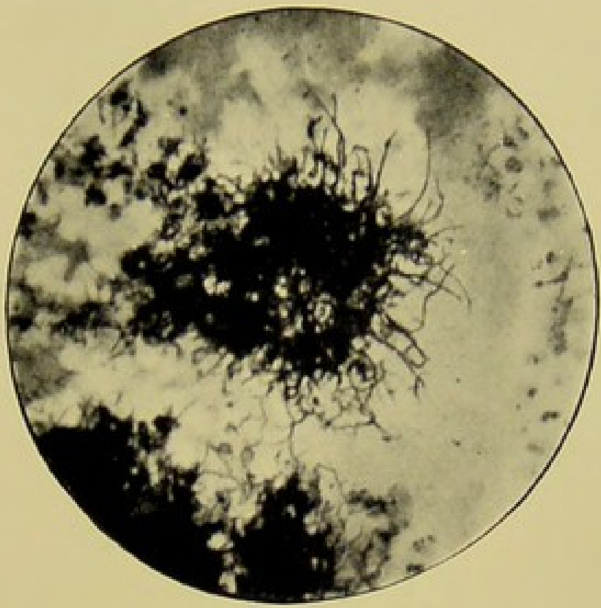
in consistence and on slight pressure flatten out. Examined with a high power, these granules are found to contain round, ovoid, or reniform bodies which have a rosette-like appearance, a more or less structureless centre with club-shaped bodies radially arranged around the periphery (Plate XIX., *a*). These peculiar structures are the cause of the disease, and are the form assumed in the animal body by an organism belonging to the streptothrix group termed the *Actinomyces*, or *Streptothrix bovis*, or, from its appearance, the ray fungus.

Sections of the diseased tissues show the structure of the organism still better. Gram's method usually gives good results, and it will generally be found that the following appearances can be observed: Surrounded by the round-cells are the reniform or ovoid bodies, situated at the periphery of which are radially arranged, club-shaped structures deeply stained with the gentian violet, while the central portion is unstained and structureless, or contains granular matter or calcareous particles. Various appearances may be met with in different parts of the section, according as the actinomycotic nodules are cut through their centre or periphery; when the latter is the case, the clubs are shown in transverse section and appear as closely packed, deeply stained dots. Sometimes, however, in addition to the clubs, the centre of the rosette is occupied by numerous interlacing filaments, also stained by the gentian violet.

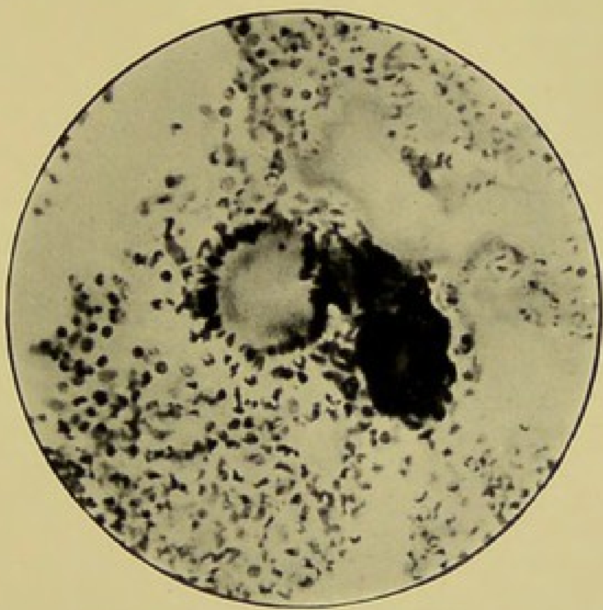
In man, actinomycosis is often associated with suppuration. If a little of the pus be examined it will probably contain tiny yellowish or sulphur-yellow granules, which, microscopically, are found to consist of tufts of fine tangled filaments, the ends of which may be continued into little swellings or clubs. In teased-up

specimens, or in sections stained by Gram's method, an appearance is observed very different from that of the bovine variety, viz. tufts of interlacing filaments stained by the gentian violet, but a complete absence of purple clubs (Plate XX., *a*). Clubs, however, are frequently present around the periphery of the filamentous tufts in a stunted condition, and they do not usually stain by Gram's method. These clubs are often seen better in fresh specimens of the pus or in unstained sections, or by staining with orange-rubin, or the Ehrlich-Biondi reagent (Plate XX., *b*). The conditions in cattle and man, at first sight so very different, are thus seen to be similar, a similarity which is further established by the occasional occurrence in cattle of filamentous tufts, staining by Gram's method, within the rosettes, and by the clubs in man now and then taking on the gentian-violet stain.

Cultural characters.—The cultivation of the *Actinomyces* can be performed by collecting the pus from a case of the disease in sterilised tubes, and subsequently turning it out into a sterilised capsule and picking out the actinomycotic granules with sterilised needles, planting these on the surface of glycerin agar, and incubating at 37° C. A certain number of the tubes will probably be uncontaminated, but in others a growth of the *Micrococcus pyogenes* var. *aureus* or other pyogenic organism, which is not unfrequently associated with the *Actinomyces*, may occur. In the uncontaminated tubes a growth begins to appear in a few days in the form of little colonies of a tough membranous consistence, somewhat wrinkled, greyish, and shining, while the agar beneath them becomes stained brownish. The growth increases and the colonies coalesce, forming a brownish, wrinkled, membranous expansion, sticking firmly to the agar and



a. Actinomycosis hominis. Section of liver showing a mycelial mass. Gram. × 500.



b. Actinomycosis hominis. Section showing a ring of stunted clubs. Gram. × 350. Same material as fig. *a* above.

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difficult to remove or break up, while the agar becomes stained brown throughout; later on the membranous growth may become dappled with yellow as though powdered with flowers of sulphur, but occasionally remains whitish. In gelatin little spherical feathery tufts develop, and sink to the bottom as liquefaction progresses. On potato a remarkable growth develops; at first brownish, it afterwards becomes almost black, and is very thick or heaped up with a much wrinkled surface, while later on it has the appearance of being sprinkled with flowers of sulphur (Fig. 49). In broth delicate woolly flocculi form. Films from young agar cultures show masses of tangled filaments, which appear to be more or less branched, and stain well with the ordinary anilin dyes and by Gram's method; with the latter the filaments often appear somewhat beaded, but no trace of rosette formation or even of clubs is ever found in cultures (Fig. 50). In pus, especially human, the filaments can sometimes be seen if stained by Gram's method with orange-rubin. Inoculated into the peritoneal cavity of rabbits and guinea-pigs the cultivated organism reproduces the disease, numerous antinomycotic nodules forming in the peritoneum and elsewhere. There is much doubt as to the mode of spread of, and the infection of man with, the disease. It does not seem to be particularly contagious, and diseased and healthy animals are often placed together without bad result; it can, however, be conveyed by

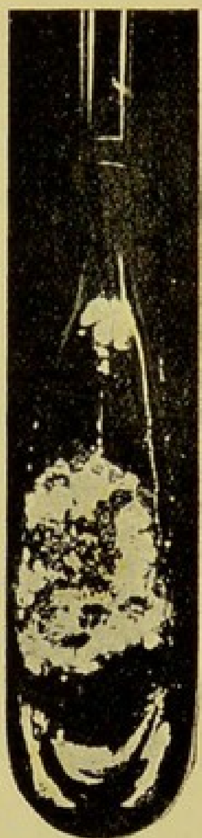


Fig. 49. — Actinomyces. Potato culture, three months old.

Brownish Colour

direct inoculation, for calves inoculated intra-peritoneally with portions of diseased tissues die after some weeks or months, with an abundant development of actinomycotic nodules, as shown by the experiments of Jone and Ponfick. Crookshank also infected a calf with the material from a human case. Feeding experiments give negative results. The view generally held is that the organism occurs on cereals, straw, or roots, and

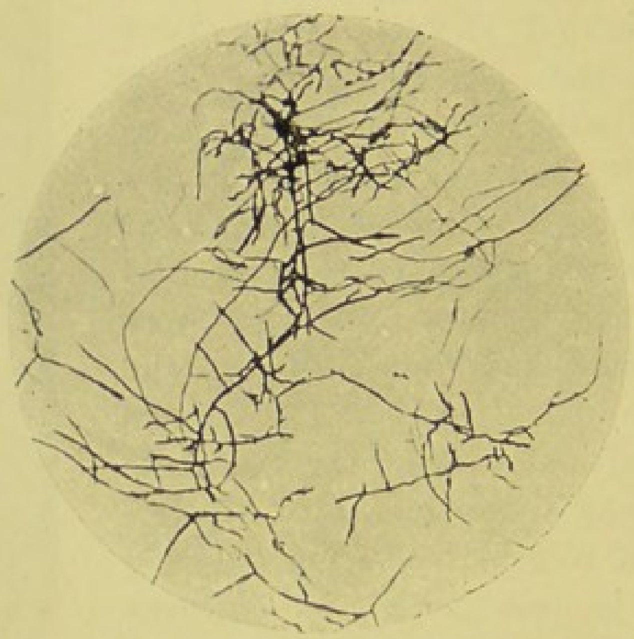


Fig. 50.—Actinomyces. Cover-glass preparation. Gram. × 750.

gains access to the system through slight scratches or wounds in the mucous membrane of the mouth, pharynx, or larynx. In man no source of infection has been traced, though cases have been reported where the disease has occurred after eating grains of barley, etc. The disease is met with not only in cattle, but also in horses and swine. In the last-named animals considerable calcification may be present in the nodules, and it may be necessary to decalcify with dilute nitric or hydrochloric acid before the rosettes can be stained.

It is important to note that tuberculin may cause a reaction in actinomycosis, similar to that which occurs in tuberculosis, and as the actinomycotic lesions are very like those which are found in the latter disease, mistakes may easily be made, and can only be avoided by a microscopical examination. It is of considerable practical importance to distinguish actinomycosis from tuberculosis, for in many cases of the former, both in man and in animals, iodide of potassium exerts a specific curative action.

By some several species of Actinomyces are believed to exist, but Homer Wright[1] considers that but one species of micro-organism is the ætiological agent, both in man and animals, the *A. bovis*.

"Farcin des bœufs," a disease of cattle occurring in Guadeloupe, and characterised by infection first of the skin and afterwards of the lymphatic glands and viscera, is due to the *S. Nocardii*.

Clinical Examination.

1. Pour out the pus or discharge into a large capsule or Petri dish so that it forms a thin layer, look for any yellowish or other granules, pick them out with a needle, and place on a clean slide in a drop of 50 per cent. glycerin. If no granules can be found a little of the discharge may be spread on a slide with a drop of 50 per cent. glycerin. Cover with a cover-glass, and apply a little pressure. Examine with a $\frac{2}{3}$-in. objective. If any actinomycotic tufts are present they will be seen as yellowish or pale brownish, spheroidal, ovoid, or reniform masses, and with a $\frac{1}{6}$-in. objective will be found to have a radiating structure from the presence of the clubs.

2. Stain cover-glass specimens of the discharge, by Gram's method, with eosin. The actinomycotic tufts will generally be found to consist of little masses of tangled filaments stained

[1] *Journ. Med. Research*, 1905.

violet and surrounded by a pink zone which has an indistinct radiating structure.

N.B.—In most instances the clubs in Actinomycosis hominis do not stain by Gram's method. The reverse is the case in Actinomycosis bovis.

3. Sections of actinomycotic tissue are best prepared by the paraffin method. If frozen, the actinomycotic nodules are very apt to fall out. Sections may be stained by any of the following ways:

(*a*) By Gram's method, with eosin or orange-rubin.

(*b*) With the Ehrlich-Biondi triple stain. Stain for from half an hour to two hours. Place in methylated spirit until the sections appear greenish, then pass through absolute alcohol and xylol. The clubs are stained yellowish-brown, and are sometimes shown in human cases when unstained by Gram's method.

(*c*) By Plaut's method. Stain in warm carbol-fuchsin for ten minutes, rinse well in water, stain in a saturated solution of picric acid in methylated spirit for five to ten minutes, rinse well in water, place in 50 per cent. alcohol for ten minutes, pass through absolute alcohol and xylol.

(*d*) Good preparations may be obtained by staining in Ehrlich's hæmatoxylin and counter-staining with orange rubin. This may also show the clubs when they are unstained by Gram's method.

Madura Disease or Mycetoma.

Madura disease, otherwise known as madura foot, mycetoma, or the "fungus disease of India," is a chronic local affection generally attacking the foot, occasionally the hand, sometimes extending up the leg, but rarely to the trunk. The disease occurs in certain districts in India, and full descriptions of it have been given by Vandyke Carter and by Lewis and Cunningham. A "madura" foot appears enlarged, and numerous sinuses with raised mammilated apertures open on the surface (Fig. 51). On making a section into the diseased tissues the bones are found to be more or less carious, while

the soft structures are tough and hypertrophied from the occurrence of chronic inflammatory changes. Numerous small cavities are present, sometimes filled by yellowish granules resembling fish-roe, and hence termed "roe-like particles," at others containing black particles of irregular shape, coal-like consistence, and variable size, exceptionally as large as a marble or walnut. The presence of the white or black granules, which may be discharged from the sinuses before mentioned, divides the disease into two classes—the so-called white and black varieties. Lewis and Cunningham

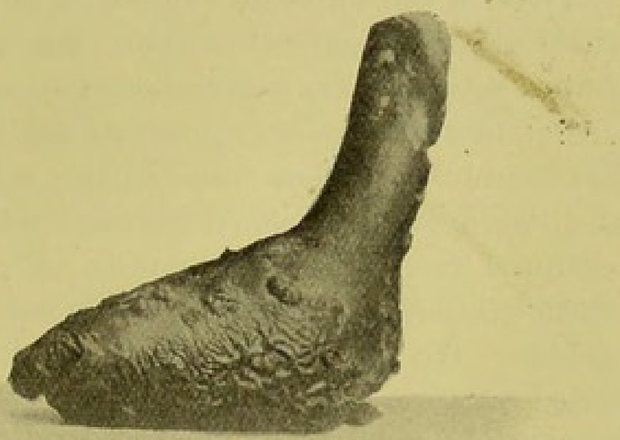


Fig. 51.—A foot affected with madura disease. (White variety.)

have also described a third variety, in which the granules are red like cayenne pepper.

Vandyke Carter[1] first called attention to the similarity between the white variety and actinomycosis in their microscopical characters. In sections stained by Gram's method more or less crescentic or reniform bodies are noticeable, divided into wedge-shaped areas, which contain masses of fine filaments stained purple. Surrounding the crescentic bodies is a zone of radially arranged elements, many of which are fan-shaped owing to branching; they are indistinct, as they do not stain with the gentian violet, but they are very sug-

[1] *Bombay Med. and Phys. Soc.*, vol. ix, 1886 (new series), p. 86. Also Hewlett, *Trans. Path. Soc. Lond.*, vol. xlii, 1893.

gestive of the club-shaped structures present in actinomycosis, and they resemble the *Actinomycosis hominis* inasmuch as they do not stain by Gram's method (Plate XVIII., *b*). By staining with hæmatoxylin and orange rubin, or with the Ehrlich-Biondi triple stain, here and there in the radial zone well-defined clubs can be demonstrated. It seems, therefore, that the radial zone is composed of degenerate club-shaped structures, and the disease evidently closely resembles actinomycosis, but seems to be due to a different species of streptothrix.

From a case of the white variety Boyce[1] cultivated a streptothrix which differed somewhat from the Actinomyces, as it grew slower, produced no pigment, and on agar formed white raised colonies with radial grooves, not unlike the tiny barnacles found on wooden piles in the sea. Vincent[2] also isolated a streptothrix, perhaps identical with that of Boyce, which differed from the Actinomyces in growing feebly in broth, in not liquefying gelatin, and in not being inoculable in the rabbit. He describes it as forming on glycerin agar umbilicated colonies, first white and afterwards red. Shattock[3] suggests that the red, cayenne-pepper-like grains occasionally met with in mycetoma may be due to colonies of the streptothrix which have produced their pigment. Microscopically, this organism (*Streptothrix maduræ*) is identical with the Actinomyces. Musgrave and Clegg in a case of the white variety isolated a streptothrix (*S. freeri*) differing from the *S. maduræ*, but identical with the *S. Eppingeri*.

The relation of the black to the white variety of madura disease has been somewhat debated. Kanthack[4] described the black variety as being probably a late stage of the white. It seems, however, that the co-existence of the two conditions in the same specimen is very rare, and Boyce and Surveyor,[5] after a critical examination of a large number of specimens,

[1] *Hygienische Rundschau*, 1894, No. 12.
[2] *Ann. de l'Inst. Pasteur*, 1893.
[3] *Trans. Path. Soc. Lond.*, vol. xlix, 1898, p. 294.
[4] *Journ. Path. and Bact.*, 1892.
[5] *Proc. Roy. Soc. Lond.*, 1893, and *Phil. Trans. Roy. Soc. Lond.*

came to the conclusion that the black variety is a distinct disease, and due to an organism belonging to the group of the higher fungi, the black particles or masses being the lignified mycelium or "sclerotium" such as is met with in ergot.

By planting out the granules from an early case of the black variety Wright succeeded in cultivating a hyphomycete.[1] It formed long branching hyphæ, but no spore-bearing organs were produced, and inoculation experiments on animals were negative. It grew on potato as a dense, widely spreading, coherent, velvety membrane, in colour pale brown with white periphery. Small drops of brown, coffee-coloured fluid appeared on the surface, and the potato became brown throughout. On agar the growth formed a meshwork of widely spreading greyish filaments; in old cultures (also in potato infusion) black hard granules, or "sclerotia," were observed. In broth little balls of radiating filaments developed.

Brumpt has distinguished no less than eight varieties of mycetoma.

It would seem that there are probably several conditions, both in actinomycosis and in mycetoma, having a general resemblance but differing slightly, and dependent upon different species of streptothrix.

Mycosis tonsillaris (Mycosis pharyngis leptothricia).

A chronic disease attacking young adults, resistant to treatment, and characterised by the presence of small, white, tough, adherent excrescences on the mucous membrane of the pharynx. Microscopically, the patches consist of collections of epithelial cells and *débris*, infiltrated with leptothrix filaments and bacteria. The disease, however, seems to be a keratosis, infection with the organisms being secondary.

But occasionally a true "mycosis" apparently occurs, readily amenable to treatment, and due to a leptothrix.[2]

[1] *Journ. Exp. Med.*, vol. iii, 1898, p. 421.

[2] See *Glasgow Medical Journal*, No. 2, 1896, p. 81 *et seq.* (Brown Kelly).

Leptothrix buccalis.

Four somewhat similar thread forms occur in the mouth, viz. *Leptothrix racemosa*, *L. buccalis maxima*, *L. innominata*, and *Bacillus maximus buccalis*. The first is very common, forms large threads, shows a peculiar beaded appearance on staining which has been regarded as sporulation, and may be a fungus form. *L. buccalis maxima* and *L. innominata* differ from each other in that the former gives a blue granulose reaction when treated with iodine and dilute sulphuric acid, while the latter does not. All these three organisms are very similar, and the filaments are either unsegmented, or the segments are of considerable length. The *B. maximus buccalis* is very like the *L. buccalis maxima*, but does not give the granulose reaction, and its segments are shorter. It is motile, flagellated, and sporing, and stains by Gram's method.

Some confusion exists respecting the thread forms of the mouth.[1]

Cladothrix dichotoma.

An organism not unfrequently met with in natural waters. It forms long threads, straight, or sometimes slightly undulating, or even spiral and apparently branched, though the branching is not dichotomous. It can be cultivated on the ordinary laboratory media at room temperature, forming on agar a brownish, wrinkled, tough, membranous layer, very adherent, and staining the medium beneath it a pale brown, not unlike the Actinomyces in these respects. It is non-pathogenic.

[1] See Goadby, *Mycology of the Mouth*.

CHAPTER XVI.

THE BLASTOMYCETES.

The Pathogenic Blastomycetes—Sporotrichosis—Thrush—Saccharomycetes and Torulæ—Yeasts and Fermentation.

Pathogenic Blastomycetes.[1]

THE Blastomycetes, or yeasts, are distinguished from the bacteria by their mode of reproduction. Whereas in the bacteria reproduction is by fission or simple division, in the Blastomycetes it is by gemmation or budding. If a cell of ordinary brewer's yeast be watched under conditions favourable to growth and reproduction, it will be found that a slight protuberance makes its appearance at one pole of the organism; this increases in size, and ultimately a daughter-cell resembling the parent is reproduced and separates off.

In some of the yeasts there is also a method of reproduction by endospore formation, and according as this occurs or not the Blastomycetes are divided into two groups:

Blastomycetes
1. Saccharomycetes, or true yeasts, in which spore formation occurs.
2. Torulæ, in which no spore formation has been observed.

Although the term "torula" has thus a definite signification, it is often loosely used to denote any yeast-cell.

There are also a few organisms composed of yeast-like cells and with multiple spores, but multiplying by fission and termed *Schizosaccharomyces*.

[1] See Le Count and Myers, *Journ. of Infectious Diseases*, vol. iv, 1907, p. 187.

In addition to reproduction by gemmation, the Blastomycetes are also distinguished from the Bacteria by their larger size, and in those forms in which endospores occur by the spores being multiple and not single in each cell. From the Hyphomycetes, or moulds, the Blastomycetes are distinguished by being unicellular, and by the reproduction being asexual. The Blastomycetes, however, are probably much more nearly allied to the Hyphomycetes than are the bacteria, for many of the moulds have a stage in which the mycelium (see next chapter) resembles an aggregation of yeast-cells, and the yeasts in old cultures form films in which the cells become much elongated, like those in the mycelium of a mould. Jörgenson and others have attempted to show that some of the yeasts are stages in the development of a fungus, but it cannot be said that this has yet been satisfactorily demonstrated. Organisms of yeast-like form, not sporing, but tending to form a mycelium under certain conditions, have been included in a group termed the *Fungi imperfecti, e.g.* torulæ and thrush.

Organisms apparently belonging to the Blastomycetes have been isolated from certain tumours, and have been regarded as having an ætiological significance in connection with malignant disease. Sanfelice cultivated yeast forms from fermenting fruits, which, on inoculation into guinea-pigs, produced death in about a month with the formation of a tumour at the seat of inoculation and embolic growths in the spleen and liver. He also obtained a similar yeast from an ox affected with carcinoma, which on subcutaneous inoculation killed guinea-pigs in about two months, and inoculated into the peritoneum in a month, with multiple embolic growths in the lungs, spleen, and mesenteric glands. A good deal of calcification was present in the growths, from which fact Sanfelice named this yeast *Saccharomyces litogenes*. Rabinowitch and also Foulerton[1] have

[1] *Journ. Path. and Bact.*, vol. vi, 1899, p. 37.

found that some of the ordinary yeasts give rise to tumour formation on inoculation, especially in the rabbit. These tumours produced by yeasts are probably granulomata and not true malignant tumours.

Curtis[1] obtained a yeast from an apparently myxomatous tumour in a young man. The organism was met with in two forms—free and encapsuled. The free form appeared in young agar cultures as round or ovoid cells measuring 3 to 6 μ in diameter, often showing budding. The encapsuled form was met with in the original tumour and in the tissues of inoculated animals, and occurred as a large sphere 16 to 20 μ in diameter, enclosing the yeast cell, the capsule being hyaline and 4 to 6 μ in thickness. On agar at 37° C. it formed whitish, opaque, creamy colonies in two to three days, becoming a thick creamy growth at the end of a week. On gelatin it formed white colonies or growth in four to five days without liquefaction, and in broth a flocculent deposit, the broth remaining clear. It was aërobic, did not grow on serum, and formed a small quantity of acetic acid and alcohol when grown in beerwort and sugar solutions. It was not pathogenic for guinea-pigs, but inoculated into rabbits, rats, mice, and dogs it produced tumours and caused death. The tumours to the naked eye appeared to be myxo-sarcomata, and in them the yeasts were found.

Busse also obtained a pathogenic yeast from a young woman who suffered from a tumour of the tibia, and ultimately died with diffused growths in the bones and organs. The yeast-like cells were observed in the affected parts, and were isolated by cultivation, and the cultures, inoculated into mice and rabbits, produced death with growths in the organs. As in Curtis's case, the cells in the tissues appeared to be encapsuled.

[1] *Ann. de l'Inst. Pasteur*, x, 1896, p. 449 (Refs.).

Gilchrist described a case of blastomycetic dermatitis. Small miliary abscesses were present in the rete and corium, in the pus of which the parasitic cells were observed. These were usually in pairs of unequal size, the largest measuring about 16 μ, surrounded by a well-defined capsule, and containing a granular protoplasm in which a vacuole was present. Clinically, the case had been regarded as one of scrofuloderma, but no tubercle bacilli could be found.

Numerous cases of blastomycetic dermatitis have now been recognised, and several instances of general systemic blastomycetic infection have been recorded.

Granulomatous tumours occurring in epidemics among horses in Japan, France, and Italy are also caused by Blastomycetes.

Sporotrichosis.[1]

A rare disease clinically resembling syphilis or tuberculosis, characterised by indurated granulomata like gummata, which subsequently break down, suppurate and ulcerate. Potassium iodide has a curative action on the condition.

In the pus of the lesions large ovoid refractile bodies suggestive of yeasts or of large spores may be detected, but no mycelium.

Cultures are best obtained on maltose agar (p. 505) from non-ulcerated lesions; agar and potato may also yield growths. The organism (*Sporotrichon Beurmanni*) grows as small raised woolly colonies, at first white, afterwards becoming brown. The growths consist of a felted mycelium of filaments with spores and yeast-like cells. It produces granulomata in inoculated mice.

[1] See Walker and Ritchie, *Brit. Med. Journ.* 1911, vol. ii, p. 1 (Bibliog.).

The botanical position of the organism is uncertain; by some it is regarded as a true fungus. It is stated to occur on decaying vegetable matter and to be the cause of epizootic lymphangitis in the horse—a disease having a superficial resemblance to farcy—in the pus of which oat-shaped bodies are found, the "crypto-coccus" of Rivolta.

Clinical Examination (Pathogenic Yeasts, etc.).

The cells can be well seen in the fresh state in the teased-up tissues mounted in water or glycerin.

Curtis recommends staining in carbol-thionine blue, and for sections, picro-carmine.

Busse's method for sections is as follows:

1. Hæmatoxylin solution for fifteen minutes.
2. Wash in distilled water.
3. Counter-stain in weak carbol-fuchsin (1 : 20) for thirty minutes to twenty-four hours.
4. Decolorise in 95 per cent. alcohol for fifteen seconds to one minute.
5. Absolute alcohol, xylol, mount in Canada balsam.

Gilchrist recommends treating the sections with 10 per cent. caustic potash solution and examining in 50 per cent. glycerin without staining.

Brayton recommends that small pieces of the tissues should be excised from the growing margin, treated with ether for two to five minutes, macerated in 20 to 30 per cent. caustic potash solution for five to ten minutes, and then examined without staining. Cultures may be readily obtained, with a little care, preferably on beer-wort gelatin or maltose agar.

Thrush.

Thrush is due to an organism (*Oidium* or *Monilia albicans*) which is usually classed among the Hypho-mycetes. It forms the whitish patches so frequently

seen on the mucous membrane of the mouth and pharynx in children and in those suffering from wasting diseases but a general infection has occasionally been produced by it. If one of these patches is removed and teased up, it will be found to consist of masses of tangled mycelial threads with yeast-like budding. The organism can be readily cultivated on all the ordinary laboratory media, and will also grow on slightly acid media such as wort gelatin. It produces whitish, membranous, adherent growths, in which it appears morphologically under two forms—as masses of tangled filaments or hyphæ and as yeast-like cells. On acid media the latter exclusively occur, on alkaline the former predominate. It liquefies gelatin, stains by Gram's method, produces an alkaline reaction by the formation of ammonium carbonate, and does not ferment lactose. Inoculated on to a damaged mucous membrane the "thrush" patches appear, subcutaneously it produces an abscess, and injected into the peritoneum a general infection, followed by death and accompanied by a sero-purulent peritonitis.

Clinical examination.—The patches may be teased up and examined in the manner described for the Hyphomycetes (p. 502). Cover-glass preparations may be stained with carbol-fuchsin or by Gram's method.

Fermentation.

The yeasts are of great importance in inducing many chemical changes, especially alcoholic fermentation, beer and wine being almost exclusively due to their activity.

Taking brewer's yeast, *Saccharomyces cerevisiæ*, as a type, the yeast cell is observed to be slightly ovoid in shape, measuring 8 to 9 μ in diameter. The protoplasm is granular, contains one or more clear spaces or vacuoles, frequently

bright, refractile globules of fatty matter, and is surrounded by a cell wall of cellulose. It has been repeatedly stated that a nucleus is present, but this is doubtful. When the yeast-cell is freely supplied with nutriment, reproduction by gemmation proceeds rapidly, and a whole string of cells may form owing to the daughter-cells budding again before they have separated from the parent. When the cell is starved, gemmation ceases, fat-globules and vacuoles increase in number, and the cell may finally become little more than a large vacuole, the protoplasm forming a thin coating over the inside of the cell wall. Within the vacuoles are often seen minute spherical bodies of a doubtful nature in rapid movement. In ordinary circumstances endospore formation does not occur, but by deprivation of nutriment, as by growing on a block of plaster-of-Paris, the cells develop spores. First the cell becomes divided by the development of membranes, the so-called "partition-wall formation," into several chambers in which the spores form. In the different yeasts the number and arrangement of the spores vary; in the *S. cerevisiæ* the typical number is four, arranged close together, three on one plane and one resting on these, like a pyramid of billiard balls.

The spores are of considerable importance in the identification of species of Blastomycetes, as the form of the cells alone and the growths on culture media are not sufficiently distinctive. In fact so little can these two characters be relied upon that in order to isolate in pure cultivation it is necessary to grow from a single cell. This can be done by making a miniature plate cultivation with wort-gelatin on a large sterilised cover-glass, and, after the layer of gelatin has set, mounting, gelatin downwards, on a large cell on a glass slide. The cover-glass should be divided into small squares by cross-lines etched on the glass and numbered. The preparation is carefully examined with a $\frac{1}{6}$ or $\frac{1}{8}$ inch objective, and the positions of single isolated cells are noted. This is not a difficult matter on account of the comparatively large size of the yeast-cells, and their position is determined by the numbered squares on the cover-glass. The preparations are

kept in a moist chamber in a warm place, and when visible colonies have developed, those which are derived from a single cell can be inoculated into tubes or flasks of a suitable culture medium.

It is found that the various yeasts form spores in different periods of time when grown under similar conditions, and on this fact is based what is known as the analysis of yeast—a most valuable method, which we owe to Hansen. The chief "diseases" of beers and yeast—i.e. abnormal fermentations giving rise to inferior products—are due to admixture of certain "wild yeasts," as they are termed, with the brewer's yeast, chiefly the *S. ellipsoideus* and *S. pastorianus*; and, in order to detect these "disease" species, the analysis consists in determining at what time ascospores appear. The mode of procedure is as follows:

The yeast is sown in a flask of sterile wort, and incubated at 25° C. for twenty-four hours. The yeast revives, and from the deposit of young cells two cultures are made on plaster-of-Paris blocks. These cultures are kept, one at 25° C., the other at 15° C., and are examined twice daily. In an uncontaminated brewing yeast ascospores should not be detected in less than thirty hours in the culture kept at 25° C., and seventy-two hours in that kept at 15° C. The plaster-of-Paris blocks are sterilised by careful flaming in the Bunsen, and are then placed in sterile glass capsules with lids, containing sufficient sterilised water thoroughly to moisten the whole of the blocks; unless this is done no growth occurs. By this method of analysis as little "wild yeast" as one two-hundredth of the whole can be detected.

Besides the distinct species of yeasts, there are also a number of varieties employed in brewing, etc., differing but slightly in morphological and cultural characters, yet giving rise to varied products. These varieties may be divided into two groups—the surface, high or top, and the sedimentary, low or bottom, fermentation forms. In this country beer is brewed by fermenting an infusion of malt ("wort") with yeast, which, during fermentation, *rises to the surface*, and belongs to the first group; while the German beers are

obtained by yeast, which *sinks to the bottom*, and belongs to the second group. The floating of the yeast in the high fermentation process seems to be due to the attachment of minute bubbles of carbonic acid gas to the cells, and it has not yet been possible to convert the one form into the other.

Characters of some of the more important yeasts.—Hansen divided the important yeasts into groups having the same general characters, and distinguishes the varieties in each by Roman numerals (I., II., etc.)

CEREVISIÆ GROUP.—These are the yeasts producing the normal fermentations resulting in beer, etc. They are round or slightly ovoid cells, and four ascospores are produced. In old cultures long sausage-shaped or even filamentous cells may be met with.

S. cerevisiæ I. and II.—These are bottom fermentation forms in use at the Old Carlsberg Brewery; the cells of No. II. are rounder and slightly larger than those of No. I., and ascospore formation is more abundant.

There is also a top fermentation form described by Hansen (*S. cerevisiæ I.* top), which is the yeast employed in the breweries of London and Edinburgh.

The yeasts of the *cerevisiæ* group can invert cane sugar, select dextrose from lævulose, and ferment maltose, but they cannot ferment lactose, nor decompose malto-dextrin.

PASTORIANUS GROUP.—These are wild yeasts. The cells are elongated or sausage-shaped, and six or eight ascospores are produced in a cell.

S. pastorianus I.—A bottom fermentation yeast producing a bitter taste in beer.

S. pastorianus II.—A feeble top fermentation form. Surface cultures on yeast-water gelatin have smooth edges, which disguishes it from the next species:

S. pastorianus III.—A top fermentation form producing turbidity in beer. Surface cultures on yeast-water gelatin have woolly margins.

ELLIPSOIDEUS GROUP.—These are wild yeasts. The cells are usually ovoid or pear-shaped, sometimes round, rarely elongated.

Five or six ascospores are produced in a cell.

S. ellipsoideus I.—A bottom fermentation yeast occurring on ripe grapes.

S. ellipsoideus II.—A bottom fermentation yeast causing turbidity in beer.

Both the *pastorianus* and *ellipsoideus* groups resemble the *cerevisiæ* group in their chemical actions, but they are able in addition to decompose malto-dextrin.

S. anomalus is a yeast forming small ovoid cells. It is curious in that the spores are hemispheres with a projecting rim at the base like a bowler hat.

Another point in the identification of species of yeasts is the period of formation of films. If the yeast is grown in wort with free access of air and is undisturbed, *e.g.* in a beaker capped with filter-paper, after a varying period a film composed of a zooglœa mass of cells appears on the surface.

If yeast, or disintegrated yeast-cells, be injected into animals, the blood acquires specific agglutinative properties, agglutinating the yeast-cells of the species with which the inoculation has been carried out.[1]

On the yeasts of fermentation, see Jörgensen, *Micro-organisms and Fermentation*, 4th ed., 1911 (C. Griffin & Co.), (full bibliog.) ; Klöcker, *Fermentation Organisms*.

Examination of Yeasts.

The yeasts can be readily examined in the fresh state in hanging-drop preparations. The cells should be young or they will not be of the typical form ; a two or three days old culture in wort or grape-sugar solution may be used. The yeasts grow well at 20°-30° C. on the ordinary gelatin, agar, and potato, but wort gelatin or agar is to be preferred. The elongated cells, common to all old cultures of yeasts, may be obtained from the films which form on wort cultures in wide flasks or beakers after two or three weeks.

[1] See Macfadyen, *Centr. f. Bakt.* (1te Abt), xxx, 1901, p. 368.

In order to stain yeasts, a dilution of the culture should be made in a watch-glass of water, so that the cells may be isolated, as they become distorted if groups form in the preparations.

If the yeast has been grown in wort, it is best, before staining, to pour off the fluid from the deposit of cells at the bottom of the flask or test-tube, add some physiological salt solution and shake, then allow the vessel to stand for an hour for the cells to sediment, and the process of washing may be repeated once. Films may be prepared in the ordinary way and stained for five minutes in Löffler's methylene blue, washed in water, dried, and mounted. Or the films, after air-drying, may be fixed by immersion in equal parts of alcohol and ether for ten minutes, dried in the air, and stained as before. The preparations can also be stained in gentian violet or fuchsin, or by Gram's method.

Ascospores may be double stained by preparing films of a sporing culture in the ordinary way, staining with carbol-fuchsin for two minutes, rinsing in water, decolorising in alcohol for half to one minute or longer if necessary, rinsing in water, counter-staining with Löffler's blue for five minutes, washing, drying, and mounting. The spores are red, the remainder of the cells blue.

CHAPTER XVII.

THE HYPHOMYCETES—ASPERGILLOSIS—RINGWORM.

The Hyphomycetes.

The Hyphomycetes are an important group of the true fungi, and include those forms which are commonly known as moulds. They are multicellular individuals, composed of filaments which may be simple or branched, jointed or unjointed. These filaments are termed hyphæ, and are formed by the end-to-end union of elongated cells. When the hyphæ project upwards into the air they are known as aërial hyphæ, and when downwards into the fluid or medium on which the organism is growing as submerged hyphæ, and the compact tufts or masses resulting from interlacing hyphæ are termed mycelia. A mycelium may form a hard lignified mass or pseudo-parenchyma, which is known as a sclerotium, such as is met with in ergot and in the black variety of mycetoma. In addition to being multicellular, the higher development of the Hyphomycetes is seen in the specialisation of certain parts for the function of reproduction. Although all the species multiply asexually, in most, if not in all, a sexual method occurs also. *Mucor mucedo*, *Penicillium glaucum*, and *Aspergillus niger* may be taken as types and more fully described.

Mucor mucedo.

Mucor mucedo, the common white mould which appears like tufts of cotton-wool on various substances, may be obtained by exposing some moistened bread or horse-dung to the air

for a short time, and then keeping it moist under a bell-jar. It consists of a mycelium composed of hyphæ, and its fluffy appearance is caused by aërial hyphæ. The aërial hyphæ are at first of even diameter throughout, but later on their free ends become swollen and ultimately form spherical bodies, which become filled with spores and are known as sporangia. In the early stage the whole organism forms but a single cell, the protoplasm of which is granular and contains vacuoles and numerous small nuclei. As it grows, and the sporangia form, these become separated by a septum from the hyphæ, and when it becomes older still the mycelial hyphæ may be divided into elongated cells. The development of a sporangium takes place as follows: The distal end of an aërial hypha swells, and immediately below the swollen part a division occurs in the protoplasm and a cellulose septum is formed, so that the swollen part is separated off from the rest of the hypha, forming the rudimentary sporangium. The sporangium continues to grow, and its protoplasm undergoes multiple fission into numerous ovoid masses, the spores, each of which becomes surrounded with a cellulose capsule. The septum separating the sporangium from the hypha projects upwards into the interior of the sporangium as a club-shaped knob known as the columella. When the sporangium is ripe the slightest touch causes its wall to rupture, so liberating the spores. When placed under favourable conditions the spore germinates, and the buds increase in length and ultimately form hyphæ.

Occasionally a process of conjugation occurs. Two adjacent hyphæ send out lateral branches which come in contact with one another, and a septum forms in each, separating a small portion of protoplasm from the rest of the hypha. The apposed walls of the two cells become absorbed and the contents mingle. The mass of protoplasm so formed becomes surrounded with a thick cell-wall, giving rise to an inactive spore-like body known as a zygospore, which under favourable conditions develops like an ordinary spore.

Certain *Mucors* form appreciable amounts of alcohol.

Penicillium glaucum.

This forms the bluish-green mouldy patches familiar to everyone. It is by far the commonest of ali species, and may be obtained from moist bread or jam or by exposing a gelatin plate to the air for a short time. If the mouldy patch be rubbed a fine greenish dust comes away. This dust consists of myriads of spores; if a little of it be transferred with a moistened needle to a gelatin plate, or, better still, to a hanging-drop preparation, the growth of the organism can be studied. After two or three days little *white* specks will be observed, which microscopically are found to consist of tufts of delicate interlacing hyphæ; these, becoming interwoven, ultimately form a tough mycelium. The patches of growth are circular, and the hyphæ will be found to radiate from the centre. As the patch increases in size it changes in colour, becoming bluish-green, though the margin for some time still remains white. From the upper surface of the mycelium delicate aërial hyphæ grow upwards, and from the under surface short submerged ones project downwards.

The hyphæ are composed of elongated cells arranged end to end, the cell-walls of which consist of cellulose enclosing a more or less vacuolated protoplasm containing several nuclei.

The aërial hyphæ are unbranched filaments, but as development proceeds the distal ends branch dichotomously, the branches remaining short and nearly parallel to one another, so that a kind of brush is produced. The ultimate branches are known as sterigmata. The ends of the sterigmata become constricted so that little globular masses, the spores, are formed; this process is repeated until a chain of spores results, *the proximal one* being *the youngest*. A spore when placed under favourable conditions germinates, a little bud appearing, elongating, and forming a hypha, just as in *Mucor*.

Brefeld, by sowing spores on moist bread, inverting the bread, and examining at intervals, observed a sexual method of reproduction in *Penicillium*. Two sets of spiral cells develop on a thick hypha, they intertwine, their contents

probably mingle, and from the union or carpogonium a tube-like hypha develops, which becomes surrounded and enclosed by branching hyphæ from the mother cell. By further development and thickening of the cell-walls a sclerotium forms; it is a hard solid body, yellowish in colour, and resembles a grain of sand, the carpogonium being at the centre. If placed in favourable conditions the sclerotia germinate after some time. Two forms of hyphæ are produced, one thick, the other thin; the latter become much twisted. The thick hyphæ become branched, and ultimately a number of pear-shaped bodies are produced. The contents of these bodies then become broken up and form spores; the bodies are known as asci and the spores as ascospores. From the ascospores the ordinary mycelial form again develops.[1]

Aspergillus niger.

Aspergillus (several varieties) is occasionally met with; it can be recognised by the rounded sporangia with radial markings which are supported on aërial hyphæ given off from the mycelium. A process of sexual reproduction occurs very like the one observed in *Penicillium*. *Aspergillus niger* grows well on the ordinary laboratory media, producing on potato a powdery, sooty growth after a time.

With the exception of the ringworm and allied fungi, which produce parasitic skin affections, the Hyphomycetes are not of very great pathological importance. In the ear and nose mucors and aspergilli may be met with, but in these situations they are epiphytes rather than parasites, and the same species occur in bronchiectases and pulmonary vomicæ. Occasionally, however, a pneumono-mycosis has been met with, the mycelium of the fungus ramifying in the lung tissue and setting up irritative and other changes. "Pneumono-mycosis" or "pulmonary aspergillosis" is

[1] See Brefeld, *Quart. Journ. Microscop. Soc.*, vol. xv, p. 342.

especially a trade disease among bird-rearers. Grain is taken into the mouth and the bird is fed with it, and in the course of this operation the mould spores are inhaled. The course of the disease is much like chronic bronchitis or pulmonary tuberculosis. The species met with in this condition seems generally to have been the *Aspergillus fumigatus*.

The black variety of madura disease, as already stated (p. 484), is due to a fungus form.

Cultivation and Examination.

The Hyphomycetes can be cultivated on the ordinary laboratory media, but wort-agar, or wort-gelatin, potato, bread, or maltose agar is to be preferred.

They can be examined by removing a portion of the growth, teasing up gently with needles in a little 50 per cent. alcohol containing a trace of ammonia, removing the surplus fluid with blotting-paper, and mounting in Farrant's solution or in glycerine jelly. If desired, they may be stained by the irrigation method with fuchsin.

In the tissues they may be stained with hæmatoxylin or methylene blue, or by Gram's or by Weigert's method.

Ringworm.

The ringworm fungi must probably be included in the group of the Hyphomycetes. Human ringworm, formerly regarded as a single disease, has been proved to comprise at least two affections through the researches of Sabouraud. These two forms are distinguished from each other clinically and by differences in the parasitic organisms.

The first variety is an affection of early childhood,

forming 80 to 90 per cent. of the ringworms met with in London; it never attacks the scalp of adults, never affects the beard or nails, is very intractable, and frequently epidemic. The parasite is characterised by small round or ovoid spores measuring 3 μ to 4 μ in diameter. Affected hairs are generally broken off, forming relatively long stumps, greyish in colour, and possessing a whitish sheath. When suitably prepared

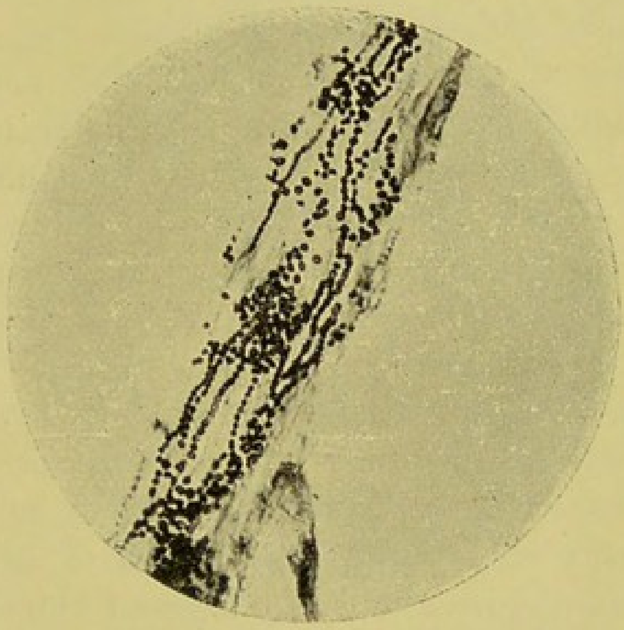


Fig. 52.—Ringworm in a hair. × 350.

in potash this sheath is seen to be composed of the spores agglomerated together without apparent order, and the hairs themselves are filled with delicate parallel mycelial threads (Fig. 52). The fungus is named the *Microsporon Audouini*.

The second variety comprises the ringworms with large spores, and is divided into two groups by Sabouraud. The first of these groups is exclusively of human origin, and has a marked tendency to affect the interior of the hairs only, and hence the organism has

been termed the *Trichophyton megalosporon endothrix*. The other group is of animal origin, and the spores are met with chiefly on the outside of the hairs, and the fungus is hence termed the *Trichophyton megalosporon ectothrix*.

The *endothrix* form occurs later in childhood, is not so persistent as the *Microsporon*, and does not attack the nails or beard. Microscopically, the fungus is seen to consist of beaded threads, which are rounded or ovoid spores arranged end to end. The *ectothrix* form rarely attacks the scalp, but is responsible for all the tinea sycosis and ringworm of the nails and half the cases of tinea circinata. Suppuration is common in this form. Microscopically appearances differ; generally the spores are arranged in chains, but the sporulation is less regular than in the *endothrix*. The spores in the *endothrix* and *ectothrix* varieties measure $4\,\mu$ to $12\,\mu$ in diameter.

The ringworm fungi can be readily cultivated on all the ordinary media—beer-wort agar and beer-wort gelatin being especially favourable. They form whitish fluffy growths with rapid liquefaction of gelatin. In order to obtain cultivations the diseased hairs or stumps are removed by forceps and placed on a sterile glass slide. The aërial portion of the hair is then cut away by means of a sterile scalpel, and the diseased portion is divided into small fragments. These can be picked up with a moistened platinum needle and transferred to the culture media, preferably beer-wort agar. In some cases a pure culture is thus obtained, but in others further treatment is necessary. When the *Trichophyton* or *Microsporon* has thrown up its aërial hyphæ the plug of wool is removed from the tube and the mouth well flamed; the tube is then held inverted over a Petri dish containing solidified maltose agar.

A sharp tap or two is given to the tube, sufficient to cause the spores to drop, and the dish is re-covered. A growth of the organism from single isolated spores thus ensues, and pure cultures can be obtained (Blaxall).

The various forms of the ringworm fungi can be differentiated by cultures, but it is necessary when comparing them to employ media of identical composition, because slight differences in the latter are

FIG. 53.—Culture of the ringworm organism. Endothrix form.

liable to induce marked changes in the characters of the cultures. A favourite medium, used by Sabouraud and by Blaxall, is maltose agar:

Peptone	0·5 grm.
Maltose	3·8 grm.
Agar-agar	1·3 grm.
Water	100 c.c.

Blaxall found that different samples of maltose materially influenced the characters of the cultures.

Characters of the cultures.—Cultures are incubated at 30° C. The colonies of the *Microsporon* do not show

any growth until about the seventh day; little white downy tufts then appear. The fully developed growth on maltose agar forms a large white downy patch with a small central boss; on potato, white downy patches appear with brown discoloration.

The *endothrix* variety commences to grow in six or seven days, and on maltose agar in about a month forms a rounded patch with a central crateriform depression, the whole being dusted with fine white powder (Fig. 53); on potato, powdery stars develop tinged with yellow and usually without discoloration of the medium.

The cultures of the *ectothrix* form are variable. They commence on the third or fourth day; some develop whitish, smooth, or wrinkled growths; others, from the dog, form dry, brown, wrinkled, powdery growths; others, of bird origin, form purplish growths.

Microscopically, all the fungi show masses of mycelial threads with spores. They stain with the ordinary anilin dyes and also by Gram's method, and can be mounted in glycerin jelly in the manner described at p. 502.

Macfadyen found that the ringworm organism produces an active peptonising enzyme, and seems to increase the solubility of keratin when grown on it; no inverting enzyme could be isolated.

Clinical Examination.

The hairs should be treated first with ether and then with caustic potash solution of about 7 per cent. strength. In this reagent they may remain for from a few hours to a few days; they are then floated on to a slide and carefully covered with a cover-glass. Permanent preparations may be mounted in Farrant's solution or in glycerine jelly.

Hairs, after treatment with ether, may be stained by the following method:

(1) Stain in anilin-gentian violet for one to two minutes, and blot.

(2) Treat with Gram's iodine solution for one to two minutes, and blot.

(3) Decolorise carefully (watching microscopically) with anilin oil containing 1 per cent. of hydrochloric acid.

(4) Treat with anilin oil and then with anilin oil and xylol.

(5) Clear in xylol, and mount in Canada balsam.

Erythrasma.—Due to infection with a fungus (*Microsporon minutissimum*), very difficult to cultivate, which occurs as extremely long, fine filaments.

Favus.—Favus is due to a fungus discovered by Schoenlein in 1839—the *Achorion Schoenleinii*. It is seen as a mycelial growth with spores in the patches. The organism grows well on maltose agar, forming fluffy, woolly, moss-like colonies with radiating outgrowths, first grey and then yellowish. It occurs on mice and other animals.

Dhobie itch.—Castellani has isolated three trichophyton-like organisms in this disease.

Pityriasis alba.—In this disease Unna's "bottle bacillus" is invariably present. It occurs as large round or oval bodies like yeast-cells, which may occasionally show budding.

Pityriasis versicolor.—In the epidermal scales of this skin affection a fungoid organism (*Microsporon furfur*) is present. It occurs as short and thick curved hyphæ between which are masses of large coarse spores. It has not been cultivated (or very rarely).

Pinta.—A skin disease met with in South America. In the scales short mycelial filaments with large (8-12 μ) spores are seen. Various organisms have been cultivated belonging to the genera *Penicillium* and *Aspergillus*.

Piedra.—A disease of the hairs met with in South America. The nodosities on the hairs are composed of masses of very large refractile spores.

CHAPTER XVIII.

THE PROTOZOA.[1]

The General Structure of the Protozoa — Pathogenic Amœbæ — Trypanosomata—Leishman-Donovan Body—Spirochaetæ—Coccidia— Malaria.

The Protozoa are an important group of unicellular organisms, regarded as animal in nature, and sharply and definitely distinguished from the rest of the animal kingdom, to which the names of metazoa and enterozoa are applied. The latter consists of many cells, differentiated to perform different functions, and arranged in two layers—endoderm and ectoderm—around a central cavity, the enteron.

"It is true that some protozoa consist of aggregates of cells, and should therefore be entitled to be called multicellular; yet an examination of the details of structure of these cell-aggregates and of their life-history establishes the fact that the cohesion of the cells in these instances is not an essential feature of the life of such multicellular protozoa, but a secondary and non-essential arrangement. Like the budded 'persons' forming, when coherent to each other, undifferentiated 'colonies' among the polyps and corals, the coherent cells of a compound protozoon can be separated from one another and live independently; their cohesion has no economic significance. Each cell is precisely the counter-

[1] See Ray Lankester's *Treatise on Zoology*, Part I, first and second Fascicles, 1907 and 1909; Minchin in Clifford Allbutt's *System of Medicine*, ed. 2, vol. ii, pt. ii; Hartog in *Cambridge Natural History*, vol. i.

part of its neighbour; there is no common life, no distribution of function among special groups of the associated cells, and no corresponding differentiation of structure. As a contrast to this, we find in the simplest enterozoa that the cells are functionally and structurally distinguishable into two groups—those which line the enteron or digestive cavity, and those which form the outer body wall. The cells of these two layers are not interchangeable, but are fundamentally different in properties and structure" (Ray Lankester). It is true that in some instances there may be a difficulty in deciding whether an organism is vegetable or animal, and Haeckel proposed to include all indeterminate unicellular organisms in a distinct kingdom, the Protista.

The cytoplasm of a protozoon is commonly differentiated into an outer, clearer, denser layer or ectosarc, and an inner, granular, more fluid portion, the endosarc. The cytoplasm is sometimes naked, or may be covered with a cuticle, usually protein in nature. The cytoplasm contains a well-marked nucleus, sometimes a secondary nucleus, and occasionally subsidiary chromatin particles or *chromidia*. A contractile vacuole, which is an excretory organ, is frequently present.

In most protozoa reproduction takes place by simple division or fission, and by a process of spore-formation; in others reproduction is exclusively by spores, which are often formed by a complicated process of development. In many of the protozoa a simple form of sexual reproduction by conjugation occurs. Two dissimilar cells (*gametes*) are produced, the larger comparable to female cells or ova and termed *macrogametes*, the smaller comparable to male elements or spermatozoa and termed *microgametes*. The cells from which the gametes are derived are known as *gametocytes*. The gametes conjugate and form a zygote, which usually divides into a number of spores from which the adult is reproduced.

In certain cases sexually differentiated individuals reproduce by fission without conjugation; this phenomenon is termed *parthenogenesis*.

Various classifications of the Protozoa have been suggested. Bütschli divides them into four classes: I. The Sarkodina

(p. 510); II. The Mastigophora (p. 516); III. The Infusoria (p. 536); and IV. The Sporozoa (p. 538).

Class I.—Sarkodina.

There are Protozoa in which the cell protoplasm is naked, and locomotion and ingestion of food are performed by means of *temporary* protoplasmic processes or pseudopodia.

The Sarkodina includes a number of forms of very varied morphology and habits, such as the Amœbæ, Heliozoa, Radiolaria, and Foraminifera, the three latter groups being characterised by the presence of a siliceous or calcareous skeleton or shell.

Pathogenic Amœbæ.[1]

Three species of *Amœbæ* seem to be parasitic in man, and the generic name of *Entamœba* has been given to them. One, the *E. buccalis*, occurs in the mouth in dental caries, the other two inhabit the intestine. One of the latter, the *Entamœba coli* (*Amœba coli*, Lösch), occurs in the upper part of the large intestine and appears to be harmless; the other, the *Entamœba histolytica*, is regarded as the cause of the amœbic or tropical dysentry.

The *Entamœba histolytica* is met with in the fæces in these cases, and also in the pus of the so-called tropical abscess of the liver. It is especially abundant in the mucoid material during the acute stage. The *E. histolytica* is a large protoplasmic mass measuring 25 to 35 μ in diameter, possessed of slow amœboid movement, and having a clearer outer zone or ectosarc and a granular endosarc. The pseudopodia are always

[1] Councilman and Lafleur, *Johns Hopkins Hosp. Reps.*, vol. ii, 1891; Schaudinn, *A.K. Gesundheitsamte*, xix, p. 547; Strong, Musgrave, Clegg, Thomas and Woolley, Bureau of Gov. Laboratories, Manila, *Bulls.* 18 and 32.

blunt, never pointed (Fig. 55). In the endosarc highly refractile granules occur, and it often contains blood-corpuscles and a vacuole (Fig. 54, *b*). A nucleus can also be demonstrated, but being poor in chromatin, stains with difficulty (Fig. 54, *a*). According to Schaudinn, the *E. coli* differs from the *E. histolytica* in that the ectoplasm is not distinctly seen except during the formation of a pseudopodium and the nucleus stains deeply. The development of the two forms is also

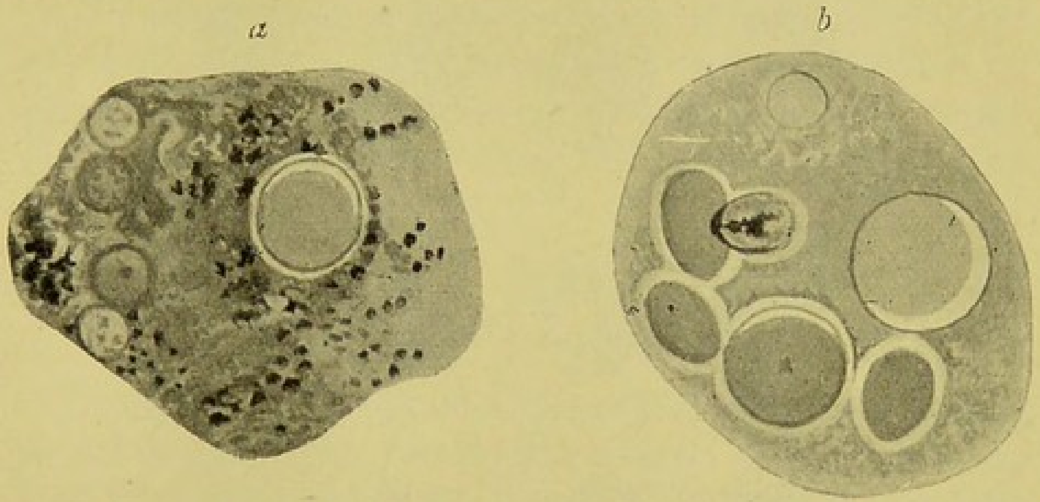


Fig. 54.—*Amœba histolytica.* (After Councilman and Lafleur.)

different. *E. coli* multiplies by simple binary fission, and also by multiple fission into eight small amœbæ. Encystment may also occur, with repeated binary division of nucleus and protoplasm, part of the nucleus being cast off, and ultimately the cyst contains eight nuclei around which the protoplasm collects, so that, if swallowed, eight small amœbæ are set free.

The *E. histolytica* multiplies by binary fission, and also by irregular gemmation, so that an indefinite number of small amœbæ is formed. Instead of encystment, as in the *E. coli*, resistant spores are formed. The nucleus gives off chromidia, some of which, together with portions of the ectoplasm, are

extruded and become spores surrounded by tough capsules. Infection of a fresh host apparently occurs only with material containing these spores.

The presence of the amœba in the pus, and especially in the wall, of tropical abscesses is of considerable diagnostic significance, and the parasite is considered to be the ætiological agent in amœbic or tropical dysentery (see "Dysentery"). The amœbæ are not

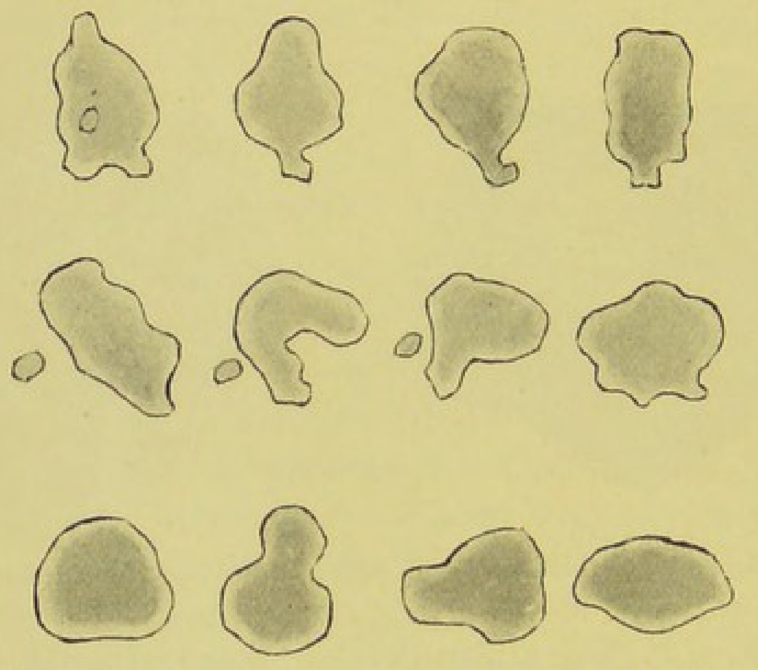


Fig. 55.—Changes in form of an *Amœba histolytica* observed on a warm stage, and drawn at intervals of one minute. (Semi-diagrammatic by the writer.)

usually observed in the abscess pus at the time of operation, but make their appearance in the discharge about the third day, *i. e.* when the wall of the abscess-cavity is contracting. In the true tropical abscess the ordinary pyogenic organisms are absent, unless a secondary infection has occurred, which is the exception. The abscess is usually single, and Rogers suggests that the amœbæ reached the liver by adhesions between it and the bowel. The amœbæ may be cultivated on

ordinary or on water agar provided some bacterium is present at the same time, *e. g. B. coli*, cholera vibrio, etc. Material rich in amœbæ may be smeared over agar plates, which are grown at 25°–30° C. for twenty-four to forty-eight hours, and are then examined with a low power. At any spot where isolated amœbæ are observed, with a little dexterity the organism may be lifted up with a fine needle and be transferred to a fresh plate, and by a repetition of the process pure cultures may be obtained. The cultivated amœbæ are pathogenic for monkeys, and induce abscess on inoculation into the liver. Musgrave and Clegg (*loc. cit.*) are of opinion that all amœbæ are, or may become, pathogenic.

Clinical Diagnosis.

1. A drop of the dysenteric discharge (the mucous portions should be chosen from the stools), pus, or, better, a scraping from the wall of the abscess, diluted, if necessary, with a little warm (37° C.) physiological salt solution, is placed on a slide, covered with a cover-glass, and examined microscopically with a $\frac{1}{4}$- or $\frac{1}{6}$-inch objective. The amœbæ will be readily recognised, and may be examined more critically with a $\frac{1}{12}$-inch oil-immersion. To be certain that the bodies are amœbæ, the amœboid movements must be observed by keeping the preparation on a warm stage.

The stools should be fresh, unmixed with urine, collected in a warmed bed-pan, and kept at blood-heat until examined, which should be done as soon as possible.

2. The *living* amœbæ in the stools may be stained by the irrigation method with a weak ($\frac{1}{2}$–1 per cent.) aqueous solution of neutral red. Preparations may also be stained by irrigation with methylene-blue and Beale's carmine; the latter stains the nucleus, the former does not. The preparation may be rendered permanent by washing away the excess

of stain, and running in some 50 per cent. glycerin by irrigation.

3. Probably Heidenhain's iron-hæmatoxylin method is the best for staining this and other protozoa:

(*a*) Make smears of the material and drop while *wet* into the fixative—two parts of saturated aqueous mercuric chloride solution, one part of alcohol, with a few drops of glacial acetic. They remain in this for ten minutes.

(*b*) Wash in weak spirit and then in weak spirit coloured with iodine, and finally wash in distilled water.

(*c*) Treat with 4 per cent. iron-alum solution for six to ten hours.

(*d*) Stain in Heidenhain's hæmatoxylin for at least six hours.

(*e*) Differentiate in 1 per cent. iron-alum, watching microscopically.

(*f*) Wash well in tap-water, pass through alcohol and xylol, and mount.

4. Twort's stain may be used for sections. The stain (which is a compound neutral red and light green preparation) is best made by rubbing up 0·25 grm. of the stain (Grübler's) with some clean sharp sand in a mortar; this prevents the stain going into a sticky mass when the alcohol is added. To the powder so obtained is now added some purest methyl alcohol (Merck's), acetone-free, containing 5 per cent. by volume of glycerin. Rub up well to obtain a saturated solution; then pour off and add a further quantity of alcohol-glycerin solution, and repeat the trituration; about 100 c.c. stain can be made from the quantity given.

The solution, when filtered, should be kept in a well-stoppered bottle (and *if* a completely saturated solution has been obtained, add 10 per cent. more alcohol-glycerin mixture). The stain may be purchased ready for use.

Tissues to be examined should be fixed in Müller's fluid containing 10 per cent. of formalin, but on no account should 10 per cent. formalin alone be used.

Paraffin sections (after xylol, alcohol and distilled water) are stained for about five minutes with the stain made up by

mixing one part of distilled water with two parts of the glycerin-alcohol stain solution. Sometimes in staining such organisms as glanders ten minutes may be necessary, especially if insufficient stain is in solution and the room temperature is low. Rinse in distilled water.

Fix for half to one minute in Unna's glycerin-ether mixture —2 per cent. in distilled water. Rinse in distilled water.

Differentiate and dehydrate in absolute alcohol. Should there be much precipitate, this can easily be removed by a few drops of methyl alcohol, or better by a mixture of equal parts of absolute alcohol and xylol. Pass through xylol and mount.

Various elements stain different colours, viz. chromatin of nuclei, purple red; mucoid and colloid degenerations, bright orange red; fetal cartilage, orange red; fibrous tissue, blue-green; erythrocytes, light grass-green. Micro-organisms stain bright red and stand out in marked contrast to the green connective tissue containing them.

Animal parasites, *e.g.* amœbæ, also stain well. The stain has the advantage of leaving all the tissues sharply differentiated.

Allusion may here be made to the Mycetozoa (Myxomycetes). These are masses of protoplasm resembling huge amœbæ, which are found on decaying vegetable matter. By some they are regarded as vegetable, by others as animal, in nature, and belonging to the *Amœbæ* of the Sarkodina.[1] Some important plant diseases, such as the "finger-and-toe" of cabbage roots, are due to their activity. The finger-and-toe disease is due to an amœboid parasite (*Plasmodiophora brassicæ*, by some included among the *Amœbæ*), the cycle of which begins with spores from which small flagellulæ are set free. Similar organisms have been supposed to be present in cancer.

[1] See Ray Lankester's *Treatise on Zoology*, Pt. 1, First Fascicle, p. 37.

Class II.—Mastigophora.

These are protozoa in which one or more permanent organs serving for locomotion or food capture are present in the form of flagella. As a rule the body is limited by either a cuticle or a differentiation of the protoplasm into a firmer external portion or *periplast*. One, two, or more flagella may be present, and when multiple are arranged in various ways. Food-vacuoles may occur in the protoplasm, also contractile vacuoles, but not in the parasitic forms. Various other granules, including *chromatophores*, which generally contain chlorophyl, may be present. The nuclear apparatus is usually double, consisting of a large principal or macronucleus, and a small or micronucleus or blepharoplast; the latter is not, as in the Infusoria, composed of generative chromatin, and is in relation with the locomotor apparatus. An undulating membrane, a thin protoplasmic membrane attached to one aspect of the body like a dorsal fin, may be present. *Euglena* is a common form in ditches, and *Noctiluca* is the chief cause of phosphorescence in the sea; both are uniflagellate. *Volvox* is also placed by some in this group. The chief parasitic genera are:

Trypanosoma and *Trypanoplasma*, both of which have an undulating membrane, but the former has one flagellum, the latter two flagella, one at each end of the body, but both starting from the blepharoplast. *Spirochaeta* (see p. 522).

Herpetomonas, like *Trypanosoma*, has a single flagellum, but no undulating membrane.

Crithidia has a pear-shaped body with single flagellum.

Trichomonas, also somewhat pear-shaped, with three short flagella and an undulating membrane.

The trypanosomes and other forms living in the blood are known as hæmoflagellates.

Trypanosomata.[1]

The trypanosomes are all parasitic in the blood of verte-

[1] For current literature on Trypanosomes and trypanosome diseases see *Sleeping Sickness Bureau Bulletin* (Royal Society).

brates, and a blood-sucking invertebrate is almost invariably concerned in their transmission. In the case of each pathogenic trypanosome, some indigenous wild animal, tolerant to that form, serves as a reservoir from which infection is derived. A trypanosome has a slender, flexible, flattened body, one extremity of which is pointed, the other passes into a single flagellum. A delicate undulating membrane passes along one edge of the body. The organism lives in the plasma, in which it is actively motile, the flagellated end being usually anterior, and measures 15–30 μ, or even 40–50 μ, in length. The protoplasm of the organism is finely granular, and near the centre of the body is a large macronucleus, and generally between it and the non-flagellated end is a smaller micronucleus or blepharoplast. From the latter a chromatin filament starts, runs along the free edge of the undulating membrane and passes into the flagellum. Reproduction takes place by longitudinal division, occasionally probably by transverse division, and amœboid and plasmodial masses may be found in the internal organs and bone-marrow. The trypanosomes have great morphological similarity, which renders them practically indistinguishable by structural characters. They can usually be differentiated into three forms—indifferent, male, and female—which in some cases may all occur together, but only become fully differentiated in an invertebrate host. The males are slender, active, only slightly granular, and with an elongated nucleus; the females are bulky, sluggish, granular, and have a rounded nucleus; the indifferent forms are intermediate. The males usually soon die off unless they conjugate; the indifferents are more hardy, the females most so. The sexual forms conjugate in an invertebrate host, but if the males have died off, both male and female forms may be reproduced from the females by a process of parthenogenesis.

Trypanosoma gambiense.

In human trypanosomiasis and sleeping-sickness of West and Central Africa, a trypanosome, *Tr. Gambiense* is the causative agent (Plate XXI., *a*). It is usually pre-

sent, though scanty, in the blood, but can often be found in numbers in the fluid aspirated from the enlarged cervical glands. In the later stages, when cerebral symptoms ensue, it is found in the cerebro-spinal fluid, but scantily, centrifugalisation being necessary in order to demonstrate the parasites. The *Tr. Gambiense* is pathogenic to monkeys, and to a less extent to white rats and guinea-pigs. Cattle and certain antelopes may act as reservoirs for the parasite. It is conveyed by a tsetse-fly (*G. palpalis*), possibly by other tsetses.

The tsetse (and possibly other biting flies) may rarely convey the disease by direct inoculation. Generally a cycle of development is passed in the tsetse. The stages of this are not known with certainty, but Roubaud has observed multiplication of the parasites in the fly and the development of *Herpetomonas* forms. According to the observations of Kleine and Bruce, the flies become infective about thirty-four days after feeding and remain infective for 70–80 days, an l probably for the rest of their lives.

In Rhodesia, a human trypanosome has been found which is probably distinct from *Tr. gambiense*. The *G. palpalis* does not occur in the district, and the macronucleus of the parasite is situated between the blepharoplast and the posterior end.

In Brazil another human trypanosome-like parasite has been discovered by Chagas (*Tr.* or *Schizotrypanum cruzi*), and is conveyed by a bug (*Conorhinus megistus*).

Tr. Brucei is the causative parasite of nagana or tsetse-fly disease of horses in Africa.

Nagana is met with in large tracts of country in Zululand and West Africa. It especially attacks the equines—horse, mule, and ass—in which it is very fatal. The animals become anæmic and emaciated, there is a discharge from the eyes and nose, staring coat, swelling of the legs and neck, and fever. The animal dies two

to six weeks after infection. Oxen are also attacked, but a small proportion recover. The dog, cat, rabbit, guinea-pig, mouse, and rat may be infected by inoculation with the fresh blood of a diseased animal. In infected animals the trypanosome is generally abundant in the blood and spleen. The *Tr. Brucei* can be cultivated, though with difficulty, on rabbit-blood agar—melted sterile agar cooled to 45° C. + sterile defibrinated rabbit's blood warmed to 45° C., mixed and allowed to solidify in the sloping position (Novy and McNeal). The disease is conveyed through the bites of a tsetse-fly (*Glossina morsitans*). The trypanosome is believed to live in the big game, from whence it is transmitted to horses entering the infected localities. The blood loses its infective properties usually within twenty-four hours of being withdrawn.

Surra attacks horses in Burma, Mauritius, and the Philippines, and is pathogenic to the same animals as nagana, and in the blood a parasite (*Tr. Evansi*) similar to that in nagana, but more active, was observed by Evans. Surra is probably spread by certain biting flies belonging to the *Tabanidæ*.

The tsetse flies (*Glossina*) belong to the house-fly order (Muscidæ), and have a general resemblance to a house-fly, but when at rest the wings fold completely over each other. The proboscis is long and straight and the wing venation is characteristic, especially the fourth longitudinal vein, which makes two bends. Instead of laying eggs, the female extrudes a single full-grown larva. They are confined to Africa and Arabia; some sixteen species have been differentiated, and they occur in the vicinity of water on the edge of forest land ("fly-belts").

Tr. equinum attacks horses in South America, causing weakness and paresis of the hindquarters ("*mal de caderas*"). Cattle are immune, most other animals susceptible.

Tr. Theileri, the largest trypanosome known (50–60 μ in length), is found in cattle in South Africa, and is not pathogenic to any other animal.

Tr. dimorphum occurs in two forms, large and small, in horses in Africa. Is pathogenic to most animals.

Dourine, a venereal disease of the horse met with in North Africa, Spain, and Hungary, is due to the *Tr. equiperdum*, which is conveyed by direct contact, and is mainly confined to the lesions, being scanty in the blood. It is pathogenic to the ordinary laboratory animals.

In rats a non-pathogenic trypanosome was found by Lewis (*Tr. Lewisi*). It is especially met with in sewer-rats, but also occurs in field-rats (Crookshank). It is somewhat shorter and thinner than the *Tr. Brucei*, and there are other small differences between the two forms. With the exception of rats and mice, and to a less extent guinea-pigs, other animals cannot be infected with the *Tr. Lewisi*. It may be kept alive for long periods in the blood placed in a refrigerator, whereas the *Tr. Brucei* soon dies under the same conditions. The two forms do not protect against each other. The *Tr. Lewisi* is readily cultivated on rabbit-blood agar and is probably transmitted by the rat-flea, in which it seems to penetrate into the epithelial cells of the gut and undergoes a process of multiplication.[1]

A number of other trypanosomes have been found in the lower animals, birds, fish, reptiles, and amphibians. A large and characteristic one is generally present in the blood of the eel.

The trypanosomes are usually agglutinated when mixed with the serum from an infected animal.

Hewlett was unable to obtain any toxic or immunising substance from ground-up trypanosomes (*Tr. Brucei*)[2]

Levaditi and Twort[3] have found that the filtrate of broth cultures of *B. subtilis* is markedly trypanocidal *in vitro* but not *in vivo*.

[1] Minchin and Thompson, *Brit. Med. Journ.*, 1911, vol. ii, p. 361.

[2] *Proc. Roy. Soc. Lond.*, B. vol. lxxxiv, 1911, p. 56.

[3] *Comp. Rend. Soc. Biol.*, vols. lxx and lxxi, 1911.

Examination of Trypanosomes, etc.

The trypanosomes, if numerous, are readily observed in the fresh blood. A very shallow cell may be formed on a slide by ringing with melted paraffin. For stained preparations the Leishman stain (see "Malaria") or the Heidenhain method (p. 514) may be employed.[1]

Leishmaniosis.

This term is applied to a group of diseases, caused

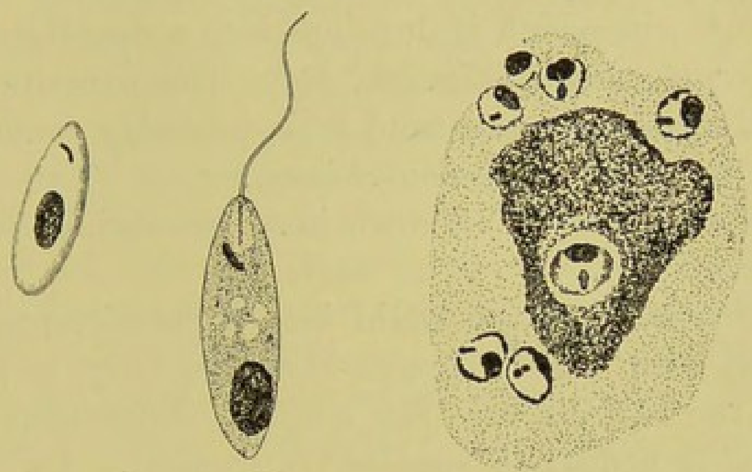


FIG. 56.—*a*. The Leishman-Donovan body. *b*. The flagellated form developing in citrated blood. *c*. Seven parasites in a large mononuclear leucocyte. (After James, Patton, and Rogers.)

by a similar parasite, and widely distributed in tropical and sub-tropical countries of the old and new world.[2]

In kala-azar or tropical splenomegaly, a disease met with in India, Assam and the East, a small parasite, the Leishman-Donovan body, occurs in large numbers in the spleen and liver, also in the lymphatic glands, lungs, and intestinal submucosa, and in large mononuclear leucocytes and endothelial cells. The bodies are small (2–3 μ), round, ovoid, or oat-shaped masses of

[1] For a special method of staining, see Plimmer, *Proc. Roy. Soc. Lond*, B. vol. lxxix, 1907, p. 102.

[2] See Hewlett, *Practitioner*, 1911, July, p. 109.

protoplasm, apparently encapsuled, and contain two chromatin masses — one large and oval, staining pale red with Leishman's stain, the other small and rod-shaped, and staining deep red with Leishman (Fig. 56, *a*). They sometimes occur in masses (Fig. 56, *c*). Leishman considered the bodies to be degenerate trypanosomes, but the organism is now considered to belong to a distinct genus, and is termed *Leishmania Donovani*. Rogers succeeded in cultivating it in citrated blood at 20°–25° C., in which it develops into a flagellated form like *Herpetomonas* (Fig. 56, *b*).[1] The parasite is not inoculable into animals, and it is probably transmitted to man by a bug (? a *Conorhinus*).

The bodies are well shown in smears stained with the Leishman stain.

In Oriental sore, or Delhi boil, a parasite practically identical with the Leishman-Donovan body is present, but as the two diseases run a totally different course, it is probably a distinct species (*L. tropica*). On cultivation it develops a flagellated form. The disease has a seasonal prevalence, and Wenyon suggests that it is conveyed by a mosquito, *Stegomyia*, sp.

In N. Africa Nicolle has observed a Leishmaniosis of children due to another species (*L. infantum*). It is transmissible to the dog and monkey, and can be cultivated. The disease has recently been found all along the Mediterranean littoral.

Spirochaetosis.

Diseases caused by infection with spirochaetes.—The spirochaetæ are delicate, undulating, or somewhat spirillar, filiform parasites, occurring in the blood of man, mammals, birds, shell-fish, etc. The filaments taper to a point at the ends, are flexible and motile, coiling and uncoiling, are described as

[1] *Brit. Med. Journ.*, 1907, vol. i, p. 427 *et seq.*

having two nuclear masses, and some possess an undulating membrane, like trypanosomes, but in the smaller forms no definite structure can be made out. They are now generally regarded as protozoa, but some still consider them to be bacteria. Bacterial cells are never pointed, nor do they show the coiling movements of spirochaetes; motility is produced by flagella, which are absent from most spirochaetes (statements to the contrary are due to errors of observation and technique), and periodicity is not exhibited by bacteria. Spirochaetes multiply by longitudinal fission, while fission in bacteria is transverse; they react in some cases to drugs (*e. g.* "606") like trypanosomes, are much more sensitive to the action of immune sera than bacteria are, and are transmitted by insects. No spirochaete has yet been cultivated.

Schaudinn believed that many so-called spirochaetes may be connected with the trypanosomes. In *S. plicatilis* he described the presence of a thread-like nucleus and of chromidia, and of an undulating membrane, but flagella are absent. In the little owl minute slender trypanosomes occur; these later penetrate leucocytes, and develop into relatively very large trypanosome forms (which have been termed *Leucocytozoa*). These intra-corpuscular forms are male and female gametocytes, the male being smaller and more slender than the female. If taken into the gnat's stomach, the male gametocytes give rise to eight microgametes by a process of sporulation, which fertilise the macrogamete, and the resulting zygote ultimately forms by sporulation an immense number of spirochaetes.

In the case of a *Halteridium* parasite of the little owl (*Athene noctua*), Schaudinn claimed to have shown that it is a stage of a trypanosome (*T. noctuæ*) which is disseminated by the common gnat. His observations have not been confirmed, and Novy and McNeal believe that Schaudinn was dealing with a double infection of both a trypanosome and a *Halteridium*, not that one was transformed into the other.

Spirochaeta recurrentis (*Obermeieri*).—Found in the blood-plasma, not in the corpuscles, in relapsing fever

during the febrile paroxysms. It is very slender and delicate, measuring 12 to 16 μ in length, and actively motile. It is said to be conveyed by the bed-bug or by pediculi—but this is uncertain—and is inoculable into monkeys, and, less readily, into rats. It has not been cultivated (Plate XXI., *b*).

It is probable that the spirochaetes of relapsing fever in different countries are distinct species.

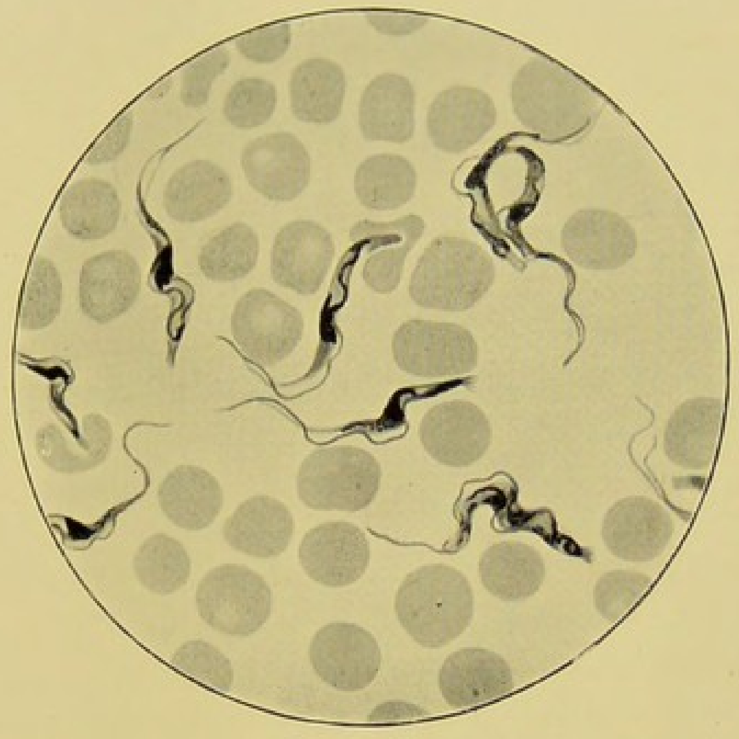
Spirochaeta Duttoni.—Found in the blood-plasma in African relapsing, or tick, fever. It closely resembles the *S. recurrentis*, but is more readily inoculable into rats, mice, and guinea-pigs, and the one does not protect against the other. It is conveyed by a tick, *Ornithodoros moubata.*

Blood spirochaetes have been found in many animals, *e. g.* cattle (*S. Theileri*), mice (*S. muris*), fowls (*S. gallinarum*), and geese (*S. anserina*).

Spirochaeta pertenuis.—Castellani[1] has found in the yaws (frambœsia) granulomata a delicate spirochaete resembling the *S. pallida* of syphilis closely, but even more delicate and difficult to stain than the latter organism, and named the *S. pertenuis.* It is present also in the spleen and lymphatic glands in the disease and in inoculated monkeys. Rabbits can be inoculated in the testicle.

Some observers have supposed yaws to be a manifestation of syphilis, but (1) syphilitic patients can be inoculated with yaws; (2) syphilis may supervene on yaws; (3) Neisser and Castellani have shown that monkeys inoculated with syphilis are not immune to yaws, and *vice versâ*; and (4) Castellani[2] has shown that the yaws antigen and anti-bodies are distinct from the syphilis antigen and anti-bodies.

1 *Brit. Med. Journ.*, 1907, vol. ii, p. 1511.
2 *Journ of Hygiene*, vol. vii, 1907, p. 558.



a. Trypanosoma Gambiense. Smear of blood of inoculated rat.
× 1500.

b. Spirochaeta recurrentis (Obermeieri). Smear of blood.
× 1500.

To face page 524.

Spirochaetes are also present in the ulcerating granuloma of the pudenda of Guiana (Wise) and Australia, in malignant growths, in ulcers, in the mouth (p. 474), and in Vincent's angina (p. 311).

Blood-smears may be stained with Leishman's stain.

(On Spirochaetosis, see Nuttall, *Journ. Roy. Inst. Public Health*, vol. xvi, 1908, p. 449.)

Duval and Todd[1] state that multiplication of *S. Duttoni* takes place *in vitro* in a culture medium made with hens' eggs and mouse blood. Leishman believes that certain chromatin bodies present in the eggs and nymphs of the ticks are the developmental forms of the spirochaetes.

Trichomonas vaginalis.—This parasite is found in the acid vaginal mucus in 50 per cent. of those examined. It must not be mistaken for a spermatozoon. It is a pear-shaped body, measuring 12 to 30 μ in length, and from the blunt end three flagella are given off.

A much smaller species, *T. intestinalis*, measuring 4 to 15 μ, has been met with in the intestinal canal of man in conditions associated with diarrhœa.

Syphilis.

Various bacterial organisms have been described in this disease, *e. g.* by Lustgarten, Eve and Lingard, Van Niessen, de Lisle and Jullien, etc., and bodies regarded as protozoa by Siegel, de Korté, and others. In March, 1905, Schaudinn[2] noted the constant presence of a spiriform organism or spirochaeta (*S. pallida*, or *Treponema* or *Spironema pallidum*) in various lesions in acquired and congenital syphilis. The *T. pallidum* varies from 6 to 15 μ in length, averaging 8–9 μ (Plate

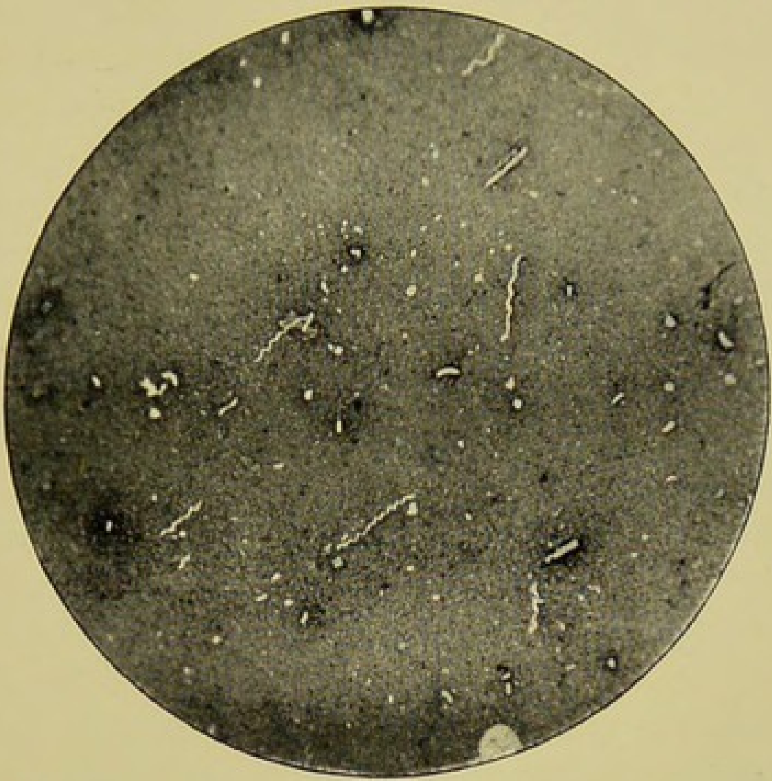
[1] *Lancet*, 1909, vol. i, p. 834.

[2] *Arbeit. a. d. Kaiser. Gesundheitsamte*, xx, 1905.

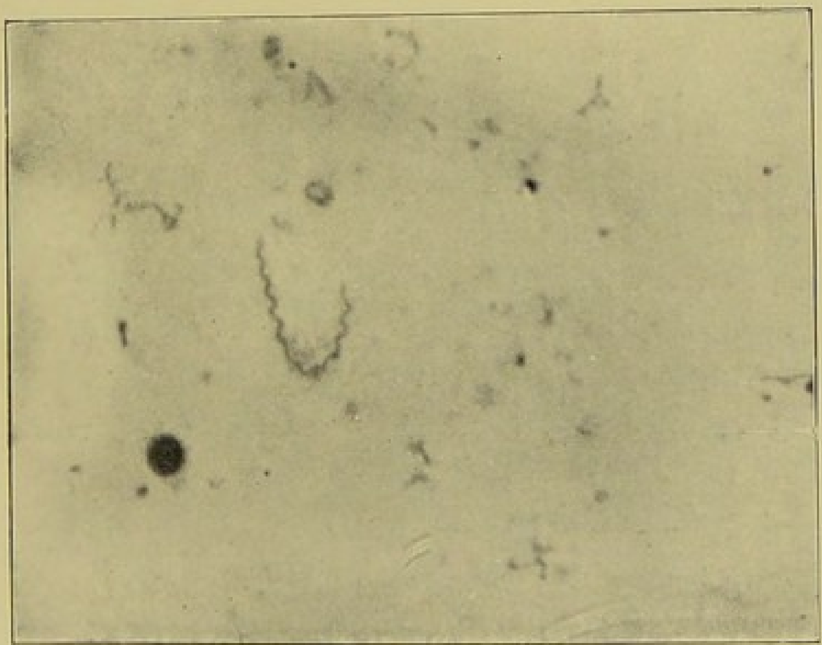
XXII., *a* and *b*). It is much more attenuated than the majority of spirochaetes, having a maximum thickness of 0·3 μ, has from three to twelve, usually from six to eight, twists, forming a close, regular, and narrow spiral, is actively motile, possessing a single delicate flagellum at either end, and it may have an undulating membrane. It stains feebly and with difficulty. Another spirochaete, the *S. refringens*, frequently accompanies, and must not be mistaken for, the *T. pallidum* in ulcerating lesions; the former is more refractile and coarser, has fewer twists and forms a wider spiral, and stains deeper and more readily than the latter. The *T. pallidum* is found generally in all primary and secondary lesions of syphilis, *e.g.* the primary sore and adjacent lymphatic glands, in the papular and roseolar eruptions, in condylomata and mucous patches. It has also occasionally been found in the spleen and blood. In congenital syphilis the *T. pallidum* is met with in the bullous eruptions, blood, and organs, and is particularly abundant in the spleen and liver (Plate XXIII., *a*).

Tertiary lesions are generally considered to be non-infective, and the *T. pallidum* is usually difficult to find in them. It has, however, been detected in the peripheral portions of gummata and in syphilitic aortitis, and may persist in the body for years after the primary lesion.

The *T. pallidum* is now universally regarded as the specific organism of syphilis, being present not only in the human lesions but in experimental lesions of inoculated apes (see below). It must be recognised that spirochaetes are of frequent occurrence in various non-syphilitic ulcerating and other lesions, *e. g.* in the mouth and in pyorrhœa, in yaws and ulcerating granuloma (in these two they may be specific forms), in ordinary ulcers and in carcinomatous tumours. Generally



a. *Treponema pallidum* from condyloma (*T. pallidum* with *Spirochaeta refringens*). Indian-ink method. × 1000.



b. *Treponema pallidum*. Smear from condyloma. Giemsa. × 1500.

To face page 526.

the *T. pallidum* can be distinguished microscopically from the other species, but care is necessary.

The *T. pallidum* has not been cultivated *in vitro* in spite of numerous attempts. By placing material from a *rhesus* monkey inoculated with syphilis into collodion sacs and introducing them into the peritoneal cavity of another monkey, and examining the contents of the sacs a month after the operation, a great multiplication of the organism was found to have taken place.[1]

Metchnikoff and Roux (also Grünbaum) found that the chimpanzee is very susceptible to syphilis, and can readily be inoculated from man, the *T. pallidum* being found in the lesions.

Macacus rhesus is also somewhat susceptible, likewise the *M. cynomolgus* and the Chinese bonnet monkey, but not the mandril. By several passages through a *rhesus* monkey the syphilitic virus becomes attenuated, so that in man it produces merely a local lesion.[2] Bertarelli[3] states that syphilis may also be inoculated on the eye of the rabbit.

Attempts by Metchnikoff and Roux to prepare an anti-syphilitic serum by inoculating apes and goats with syphilitic virus proved unsuccessful (as did earlier experiments with other animals by Héricourt and Richet). The syphilitic virus as ordinarily introduced into man by sexual intercourse probably takes some hours to become generalised, for Metchnikoff found experimentally in apes that if the seat of inoculation were treated with a calomel ointment up to eighteen hours after inoculation infection was prevented.

The syphilitic virus does *not* pass through a Berke-

[1] Levaditi and McIntosh, *Ann. de l'Inst. Pasteur*, xxi, 1907.
[2] Metchnikoff, *Journ. of Prev. Med.*, 1906, August.
[3] *Centr. f. Bakt.* (Orig.), xliii, 1907.

feld filter, and hence is not ultra-microscopic. It is readily destroyed by heat (52° C.) and antiseptics. Treatment with mercury and with "606" (salvarsan) cause diminution or disappearance of the spirochaetes.

Examination for the T. pallidum.

1. *Examination in fresh preparations.*—Scrapings from the deeper layers of the chancre, etc., may be emulsified in physiological salt solution and examined microscopically, particularly with dark-ground illumination (p. 146) with special condenser.

Another useful method is the *Indian-ink method.* A scraping is obtained from the lesion as above, and the fluid thus obtained is placed on a slide and an equal quantity of ink added. The ordinary commercial Indian inks may be used, Günther Wagner's being particularly good (p. 82). The serum and the ink are then rapidly and thoroughly mixed and smeared over the slide so that a pale brown colour results. The material dries in a minute or slightly less, and may be examined directly with the oil-immersion lens, or the wet preparation may be covered with a cover-glass and examined.

The preparations keep for a considerable time. The preparation shows the red blood-cells as large clear circular areas in a brownish-black field, the bacteria and *débris* present appearing as white rods, dots, etc., and spirochaetes, if present, as clear white spirals (Plate XXII., *a*).

It is particularly important in using this method that in so far as possible serum alone be used, and that a minimal amount of mucous material or fibrin be mixed with the ink. The presence of mucus results in the taking up of a large amount of the colouring matter of the ink, with the result that a smear of the requisite colour and thickness cannot be made. If too much serum is used the albuminous material appears to precipitate the colour from the fluid and a finely granular appearance is seen microscopically, which is practically worthless for diagnostic purposes. Again, if too much ink is used,

PLATE XXIII.

a. Treponema pallidum. Section of liver of fetus (congenital syphilis). Levaditi's method. × 1500.

b. Coccidium oviforme. Section of rabbit's liver. × 350.

To face page 528.

the surface of the smear is increased in size to such an extent that the task of examining it thoroughly is greatly lengthened.

Coles[1] notes a useful point in the recognition of the treponema, namely, that if the number of turns of the spiral of the syphilitic spirochaete in the length of the diameter of a red blood-cell be counted, these will be found to be from six to seven. The distance from the top of one spiral to the next is from 1 to 1·2 μ. The red blood-cells measure about 7·5 μ in diameter, so that on the average six or seven turns will be equal to that of a red blood-cell. The treponema varies in length from 6 to 15 μ, or even more, and consequently contains from six to fourteen and sometimes twenty or more turns of the spiral. This measurement of the length of the spiral is usually possible, and is of the greatest value in identifying the treponema.

2 *Stained preparations.*—*Smears* from chancres, etc., may be stained by the *Giemsa method.* The Giemsa stain is a solution of an azur-blue eosin compound in equal parts of glycerin (Merck, *puriss.*) and methyl alcohol (Merck or Kahlbaum).

The smears are fixed for ten minutes in absolute alcohol. The preparations are then stained in a dilute solution of the Giemsa solution for two to twenty-four hours, washed in distilled water, dried, and mounted. (The dilute Giemsa is prepared by adding one drop of the Giemsa stain to a cubic centimetre of distilled water, and rendering alkaline with one drop of 0·01 per cent. potassium carbonate solution.) The preparations may also be stained in the undiluted Giemsa stain for half to six hours. Leishman's solution may also be used.

Sections may be stained by Levaditi's method :

(1) Fix pieces of tissue about 1 mm. thick in 10 per cent. formalin for twenty-four hours.

(2) Wash in water, and harden in 96 per cent. alcohol for twenty-four hours.

[1] *Brit. Med. Journ.*, May 8th, 1909.

(3) Wash in distilled water for some minutes (until pieces sink).

(4) Place in 3 per cent. silver nitrate solution at 37° C. for three to five days in the dark.

(5) Wash in distilled water for some minutes, and then place in the following solution at room temperature for twenty-four to forty-eight hours.

Pyrogallic acid	2–4 grm.
Formalin	5 c.c.
Distilled water	100 c.c.

(6) Wash in distilled water, dehydrate in absolute alcohol, clear in xylol, embed in paraffin, cut, and mount.

The spirochaetes are stained black or brown (Plate XXIII., *a*), the tissues yellow.

Some have asserted that the spirochaetes seen in the tissues after staining by this method are artifacts or are composed of filaments of elastic tissue.[1]

3. *The Wassermann reaction or antigen test.*[2]—This has been largely applied in the diagnosis of syphilitic conditions, and as a confirmatory test of the presumably syphilitic nature of such conditions as tabes dorsalis and general paralysis of the insane. The test is based on complement-fixation (p. 190). In this method an organism (the "antigen") fixes its homologous immune body, and the complex then takes up complement; this is demonstrated by the use of a hæmolytic system (p. 191).

As a matter of fact, however, the Wassermann reaction, as it is preferably termed, is apparently not a true antigen reaction,[3] for the substance used as antigen is soluble in alcohol, and various non-specific bodies may be employed as antigen. Moreover, the substances which act as amboceptor and fix the complement are probably lipoid in nature, and are derived by a peculiar degeneration or breaking down of the

[1] See Saling and Mühlens, *Centr. f. Bakt.* (Orig.), xlii and xliii.

[2] One of the best *resumés* on this subject is by Gomes, *Archivos do Instituto Bacteriologico Camara Pestana*, vol. iii, Fasc. II, p. 143. (In French. Lisbon, 1911. Full bibliography).

[3] See Emery, *Lancet*, 1911, vol. i, p. 564.

tissues in syphilis. Again, the reaction is not confined to syphilis: it may also be obtained with the syphilitic "antigen" in trypanosomiasis, yaws, leprosy, and the early stage of scarlatina. This does not, however, militate against its value in the diagnosis of syphilitic conditions.

In the original method a fresh salt-solution extract of the liver of a syphilitic fetus was used as the "antigen." Levaditi employed a similar extract of the dried and powdered liver. Now an alcoholic extract of the liver is made use of. The test-substance was the blood-serum or cerebro-spinal fluid of the patient, inactivated by heating to 56° C for half an hour. The complement was guinea-pig serum, and the hæmolytic system sheep's corpuscles, and a serum hæmolytic for these corpuscles.

This is probably the best method, but is now not generally employed on account of the difficulty of obtaining syphilitic fetal liver, and other substances act nearly as well as the antigen, *e. g.* alcoholic extract of heart-muscle, sodium taurocholate, glycocholate, or oleate, lecithin, cholesterin, etc.

At present alcoholic extract of heart-muscle[1] (human or guinea-pig) is commonly employed. The complement may be that contained in the serum to be tested, and the hæmolytic system may be (1) normal human serum and sheep's corpuscles,[2] (2) the serum of a rabbit injected with human corpuscles, and human corpuscles, or (3) serum hæmolytic for sheep's corpuscles, and sheep's corpuscles.

The following is the method devised by Emery,[3] and has the advantage of comparative simplicity.

(*a*) *The hæmolytic serum and corpuscles.*—A rabbit is injected intra-peritoneally two or three times at intervals of a week with 10 c.c. of a 50 per cent. suspension in salt-solution of well-washed human corpuscles. The serum is prepared for use by bleeding the animal to death, and collecting the

[1] Heart-muscle is peculiar in that it contains a large amount of lipoid substances.

[2] Fleming's method (*Lancet*, 1909, vol. i, p. 1512). Normal human serum is *generally* (not always) hæmolytic for sheep's corpuscles.

[3] *Lancet*, 1910, vol. ii, September 3rd.

blood aseptically. The serum is then standardised with human corpuscles and fresh human serum, so as to determine the minimal hæmolytic dose under the conditions of the experiment. It is then diluted with sterile physiological salt solution in the proportion indicated in the standardisation experiments, pipetted off into sterile vaccine bulbs ($\frac{1}{2}$–1 c.c. in each) sealed and heated to 60° C. for half an hour. If there is any doubt as to its sterility the heating should be repeated on two other successive days.

The method of standardisation will be seen from an example. The requisites are: (1) A 20 per cent. suspension of human red corpuscles in salt solution; it should have been re-washed at least three times. (2) Fresh normal human serum. (3) Physiological salt solution. (4) The serum to be tested ("immune serum") and previously heated to 60° C. for half an hour. The following mixtures were prepared:

(*a*)	Suspension 1 vol. +	serum 5 vols. +	immune serum	1 : 0	1 vol.
(*b*)	,,	,,	,,	1 : 3	,,
(*c*)	,,	,,	,,	1 : 5	,,
(*d*)	,,	,,	,,	1 : 10	,,
(*e*)	,,	,,	,,	1 : 20	,,
(*f*)	,,	,,	,,	1 : 50	,,
(*g*)	,,	,,	,,	1 : 100	,,

The mixtures of the ingredients are made by means of an ordinary Wright's opsonic pipette with a unit mark about 1 inch from the end, and the serum dilutions prepared in a similar way. The mixtures are placed in small test-tubes about $\frac{1}{8}$ in. internal diameter, well stirred with the pipette, and incubated at 37° C. for one hour. (They are also thoroughly stirred after half an hour.) The following was the result: With *a*, *b*, *c* and *d* there was complete hæmolysis; with *e* there was a trace of hæmolysis and much agglutination; with *f* there was agglutination, but no hæmolysis; and with *g* there was partial agglutination. This serum was only diluted 1 in 4 for use, so as to make sure of there being an excess of immune body in the conditions of the experiments. The five volumes of normal serum contain a large excess of complement.

(*b*) *Patient's serum.*—The blood for the test is collected in the ordinary way from a skin puncture in a Wright's capsule (Fig. 35, *d*, p. 225); about ¼ cubic centimetre of blood is ample, even if the test has to be repeated. To obtain plenty of serum it is advantageous (*a*) that the blood shall not be allowed to cool after it is collected, and (*b*) that the clot shall be separated from the sides of the vessel in which it is contained, so as to allow of free retraction. To meet the former indication it is a good plan to put the pipette in the incubator as soon as possible after it has been filled; to meet the latter shake the clot as soon as it has formed towards the curved end of the pipette and back again.

(*c*) The "*antigen*" used is an alcoholic extract of normal human heart, prepared by grinding up a weighed amount of heart muscle with four times the number of cubic centimetres of absolute alcohol that there are grammes of muscle, allowing the mixture to stand twenty-four hours, and repeating the grinding and maceration for another twenty-four hours, after which it is heated to 60° C. for one hour. It must be quite clear when used, a little being withdrawn from the top layer by means of a pipette. It is diluted with nine times its volume or more, as determined by experiments, of salt solution for use. Before use and occasionally afterwards it is important to test this extract. To be of value it should—(*a*) give complete absorption of the complement in a known case of late secondary or early tertiary syphilis, even when one volume of fresh normal serum from a healthy person is added; and (*b*) used in the conditions of the test about to be described should cause very little absorption (or destruction?) of complement in a normal blood. This is tested simply as follows: Two tubes are prepared, of which the first contains one volume of normal serum, four volumes of salt solution, and the second one volume of the same serum and four volumes of the diluted extract. These are incubated together for half an hour, and to each is then added one volume of the immune serum prepared as above, and an excess (five volumes is usually enough) of 20 per cent. emulsion of washed human corpuscles. The incubation is continued for one hour, the tubes being stirred

once or twice. At the end of that time they are centrifugalised, and a definite quantity of the clear blood-stained fluid is pipetted off and examined in a hæmoglobinometer. In a good extract there should be *no* difference between the two, and this is sometimes the case. Usually there is a slight difference, which naturally tends to interfere with the accuracy of the reaction. If there be a marked difference between the amounts of hæmoglobin liberated in the two tubes, the extract should be discarded or re-tested at a higher dilution.

(*d*) *Salt solution.*—A 0·85 per cent. solution of sodium chloride in distilled water.

(*e*) *The corpuscles.*—A 20 per cent. suspension of human corpuscles, washed three times in the salt solution.

The apparatus required are (1) a Wright's pipette, somewhat wide, *i. e.* 1 mm. internal diameter, with a 1-unit mark about one inch from the end, and a 4-unit mark; (2) a series of small test-tubes like Durham's tubes, *i. e.* about $\frac{1}{8}$ inch diameter and $1\frac{1}{2}$ to 2 inches long; (3) an incubator. The ordinary blood-heat incubator may be used, but a water-bath is preferable. Emery has modified Hearson's opsonic incubator for the purpose, which is very convenient, but a tin filled with water, and having on top of it a piece of *paraffined* cardboard pierced with holes of appropriate size to contain the small test-tubes will, with a little care in regulating the temperature to 37°–38° C., serve every purpose. All the constituents, antigen solution, sera, salt solution, etc., are contained in larger tubes, and are kept warm in the bath, as well as the small tubes for the tests: for each test two small tubes are required.

The process is carried out as follows: Prepare a Wright's pipette with a 1-unit and a 4-unit marks. Place in one tube 4 units of salt solution; this is to serve as a control in order to make sure that the serum to be tested contains sufficient free complement and that the hæmolytic system is in working order. In the other tube 4 units of the diluted extract are placed and the pipette is carefully washed out.

One unit of the serum to be tested is now added to each of the two tubes, that containing the salt solution receiving

its addition first, then that containing the extract; this is to avoid carrying over a little extract into the control. In each case the fluids are thoroughly mixed by repeatedly sucking them into the pipette and expelling them, and in each case it is advisable to see that the mixture forms a continuous column. The pipette is then rinsed out with salt solution, and the process is repeated with as many sera as are to be tested.

The hæmolytic system is then prepared. Take one unit of suspension of red corpuscles which have been washed three or four times, and mix them with four times their volume of the prepared rabbit's serum. This will be more than enough to saturate them with amboceptor and also with agglutinin. Place them in the incubator or bath so as to hasten the combination of the corpuscles and the antibodies.

The final stage of the test consists in the addition of these sensitised and agglutinated corpuscles to the tubes containing the diluted serum. When a bath is used the combination in the extract tube will be complete in five minutes after it has reached 38° C., and almost complete in two and a half minutes. Of course if the tubes are incubated in air these times are greater, since the tubes and their contents take an appreciable length of time to become heated to this temperature; but when the two substances are placed in a narrow tube surrounded with warm water the combination is very rapid, and if several sera are being tested, the reactions will be complete in the first tubes by the time the mixtures have been made in the last tubes.

If hæmolysis occurs the reaction is negative, *i. e.* the serum is not syphilitic; if no hæmolysis occurs the reaction is positive, *i. e.* the serum is syphilitic.

The examination of a very large number of cases of syphilis by different observers indicates that the test is of very considerable value and diagnostic significance. In conditions such as tabes dorsalis and general paralysis of the insane, which on other grounds are generally regarded as due to syphilis, 52 per cent. give the reaction. A *positive* reaction may be said to show a positive, and probably active, syphilitic

infection, but a *negative* reaction does not necessarily exclude syphilis. A course of mercurial treatment may render the reaction negative.

(4) *Porges' reaction.*—If syphilitic serum be added to a solution of lecithin or other lipoid substances, in many cases it gives a white precipitate. Normal or non-syphilitic serum gives no precipitate. This has been tried extensively as a substitute for the Wassermann reaction, but it is not so delicate.

Class III.—Infusoria (Ciliata).

The Infusoria are protozoa the locomotive organs of which consist of cilia, and in which the nuclear apparatus is differentiated into a vegetative macronucleus and a generative micronucleus. The cytoplasm is enclosed within a cuticle, an oral aperture is present in the form of a slit or pore, and waste matter is extruded by a pore, constant in position, but, as a rule, visible only when in use. A contractile vacuole is generally present. Reproduction usually takes place by fission, which is preceded by division of the two nuclei, the micronucleus by mitosis, the macronucleus by direct division.

The Infusoria are not of much pathological importance, but are common in ponds and ditches, *e. g. Paramecium* and *Vorticella.*

Balantidium (Paramecium) coli.

This is an intestinal parasite of swine, occasionally met with in man in conditions associated with chronic diarrhœa and dysentery.

It is somewhat ovoid in shape, the ends being bluntly pointed, is covered with cilia, measures 65 to 85 μ in length, and has a superficial resemblance to the ordinary *Paramecium.*

According to Saville Kent, the *Balantidium coli* is to be distinguished from the ordinary forms of water paramecia by the following characters: The *Bal. coli* is somewhat spindle-

shaped or ovoid, and bluntly pointed at each end, one and a half to twice as long as broad, measuring $\frac{1}{360}$ in. to $\frac{1}{168}$ in. in length; the *paramecium* is more cylindrical, four times as long as broad, measuring $\frac{1}{120}$ in. to $\frac{1}{96}$ in. in length. The oral aperture in *Bal. coli* is near one extremity (Fig. 57); in *paramecium* it is situated at about the middle of the ventral surface. In *Bal. coli* the cilia round the oral aperture are as long again as those over the body generally; in *paramecium* the whole of the cilia are of the same length

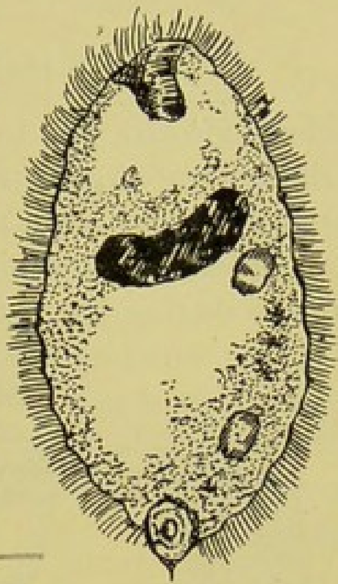


FIG. 57.—*Balantidium coli.*

The *Bal. coli* seems undoubtedly sometimes to be a cause of dysentery.[1]

Examination of Flagellated and Ciliated Forms.

(1) These may be examined fresh in the fluid in which they are present, by mounting on a slide, and covering with a coverglass, one edge of which rests on a bristle to avoid pressure.

(2) Permanent mounts may be made by the Heidenhain method (p. 514).

(3) Films may be made in the ordinary way, and stained with weak carbol-fuchsin or Leishman's stain. (The organisms are apt to be distorted.)

(4) The following method, devised by Rousselet (*Journ. Quekett Microscop. Club*, 2nd series, vol. vi, no. 36, p. 5, March, 1895) for preserving rotatoria, may be tried. In those forms which are non-contractile, kill by adding a drop of ¼ per cent. osmic acid, wash immediately in water, and preserve in 2½ per cent. formalin. Contractile forms may be first narcotised

[1] Strong and Musgrave, *Johns Hopkins Hosp. Bull.*, vol. xii, 1901, p. 31; Bureau of Gov. Laboratories, Manila, *Bull.* 26, 1904.

by adding a drop or two of 2 per cent. cocaine solution, then killed with the osmic and preserved as before.

Class IV.—Sporozoa.

The sporozoa are exclusively endoparasitic protozoa, the adult lacking organs for locomotion or the capture of food, and multiply by some method of sporulation, often very complex. Binary fission is almost unknown in this group. A parasite during the nutritive or "trophic" phase, when it is absorbing nutriment and growing at the expense of its host, is termed a *trophozoite*; when it is mature and ready for sporulation it is termed a *sporozoite* or *schizont*. The spores are of various kinds, and may develop outside the body or in a second host.

Order.—Coccidiidea.

The Coccidiidea, with a single exception, are intra-cellular during the trophic stage, and present a dimorphism or alternation of generations; the one is endogenous and asporular, determining the reproduction of the parasite within the host, the other exogenous and sporular and permitting of infection.

Coccidial Disease of Rabbits.

This is a disease caused by a sporozoon, the *Coccidium oviforme* or *cuniculi*, and often met with in warrens and hutches; in some of the former as many as 90 per cent. of the animals may be affected. The young animals suffer most, and become infected when they cease to suckle and commence to eat green food, the adult animal as a rule resisting the disease. The affected animals waste, suffer from enteritis, and a large proportion die in from one to three weeks, the condition being known as "wet-snout" among the keepers. The parasites occur in the intestine, bile-ducts, and liver in large numbers. Each parasite is ovoid in shape, measuring

36 μ in length and 22 μ in breadth, is enclosed in a firm translucent cyst, which encircles a very granular protoplasm. Sometimes this protoplasm becomes condensed so as to form a spherical mass lying free within the cyst (Fig. 58, *c*). In the intestine and bile-ducts the parasites are attached to the epithelial cells, and in the liver, if the animal lives beyond the acute stage, set up some remarkable changes. The affected liver is studded with greyish-white nodules varying in size from a pin's head to a pea. On making sections and examin-

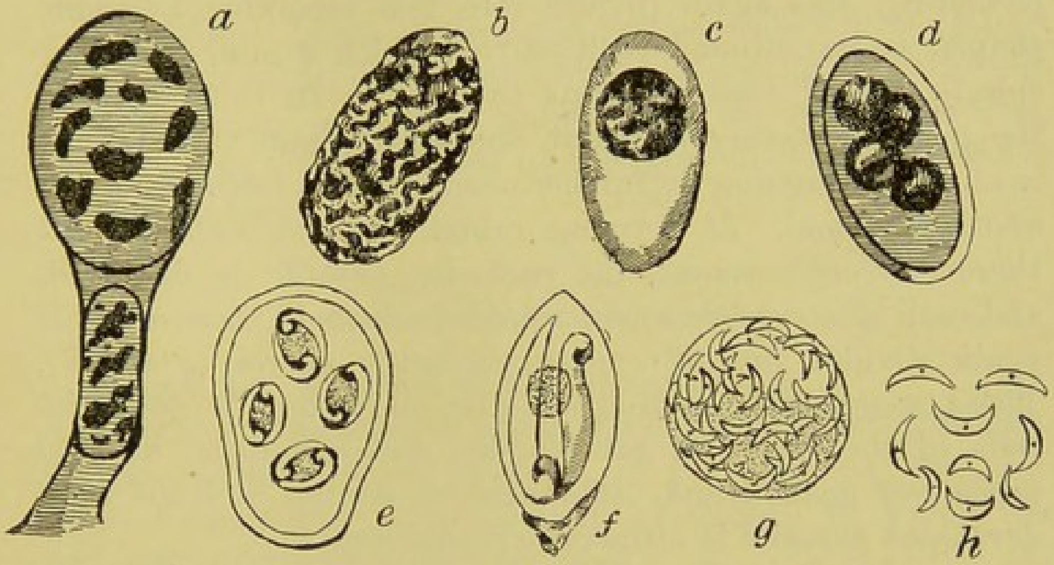


Fig. 58.—*Coccidium Oviforme* of rabbit: *a*, Coccidium attached to an epithelial cell. *b–g*, Stages in the life-cycle. *h*, Free spores.

ing them microscopically, it is found that these nodules consist of dilated bile-ducts filled with a much hypertrophied and convoluted mucous membrane, which forms branched projections covered with cubical epithelium, among which the parasites occur in great numbers (Plate XXIII., *b*). A curious fact is that subcutaneous or intra-venous inoculation, or inoculation into the liver of a healthy rabbit with the coccidia from another rabbit, fails to induce the disease.

The coccidium has a complicated life-history, and infection only seems possible in one of the stages. In order to study the life-cycle the parasite must be placed under suitable con-

ditions, and an infusion of rabbits' fæces, kept at the ordinary temperature, is perhaps as good a cultivating medium as any, the changes being watched by means of interlamellar films. When the coccidia are observed under these conditions, the first change is apparently the formation of micro- and macro-gametes, fusion of these, and the formation of a zygote or oocyst (Fig. 58, *b*). The protoplasm of this then condenses so as to form a sphere lying free within the cyst (*c*), a stage sometimes observed in the animal. The sphere then divides into four smaller spherules (*d*). Each spherule becomes elongated, and again divides into two somewhat crescent-shaped bodies, around each pair of which a new, somewhat spindle-shaped capsule forms (*e* and *f*). In this condition the parasite is very resistant, and may remain alive for six months, undergoing no further change unless introduced into another animal. If a young rabbit swallows with its food these crescentic spores, the enclosing capsule is dissolved, and each crescent becomes a rounded amœboid mass, and this again divides up into many crescentic spores (*g* and *h*). These spores are apparently motile, and enter the epithelial cells of the intestine, gall-bladder, and bile-ducts, where a process of growth and differentiation occurs, and the fully developed parasite is ultimately reproduced.

Coccidial disease, or, as it is sometimes termed, psorospermosis, is occasionally met with in animals, as the sheep, and a wasting disease of young pheasants due to coccidia has been described by McFadyean.[1]

In man, coccidial disease has been described (but rarely) in the liver, gall-bladder, ureter, etc.[2]

Rixford and Gilchrist[3] described two cases of protozoan infection of the skin and organs, accompanied by great destruction of tissue and ending in death. The organisms were spherical, 7 to 27 μ in diameter, surrounded by a thick capsule, enclosing granular bioplasm (*C. immitis*).

[1] *Journ. Comp. Path. and Therapeut.*, 1895.
[2] *Journ. Comp. Path. and Bact.*, 1898, June, p. 171.
[3] *Johns Hopkins Hosp. Reps.*, vol. i, 1896, p. 209.

The Ruffer-Plimmer bodies of cancer were at one time believed to be coccidia (p. 561).

The term "psorospermosis" has been applied to human infection with coccidium, Sarcosporidia (p. 561), etc.

Examination.

(1) The coccidial forms are readily examined in the fresh state. The only bodies they are likely to be mistaken for are certain ova.

(2) Paraffin sections of rabbit's liver containing coccidia may be stained much in the same way as tuberculous tissues —viz. warm carbol-fuchsin ten minutes, decolorise *cautiously* in 5 per cent. acid, and counter-stain in methylene-blue. Sections may also be stained in the Ehrlich-Biondi stain for one to two hours.

Order.—Hæmosporidia.

The general characters of this group are:

(1) Life at the expense of the red blood-corpuscles, at least during a portion of the life-cycle.

(2) Endogenous multiplication by spores, by which the life-cycle is repeated within the host.

(3) Development of a form which becomes free in the plasma, and which is the commencement of a sexual cycle to be completed in a second host.

(4) Inoculability, but only from one animal to another of the same species.

The group includes the malaria parasite and similar parasites in mammals and birds, the hæmogregarines, *Drepanidium* of the frog, and perhaps the Piroplasmata.

Malaria.

Malaria is caused by parasitic protozoa, placed in the genus *Plasmodium* (*Hæmamœba*), the credit of the discovery of which must be given to Laveran, who

described the parasite as occurring in four phases, viz. (1) spherical bodies, (2) flagellated bodies, (3) crescentic bodies, and (4) segmented or rosette bodies.

The parasites cannot be cultivated, but inoculation of healthy individuals with the blood of malarial patients reproduces the disease, and the same structures or parasites are found in the blood of these infected persons. Inoculation experiments on all animals except man have proved negative, and in the latter the inoculation must be intra-venous.

In the various forms of malarial fever the parasites have the same general characters, though there are distinct differences between them, by which they can be recognised and the type of fever differentiated. In each there is an endo-corporeal cycle within the host, through which the recurrent attacks are developed; there is also an extra-corporeal cycle of development outside the body of the host, whereby the infection of fresh individuals becomes possible. Each of these cycles needs separate description.

If the blood of a malarial patient is examined an hour or two before, or at the very commencement of, the febrile paroxysm, the parasite will be recognised as a pale, ill-defined mass of protoplasm within the red corpuscles, of which a variable proportion are infected, the size of the parasite varying in the different types of fever. When some hours old a variable number of blackish pigment-granules of melanin make their appearance. These subsequently coalesce into smaller groups, and the latter again into one or two larger, more or less centrally disposed, masses. The parasites exhibit more or less amœboid movement, and the melanin granules are frequently in a state of tremor. Later on most of the parasites (now schizonts) become divided into a variable number of segments,

which separate and become spherical, the blood-corpuscle breaks down, the spherical bodies or spores are set free, and a certain number of them, again becoming attached to red corpuscles, develop into the first stage of the parasite. The melanin granules and some of the spores are ingested by phagocytes, and the melanin is deposited in the spleen and liver for a time.

The parasite, termed a *plasmodium*, or better, an *amœbula*, contains a vesicular nucleus and a nucleolus, and the melanin granules are present in the surrounding protoplasm. When segmentation occurs, each segment contains a portion of both the nucleolus and the protoplasm. The maturation of each "brood" of parasites is coincident with a fresh febrile paroxysm. In the sub-tertian (pernicious) forms of malarial fever there exist in the blood for some time after the subsidence of the acute paroxysms well-marked non-motile, crescentic or sausage-shaped bodies, with rounded ends, the so-called "crescentic bodies" or "crescents"; their longer diameter is greater ($\frac{1}{3}$) than that of a red corpuscle, their protoplasm is finely granular, and contains at about the centre several well-marked pigment-granules. In the crescentic forms the extremities of the crescent often appear to be joined by a delicate membrane (Fig. 64, *f* and *j*); this is the remains of the blood-corpuscle in which the parasite has developed.

When a "wet" specimen of malarial blood from a case of pernicious or sub-tertian malaria is kept under observation (p. 555), it not infrequently happens that after a time the so-called flagellated "bodies" make their appearance. These consist of a central protoplasmic mass attached to which are from one to six delicate flagella measuring 20–30 μ in length (Fig. 59, *c*). The flagella are actively motile and disturb the corpuscles, but the body itself does not move much,

Frequently one or more of the flagella break away and swim free, remaining active for several hours. The flagellated bodies are never seen in the freshly drawn blood, and Ross has found that flagellation does not occur if the finger be pricked through a spot of vaseline, the blood remaining covered with the film of grease. Careful observation has shown that the flagellated bodies develop from "crescents" in subtertian malaria, and from special rounded parasites,

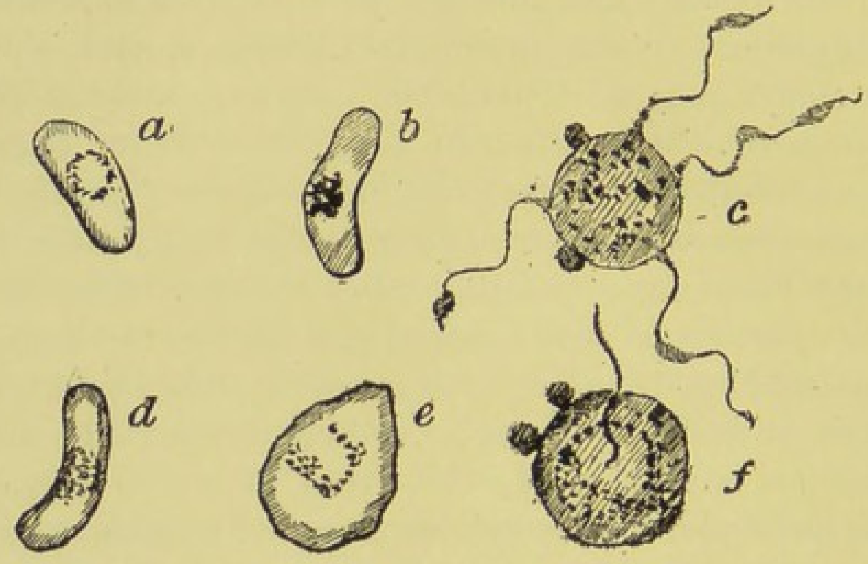


FIG. 59.—Development of the malaria parasite in the mosquito. *a*, *b*, and *c*, the male gametocyte; *d*, *e*, and *f*, the female gametocyte; *f*, fertilisation of the female gametocyte by a microgamete. (After Ross and Fielding-Ould.)

difficult to distinguish from the schizonts, in the benign tertian and quartan fevers.

Various theories were held in the past as to the nature of these flagellated bodies. Through the brilliant researches of Ross, which have been confirmed and extended by observers in all parts of the world, it is now known that these cells are sexual elements. The flagellated body represents the male cell or "male gametocyte," the flagella ("gametes") being analogous to the spermatozoa of higher animals. The female

cells or female gametocytes or gametes are non-flagellated, and are fertilised by the entrance of one of the flagella of a male gametocyte. *This fertilisation takes place in the stomach* (middle intestine) *of certain species of mosquito*, and after fertilisation a series of changes ensues resulting in the formation of spore-like bodies, which are injected when the insect bites its victim, and thus the infection of fresh individuals with the malaria parasite takes place. The first demonstration of the nature of "flagellated bodies" was given by Opie and MacCallum on the *Halteridium*, a parasite of pigeons (p. 556), and this forms a good example of the value of abstract research to practical medicine (see p. 556). Ross also followed the development of the malaria-like *Proteosoma* of sparrows, etc., in the mosquito, *Culex fatigans*. The development of the malaria parasite of man in the mosquito is as follows, according to Ross and Fielding-Ould.[1] It is not known what determines whether an amœbula will become a sporocyte or a gametocyte. When the sexual cells or "gametocytes" are ingested with the blood by the mosquito, they pass into the middle intestine. Within a few minutes the corpuscles enclosing them break down, the parasites are set free, and quickly become spherical or ovoid (Fig. 59, *c*, *e*, and *f*). One or two spherical granules are often attached to the naked parasites, and may represent polar bodies (Fig. 59, *c* and *f*). Very soon the male cells become flagellated (Fig. 59, *c*), and before long the flagella or "microgametes" break away from the parent cell, and by their own motility make their way through the liquor sanguinis. Should one come in contact with a female cell or "macrogamete," it fuses with the latter, uniting with the nucleus (Fig. 59, *f*),

[1] *Thompson Yates Laboratories Report*, vol. iii, pt. vol. ii, 1901, p. 183.

fertilisation is completed, and a "travelling vermicule" or "ookinet" results; this passes into the outer wall of the mosquito's stomach, where it becomes encysted and forms a "zygote" (Fig. 60, *a*, *b*). At this period the zygote is about 7–8 μ in diameter. If development proceeds, it acquires a distinct capsule and begins to

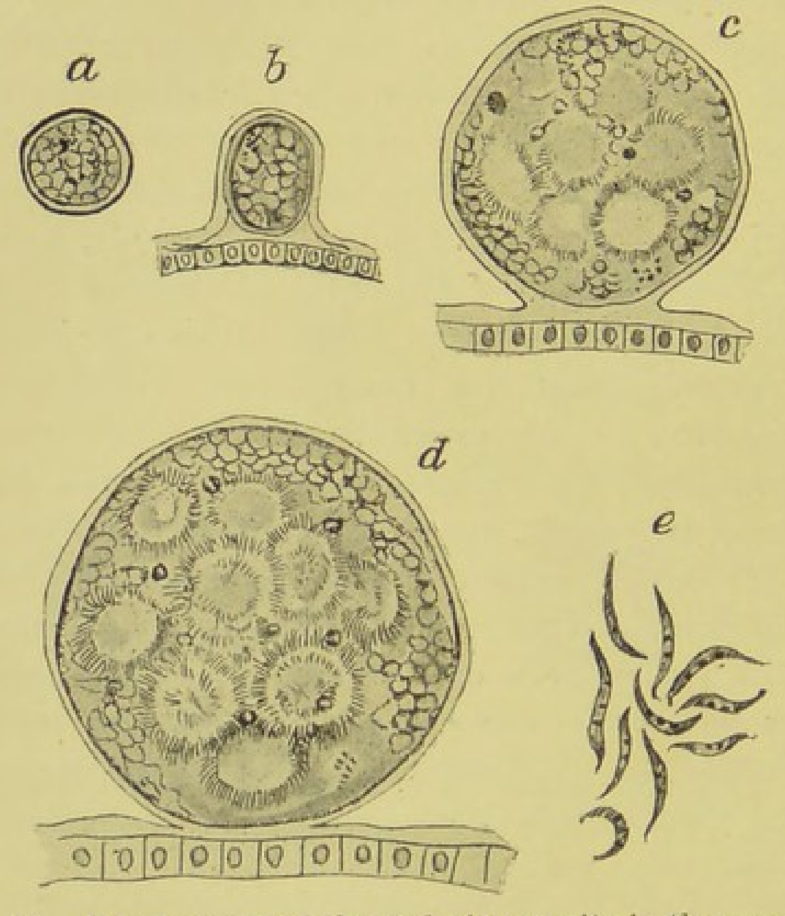


FIG. 60.—Development of the malaria parasite in the mosquito. (After Ross and Fielding-Ould.)

grow rapidly, and when mature at the end of a week or more, according to the temperature, is 60 μ in diameter, and projects into the body-cavity of the insect (Fig. 60, *b*). Its substance next divides into eight to twelve portions, or "zygotomeres," then each zygotomere becomes a spherical body, or "blastophore" (Fig. 60, *c*), and each blastophore develops upon its

surface a number of spindle-shaped, radially disposed bodies, or "zygotoblasts" (Fig. 60, *d*). When the zygote reaches maturity the blastophores disappear, leaving its capsule packed with large numbers ("thousands") of free zygotoblasts. The capsule then ruptures, and the zygotoblasts are poured into the

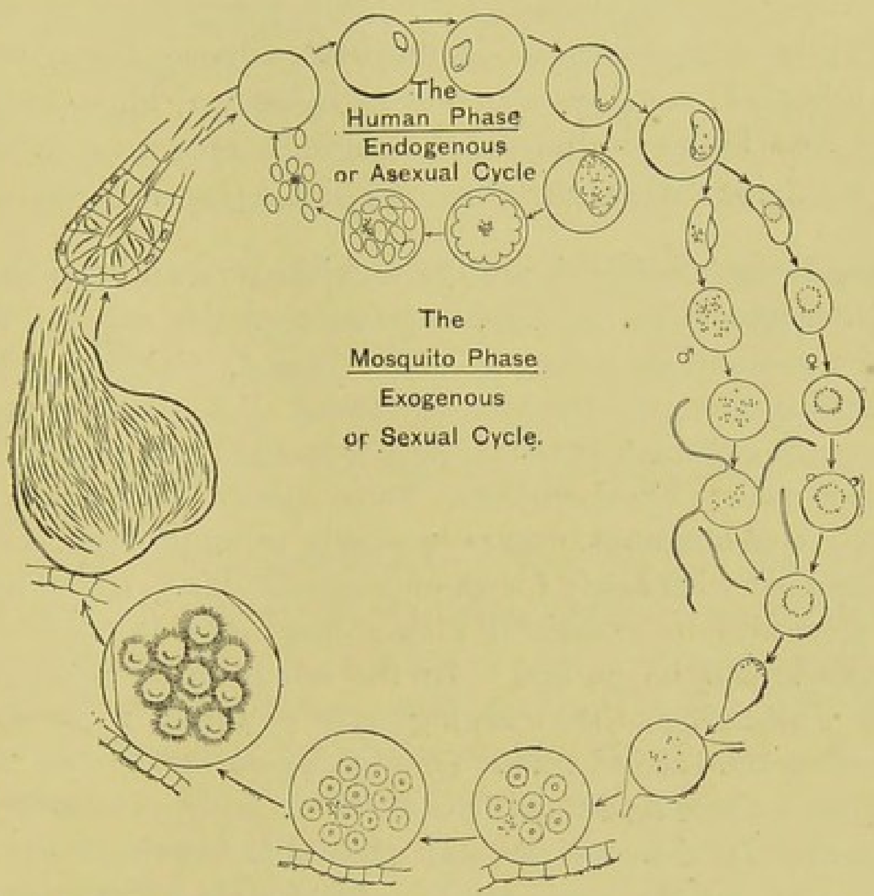


FIG. 61.—Diagram of the asexual and sexual cycles of the malaria parasite.

body-cavity of the mosquito. The "blasts" measure 12–16 μ in length, taper at each extremity, and possess a central nucleus (Fig. 60, *e*), and they make their way to all parts of the body of the host, and accumulate in the salivary or poison glands, whence they are discharged by the middle stylet (hypopharynx) of the proboscis, when the insect "bites," into the circulation

of a fresh vertebrate host. Here, presumably, the blasts become attached to erythrocytes and develop into amœbulæ. The diagram[1] (Fig. 61) represents in graphic form the asexual and sexual cycles of reproduction of the malaria parasite.

So far as is known, malarial infection is conveyed only through the bite of infected mosquitoes of the sub-family *Anophelinæ*. It has been repeatedly proved that infected mosquitoes convey infection, and that if mosquitoes be excluded human beings may live in the most malarious districts without contracting the disease.

Mosquitoes (*Culicidæ*) are distinguished from other mosquito-like insects by the fringe of scales on the wings. The common mosquitoes belong to the sub-family *Culicinæ*. The *Anophelinæ* are usually less abundant (but there is great variation in different districts), and bite mainly at night; the females alone are blood-suckers. Some species breed in natural collections of stagnant, others in slowly running fresh, water well supplied with lowly forms of vegetable life. If the head of a mosquito be examined with a hand-lens, three sets of appendages will be noticed. In the middle is the stout proboscis containing the stinging and suctorial apparatus; situated at the base of this are two palpi, one on either side, and outside these again are two antennæ, which are more or less hairy. In *Anophelinæ*, both male and female, the palpi are as long as the proboscis; in the *female Culex* (also in *Stegomyia* and many other genera) they are short and stumpy. In *Anophelinæ* the scales on the veins of the wings are usually arranged in alternating light and dark patches, giving a speckled or dappled appearance, different as a rule from anything seen in *Culex*. (Some *Culices* have a similar arrangement, and it is wanting in *A. maculipennis* and *bifurcatus*.) The front or costal margin of the wing in *Anophelinæ* is almost always marked with dark blotches. *Anopheles*, as a

[1] This figure is reproduced by permission from Daniels' *Laboratory Studies in Tropical Medicine* (Bale, Sons & Danielsson, 1908).

whole, is a more slender insect than *Culex*, and when at rest its body is all in one line, whereas *Culex* is angular or hump-backed. The important species known to carry malaria are *Anopheles maculipennis* in Europe, N. Africa, and N. America, *A. bifurcatus* in Europe, *Myzomyia funesta* and *Pyretophorus costalis* in Central and W. Africa, and *Cellia argyrotarsis* in tropical America. Other species, e. g. *Myzorhynchus sinensis*, *Cellia Kochii*, and others, are less important carriers.

(On Mosquitoes, see Theobald, *Brit. Museum Monograph*, and Allbutt's *System of Med.*, ed. 2, vol. ii, pt. 2; Giles, *Handbook of the Gnats and Mosquitoes*; Daniels, *Laboratory Studies in Tropical Medicine*, ed. 3, 1908.)

There are probably at least three species of malaria parasite[1] occurring in the various types of malarial fever in man, though some authorities (*e.g.* Laveran) regard the forms as varieties of a single species, and the following are the differential characters between them :

(1) *Benign quartan fever* (Fig. 62).—The quartan parasite (*Plasmodium malariæ*) completes its asexual life-cycle in seventy-two hours; there are two complete days without an attack, and reckoning the day of the previous attack, an attack occurs every fourth day, hence the name "quartan." It commences as a small amœbula, which is feebly motile. It enlarges, becomes pigmented, and motility ceases, the pigment-granules being numerous and coarse. The parasite finally occupies nearly the whole of the corpuscle, which, however, is but little altered (*a–d*).

Towards the end of the apyrexial period the pigment collects in the centre, and segmentation takes place with the formation of a symmetrical rosette (*e*), and afterwards of six to twelve spores (*f*). The

[1] Hewlett, *Trans. Fourteenth Internat. Congress of Hygiene*, vol. ii, 1908, p. 141.

quartan parasite does not form crescents, and the flagellated bodies (*h*), which are rarely seen, are developed from large pigmented parasites.

(2) *Benign, or spring, tertian fever* (Fig. 63; Plate XXIV., *a*).—The benign tertian parasite (*Plasmodium vivax*) completes its asexual life-cycle in forty-eight hours, an attack occurring every other day, or, reckoning the day of the previous attack, every third day. In the early stage it resembles the quartan, but shows much more active amœboid movement. The pigment-granules are also finer than in the quartan,

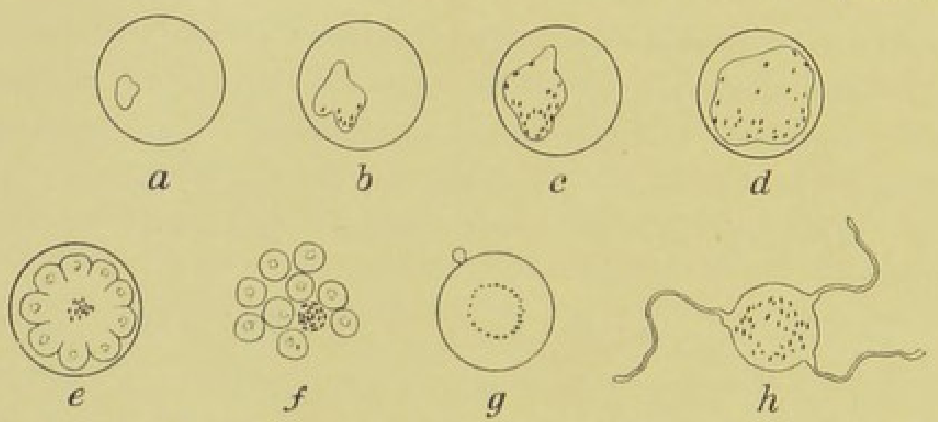
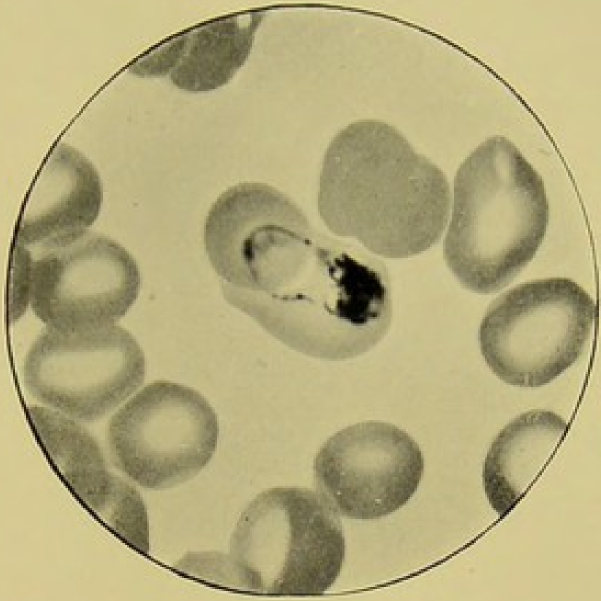


Fig. 62.—The quartan parasite: *a*, *b*, *c*, *d*, amœbulæ; *e*, sporocyte; *f*, free spores; *g*, female gametocyte with so-called polar body; *h*, male gametocyte. (After Rees.)

and incessantly change their position. The parasite finally invades the whole corpuscle, which becomes enlarged and pale. Enlargement of the corpuscles is a marked feature in the benign tertian infection (*d*).

Segmentation takes place, but is unsymmetrical (*e*), resulting in the formation of a grape-like cluster of twelve to twenty spores (*f*). As in the quartan, no crescentic bodies are developed, and the gametocytes (*g*, *h*) are similar to, but larger than, the quartan (Plate XXIV., *b*).

(3) *The æstivo-autumnal, malignant, pernicious, or sub-tertian, fevers* (Fig. 64).—This parasite (*Laver-*



a. Malaria. Parasite of benign tertian fever. Smear of blood. × 1500.

b. Malaria. Gametocyte of benign tertian parasite. Smear of blood. × 1500.

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ania malariæ) (or parasites, for it has been divided into three species by the Italian observers, viz. the pigmented and the unpigmented quotidian and the

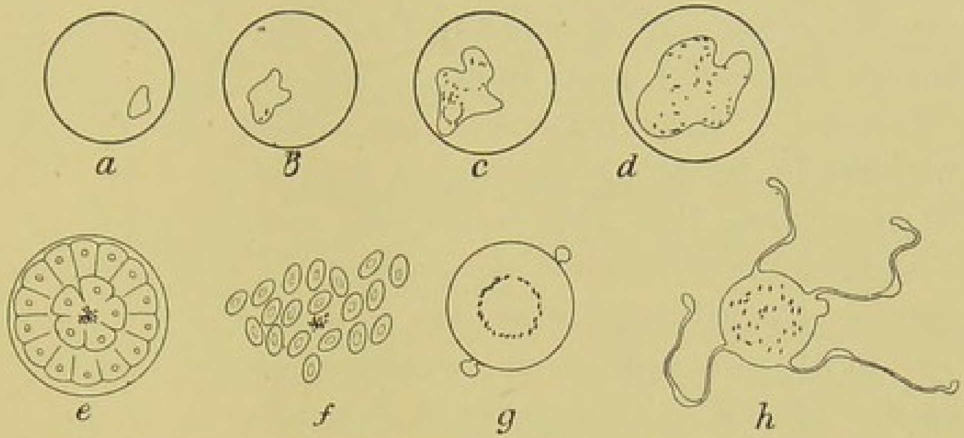


FIG. 63.—The benign tertian parasite: *a*, *b*, *c*, *d*, amœbulæ; *e*, sporocyte; *f*, free spores; *g*, female gametocyte with so-called polar bodies; *h*, male gametocyte. (After Rees.)

malignant tertian, but this is not generally accepted) is much smaller than the quartan or benign tertian, and when it reaches the stage of multiplication it dis-

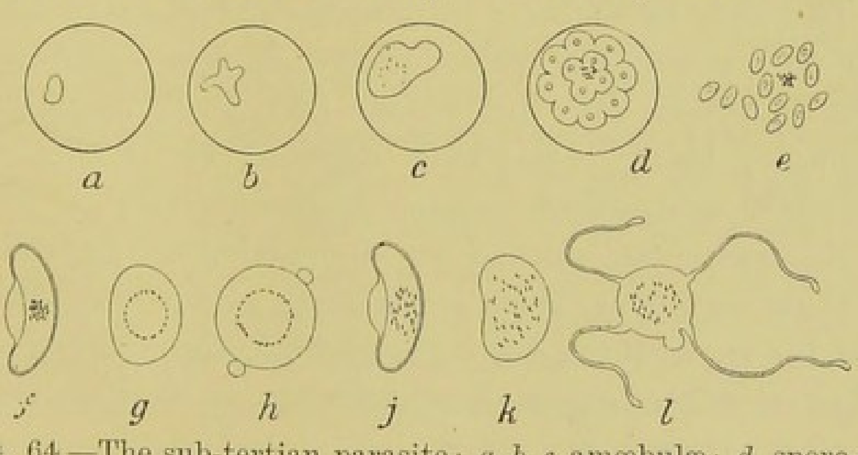


FIG. 64.—The sub-tertian parasite: *a*, *b*, *c*, amœbulæ; *d*, sporocyte; *e*, free spores; *f*, *g*, *h*, female gametocyte; *j*, *k*, *l*, male gametocyte. (After Rees.)

appears from the peripheral blood and collects in the internal organs, spleen, liver, cerebral capillaries, and bone-marrow. It is actively amœboid, seems to change its position within the corpuscle, and the pigment-

granules are very fine in the young parasites, but early aggregate into large clumps. The fission forms (*d*, *e*) are only met with in the internal organs. Multiple infection of the corpuscles may also occur. The corpuscles often suffer severely from the infection, some being shrivelled and spinous, others dark in colour, "brassy"; they may also be altered or destroyed without being actually invaded by the parasite. It is in this form that the crescentic bodies appear (*f*, *j*). These, however, are not met with at the very commencement of the attack, but appear in a week or so, and may not disappear until some weeks after the termination of the attack. This parasite is met with in the subtertian, or so-called malignant, types of fever, which are characterised by irregularity of the fever, considerable blood destruction, often accompanied by hæmoglobinuria, and cachexia; coma is another complication in certain instances, probably caused by massing of the parasites in the cerebral capillaries.

The cure of malaria by quinine is regarded as being due to a poisonons action on the parasites analogous to that exerted on numerous protozoa, amœbæ, for example, being injuriously affected by so little as a 1–50,000 solution of quinine hydrochlorate.

No toxin can usually be demonstrated in the blood of those suffering from a malarial attack, but Rosenau and his co-workers have found that the filtered blood, *taken when the temperature is rising*, produces a malaria-like paroxysm.[1]

A malaria-like parasite (*Plas. Kochii*) occurs in apes, in which it produces fever.

The nature of Blackwater fever, so called from the presence of hæmaturia and hæmoglobinuria, has given rise to much discussion. By some it is considered to be a disease

[1] See Hewlett, *loc. cit.*, p. 144.

sui generis, of unknown ætiology. By others it is regarded as a form of malaria, either of an intense type, or in which the kidneys are especially involved, or as due to malarial infection *plus* quinine. It may be that under particular conditions, of the nature of which we are at present ignorant, hæmolysins may be set free and cause hæmolysis, the blood-pigment being eliminated by the kidneys.[1]

Clinical Examination.

The blood of malarial patients may be examined either in the unstained or stained condition.

Examination in the unstained condition.—The finger or lobe of the ear is pricked, and a droplet of blood taken up on a clean cover-glass, which is then placed upon a slide, so that the droplet of blood spreads out into a thin layer between the two glasses. The cover-glass may then be ringed with oil or vaseline to prevent evaporation. A little practice is required to judge the right quantity of blood. The preparation should be examined with a $\frac{1}{12}$-inch oil-immersion lens.

Examination in the stained condition.—To prepare stained specimens the finger or ear is pricked as before, and a droplet of blood taken up on a cover-glass; another cover-glass is applied, and the two are separated so that each is smeared with a thin film of blood; several are prepared in this manner. Manson recommends picking up a droplet of blood on an oblong slip of fine clean tissue or cigarette paper. The charged surface of the paper is then applied to a clean glass slide; in a second or so the blood will have formed a thin film between the slide and the tissue paper. The latter is then withdrawn, leaving a very thin film on the glass, and may be applied to a second slide, and, in like manner, to three or four in succession. A piece of gutta-percha tissue may be similarly used. Or a droplet of blood may be picked up on a slide near one end, and the edge of a second slide held at an angle of 45° being applied to it, the blood is spread by *pushing* the second slide over the first one, or the droplet of blood on a

[1] See Hewlett, *loc. cit.*, p. 145.

slide may be spread by touching it with a needle held flat on the slide and drawn evenly along the surface of the slide. Whatever method is adopted, the film is allowed to dry in the air, and may then be fixed (not if Leishman's stain is used) by heat, preferably at 110° C. for one hour, as over-heating ruins the preparations. It is much simpler and better to fix in a mixture of equal parts of absolute alcohol and ether for not less than ten minutes, preferably for half an hour; this gives excellent results. In hot countries a saturated solution of corrosive sublimate may be used. The methods detailed at p. 99 may also be employed.

As regards staining, this is usually carried out with Leishman's stain (No. 13, p. 104). The blood films, *unfixed*, are flooded with a few drops (5–10) of the stain, which is spread by tilting, no attempt being made to check evaporation. After half a minute about double the quantity of distilled water is added, allowed to mix with the stain on the film, and staining is continued for five, or in some cases for ten, minutes. The film is then washed in distilled water, some of the water is allowed to remain on the film for one minute, and it is then dried and mounted. Jenner's or Giemsa's blood-stain may be similarly used.

Staining may also be done in a half-saturated aqueous solution of methylene-blue or in Löffler's blue for half an hour, washing in water, and counter-staining with a very weak eosin solution for a few seconds, washing, drying, and mounting. Manson recommends treating the films with a very weak acetic acid—two or three drops to the ounce of water—to wash out the hæmoglobin, and, after washing, staining in the following solution for half a minute:

Borax	5 parts
Methylene-blue	0·5 part
Water	100 parts

washing, drying, and mounting in xylol balsam.

Hæmatoxylin (Ehrlich's, or Mayer's hæmalum) is preferable for permanent preparations, and in hot countries, where methylene-blue rapidly fades. The preparations may be counter-stained with a weak solution of eosin.

Ross recommends for rapid diagnosis the use of *thick* blood films, from which the hæmoglobin is first removed with very dilute acetic acid; the films are then stained with Leishman's stain, and examined with a $\frac{1}{6}$-inch objective. Practice is required for this method.

In order to demonstrate the flagellated organisms Manson recommends the following procedure: Thirty or forty strips of thick blotting-paper (3 inches by $1\frac{1}{2}$ inch), each having an oblong hole ($\frac{7}{8}$ inch by $\frac{2}{3}$ inch) cut lengthways in the centre, are prepared, moistened with water, and laid on a sheet of window glass. A patient is selected in whose blood the crescentic form is plentiful, and a minute droplet of the blood, about the size of a pin's head, is expressed from a prick. A clean slide is then breathed on, and the droplet of blood picked up on it and spread out with a needle so as to cover an area $\frac{3}{4}$ inch by $\frac{1}{2}$ inch. The slide is immediately inverted over a blotting-paper cell and pressed down sufficiently to secure perfect apposition. The rest of the paper cells are similarly covered with blood-charged slides. In from half to three quarters of an hour the slides are removed and dried by gentle warming, and then fixed with absolute alcohol for five minutes. The alcohol is allowed to evaporate, and the films are treated with a few drops of 15 per cent. acetic acid to dissolve out the hæmoglobin. The slides are then washed in water and stained with weak carbol fuchsin (20 per cent.) for six to eight hours, washed in water, dried, and mounted.

N.B.—Negative results in the examination for the malaria parasite must be accepted with caution unless repeated. A single undoubted parasite is sufficient to establish the diagnosis. Quinine causes the disappearance of the parasite. The parasites in the sub-tertian fever disappear during the apyrexial intervals (except the crescents), and are most likely to be found at the commencement of the attack—*i.e.* when the temperature is rising. The parasites of the other forms are larger and more obvious during the apyrexial intervals.

[For further particulars on Malaria and on the demonstration of the malaria parasite, see Daniels' *Laboratory Studies in Tropical Medicine*, 1908.]

Plasmodium præcox.

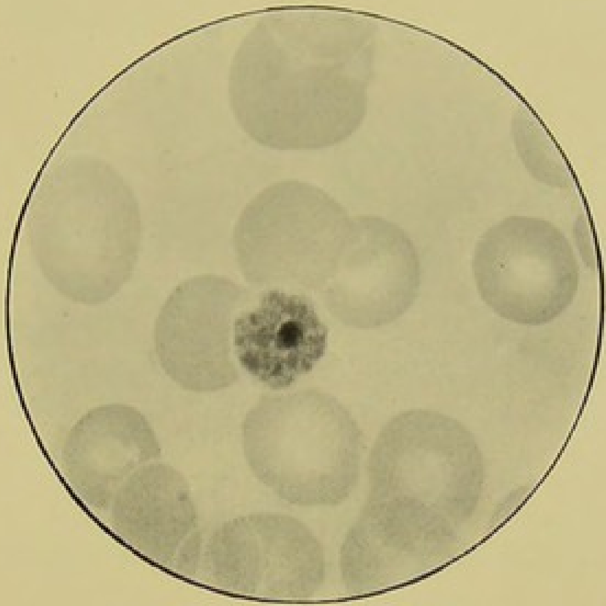
Syn. *Proteosma Grassii*, *Hæmamæba relicta*.

This parasite (commonly called "proteosoma") is met with in sparrows and other birds, in which it invades the red blood-corpuscles, and its structure and development are practically identical with those of the benign malarial parasites of man. It grows from a minute granule into an amœboid plasmodium, which ultimately segments and forms a rosette. In some specimens of blood flagellated male gametocytes make their appearance, similar to those of malaria, the flagella break away from the main mass, fertilise other non-flagellated or female cells, and a series of changes ensues analogous to those occurring in the malaria parasite (p. 543). The fertilisation and development of the fertilised cell take place in the stomach of a mosquito (*Culex fatigans*), by which the infection is transmitted to other birds.

Halteridium Danilewskyi.

This is an elongated, curved parasite (also known as *Hæmoproteus* or *Hæmamæba Danilewskyi*), found in the red corpuscles of certain birds (pigeon, crow, etc.), and embracing the nucleus (Plate XXV., *b*). By some it is included among the malaria-like parasites (*Plasmodium*). At an early stage it much resembles the proteosoma, but as it grows it becomes elongated, pigment-granules appear, and are either scattered throughout the protoplasm or collect in two groups, one at each extremity. Finally, the parasite occupies nearly the whole of the corpuscle, dislocating its nucleus. The fully grown parasites may be differentiated into two forms, one of which remains almost completely unstained when treated with methylene-blue, the other staining deeply with this dye (Opie). When the blood is withdrawn, the corpuscles disintegrate and liberate the contained parasites, which assume a circular outline, and a certain number become flagellated. *It is only the non-staining form which becomes flagellated.* These two varieties of the parasite are the male and female

PLATE XXV.



a. Malaria. A tertian "rosette." Smear of blood. × 1500.

b. *Halteridium Danilewskyi*. Smear of pigeon's blood. × 1500.

To face page 556.

cells respectively, and the fertilisation of the female cell by a free flagellum has been actually observed by MacCallum.[1] It can hardly be doubted that the development of the fertilised cells takes place in some insect, but the definitive host has not yet been discovered with certainty.

The presence of these parasites induces rise of temperature, deposition of melanin, and changes in, and enlargement of, the spleen and liver, analogous to those occurring in malaria in man. The *Halteridium* parasite, according to Schaudinn, is a stage in the life-cycle of a trypanosome (see p. 523).

The Piroplasmata.[2]

Syn. *Pyrosoma, Babesia.*

The Piroplasmata form a somewhat anomalous group, but are usually included in the Hæmosporidia of the Sporozoa. They differ from the *Plasmodia* in the following respects: absence of pigment, non-fragmenting of the nucleolus, division into two or four only, and frequency of extra-corpuscular forms. They cause many diseases in animals, are conveyed by ticks, but are unknown in man. (A piroplasma was described as the causative organism of Rocky Mountain spotted fever by Wilson and Chowning, but the observations appear to be erroneous, see p. 575). The body of a piroplasma is typically pear-shaped (Plate XXVI., *a*), but rounded and rod forms occur. Two nuclear masses are present, one larger than the other.

The developmental cycle in the ticks has not been worked out, but Koch has observed peculiar rayed forms with *P. bigeminum*, and Christopher[3] various developmental forms with *P. canis*. Miyajima states that a piroplasma of Japanese cattle (apparently *P. parvum*) in blood broth develops into typical trypanosome forms.[4]

[1] *Journ. Exper. Med.*, vol. iii, 1898, pp. 79, 103, 117.

[2] See Hewlett, *Trans. Fourteenth Internat Cong. of Hygiene*, Berlin, vol. ii, 1908, p. 146; Minchin in Allbutt's *System of Med.*, ed. 2, vol. ii, pt. 2, p. 86.

[3] *Brit. Med. Journ.*, 1907, vol. i, p. 76.

[4] *Philippine Journ. of Science*, vol. ii, 1908, p. 37.

Piroplasma bigeminum.—This is the parasite of the well-known Texas fever of cattle, a disease which is characterised by fever emaciation, anæmia, hæmoglobinuria, and enlargement of the liver and spleen.

The disease causes considerable loss among cattle, and is met with in various parts of the world, America, Australia, South Africa, Malaya, the Philippines, the Roman Campagna, Greece, Roumania, and North Ireland.

In the acute type of the disease a small proportion (1–5 per cent.) of the red corpuscles in the peripheral circulation contain pairs of pyriform bodies 2–4 μ in length and 1·5–2 μ in largest diameter. One end of each body is rounded, and the body gradually tapers to a point at the other end, and the pair lie close together, their tapering ends directed towards each other. A dark spherical body is present at the rounded end of the parasite.

Some of the young parasites exhibit amœboid movements when the blood is examined on a warm stage. In the internal organs the parasites are more numerous; in the kidney and liver 10–25 per cent. of the corpuscles contain them, in the heart-muscle 50 per cent. In the mild type 5–50 per cent. of the corpuscles in the circulating blood may be infected at one time or another, and the parasite appears in some cases as a coccus-like body at the periphery of the corpuscle. This appears to become enlarged and spindle-shaped, then to taper in the middle, divide, and so give rise to the pyriform bodies. Occasionally minute free coccoid bodies are seen in the plasma, and at times two to five minute (0·5 μ) coccoid cells are present in the red cells. After death the pyriform bodies seem to become spherical or angular.

Sexually differentiated gametes are not known with certainty, but flagellated forms have been described.

The disease is transmitted through the bites of ticks (*Rhipicephalus annulatus, R. australis*). The female tick, after biting an infected ox and sucking its blood, falls off and lays its eggs; the eggs hatch in two to six weeks' time, and the daughter ticks transmit the disease to other animals through

their bites.[1] The disease may be to some extent controlled by prophylactic measures designed to destroy the ticks, and to prevent infection thereby.

A partial immunity is enjoyed after an attack of the disease, but by repeated attacks the immunity may be rendered absolute. By inoculation with the blood of an affected animal in which the fever has subsided, a transient illness in the inoculated animal is produced together with partial immunity, and by a second or third inoculation the immunity may be much increased. The mortality from such a procedure amounts to 3–5 per cent.[2]

P. parvum causes Rhodesian red-water of cattle. It is not directly inoculable, and is conveyed by the tick *R. appendiculatus.*

P. equi causes biliary fever in horses.

P. canis causes epidemic jaundice in dogs (Plate XXVI., *a*). It is conveyed by the ticks *Hæmaphysalis leachi* in South Africa, *R. sanguineus* in India, and *Dermaceutor reticulatus* in Europe.[3] (On Ticks, see Nuttall, *Journ. Roy. Inst. of Public Health*, vol. xvi, 1908, p. 385.)

Hæmogregarina.

The Hæmogregarines (which must be distinguished from the Gregarines) are unpigmented parasites, not amœboid, typically having an elongated body or vermicule, occurring in the blood, mostly in cold-blooded vertebrates (Plate XXVI., *b*), but several species have of late been found in mammals (dog, jerboa, palm squirrel), though not in man. In the dog, the parasite (*Leucocytozoon canis*) occurs as an elongated, curved or doubled-up body in the polymorphonuclear leucocytes. It is encapsuled and contains a single granular nucleus.

[1] See Smith and Kilborne, *Texas or Southern Cattle Fever*, United States Dep. Agricult. *Bull.* No. 1, 1893.

[2] See Tidswell, *Report on Protective Inoculation against Tick Fever*, New South Wales, Dep. Pub. Health, vol. i, 1898; vol. ii, 1900.

[3] See Nuttall and Graham-Smith, *Journ. of Hygiene*, vol. iv to viii, 1904–8.

Encystment with sporulation occurs in the bone-marrow, and a sexual development is stated to occur in a tick.

Hæmogregarina (*Drepanidium*, *Lankesterella*) *ranarum* inhabits frogs (*Rana esculenta*), and possesses both an intra- and an extra-corpuscular phase. In the former the parasite occurs as an elongated gregarine-like body within the red corpuscles, which increases in size until its length is 10–15 μ; it then divides into numerous small or a few large gymnospores. In the first case the spores may number fifty, are 3–5 μ in length, occur in May or June, and are exclusively within the erythrocytes; in the latter case the spores measure 5–8 μ in length, are five to fifteen in number, and develop within cells in the blood-forming organs. The extra-corpuscular phase, commencing within the corpuscles, ends in an elongated organism possessing a vermicular movement, and free in the plasma. Similar parasites are frequent in the lower vertebrates, *e.g.* snakes (Plate XXVI., *b*).

Order.—Myxosporidia.

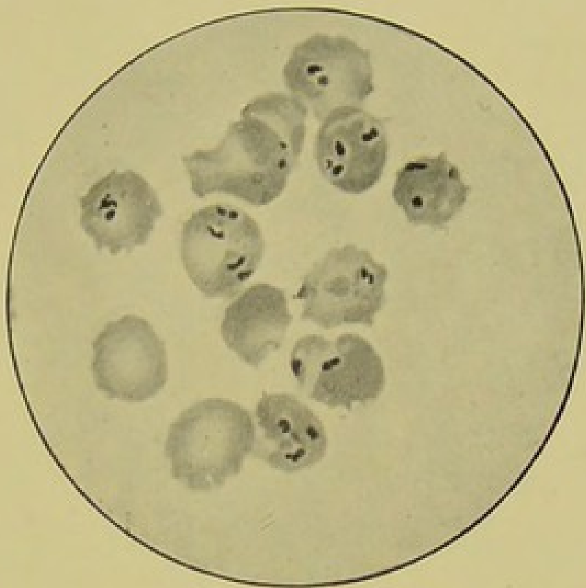
In this group the trophozoite is amœboid, and the species are almost exclusively parasites of fish, in the young stage being intra-cellular (" fish psorosperms ").

Order.—Microsporidia.

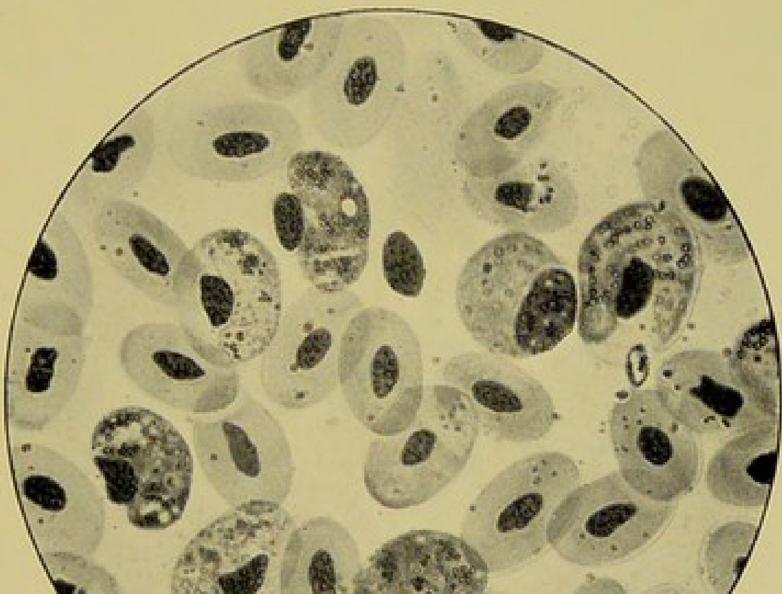
The Microsporidia are cell parasites of invertebrates, especially anthropods, and the trophozoite is more or less amœboid.

Nosema bombycis causes pébrine, a disease of silkworms, which is of considerable importance commercially, for the silk industry in France was once threatened with extinction owing to its ravages. The worms do not grow normally, cease to eat, and die, or form abnormal pupæ. Within the body of the affected worms a large number of roundish, highly refractile corpuscles are found. Pasteur ascertained that the disease was propagated by healthy worms eating with their food the excreta of infected ones. The moths were thus

PLATE XXVI.



a. Piroplasma canis. Smear of blood. × 1500.



b. Hæmogregarine of cobra. Smear of blood. × 1000.

To face page 560.

infected, and laid infected eggs. By allowing each moth to lay its eggs separately, and subsequently examining the body of the moth microscopically, he was able to separate the healthy from the diseased, and the eggs of the former were kept, while those of the latter were destroyed. According to Pfeiffer,[1] when the worms eat the excreta containing the corpuscles mentioned above, these lose their capsule and form large amœboid masses which penetrate the muscles and blood-corpuscles. The amœboid masses then become encapsuled and are yellow and granular. Later on the bright roundish corpuscles form within them.

Another disease of silkworms is known as flacherie, but is due to a bacterium, *Micrococcus bombycis*. It is contagious, and can be transmitted by inoculation.

Order.—Sarcosporidia.

The parasites belonging to this order are not thoroughly worked out. They complete their life-history in the substance of striated muscular fibres : such are the well-known Miescher's corpuscles. Few instances of this class of parasite are recorded in man, but it occurs in the monkey[2] and also in the ox. T. Smith[3] describes the characters and development of a species found in mice.

[1] *Zeitschr. f. Hyg.*, vol. iii, 1888, p. 3.
[2] De Korté, *Journ. of Hygiene*, vol. v, 1905, p. 451.
[3] *Journ. Exper. Med.*, vol. vi, No. 1, 1901, p. 1.

CHAPTER XIX.

Scarlet Fever—Hydrophobia—Infantile Paralysis—Typhus Fever—Yellow Fever—Dengue—Phlebotomus Fever—Vaccinia and Variola—Malignant Disease.

Scarlet Fever.

VARIOUS organisms have been described in scarlet fever—a bacillus by Eddington, a streptococcus by Fränkel and Freudenberg, protozoa by Mallory and others. The disease may be milk-borne, and in the historic Hendon outbreak a streptococcus was claimed by Klein to be the specific infective agent, but the researches of Crookshank and others seem to disprove this.

In 1885 an epidemic of scarlet fever occurred in Marylebone, and was traced to infection conveyed by milk supplied from a farm at Hendon. The infection could not be traced to any human source, and it was therefore concluded that the cows themselves were affected with scarlet fever, and infected the milk. A vesicular eruption was found on the udders and teats of the cows, and this was regarded as the local manifestation of bovine scarlatina. From the vesicles and crusts Klein isolated a streptococcus which, although closely resembling the *Streptococcus pyogenes* (as then known), differed slightly from it; on inoculation into calves it produced death, with lesions of the kidney resembling those of the human disease. Klein also

isolated the same streptococcus in five out of eleven cases of the disease in man. The conclusions which Klein and Power came to were, therefore, that scarlet fever is communicable to, and may exist in cows, the milk thereby becoming infected and conveying the disease to man, and that a streptococcus is the specific infective agent.

The Hendon outbreak was reinvestigated by Axe and Crookshank.[1] Axe found that, so far from there being no source of human infection, cases of scarlet fever had occurred near the dairy within a short time of the outbreak, and the eruptive disease of the cow was shown by Crookshank to be cowpox, while the so-called streptococcus of scarlet fever he regarded as a variety of the *S. pyogenes*. The existence of bovine scarlet fever is entirely discredited by the veterinary profession, both here and on the Continent.

In 1909 a milk-borne epidemic occurred in certain districts in London and Surrey, and was traced to milk derived from one farm. The outbreak was investigated and reported on by Hamer and Jones, who again traced it to infection of the *cows*. Hunting[2] reviews the evidence and shows how little there is to support this conclusion, as there is no doubt that the family of one of the employées on the farm were suffering from scarlatina.

Scarlatina seems to be inoculable on the chimpanzee and some of the lower apes.

Gordon[3] reinvestigated the bacteriology of scarlatina with special reference to the *Streptococcus scarlatinæ* or *conglomeratus* of Klein. He found that this organism differs

[1] On the Hendon outbreak, see *Trans. Path. Soc. Lond.*, 1888 (Refs.).
[2] *Journ. Roy. Sanitary Inst.*, vol. xxxii, 1911, p. 62.
[3] (*a*) *Rep. Med. Off. Loc. Gov. Board* for 1898–99, p. 480; (*b*) *ibid.* for 1899–1900, p. 385.

distinctly in its cultural characters from other varieties of streptococci, and that it occurs constantly in the mucous secretion on the surface of the tonsils and fauces and in the nasal, but not in the aural, discharge in scarlatina. It is also present in a somewhat modified form in the blood and tissues post mortem. It was not found in four non-scarlatinal throats examined. Gordon concluded, therefore, that the *S. scarlatinæ* or *conglomeratus* is the "specialised and essential agent" of scarlatina. It is pathogenic to mice.

Cumpston[1] investigated the biological characters of 101 streptococci isolated from scarlet fever, applying Gordon's tests (p. 248). The majority corresponded with the *S. longus* type.

Baginsky and Sommerfeld, Class and Jaques also isolated streptococcoid organisms in scarlatina, but they possessed no very distinctive cultural characters.

It seems very doubtful if streptococci are the ætiological agents in scarlet fever; they are probably secondary infective agents. It is remarkable how frequently diphtheria complicates scarlatina.

Mallory detected small bodies, 2–7 μ in diameter, staining delicately but sharply with methylene-blue, and occurring in and between the epithelial cells of the epidermis and in the lymph-vessels and spaces of the corium. He regards these as protozoa, but others consider them to be degenerated leucocytes.

The blood in the early stages of scarlatina gives the Wassermann reaction (p. 531).

Hydrophobia.[2]

Hydrophobia attacking man is invariably contracted through the bite of an animal affected with the disease. In the lower animals the disease is termed rabies, and is most frequent in the dog, but the cat, wolf, and deer

1 *Journ. of Hyg.*, vol. vii, 1907, p. 599.

2 See Stanley, *Journ of Hyg.*, vol. i, No. 2, 1901, p. 260; Marie, *La Rage*, 1901; *Scientific Memoirs Gov. of India*, Nos. 30 and 44.

are also subject to it, and other animals can be infected by inoculation. The disease may assume two forms—the raging or the paralytic. The latter is not met with in man, unless certain rare forms of acute ascending paralysis (*e. g.* Landry's) be manifestations of it. In the dog either may occur, but in rodents the paralytic form is almost always the one assumed. In man the incubation period is very variable ; it is never less than about twenty days, and possibly may be as long as two years, or even more ; the average seems to be about ten weeks. In the rabbit, after inoculation from the dog, the incubation period is about two to three weeks.

The virus resides in the central nervous system, as was shown by Pasteur. Inoculation with emulsions prepared from the medulla and with the saliva conveys the disease, but the *filtered* emulsions are usually inactive, and the other tissues and fluids of the body, excepting the lacrimals and suprarenals, are non-infective.

Remlinger[1] has found that after very complete trituration the virus may pass through a porcelain filter.

No micro-organism has been demonstrated with certainty in rabies. Negri has described the constant presence of structures—the Negri bodies—particularly in the grey matter of the hippocampus major, which he regards as protozoa. They are of varying size, apparently encapsuled, taking a homogeneous purplish colour in smears stained with eosin and methylene-blue, the smallest spherical and structureless, larger ones with a central granule or nucleus, the largest, round, ovoid or elongated, containing several (as many as eight) granules (Fig. 65). They occur abundantly in

[1] *Bull. de l'Inst. Pasteur*, iv, 1904, p. 342.

animals suffering from chronic rabies, but in the acute type are scanty, though still to be found; in "fixed virus" (p. 567) they are very small. So constantly are the Negri bodies present in rabies, and absent in non-rabic conditions, that their presence or absence forms a rapid and simple means of diagnosis.[1]

Babes states that the virus is destroyed at a temperature of 60° C., but the medulla and other infective

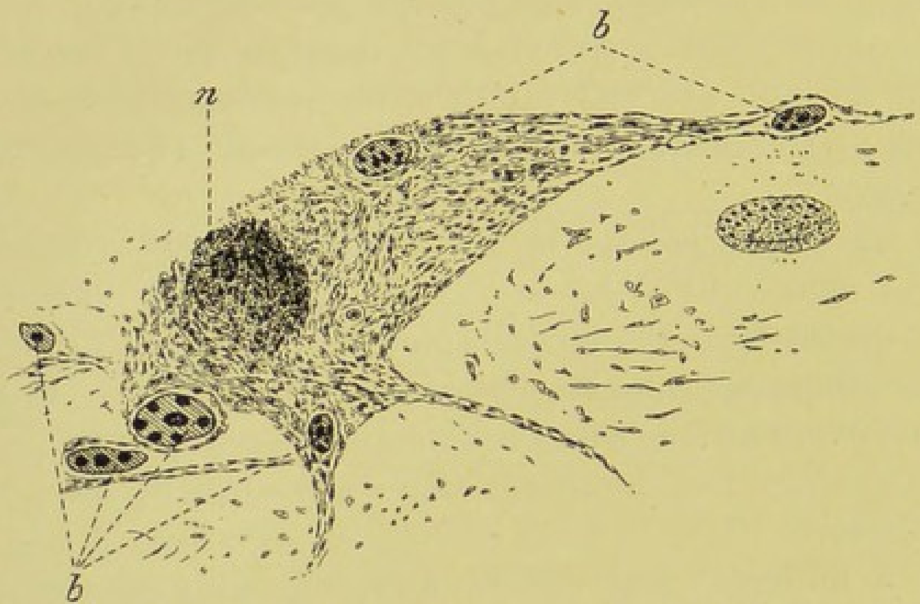


Fig. 65.—Smear from hippocampus major of rabid dog: *n*, nucleus of nerve-cell; *b*, *b*, the Negri bodies (eosin and methylene-blue). (After Williams and Lowden.)

material retain their virulence for months in glycerin. He has described certain lesions present in the medulla in cases of rabies, the so-called rabic tubercles. These consist of an invasion of the peri-ganglionic spaces by an accumulation of round-cells, with degeneration of the cells of the bulbar nuclei.

Van Gehuchten has described as pathognomonic of rabies certain lesions in the sympathetic and cerebro-spinal ganglia, especially those of the pneumo-gastric.

[1] See Williams and Lowden, *Journ. Infect. Diseases*, vol. iii, 1906, p. 452.

These ganglia consist normally of a supporting tissue holding in its meshes large ganglionic cells with distinct well-staining nuclei, each being enclosed in a capsule lined with endothelium. The changes in rabies consist in atrophy of the ganglionic cells, which become shrunken and no longer fill the enclosing capsule, and their nuclei at the same time become ill-defined and stain badly. A number of new-formed cells also appear within the ganglionic capsules. Ravenel and McCarthy studied twenty-eight cases of rabies in various animals, and consider that these capsular and cellular changes in the ganglia, taken in conjunction with the clinical manifestations, afford a rapid and trustworthy means of diagnosis of rabies, but that the absence of these changes does not necessarily imply that rabies is not present. They also consider that the rabic tubercle of Babes is present sufficiently often to furnish valuable assistance in cases where the central nervous system only is obtainable.[1]

Pasteur showed that the virus can be attenuated by desiccating the infective nerve matter, and in this way was able to prepare a vaccine which protects animals from otherwise fatal doses of the virus. Advancing a step further, he used his vaccines to treat individuals who had been bitten by rabid animals, but in whom the symptoms had not yet developed, and so inaugurated the present system of anti-rabic inoculation as carried out at the Pasteur and other institutes.

To prepare the anti-rabic vaccines, a rabbit is inoculated subdurally with an emulsion made from the medulla of a rabid dog. When the animal dies, a second rabbit is similarly inoculated from the first, and the passage through rabbits is continued until a "fixed"

[1] See *Journ. Compar. Pathol. and Therapeut.*, vol. xiv, pt. i, 1901 p. 37.

virus is obtained, with which the first symptoms appear on the seventh or eighth day, and which kills with certainty in about ten days. This having been attained, two or three rabbits are inoculated subdurally every day, so that there is a daily supply of animals dead of the disease. The spinal cord is removed with aseptic precautions, cut into convenient segments, and suspended in bell jars containing a layer of caustic potash at the bottom, which serves to desiccate them. The jars are dated, and preserved in glass cases in a dark room, kept at a constant temperature of about 23° C. In Paris the vaccine fluids are prepared by triturating portions of the dried cords in sterile broth, so as to form an emulsion—1 cm. of cord in 5 c.c. of sterile broth, of which 1 c.c. (*i. e.* 2 mm. of cord) forms a single dose. At the commencement of treatment the cords which have been dried for fourteen days are used, at the end of treatment those which have been dried for only three days; the latter are much more virulent, and would communicate the disease but for the previous treatment. The rabbits employed should all be of the same weight (2½ kilogrammes in Paris); if the rabbits are small, a slightly shorter period of desiccation of the cords would be necessary. The treatment varies in duration according to the severity of the case, which is gauged by the number and situation of the bites and by the species of animal. Bites on exposed parts are regarded as much more serious than those through clothing, and on the face, where efficient treatment is difficult, than on the hands, and wolf-bites than dog-bites.

The doses are injected subcutaneously in the flank, and do not produce much constitutional disturbance. At first there is a feeling of lassitude, and considerable muscular tenderness at the seat of inoculation, which

later on passes off. At Lille, where there are only a few cases under treatment at a time, the cords, after drying for the requisite period, are placed in pure sterile glycerin. In this they retain their virulence unimpaired for about a month. This method does away with the necessity for the daily inoculation of rabbits, a rabbit being inoculated occasionally as required. The system of dosage employed at the various anti-rabic stations differs somewhat; the following is that employed at Lille, 2 mm. of cord being emulsified in 5 c.c. of sterile broth, or physiological salt solution :

ORDINARY TREATMENT.

Day of treatment.	Days of desiccation of cord.
1 (two injections)	14 and 13
2 "	12 and 11
3 "	10 and 9
4 "	8 and 7
5	6
6	5
7	4
8	3
9 (two injections)	9 and 8
10 "	7 and 6
11	5
12	4

ORDINARY TREATMENT.

Day of treatment.	Days of desiccation of cord.
13	3
14 (two injections)	9 and 8
15 "	7 and 6
16	5
17	4
18	3

FOR SEVERE BITES, in Addition.

Day of treatment.	Days of desiccation of cord.
19 (two injections)	7 and 6
20 "	5 and 4
21	3

At Buda-Pesth a dilution method has been employed; instead of drying the cords, an emulsion is made with the fresh cord, and this emulsion is considerably diluted for the earlier doses, dilutions of 1 in 10,000 to 1 in 6000, corresponding to cords dried for from fourteen to eight days. Other systems of inoculation have also been proposed.

Undoubtedly the Pasteur inoculations will protect animals from rabies, the duration of immunity after vaccination in the dog being at least three years. In man the efficacy of the treatment can only be judged by statistics. The mortality after bites by supposed

rabid animals is variously stated, the most favourable being about 16 per cent. (Leblanc). At the Pasteur Institute, Paris, among 2730 cases treated in which the animal which inflicted the bites was proved to be rabid by inoculation experiments, nineteen deaths occurred—a mortality of 0·7 per cent. In 1905, 727 cases were treated, with three deaths; in 1906, 772 cases, with one death; in 1907, 786 cases, with three deaths, being mortalities of 0·41, 0·13, and 0·38 per cent. respectively.

The failure of the treatment may be due to two causes: (1) delay in its commencement, and (2) a short incubation period. The principle of the treatment probably depends upon the long incubation period of the disease, owing to which it is possible to forestall the disease, and to immunise the body by the inoculations before its onset. If, unfortunately, the infective material should be very virulent, and the incubation period thereby reduced to the lower limit, it may be impossible to do this before the onset of the disease, and the same is the case if the commencement of the treatment be delayed. Pasteur's system of inoculation is useless when the disease has declared itself.

By vaccinating animals by the Pasteur method by a long series of injections, and with the most virulent material, the blood-serum acquires "anti-" properties, and this "anti-rabic" serum is said to be of service in the treatment of the declared disease.

Variations from typical rabies have been described both in animals and in man under such names as "chronic rabies," "abortive rabies," etc. Harvey, Carter, and Acton[1] describe a spontaneous disease in dogs due to a general infection with *B. pyocyaneus*, which closely simulates rabies. By subdural inoculation the disease is reproduced in rabbits, with paresis

[1] *Veterinary Record*, July 22nd, 1911, p. 57.

of the hind legs and death in from sixteen to twenty-one days. The Negri bodies are absent, the course of the disease differs somewhat from rabies, and the *B. pyocyaneus* can be isolated from the brain and blood.

Diagnosis of Rabies.

In a case of suspected rabies in a dog the animal should *not* be killed immediately, but should be kept under observation until it dies, or for three or four weeks, and then killed.

1. Moderately thin smears on slides are made from (*a*) the cortex in the region of the fissure of Rolando (the crucial sulcus in the dog), (*b*) the hippocampus major, (*c*) the cerebellum. They are dried in the air, fixed for five minutes in methyl alcohol, and then stained in weak Giemsa (1 drop stain, 1 c.c. distilled water; with 1 drop of 1 per cent. potassium carbonate solution to every 10 c.c. of the dilute stain) for three hours. The stained films are then washed in running tap-water for one to three minutes, dried with filter-paper, and examined for the Negri bodies.

Or the moist films may be fixed in methyl alcohol, and without drying stained for one minute in a mixture of 10 c.c. distilled water, 3 drops of a saturated alcoholic solution of basic fuchsin, and 2 c.c. of Löffler's methylene-blue. Eosin-methylene-blue mixtures may also be used.

The cytoplasm of the bodies stains orange, pink, red, or magenta, the central nuclei are granular, and appear bluish or purplish.

2. If the Negri bodies cannot be detected inoculation should be performed. The brain should be removed as soon as possible, and if it cannot be manipulated immediately, should be placed in sterile glycerin. From the middle of the floor of the fourth ventricle a small piece about the size of a pea is removed; this is triturated and thoroughly emulsified in a sterile watch-glass by means of a sterile glass rod with a bulbous end, a little sterile broth being used to make the emulsion, and sufficient being added to measure about 10 c.c. The hair on the head of a good-sized rabbit is cut close, the

animal is anæsthetised with ether, the skin on the scalp reflected and a trephine hole made through the skull. The centre of the trephine hole should be in the middle line, and on the line drawn between the posterior corners of the eyes, the diameter of the trephine being about $\frac{3}{16}$ inch. A little of the emulsion is drawn up in a small syringe, having a fine needle, and two or three drops are injected beneath the dura mater. The operation is carried out with antiseptic precautions, the wound closed, and a little wool and collodion dressing applied.

If the material injected be from a rabid animal, the first symptoms will be noticed in from ten to fourteen days. The inoculated animal loses control over its hind legs and throws them about peculiarly when running. This increases, and in another day or so the animal is apt to fall when running, and in another day or two the hinder extremities become paralytic, and the animal is unable to move, and dies shortly. The onset of symptoms is hardly ever delayed beyond twenty-one days.

Van Gehuchten's method.—The ganglion is placed in absolute alcohol for twelve hours, the alcohol being changed once; it is then embedded, and sections are cut. These are stained for five minutes in Nissl's methylene-blue and mounted. Or the material may be fixed in 10 per cent. formalin before staining. The capsular changes are best shown by staining with hæmatoxylin and eosin.

Babes' method.—A piece of the medulla or cord is hardened in alcohol and stained with anilin red, and sections are prepared.

Infantile Paralysis.[1]

Infantile paralysis or acute anterior poliomyelitis occurs sporadically and also in epidemics.

Various organisms have been described in this disease, but recent researches, particularly by Levaditi, Land-

[1] See Levaditi, *Journ. Roy. Inst. of Public Health*, vol. xix, 1911, pp. 1 and 65 (Bibliog.); Flexner and others, *Journ. Amer. Med. Assoc.*, 1910–1911.

steiner, and Flexner, have proved that the virus is a filter-passer.

Injection of emulsions of the affected cord into the brain, spinal cord, peritoneal cavity, and blood-stream of monkeys reproduces the disease with the same clinical and pathological features as in man. The disease can be carried on from monkey to monkey by inoculation, but does not seem to be transmissible to other animals. The salivary and some of the lymphatic glands contain the virus.

Flexner has observed a case of spontaneous infection in the monkey, and found that the naso-pharyngeal mucosa is infective, so that this is probably the channel of infection in man. Human cerebro-spinal fluid was not found infective in some instances, but monkey cerebro-spinal fluid is infective (infectivity in this case may depend on the stage of the disease).

Human ascitic fluid inoculated with the filtered fluid from emulsions of cord became turbid, but no organism could be detected microscopically. Monkeys which have recovered from an attack are refractory to inoculation. A certain degree of active immunity may be established by subcutaneous injection of the virus. The serum of immunised and recovered animals possesses considerable neutralising power for the virus. Attempts are now being made to prepare a curative serum.

Some cases of the acute ascending paralysis of Landry may be forms of this disease (see also p. 565).

Buzzard, from a case of the latter disease, isolated a coccus which induced a rapidly spreading palsy on subdural inoculation into rabbits.

Typhus Fever.[1]

Many organisms have been described in this disease. Nicolle, in Tunis, has found that typhus fever of man is communicable to the chimpanzee by inoculation and from the anthropoid to the Chinese bonnet monkey. Nicolle and Conseil have found it possible directly to infect the *Macacus sinicus* and *rhesus* monkeys from human cases.

Nicolle ascertained that the blood is virulent from the commencement of infection and continues so until the day after the temperature becomes normal. The dog and rat are quite refractory. The disease appears to be transmitted by the body-louse (*P. vestimenti*), not by the flea, as suggested by Matthew Hay.

The blood from a mild case does not produce immunity on injection, nor does a mild attack itself induce any appreciable immunity. On the other hand a severe infection induces considerable immunity. Nicolle and Jæggy have not detected any microbe in affected persons or animals. As the polymorphonuclear leucocytes suffer considerably during the attack, undergoing fragmentation of the nucleus and necrosis, it is suggested that the micro-organism may be intra-leucocytic.

Other researches have been carried out in America on the typhus of Mexico, known locally as "Tabardillo." Anderson and Goldberger first showed that the *Macacus rhesus* monkey could be directly infected with Mexican typhus. Ricketts and Wilder have confirmed this, and find that typhus blood is not infective if passed through a Berkefeld filter, indicating that the micro-organism is of appreciable size. They also find that the disease is conveyed by the body-louse, and, moreover, that the

[1] See Hewlett, *Practitioner*, July, 1911, p. 112 (Refs.).

infection is hereditary in the louse, the second generation of lice derived from infected lice apparently being still infective. Neither bugs nor fleas conveyed the disease.

In the blood of typhus patients Ricketts and Wilder detected a small bacillus, measuring 2μ in length by $0{\cdot}6\mu$ in breadth, tending to stain at the poles and belonging to the group of the hæmorrhagic septicæmic bacteria. It is not numerous, and is found from the seventh to the twelfth day of the disease. It is also found in infected lice, but could not be cultivated. A similar micro-organism was also observed in Mexican typhus blood by Gavino and Girard, and by Campbell, and the latter also finds that the blood is not infective if passed through a Chamberland F filter.

Ricketts and Wilder also discuss the relationship between typhus fever and Rocky Mountain spotted fever.[1] Some years ago Wilson and Chowning made observations on a typhus-like fever occurring in limited tracts of country near the Rocky Mountains and ascribed it to a *Piroplasma*. Subsequent research, however, failed to confirm this, though the disease appears to be conveyed by a tick, and not by fleas, lice, etc. There are clinical differences between typhus and Rocky Mountain spotted fever; moreover, the guinea-pig is susceptible to the spotted fever but not to typhus, and a monkey immunised to typhus is susceptible to spotted fever. Ricketts believes that the spotted fever is due to a bacillus which can be found in the ovary of the tick and is agglutinated by the serum in dilutions of 1–500.

Cathoire has made observations on complement fixation in typhus. Using as an antigen an alcoholic

[1] The name is an unfortunate one, for this disease is quite distinct from "spotted fever"—epidemic cerebro-spinal meningitis.

extract of typhus spleen, marked complement fixation was obtained with the serum of typhus cases.

Yellow Fever.

As far back as 1889 Sternberg described a bacillus —"*Bacillus X*"—in yellow fever, a facultative anaërobic organism, very pathogenic to rabbits. In 1897 Sanarelli[1] described his *Bacillus icteroïdes*, which later investigation has proved to be an organism belonging to the Gärtner group (see p. 394).

Reed and Carroll[2] critically examined the *B. icteroïdes* and its relation to yellow fever. Their conclusions were that the *Bacillus X* belongs to the colon group, the *B. icteroïdes* to the Gärtner group, that the *B. icteroïdes* and hog-cholera bacillus produce the same lesions in animals and mutually protect against each other, that the *B. icteroïdes* causes in swine all the symptoms and lesions of hog cholera, and that the blood of hog cholera agglutinates the *B. icteroïdes* in a much more marked degree than does the blood of yellow fever.

Reed, Carroll, and Agramonte,[3] having thus shown the ætiological position of the *B. icteroïdes* to be untenable, directed their attention to the transference of yellow fever through the agency of mosquitoes. Finlay, of Havanah, suggested many years ago that yellow fever might be propagated through the intermediary of a mosquito—*Stegomyia calopus* (*fasciata*)—and with this species these investigators worked. They allowed mosquitoes to bite yellow-fever patients at various stages of the disease, and the infected mosquitoes were subsequently allowed to bite eleven

1 *Ann. de l'Inst. Pasteur*, xi, 1897, pp. 443, 673, and 753.

2 *Journ. Exper. Med.*, vol. v. pt. iii, p. 215.

3 *Philad. Med. Journ.*, October 27, 1900, p. 790.

individuals, two of whom contracted yellow fever. It is true this is not a very convincing experiment, but it is to be noted that during the period of fifty-seven days among a population of 1400 non-immune Americans there were only three cases of yellow fever, and that two of these had been bitten by contaminated mosquitoes within five days of the commencement of their attacks. The matter was put to the further test of experiment in the following manner.[1] Under the same observers a camp was established with several tents each occupied by one to three non-immune individuals, and precautions were taken to prevent the introduction of yellow fever from outside. Five individuals were bitten by infected mosquitoes, and four out of the five contracted yellow fever, no other occupants of the camp being attacked by the disease. Subsequently several non-immume individuals were exposed to yellow fever infection from soiled linen, yellow-fever discharges, etc., in a mosquito-proof hut from which mosquitoes were excluded, with entirely negative results. These experiments prove, therefore, that yellow fever is conveyed by mosquitoes only, and further work by Americans and Cubans, and by French and Brazilian Commissions, have entirely confirmed these researches and conclusions. It has been found that to convey infection, it is necessary for the mosquitoes to bite the patient during the first three or four days of the illness, but they do not become infective until about the twelfth day after feeding, and then retain their infectivity indefinitely. All these facts point to a protozoon as being the causative organism, but none has been found with certainty.

The Americans have shown that the blood-serum after filtration through a porcelain filter is still infective; the organism, therefore, is probably ultra-microscopic,

[1] *Journ. Amer. Med. Assoc.*, February 16th, 1901, p. 431.

at least at one stage. Seidelin[1] describes extremely small rounded bodies with a minute chromatin point and feebly staining protoplasm, without pigment, in the blood corpuscles. Somewhat similar, but larger, bodies may also be present in the organs and free in the plasma.

Dengue.

No organism, bacterium or protozoon, has been demonstrated in this disease. The intra-venous inoculation of filtered dengue blood into healthy individuals is followed by an attack; the organism is therefore probably ultra-microscopic. The disease can be transmitted by a mosquito, *Culex fatigans*, and this is probably the common mode of infection.[2]

Phlebotomus Fever.

A fever of short duration (three days) occurs in South Austria, the malady being somewhat like dengue. It is known locally as "pappataci," and an apparently identical disease has been described by Birt[3] in Malta under the name of "phlebotomus fever." Investigation has shown that this disease is conveyed by the bite of a dipterus fly, the sand-fly (*Phlebotomus pappatasii.*) "Canary fever," "Shanghai fever," "Chitral fever," and the seven days continued and "sand-fly" fevers of India are probably of the same nature. The virus in phlebotomus fever passes through a Berkefeld filter.

Further research must decide whether these and dengue are distinct diseases or whether they are all manifestations of dengue.

1 *Journ. Pathol. and Bacteriol.*, vol. xv, 1911, p. 282.
2 Ashburn and Craig, *Philippine Journ. of Science*, vol. ii, 1907, p. 93.
3 *Journ. Roy. Army Med. Corps*, 1910, August.

Variola and Vaccinia.

The specific contagia of these two diseases have not yet been discovered with certainty.

Variola is inoculable on man, the calf and the monkey, vaccinia on the rabbit in addition.

A large number of observations have been made with vaccine lymph, but no distinctive bacterium has been obtained except by Klein and Copeman. Usually the ordinary pyogenic organisms and many saprophytic forms can alone be isolated. Klein observed the presence of a bacillus in vaccinia, which was subsequently more fully studied by Copeman.[1] It was found in vaccine vesicles at an early stage, but at maturation could no longer be detected. It is a very fine bacillus, and these observers were unable to cultivate it. Subsequently Copeman found a similar organism in variola, and succeeding in cultivating the bacillus from both sources in eggs, and from such egg-cultures was able to inoculate calves. Klein,[2] by storing variola crusts in 50 per cent. glycerin and so getting rid of the saprophytic forms, has cultivated an organism which he terms the *Bacillus albus variolæ*. Morphologically it closely resembles the bacillus observed in vaccine lymph; it forms small white, opaque, coherent colonies on agar, but grows very feebly on gelatin. Involution forms occur, and it seems to belong to the group of diphtheria and xerosis bacilli. On inoculation into calves some approach to, but not typical, vaccinia was produced. Moreover, the inoculated calves were not immune to subsequent vaccination. Copeman,[3] by inoculating collodion cap-

[1] *Milroy Lectures on Vaccination*, 1898.

[2] *Rep. Med. Off. Loc. Gov. Board* for 1896–97, p. 267.

[3] *Brit. Med. Journ.*, 1901, vol. i, p. 450.

sules filled with beef broth with glycerinated vaccine lymph, in which the extraneous organisms had died out, and inserting in the peritoneal cavity of rabbits, observed zoogl[oe]a masses made up of bodies resembling spores which he regards as the resting stage of the specific microbe.

De Korté finds that the vesicles, both in variola and in vaccinia, are sterile before maturation, and regards the bacterial forms that have been isolated as secondary infections.

The failure to isolate a bacterial form has induced many observers to seek for a parasitic protozoon in variola and vaccinia. L. Pfeiffer in 1887 observed roundish or ovoid bodies in the lymph in both diseases, which he regarded as sporozoa. Guarnieri found small bodies, about half the size of the nucleus, in the epithelial cells of the skin in the prepustular stage of variola (*Cytoryctes variolæ*). Small shining amœboid bodies were also noticed in the epithelial cells of the corneæ of guinea-pigs inoculated with vaccine lymph. L. Pfeiffer confirmed Guarnieri's work, and also described these amœbiform parasites in the blood in variola and vaccinia, and of vaccinated calves. J. Clarke, and Ruffer and Plimmer in this country described somewhat similar appearances. Ruffer and Plimmer describe the supposed protozoon as a small round body, about 3 μ in diameter, lying within a clear vacuole in the protoplasm of the epithelial cell.

Councilman, Magarth, Brinkerkoff, Tyzzer, and Calkins[1] in America have found the Guarnieri body in variola and vaccinia in man and animals, and regard it as a protozoon and the causal agent of these diseases.

[1] *Journ. Med. Research*, vol. xi, 1904, p. 173; *Philippine Journ. of Science*, vol. i, 1906, p. 239.

Ogata found bodies which he regards as parasitic protozoa and the causative agent of the disease in variolous and vaccine lymph. Reed likewise observed small granular amœboid bodies having a diameter of about one third that of a red blood-corpuscle, similar apparently to those described by L. Pfeiffer, in the blood of vaccinated children and monkeys, but also observed them—and this is important—occasionally in the blood of normal children and monkeys.

Funck, Roger and Weil, and Calmette[1] have also observed various bodies and refractile granules in lymph. The monkey and rabbit are both susceptible to vaccinia; in the latter animal the pustules are mature on the third day and immunity is acquired by the sixth day.

Ferroni and Massari state that appearances similar to those described by Guarnieri can be obtained in corneæ inflamed by croton oil or Indian ink, and therefore believe that the so-called parasites are derived from the nuclei or from emigrated leucocytes. Salmon considers that the so-called parasites in vaccinia and variola are more or less condensed balls of chromatin of extra-epithelial origin derived from the migratory polynuclear leucocytes. According to von Prowazch these cell inclusions (the Guarnieri bodies, etc.) in this and other conditions (*e. g.* scarlatina) are not parasites, but consist of plastin and nuclease, and are derived from the cells in which they occur.

De Korté[2] has observed in the variolous and vaccine vesicles before maturation large amœboid bodies (10 μ), which he believes to be protozoa (*Sporidium vaccinale*). In vaccine lymph refractile motile granules occur in abundance, believed by De Korté to be spores.

[1] *Ann. de l'Inst. Pasteur*, xv, 1901, No. 3, p. 161.

[2] *Trans. Path. Soc. Lond.*, vol. lvi, 1905, p. 172.

The relationship of vaccinia to variola has been a very vexed question. With few exceptions (Ceely, Hime, Simpson, Klein, King, Copeman) attempts to inoculate variola on the calf have failed. In the successful cases the lymph obtained from the calf has, on inoculation upon children, produced typical vaccinia without any untoward results. The positive results obtained by the inoculation of variolous material being so few, a doubt arises whether in these cases there may not have been some fallacy, such as accidental contamination with vaccinia. Simpson, however, performed his experiments within the precincts of a smallpox hospital and away from possible vaccine infection, and Copeman[1] found that variola may be readily inoculated upon monkeys, and after several passages through these animals is easily inoculable upon the calf. He suggests, therefore, that vaccinia in the calf was originally due to infection with *inoculated* smallpox, so prevalent at the time of Jenner's discovery. A somewhat parallel instance of the attenuation of a virus by passage through another animal is recorded by Sticker and Marx in the case of birdpox, which produces an extensive smallpox-like eruption in fowls and pigeons. In fowls and in pigeons the virus retains its pathogenic properties for each bird unaltered for any number of inoculations, but the pigeon strain, after a few inoculations into fowls, completely loses its virulence for the pigeon. There seems little doubt, therefore, that vaccinia is modified variola, and the rationale of vaccination rests upon a scientific basis.

The preparation of vaccine lymph is fully described by Blaxall.[2] Calves are vaccinated with lymph under aseptic precautions, and five days later the contents of the vesicles are

[1] *Brit. Med. Journ.*, 1901, vol. i, p. 1134, and 1901, vol. ii, p. 1736.
[2] *Rep. Med. Off. Loc. Gov. Board* for 1898-99, p. 35.

scraped off, the pulp is triturated in a machine, and is then placed in six times its weight of sterilised 50 per cent. pure glycerin in distilled water, and stored for about a month in test-tubes, until agar cultivations show that extraneous bacteria have died out, when it is issued for use. It remains very active for fifty to sixty days, after which it begins to deteriorate.

Green[1] rapidly prepares vaccine lymph by killing off the extraneous organisms with chloroform vapour.

Malignant Disease.

The analogies between carcinoma and sarcoma and many infective diseases have led investigators to search for micro-organisms in these conditions.

Bacteria have been repeatedly looked for, but Shattock was unable to isolate any bacterial form from malignant disease. Doyen has isolated a micrococcus (*M. neoformans*, p. 242), but his results are not accepted.

A great impetus was given to the study of parasites in malignant disease by the publication of a paper by Russell. He observed, by certain methods of staining, small corpuscles within the epithelial cells. They were spherical in shape, 4 to 10 μ in diameter, occurring singly or in groups, were apparently homogeneous, and surrounded by a capsule. Russell regarded these structures as belonging to the "sprouting fungi" (Blastomycetes), and they have since been known by the name of "fuchsin bodies" or "Russell's corpuscles."

Subsequently structures were observed within the epithelial cells of carcinoma which were regarded by many investigators as parasitic protozoa.[2] These structures are round or ovoid, 2 μ to 10 μ in diameter, with a very distinct outline, as though encapsuled, and clear refractile contents in which is a smaller body of variable size analogous to a nucleus (Fig. 66, *a*). Occasionally the refractile contents present a radial striation or a granulation.

[1] *Rep. Med. Off. Loc. Gov. Board* for 1900-01, p. 639.

[2] See Ruffer and Walker, *Journ. Path. and Bact.*, vol. i, 1893, p. 395.

These bodies are usually single, but may number as many as eight or ten, and sometimes they invade the epithelial nucleus. The Ruffer's or Plimmer's body, however, is a structure probably analogous to the archoplastic vesicle of the cells of reproductive tissue (Fig. 66, *b*). Save for the presence of these structures, there is no proof that protozoa are present in, or are the cause of, carcinoma.

Another hypothesis of the nature of malignant disease is that it is due to a blastomycetic infection (see p. 488).

Washbourn and others have observed infective venereal tumours in dogs. These have been stated to be sarcomata, but are probably granulomata.

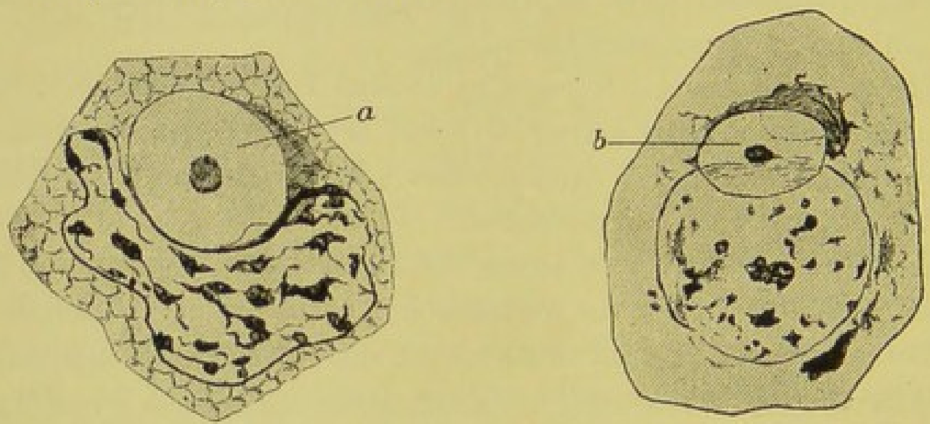


FIG. 66.—*a*, Ruffer's or Plimmer's body in a cancer-cell; *b*, the archoplastic vesicle in spermatid of mouse. (After Farmer, Moore, and Walker.)

Malignant disease occurs in all classes of vertebrates, and is generally inoculable on an animal of the *same* species as that from which it is derived, but not on other animals. The carcinoma of mice has been the subject of much investigation of late. In the writer's opinion, the trend of recent research is to show that malignant disease is not due to a micro-parasite, but is derived from the irresponsible division of cells of the normal or of embryonic tissues.[1]

The molluscum bodies have likewise been regarded as parasitic (coccidial) in nature, but with them also inoculation and cultivation experiments have failed.

[1] For further information consult *Pathology, General and Special*, ed. 2, R. T. Hewlett (Churchill, 1907).

CHAPTER XX.

SOME DISEASES NOT PREVIOUSLY REFERRED TO, WITH A DISCUSSION OF THEIR CAUSATION—MICRO-ORGANISMS OF SKIN AND MUCOUS MEMBRANES.

Appendicitis.—The following table[1] shows the usual kinds and relative frequency of the infections in appendicitis :

Micro-organism.	Acute appendicitis.	Chronic appendicitis.
Bacillus coli in pure culture	70 per cent.	90 per cent.
" with staphylococci	15 "	6 "
" " streptococci	7 "	Very rare.
Staphylococci alone	4 "	1 per cent.
Streptococci "	Very rare.	Very rare.
Other organisms or combinations	4 per cent.	3 per cent.

It is not improbable that in a still greater percentage of cases a mixture of organisms is present at first, the *Bacillus coli* subsequently crowding out the other forms. The *Bacillus proteus*, *B. pyocyaneus*, and *B. Welchii* also occasionally occur.

Castellani[2] describes a bacillus, pathogenic to guinea-pigs, isolated from a case of gangrenous appendicitis. Morphologically it resembled the Shiga-Kruse dysentery bacillus, and was non-motile, produced acid and gas in glucose and maltose and curdled milk, but did not ferment mannite, lactose, and sucrose.

Beri-Beri.—Various observers have attempted to cultivate

[1] Battle and Corner, *Diseases of the Vermiform Appendix*, 1904.

[2] *Brit. Med. Journ.*, 1907, vol. i p. 1513.

a micro-organism in this disease. Pekelharing and Winkler isolated a coccus producing a white growth and resembling the *M. pyogenes*, var. *albus*, very closely. Hunter obtained a similar coccus from two cases. A Gram-positive coccus was also isolated by Okata and Kokubo from the blood and urine.

Rost described a small motile sporing bacillus which he isolated from the blood and cerebro-spinal fluid in cases of beri-beri. The organism is also present in rice, and can be cultivated in rice-water, ascitic fluid, or on blood-serum. Inoculated into fowls it produced paresis and death.

Hamilton Wright suggests that the disease is due to an intoxication, the result of a gastro-duodenal infection with a large Gram-positive bacillus (unisolated). Daniels believes that the epidemiology of the disease is best explained on the hypothesis of a protozoan infection conveyed by lice. The writer and De Korte[1] also suggest a protozoan infection, the organism perhaps being eliminated in the urine.

Other views are that beri-beri may be a peripheral neuritis due to arsenical poisoning, or that it is caused by the absence of certain nutritive elements from polished rice, and the evidence in favour of the latter view seems to be accumulating.

BRONCHITIS.—Ritchie[2] concludes that acute bronchitis is an infective disease, but is not due to any one specific organism, the most important causal bacteria being the *D. pneumoniæ* and streptococci. In every case of acute bronchitis numerous pathogenic bacteria are present in the bronchi, which are usually sterile in health. The commonest organisms are *B. pneumoniæ*, *B. influenzæ*, and *M. catarrhalis*. Spirochaetes are present in some forms of tropical bronchitis; in others Castellani has described oidium-like and yeast-like organisms.

CHANCRE, SOFT. — An extremely small bacillus, first described by Ducrey,[3] has been found in the ulcers and buboes. It has not been inoculated successfully on animals, but can be inoculated from a chancre, experimentally, from man to

[1] *Journ. Trop. Med.*, October 1st, 1907, p. 315.
[2] *Journ. Path. and Bact.*, vol. vii, No. 1, p. 1.
[3] *Comp. Rend. Congrès Internat. de Dermatologie* (Paris, 1889), p. 229.

man. The bacillus does not stain by Gram's method, and can be cultivated on blood agar, on which it forms shining greyish colonies 1 mm. in diameter, or in guinea-pig blood.[1]

CONJUNCTIVITIS.—Conjunctivitis is of several varieties:

(*a*) *Acute contagious conjunctivitis*, due to the Koch-Weeks bacillus. This is a slender, non-motile organism, 1–1·5 μ in length, occurring singly or in pairs, both free and within the pus-cells. It is decolorised by Gram's method, and is difficult to cultivate, growing best on a serum-agar mixture, on which it forms small, punctiform transparent colonies. It is hardly pathogenic to animals, but in man sets up a typical acute conjunctivitis.

(*b*) *Chronic catarrhal conjunctivitis*, due to the Morax-Axenfeld diplo-bacillus. This organism is 2 μ long by 1 μ broad, is not stained by Gram's method, and can be cultivated on blood-serum or serum agar.

(*c*) *Gonorrhœal conjunctivitis.*

(*d*) *Diphtheritic conjunctivitis.*

(*e*) *Conjunctivitis of streptococcic origin.*

(*f*) *Conjunctivitis of pneumococcic origin.*—Usually in children, and accompanied with coryza and scanty muco-purulent discharge.

(*g*) Micrococci (*aureus* and *albus*) and *B. coli* may also occasionally cause conjunctivitis.

DIARRHŒA (SUMMER) OF INFANTS.—Booker,[2] in an elaborate paper, came to the following conclusions: "No single micro-organism is found to be the specific exciter of the summer diarrhœa of infants, but the affection is generally to be attributed to the activity of a number of varieties of bacteria, some of which belong to well-known species, and are of ordinary occurrence and wide distribution, the most important being a streptococcus and the *Proteus vulgaris*."

Lesage obtained a bacillus from the "green diarrhœa" of infants which he believed to be the cause of this complaint. It is a small, motile, non-liquefying bacillus, producing on

[1] Himmel, *Ann. de l'Inst. Pasteur*, xv, 1901, p. 928.

[2] *Johns Hopkins Hosp. Reps.*, vol. vi, 1897, p. 159 (Bibliog.).

gelatin a whitish expanded growth with crenated margins, and giving rise to a green fluorescence in the medium. The *B. pyocyaneus* may be an occasional cause.

In cases with blood and mucus in the stools, the *B. dysenteriæ* (Shiga-Kruse type) has been found to be present in America and in this country. In London, Morgan has isolated in a number of cases a bacillus which in its fermentation reactions is nearly allied to the hog-cholera bacillus (see p. 394).

Ralph Vincent ascribes the disease (which he terms "zymotic enteritis") to the ordinary organisms of putrefaction gaining access to milk and multiplying and causing alterations therein.

The stinking motions of the diarrhœa of children have been ascribed to the action of organisms belonging to the *Proteus* group, particularly *B. proteus* (*P. vulgaris*, see p. 658), which occurs in putrefying matter, sewage, and in the intestine. (This organism may also cause abscesses and cystitis, and a form of meat poisoning has been ascribed to its action.) Filtrates of cultures were found by S. Martin to produce a fall of temperature, collapse, and diarrhœa in rabbits.

Distemper of Dogs.—According to Galli-Valerio,[1] this is caused by a bacillus (*B. caniculæ*) intermediate in character between the coli-typhoid and hæmorrhagic septicæmic groups of organisms.

Evidence has also been brought forward that distemper is due to a filter passer. Probably the term "distemper" may include several different diseases.

Dysentery.—Dysentery must be regarded as a term applied to a series of clinical symptoms associated with colitis which is due to different specific agents. There are at least two forms of the disease, one, the so-called tropical or endemic dysentery, met with especially in the East, and characterised by chronicity, a tendency to relapses, amenability to treatment with ipecacuanha, and the occurrence of the single liver

[1] *Centr. f Bakt.* (Ref.), xli, 1908, p. 563. See also M'Gowan, *Journ. Pathol. and Bacteriol.*, vol. xv, No. 3, 1911, p. 372 (Bibliog.)

abscess as a sequela; the other, epidemic dysentery. met with in all parts of the world, particularly in times of war and famine, not amenable to ipecacuanha, and not followed by liver abscess. There are also probably other forms occurring in small outbreaks or sporadically. Tropical dysentery is due to the *Amœba coli*, which is found abundantly in the stools, especially in the acute stage, and also in the liver abscesses (see p. 512).

In the epidemic dysentery of Japan and other parts of the world a bacillus, or group of bacilli, has been isolated by Shiga, Flexner, Strong, Kruse, and others. This is the *B. dysenteriæ* described at p. 396.

Coli-form bacilli have been isolated from cases of dysentery. Calmette in Tonkin isolated the *B. pyocyaneus*, and this organism seems to have been the cause of a small outbreak in New York State investigated by Lartigau.[1] In Japan, Ogata isolated a fine Gram-staining, liquefying bacillus which does not seem to have been met with by subsequent observers. Spirochaetes have been found in large numbers in a form of dysentery occurring in Bordeaux.

Vedder and Duval,[2] as a result of the study of a number of cases of acute dysentery in the United States, conclude that the disease, whether sporadic, "institutional," or epidemic, is due to the *B. dysenteriæ* of Shiga.

The *B. dysenteriæ* (Shiga type) has been isolated by Eyre, McWeeney, and others from cases of ulcerative colitis or asylums dysentery in the British Isles (see pp. 397–401).

The *Balantidium coli* (p. 536) and certain parasitic worms may also induce a dysenteric condition.

Skin Diseases: *Acne.*—In the acne pustules, the *M. pyogenes* var. *aureus*, with or without var. *albus*, is almost invariably present, and a staphylococcic vaccine generally acts extremely well. In the comedoes a Gram-positive, Hofmann-like bacillus (*B. acnes*) is present in considerable numbers, and may be the cause of the comedo. This organism

[1] *Journ Exper. Med.*, vol. iii, No. 6, p. 595.
[2] *Ibid.*, vol. vi, 1902, No. 2, p. 181.

was cultivated by Fleming on a neutral agar to which glycerin and oleic acid are added. Südmersen and Thompson[1] cultivate on an acid (+ 40) serum-agar. The organism is anaërobic, at least at first, and will grow in glucose-agar stabs. In culture the organism is diphtheroid. A vaccine prepared with it is of service in the comedo stage.

Eczema is produced by the action of the pyogenic cocci (*M. pyogenes*, var. *aureus* and *albus*). Virulent cultures of these organisms, with or freed from their toxins, seem, however, to produce an impetigo rather than eczema. But the filtered cultures, *i.e.* toxins, are harmful to the skin, and when applied to it for one or two days by means of moist warm pads a typical papular or vesicular eczema ensues. Probably in the human subject in addition to the micro-organisms some peculiarity in the soil is necessary for the disease to develop.[2] In so-called seborrhœic eczema, a non-liquefying micrococcus which forms butyric acid has been isolated.

Impetigo.—The large vesiculo-bullous eruption of impetigo contagiosa is caused by the *Streptoccocus pyogenes*; the small pustule in the neighbourhood of hair-follicles, impetigo of Bockhart, is caused by the *M. pyogenes* var. *aureus*. The *B. diphtheriæ* may also cause an impetigo (p. 285).

Pemphigus.—A diplococcus has been isolated in acute pemphigus by Demme, and in the chronic form by Dähnhardt. Bulloch and Russell Wells, in this country, seem to have isolated an identical organism, and the following description of it is taken from their papers. Cocci 0·8 to 1·5 μ in diameter. mostly arranged as diplococci, and staining by Gram's method, On surface agar the organism forms a thick, white, shining growth. In stab agar the growth has a "nail-shaped" appearance. The colonies on agar are at first round, but later, in seven days, they throw out lateral projections and assume a rosette appearance. On gelatin the growth is slow and slight, with some, but not marked, liquefaction. On blood-serum the growth resembles that on agar. On potato a whitish, semi-transparent film forms. Milk is curdled. In broth it

[1] *Journ. of Pathol. and Bacteriol.*, vol. xiv, 1910, p. 224.

[2] Whitfield, *Practitioner*, February, 1904, p. 202.

causes a general turbidity, with a whitish sediment, and sometimes a pellicle, which soon sinks. Guinea-pigs and mice inoculated or vaccinated with the organism died in four to eight days, fine hæmorrhage, occurring in the lungs, and the cocci being obtained from the blood. No bullæ appeared on the skin. Th *B. pyocyaneus* may cause dermatitis and bullous eruptions (see p. 250).

The pyogenic cocci or their toxins may produce various bullous eruptions, *e. g.* pemphigus neonatorum and contagiosus and hydroa gestationis.[1]

Herpes zoster.—Pfeffer observed bodies in the cells of the vesicles which he believed to be protozoa. Gilchrist, however, regards these merely as altered nuclei.

Foot and Mouth Disease.—Various organisms have been described in this disease, but a German commission comprising Löffler and Abel[2] stated that they were unable to prove its ætiological significance. Löffler and Frosch have determined that the organism must be a very minute one, as it passes through the smallest pored porcelain filter.

Malta Fever.[3]—*Synonyms :* Rock, Mediterranean or undulant fever. A disease met with especially on the Mediterranean littoral, but also in South Africa, India, China, the Philippines, and the subtropical countries of America, and clinically often simulating typhoid fever.

A minute micrococcus (*M. melitensis*), first described by Bruce, is the cause of the disease.

Microscopically, the organism from cultures occurs as a coccus, single, in pairs, or in short chains; it is easily stained by the ordinary anilin dyes, but is Gram-negative. In hanging-drop cultures it shows decided movement, which may be only an active Brownian movement, but is perhaps a true motility inasmuch as Gordon has described the presence of flagella (other observers have failed to find them). The organism may be isolated from the spleen of a cadaver.

[1] *Brit. Med. Journ.*, 1902, vol. i, p. 73.

[2] *Centr. f. Bakt.*, xxiii, 1898, March.

[3] See *Reports of the Mediterranean Fever Commission* (Royal Society), pts. i–vii, Harrison & Sons, 1904–1907.

On agar it grows as minute transparent colonies, which first appear when inoculated from the spleen in 90 to 125 hours. In thirty-six hours more the colonies become amber-coloured, and later still, in four to five days, they become opaque, of a slightly orange colour, and round, with granular margins. On gelatin a whitish growth slowly forms without liquefaction, and in broth a diffused cloudiness forms, with a white deposit and without film-formation. Litmus milk becomes alkaline without curdling. Alkali is also produced in glucose media, but galactose, maltose, and saccharose are unchanged (see table, p. 261). The distribution of the *M. melitensis* in the body corresponds closely with that of the *B. typhosus*; thus it is abundant in the spleen, relatively scanty in the blood, and is excreted in the urine.

The *M. melitensis* maintains its vitality outside the body in the dry state in dust or on clothing for two to three months, in tap- or sea-water for a month. The thermal death-point is about 55° C.

Inoculated into animals no result usually ensues; in the monkey, however, a febrile condition is produced, with enlarged spleen, sometimes terminating in death, the course of the temperature resembling that of the disease in man. By intra-cerebral inoculation Durham found that the organism becomes pathogenic for the rabbit and guinea-pig, otherwise it is without effect. For the diagnosis of the disease the agglutination reaction is most valuable. It may be carried out by the microscopic method, a forty-eight-hours' broth culture being employed, the details of the process being the same as described at p. 198. Dilutions of 1 in 30, 1 in 50, and 1 in 100 should be prepared, as well as controls with normal serum, for old laboratory strains sometimes agglutinate with normal serum in dilution of 1 in 20 or 30 (see p. 196. Neglect of this precaution led Bentley to ascribe kala-azar to a Malta fever infection). The organism being minute, it is necessary to use the $\frac{1}{12}$-inch oil-immersion, the $\frac{1}{6}$-inch with a high eyepiece and draw-tube extended, or better, a $\frac{1}{8}$-inch dry objective. The sedimentation method is preferable.

The disease may be conveyed to monkeys by contact, by inhalation of infected dust, and by feeding. Mosquitoes and other insects do not seem to convey it.

The investigations of the Mediterranean Fever Commission have shown that the main source of infection of man is by goat's milk. Goats may be infected (and are largely so in endemic districts, *e.g.* Malta and South Africa) without showing any symptoms, and excrete the organism in large numbers in their milk. Since the goat's milk has been boiled the incidence of the disease in Malta has fallen from 663 cases in 1905 to 7 cases in 1907 in the Army, and in the Navy there were no cases in 1907 (Bruce).

Toxin, vaccine, and serum therapy.—The *M. melitensis* forms no extra-cellular toxin, but Macfadyen obtained an endotoxin by disintegration. Attempts to prepare an anti-serum have not been successful. A vaccine prepared with cultures killed by heat (see p. 232) has been used in the chronic form of the disease by Bassett-Smith[1] and others with some amount of success.

Mastoid Disease.—See "Otitis Media."

Measles.—Doehle and Behla described small flagellated bodies which they believed to be protozoa in this disease. Canon and Pielicke found small bacilli in the blood, which Tchaïkovsky confirmed. They are motile, do not stain by Gram's method, and can be cultivated on agar and serum, on which they form delicate colonies. Czajkowski has found a similar organism. Lesage[2] cultivated a small micrococcus from the nasal mucus and blood, which produced a fatal hæmorrhagic septicæmia in animals. The influenza bacillus is present in many cases.

Meningitis may be caused by *D. pneumoniæ* (60 per cent. of acute cases), *D. intracellularis*, Still's diplococcus, *B. tuberculosis*, gonococcus, and micrococci and streptococci.

Mumps (Epidemic Parotitis).—Mecray and Walsh isolated from the parotid and blood in some cases of mumps

[1] *Journ. of Hygiene*, vol. vii, 1907, p. 115.
[2] *Compt. Rend. Soc. Biol.*, 1900, p. 203.

a coccus resembling that described by Laveran and Catrin. It occurs chiefly as a diplococcus, but also in large groups. The colonies form circular, white, shining points, with slow growth and gradual liquefaction. On potato a white growth occurs; on blood-serum a plentiful cream-coloured growth; and in litmus milk production of acid with coagulation.

NOMA AND CANCRUM ORIS.—Grawitz in 1890 observed bacilli in the affected tissues in this disease, others fusiform bacilli with or without other organisms; Comba considered that there was probably no specific organism; Durante found the *M. pyogenes*, var. *aureus*, with *B. proteus*, and Ravenna the same micrococcus with the typhoid bacillus. Diphtheroid bacilli have also been isolated. Weaver and Tunnicliff[1] in a case of cancrum oris observed the presence of fusiform bacilli and spirilla. Hellesen[2] isolated a diplococcus from a case of noma. The organism is not unlike the pneumococcus, but possesses no capsule, is Gram-positive, gives a general turbidity in broth with acidity, forms no gas from glucose, curdles milk with acid production, and forms punctate, whitish-grey, translucent colonies on surface agar. On inoculation into animals a specific necrosis was produced.

Bishop and Ryan, in two out of three cases, isolated an organism which culturally and morphologically resembled the diphtheria bacillus, but which only produced some local inflammation on inoculation into guinea-pigs. In the third case the *M. pyogenes*, var. *aureus*, and the *Streptococcus pyogenes* were isolated. Guizzetti, and Freymuth and Petruschky have isolated the Klebs-Löffler bacillus in noma.

OPPLER-BOAS BACILLUS.—Met with in the stomach, particularly in cases of carcinoma, and its detection is suggestive of this condition. The bacilli occur in masses, are long and filiform and non-motile, and frequently join one another in angles. They measure usually 6–8 μ in length, but vary between 3 and 10 μ. The organism has been cultivated, and is a facultative anaërobe, non-sporing and

[1] *Journ. Infectious Diseases*, vol. iv, 1907, p. 8 (Bibliog.).

[2] See *Lancet*, 1908, vol. i, p. 955.

Gram-positive. It curdles milk and forms lactic acid from various sugars.

Otitis Media.—The *Diplococcus pneumoniæ* is perhaps the commonest organism met with; next in frequency comes the *Streptococcus pyogenes*, and then the pyogenic cocci. In scarlatinal otitis media, Blaxall found the *S. pyogenes* to be always present, and generally accompanied by other organisms, pyogenic cocci, etc. In thirty-seven cases of mastoid disease Blake found the following organisms, and remarks that as a rule the same were found in the middle ear:

Streptococcus	12
Staphylococcus	5
Diplococcus (? *pneumoniæ*)	6
Streptococcus and diplococcus	5
Streptococcus and *Bacillus fetidus* (? colon bacillus)	3
Streptococcus and *Bacillus pyocyaneus*	1
Streptococcus and diplococcus	1
Streptococcus, micrococcus, and diplococcus	2

In two of the cases no organisms could be isolated.

Ozæna (Atrophic Rhinitis).—Löwenberg described in this disease encapsuled bacilli somewhat resembling the pneumo-bacillus morphologically. Some Italian observers found bacilli apparently identical with the diphtheria bacillus. Abel[1] described a bacillus somewhat resembling the pneumo-bacillus. It is this organism which produces the atrophy of the mucous membrane, but the fetor is due to the decomposition of the secretions produced by other organisms.

Perez[2] isolated an organism in ozæna (*Cocco-bacillus fetidus ozænæ*) which has the following characters: it is a short bacillus with rounded ends, non-motile, does not stain by Gram's method, does not liquefy gelatin, does not ferment lactose nor curdle milk, but forms indole and ferments urea. Its cultures are foul-smelling, and it is pathogenic for guinea-pigs, mice, rabbits, and pigeons.

Peritonitis.—Treves gives the following table of the micro-organisms found in peritonitis:

[1] *Zeitschr. f. Hyg.*, xxi, p. 89.

[2] *Ann. de l'Inst. Pasteur*, xiii, 1899, p. 937, and xv, 1901, p. 409.

	Fränkel.	Tavel and Tanz.	
	Found alone.	Found alone.	Found in association.
Bacillus coli communis .	11	15	16
Streptococcus . . .	7	3	15
Staphylococcus . . .	1	2	6
Pneumococcus . . .	1	0	2
	20	20	39

Dudgeon[1] believes the *B. coli* is frequently a secondary agent and not the primary infection. He finds that the *M. pyogenes*, var. *albus*, is very commonly present from the first, and may exert a protective action by determining the occurrence of phagocytosis.

Psilosis or Sprue.—Carnegie Brown[2] considers this disease to be due to an abnormal fermentation in the intestine brought about by some organism, bacterial or protozoan, which has not yet been isolated.

Puerperal Fever.—This condition may be either a localised infection with intoxication (sapræmia), or a localised infection with general infection (puerperal septicæmia); in both the primary seat of infection may be perinæal or vaginal lacerations, or the contents of the uterus or the placental site. The infecting organisms may be *S. pyogenes*, pure (20 per cent.), or with other organisms (30 per cent.), occasionally the *D. pneumoniæ*, *B. coli*, *M. pyogenes*, var. *albus*, *M. pyogenes*, var. *aureus*, *M. gonorrhœæ*, *B. Welchii*, and diphtheroid bacilli. These are rarely alone, but generally occur with one or other of the organisms named. The *B. diphtheriæ* may exceptionally be met with.[3]

Purpura.—Hæmorrhagic septicæmia may be caused by a

[1] *Bacteriology of Peritonitis* (Constable, 1905).

[2] *Sprue and its Treatment* (Bale, Sons, & Danielsson, 1908).

[3] See Foulerton, *Practitioner*, March, 1905, p. 387.

number of capsulated bacilli allied to the *B. pneumoniæ* of Friedländer[1] (see pp. 271, 427), as well as by streptococci and pyogenic cocci. Paratyphoid infection may be accompanied with purpura.

Pyorrhœa Alveolaris (Rigg's disease).—Goadby[2] has found the following organisms to be probably causative in this disease: *M. citreus granulatus*, *M. pyogenes*, var. *aureus*, streptococci, *M. catarrhalis*, and diphtheroid bacilli, and has used vaccine treatment with success. Eyre and Payne[3] have found similar organisms.

Rheumatism (Acute).—The opinion has gained ground of late years that acute rheumatism is an infective disease. A number of observers have isolated streptococci and micrococci in this disease, and Singer regards the disease as merely an attenuated form of pyæmia. Menzer considers that rheumatic fever is not due to any one organism, but is a particular reaction in predisposed persons to various microbes, especially streptococci. In 1897 Achalme isolated an anaërobic anthrax-like bacillus from several cases. This bacillus agrees in all its characters with the *B. Welchii* (*enteritidis sporogenes*), and is believed by the writer[4] to be identical with the latter; it is probably a terminal infection or a contamination. Poynton and Paine[5] in 1899 obtained from eight successive cases a diplococcus (*D. rheumaticus*) which in broth develops into a streptococcus. Injected intra-venously into rabbits the diplococcus frequently produces enlargement and inflammation of the joints with effusion, and occasionally valvulitis and endocarditis. In man the organism was demonstrated in the vegetations, pericardium, tonsils, and rheumatic nodules, and has been isolated from the blood, pericardial fluid, cardiac vegetations, and tonsils.

[1] See Howard, *Journ. Exp. Med.*, vol. iv, 1899, p. 149 (Bibliog.).

[2] *Proc. Roy. Soc. Med.*, February, 1910 (Odontological Section).

[3] *Ibid.* December, 1909.

[4] *Trans. Path. Soc. Lond.*, vol. lii, pt. ii, 1901, p. 115.

[5] *Lancet*, 1900, vol. ii, p. 861 *et seq.*; *Trans. Path. Soc. Lond.*, vol. lv, 1904, p. 126.

Andrewes and Horder found that two strains of the *D. rheumaticus* corresponded with the *S. fæcalis* (p. 248).

Beattie[1] also obtained a streptococcus from the synovial membrane of cases of acute rheumatism, which regularly produced arthritis, and occasionally endocarditis, in rabbits. Goadby has observed similar effects with a streptococcus obtained from the mouth.

The manner in which typical acute rheumatism generally reacts to salicylates suggests a protozoan organism, if an organism be the cause.

Rheumatoid Arthritis (Arthritis Deformans).—Schuller described a small bacillus in this disease.[2] Blaxall[3] found in the synovial fluid, and occasionally in the blood, a minute bacillus measuring 2 μ in length. It possessed marked polar staining, was decolorised by Gram's method, and could only be stained by prolonged (3–5 days) immersion in anilin methylene blue. The organism can be cultivated on agar, on serum, and in broth. In a clear broth, after three days, minute shining, yellowish particles appear and increase in amount, giving rise on shaking the flask to an appearance of "gold dust." Inoculation experiments on animals failed.

Poynton and Paine[4] isolated a diplococcus (? a form of their *D. rheumaticus*) from an osteo-arthritic joint, which produced arthritis, with osteo-arthritic changes, when injected intra-venously into rabbits.

Osteo-arthritis is probably not a single disease.

Rhinoscleroma.—A bacillus has been described in this disease. It is a short rod, with rounded ends, encapsuled, and frequently linked in pairs. The organism is non-motile, does not stain by Gram's method, and forms on gelatin a whitish growth without liquefaction like that of Friedländer's pneumo-bacillus. Milk is not coagulated. The organism is slightly pathogenic. It is doubtful if it is the causal agent.

Rinderpest.—Simpson, Koch and Eddington described

[1] *Journ. Pathol. and Bacteriol.*, vol. xiv, 1910, p. 432.

[2] *Berlin klin. Woch.*, September 4th, 1893.

[3] *Lancet*, 1896, vol. i, p. 1120 (Bibliog.).

[4] *Brit. Med. Journ.*, 1902, vol. i, p. 79.

bacilli in this disease, but Nicolle and Adil-Bey have found that the virus passes through a porcelain filter, and the organism therefore is probably ultra-microscopic.

Trachoma.—Various organisms have been observed in this disease, *e. g.* a diplococcus by Sattler, gonococcal-like organisms by Lindner and others (it is even suggested that the organism may be an "involuted" gonococcus), and intra-cellular bodies (? protozoa) by Prowazek, etc.

Griffith regards trachoma as being caused by the Koch-Weeks bacillus.[1] The Morax-Axenfield diplobacillus and the pneumococcus have also been isolated from cases. The causative organism cannot yet be said to be known.

Micro-organisms of the Skin and Mucous Membranes.

Skin.—In the normal clean skin micro-organisms are scattered here and there in cracks of the horny layer and in crevices around hairs and glands, but such skin is not swarming with microbes. The *S. pyogenes* and *M. pyogenes*, var. *aureus*, *albus*, and *citreus*, and the *M. epidermidis* (*albus*) of Welch,[2] are the commonest (see p. 240). Equally common on the skin and scalp is the scurf micrococcus isolated by Gordon (see table, p. 241). Sarcinæ, bacilli, and moulds occur also. On the skin of the groin, scrotum, and vulva the smegma bacillus occurs. From sweating feet various organisms have been isolated, which on culture evolve a disagreeable odour, among which is the *Bacterium fetidum* of Thin.

Conjunctivæ.—Some observers have stated that the conjunctiva is generally sterile. A certain number of organisms are, however, usually present, though they are not numerous, and if artificially inoculated the excess is rapidly eliminated. The *B. xerosis* can often be isolated.

[1] *Thompson Yates Lab. Rep.*, vol. iv, pt. i, 1901, p. 139.

[2] On the *rôle* of cocci in the pathology of the skin, see *Brit. Med. Journ.*, 1901, vol. ii, p. 794.

Randolph[1] states that the normal conjunctiva always contains organisms, the commonest species being the *Micrococcus epidermidis* (*albus*) of Welch.

Lawson[2] found the normal conjunctiva to be sterile in 20 per cent. of cases and pyogenic cocci to be rare, and, when present, non-virulent.

Nose.—In the anterior nares, crusts and vibrissæ micro-organisms are present in great abundance, but, contrary to the usual opinion, StClair Thomson and the writer[3] showed that the mucous membrane of the interior of the nose is comparatively sterile, and when organisms are present they are very scanty compared with the number of organisms inspired.[4] Moreover, organisms artificially deposited were found to be rapidly disposed of. After two hours, for example, *prodigiosus* inoculated on to the inferior tubinate could not be detected by cultivation. Wurtz and Lermoyez asserted that the nasal mucus is germicidal, but StClair Thomson and the writer[5] were unable to confirm this, though it may have an inhibitory action.

Air-passages.—Below the larynx under normal conditions the air-passages are free from micro-organisms. Expired air is also free from organisms, and the air from the naso-pharynx after passing through the nasal cavities is deprived of the majority of its organisms.[6]

Mouth.—Micro-organisms of all kinds are present in the buccal cavity in the greatest abundance—leptothrix, bacilli, pyogenic cocci, sarcinæ, and spirilla are almost always to be found. The *Streptococcus pyogenes*, *M. pyogenes*, var. *aureus*, and *Diplococcus pneumoniæ* are frequently present. Certain

[1] *Archives of Ophthalmol.*, vol. xxvi, 1897, p. 379.

[2] *Trans. Jenner Inst. Prev. Med.*, vol. ii, p. 56; also Griffith, *Thompson Yates Lab. Rep.*, vol. iv, pt. i, 1901, p. 99.

[3] *Medico-Chirurg. Trans*, vol. lxxviii, 1895 (Bibliog.).

[4] Other observers, however, have not altogether confirmed this. See Iglauer, *Laryngoscope*, 1901, November, p. 363.

[5] "The Fate of Micro-organisms in Inspired Air," *Lancet*, 1896, January 11th.

[6] *Ibid.*

organisms have their normal habitat in the mouth, are difficult to cultivate, and are of considerable importance in the production of dental caries.[1] Well-defined micrococci and streptococci also occur in the saliva (*M. salivarius*, p. 241, and *S. salivarius*, pp. 248 and 639). The normal saliva is germicidal to some extent. (See also pp. 485, 486.)

Stomach and intestine.—Although a vast number of organisms gain access to the stomach, a large number are destroyed by the acid gastric juice. At the same time a considerable proportion are able to survive—sarcinæ, and lactic and butyric acid bacilli. In normal nurslings the mouth and stomach contain few bacteria—a few cocci, and some bacilli of the *B. coli* and *B. lactis aërogenes* groups. The small intestine contains remarkably few organisms of the same types. In the large intestine bacteria are extremely numerous, particularly Gram-positive ones. These are mostly slender, slightly curved bacilli of moderate size, the *B. bifidus* of Tissier, which often has a bifid extremity, also a somewhat similar organism, *B. acidophilus* of Moro, but capable of developing in an acid medium, a few *B. Welchii*, and a diplococcus. The Gram-negative forms are *B. coli*, *B. lactis aërogenes*, and cocci. In bottle-fed children the same organisms occur, but the preponderating organisms are Gram-negative of the *B. coli* type, with many cocci and streptococci. In childhood and adolescence organisms of the *bifidus* type become less numerous but putrefactive anaërobes become more so, particularly *B. Welchii* and *B. putrificus* (*coli*) of Bienstock; the latter is a long, slender, Gram-positive bacillus with large terminal spores. During adult life the putrefactive anaërobes tend to become still more numerous, and the putrefactive decompositions they produce are regarded by Metchnikoff as standing in causal relation to old age. In the healthy adult the stomach, duodenum and jejunum contain relatively few organisms, from the lower ileum to the rectum the intestinal contents are crowded with bacteria, and the greatest number of anaërobic organisms occur here and putrefactive changes are

[1] See Goadby, *Mycology of the Mouth*.

most in evidence.[1] Kendall[2] has described the presence of a bacillus (*B. infantilis*) in large numbers in a condition of infantilism, associated, according to Herter, with chronic intestinal infection. The organism is a Gram-positive, motile, sporing bacillus belonging to the *subtilis* group. It is aërobic and facultatively anaërobic, grows readily on the ordinary culture media, and ferments dextrose and saccharose with the production of acid only, but lactose is hardly attacked. In a dog and a monkey diarrhœa was produced by feeding with it.

Urinary and genital organs.—The meatus urinarius and distal portion of the urethra contain a few organisms, which increase in number in inflammatory conditions, and Gram-negative cocci may be found (see p. 261). The deeper portion of the urethra, however, is free from organisms, and the bladder is sterile. The genital tract in the female up to the middle zone of the cervix contains organisms, but the uterus and Fallopian tubes are normally sterile. The *B. vaginæ* of Döderlein, a large Gram-positive bacillus capable of growing in an acid medium, is frequently present in considerable numbers in the vagina.

[1] See Herter, *Bacterial Infections of the Digestive Tract*, 1907.

[2] *Journ. Biolog. Chemistry*, vol. v, p. 419.

CHAPTER XXI.

THE BACTERIOLOGY OF WATER, AIR, AND SOIL, AND THEIR BACTERIOLOGICAL EXAMINATION—SEWAGE—BACTERIOLOGY OF MILK AND FOODS.

Some of the Commoner Organisms found in the Air, Water and Soil.

Bacterial Content of Waters and the Factors influencing it. Filtration, etc.

The bacterial flora of natural waters is a very varied one. The organisms met with in surface waters, such as streams, ponds, and shallow wells, are derived from the air and soil through which the water has passed, and if not contaminated from human or animal sources, from the air of towns, from sewage or manure, consist mainly of non-pathogenic bacilli, the majority of which are chromogenic and non-liquefying, and develop best on culture media at a temperature of 18° to 22° C. or thereabouts, not at blood heat; also of some sarcinæ and a few micrococci; *B. coli* and *B. Welchii* are usually absent. When, however, the water passes through cultivated lands, or receives sewage, the number of organisms is enormously increased; a large proportion of them liquefies gelatin and develops at blood heat, and *B. coli* and *B. Welchii* appear more or less numerously. Whereas water from shallow wells has a bacterial content nearly as great as the surrounding

surface water, that from deep wells, especially in the chalk, is remarkably free from organisms. The following table illustrates the number of organisms that may be met with in water from different sources:

Source.	Number of organisms per cubic centimetre.
Freshly fallen snow	34–38
Ice	(very variable) 30–1700
Rain water (Paris)	4–5
Rhone, above Lyons	75
Rhone, below Lyons	800
Rhine, at Mühlheim	average about 20,000
Thames, at Hampton (Frankland)	(variable) 2000–90,000
Deep well in the chalk (Kent Company)	3–19
Surface well	1200
Spring water, Reigate (Frankland)	8
Lake of Lucerne	8–50
Loch Katrine (Frankland)	74
Filtered water supplied to London (Houston)	average rarely exceeds 100
Sewage (Frankland)	26,000,000

The number of bacteria in a natural water varies considerably with its source, at different seasons, and under different climatic conditions. The table[1] on p. 605 illustrates the seasonal variation in certain *raw* London waters.

The following factors modify the number of organisms present in the water:

(1) *Storage of unfiltered water.*—A large storage capacity permits of the water being admitted when the source (river, etc.) is in its best condition, so that foul

[1] Houston, *Fifth Ann. Rep. Metropol. Water Board*, 1911.

Micro-organisms in Raw Waters (Average Number per c.c.).

Gelatine at 20°–22° C.; counted on third day. Agar and neutral red bile-salt agar at 37° C.; counted after 20–24 hours.

1910–11. Month.	River Thames at Hampton.						River Lea at Ponder's End.						New River at Hornsey.					
	Routine samples.	Comparable samples.*			Ratio, Cols. 4 to 3.	Ratio, Cols. 5 to 4.	Routine samples.	Comparable samples.*			Ratio, Cols. 10 to 9	Ratio, Cols. 11 to 10	Routine samples.	Comparable samples.*			Ratio, Cols. 16 to 15	Ratio, Cols. 17 to 16
	Gelatine.	Gelatine.	Agar.	Bile-salt agar.			Gelatine.	Gelatine.	Agar.	Bile-salt agar.			Gelatine.	Gelatine.	Agar.	Bile-salt agar.		
Av. for 1909–10	**5268**	**5310**	**495**	**63**	**1 : 11**	**1 : 8**	**37071**	**35217**	**837**	**86**	**1 : 42**	**1 : 10**	**2801**	**2786**	**129**	**11**	**1 : 21**	**1 : 12**
1910. April . .	3109	2350	139	2	1 : 17	1 : 70	10676	15800	389	7	1 : 41	1 : 55	3300	2700	57	[Less than 1]	1 : 47	1 : 570
May . .	[1522]	2272	[94]	[1]	1 : 24	1 : 94	2317	3800	[139]	[2]	1 : 27	1 : 70	1009	1098	[47]	[Less than 1]	1 : 23	1 : 235
June . .	2721	[1320]	400	20	1 : 3	1 : 20	[1672]	1950	345	15	1 : 5	1 : 23	693	555	61	3	1 : 9	1 : 20
July . .	2589	1625	208	14	1 : 8	1 : 15	4258	3675	752	38	1 : 5	1 : 20	935	1060	89	4	1 : 12	1 : 22
August . .	2702	2100	600	25	1 : 3	1 : 24	2886	[1340]	275	39	1 : 5	1 : 7	[573]	[462]	91	3	1 : 5	1 : 30
September .	3035	6740	111	7	1 : 61	1 : 16	4248	4750	310	16	1 : 15	1 : 19	761	1090	61	1	1 : 18	1 : 61
October . .	3736	1990	165	11	1 : 12	1 : 15	3735	2800	255	13	1 : 11	1 : 19	1398	1180	65	2	1 : 18	1 : 32
November .	17932	14325	522	*59*	1 : 27	1 : 9	42677	*74300*	*1228*	24	1 : 60	1 : 51	*7327*	*6675*	287	*15*	1 : 23	1 : 19
December .	*22939*	*20225*	*760*	42	1 : 26	1 : 18	*52322*	36950	942	28	1 : 39	1 : 33	6805	5400	159	10	1 : 34	1 : 16
1911. January . .	10438	9360	340	36	1 : 27	1 : 9	12619	11000	360	15	1 : 30	1 : 24	2372	1900	70	3	1 : 27	1 : 23
February .	8035	7325	350	9	1 : 21	1 : 39	11490	8375	152	5	1 : 55	1 : 30	2460	2800	142	2	1 : 20	1 : 71
March . .	9300	6825	415	12	1 : 16	1 : 34	24648	21750	670	23	1 : 32	1 : 29	2630	3475	185	14	1 : 19	1 : 13
Av. for 1910–11	**7324**	**6184**	**339**	**20**	1 : 18	1 : 17	**14451**	**14709**	**467**	**19**	1 : 31	1 : 24	**2512**	**2273**	**106**	**4**	1 : 21	1 : 26
Total samples	246	52	52	52			246	52	52	52			247	52	52	52		

* All these samples were tested by the three tests (gelatine, agar, and bile-salt agar). The highest and lowest numbers are indicated by italics and brackets respectively.

water, in flood time or drought, may be avoided. Moreover, storage alone usually markedly diminishes the number of organisms, partly by subsidence, partly by lack of aëration, and partly probably owing to the struggle for existence going on among them (see also p. 381).

(2) *Thickness of fine sand in the filter-beds.*—Efficient sand filtration removes quite 99 per cent. of the organisms originally present. The fine sand only has to be taken into account in estimating the removal of organisms and efficiency of a filter bacteriologically. It probably should form a layer not less than 3 ft. to 3 ft. 6 in. in thickness. Moreover, a filter-bed is not efficient at first, but becomes so when the surface film forms, composed of sedimented particulate matter, and of a zooglœal mass of bacteria and algæ.

(3) *The rate of filtration.*—The removal of organisms is less perfect when the rate of filtration is increased; this should not exceed about 1·5 gallons per square foot per hour.

(4) *The renewal of the filter-beds.*—New, or recently cleaned, filter-beds allow a greater number of organisms to pass through. The beds must be cleaned from time to time by raking up and clearing away the surface layer of sand, for as time goes on the rate of filtration becomes slower and slower, though the bacterial efficiency of the filter-beds does not appear to be reduced by prolonged use. The normal bacterial efficiency seems to be rapidly regained after cleaning—within two or three days.

Besides storage and filtration, sedimentation in the presence of fine particles, either naturally present or artificially added, may also effect a marked removal of micro-organisms from water. Thus, by the addition of alum, an old method of clarifying turbid water, a large

Effect of Storage on the Bacterial Content of Water (Houston, 1908).

DESCRIPTION OF THE SAMPLE.	Average number of microbes per c.c.			*B. coli* test (typical *B. coli*) (percentage results).							
	Gelatin at 20°–22° C. 3 days.	Agar at 37° C. 2 days.	Bile-salt Agar at 37° C. 2 days	Negative 100 c.c. %	+ 100 c.c. %	+ 10 c.c. %	+ 1 c.c. %	+ ·1 c.c %	+ ·01 c.c. %	+ ·001 c.c. %	+ ·0001 c.c. %
Raw Thames *before* storage { Average of 8 months ended March 31st, 1908	**4218** (165 samples)	**308** (59 samples)	**47** (58 samples)	—	**1·8**	**11·5**	**32·7**	**43·0**	**1·30**	**0·6**	—
Staines Stored Water (8 months) 53 samples	230	42	3	24·5	28·3	32·0	15·1	—	—	—	—
Chelsea ,, ,, (8 months) 59 samples	224	52	4	28·8	32·2	22·0	11·8	3·4	1·7	—	—
Lambeth ,, ,, (6 months) 50 samples	391	51	3	6·0	28·0	32·0	22·0	12·0	—	—	—
Raw Lea *before* storage. { Average of 8 months ended March 31st, 1908 . . .	**9442** (165 samples)	**398** (59 samples)	**33** (58 samples)	**0·6**	**0·6**	**5·4**	**41·8**	**38·2**	**9·7**	**3·0**	**0·6**
Lea Stored Water (8 months) 59 samples .	56	11	0·5	62·7	32·2	5·1	—	—	—	—	—

Micro-organisms of Filtered London Waters (Average Number per c.c.).

A = Agar at 37° C. (counted after 20-24 hours). G = Gelatine at 20°-22° C. (counted on third day). The highest and the lowest results are indicated by italics and brackets respectively.

1910-11. Month.	New River (1398 samples).		E. London (Lea) (404 samples).		Kempton Park (454 samples).		Chelsea (610 samples).		Grand Junction (1054 samples).		West Middlesex (1077 samples).		Southwark and Vauxhall (995 samples).		Lambeth (621 samples).		Thames-derived waters (4839 samples).		Grand Totals, All London Waters (7115 samples).	
	A.	G.	A.	G.	A.	G.	A.	G.	A.	G.	A.	G.	A.	G.	A.	G.	A.	G.	A.	G.
Averages, 1909-10	—	10·0	—	21·9	—	19·6	—	17·3	—	11·8	—	14·1	—	14·6	—	11·9	—	14·3	—	13·7
1910. April	3·0	11·1	2·0	18·3	2·7	26·2	1·8	*34·6*	1·0	15·7	1·8	11·6	2·0	18·6	[1·2]	11·5	1·8	17·1	2·0	15·9
May	2·0	8·1	2·0	28·7	3·8	*27·1*	2·6	16·5	1·0	12·9	3·0	16·1	5·1	*31·1*	2·5	20·2	2·9	*19·5*	2·5	17·1
June	1·8	6·8	10·0	26·0	*8·8*	24·0	2·3	[11·4]	1·8	13·2	*7·6*	15·8	*8·0*	20·7	5·8	18·3	5·5	16·7	4·6	14·3
July	2·2	8·1	7·8	14·7	3·3	19·3	1·2	14·2	2·2	[11·3]	7·3	21·2	2·3	13·3	2·4	14·2	3·2	15·3	3·0	13·1
August	1·5	8·6	6·6	23·8	3·3	30·6	1·6	14·7	1·8	14·1	3·8	20·3	5·9	18·3	3·4	20·2	3·4	19·0	2·9	15·9
September	0·9	5·8	*16·6*	*36·2*	1·1	9·1	1·8	18·6	*4·2*	14·7	3·2	14·1	2·1	11·8	1·5	14·4	2·6	13·8	2·6	[11·9]
October	[0·6]	[4·4]	2·5	[9·9]	[1·0]	7·7	2·4	25·5	[0·6]	14·7	[1·3]	11·4	2·2	11·9	1·3	20·8	1·4	14·9	[1·3]	12·1
November	4·6	15·7	1·6	27·6	1·3	[6·0]	1·7	24·5	1·3	18·1	[1·3]	[11·3]	[0·8]	[10·6]	1·3	14·0	[1·2]	13·8	1·9	14·5
December	*8·9*	*26·1*	1·3	31·1	1·1	7·6	*2·9*	27·0	2·2	*18·3*	4·0	*21·4*	2·2	13·7	*6·1*	*24·0*	3·0	18·5	4·0	*20·2*
1911. January	5·1	15·3	[1·1]	20·9	5·9	10·2	2·8	26·4	2·6	16·2	5·4	15·7	3·8	22·1	4·0	11·3	*4·0*	17·2	*4·1*	16·8
February	2·0	9·4	1·3	33·9	3·1	8·5	1·0	21·3	1·6	15·7	2·2	11·7	2·8	19·8	1·6	[8·1]	2·1	14·7	1·9	14·3
March	2·6	9·9	1·6	27·2	2·9	6·6	[0·6]	20·0	0·9	11·6	2·4	14·1	1·8	13·4	1·6	10·8	1·7	[13·0]	1·8	13·0
Averages, 1910-11	2·9	10·5	3·5	24·4	3·2	14·4	1·9	20·9	1·7	14·8	3·4	14·8	3·1	16·8	2·5	15·3	2·7	15·9	2·7	14·9

Exclusive of samples containing 100 or more microbes per c.c. Agar figures for 1909-10 not available.

number of the organisms present are carried down in the precipitate.

The Clark process of softening water may also reduce the number of organisms present, but is very uncertain (Moor and Hewlett). By the Porter-Clark rapid process, however, Nankivill believes considerable purification is effected.

The tables on pp. 607 and 608 illustrate the influence of storage and of sand filtration on the bacterial content of a water.

The Bacteriological Examination of Water.[1]

The bacteriological analysis of water affords valuable indications as to the purity or otherwise of a water, and, if properly carried out, will indicate a pollution so small in amount as to be incapable of detection by chemical methods.

The specimen of water should be collected in clean bottles of about 100–200 c.c. capacity, sterilised preferably by heat. If, however, the bottles be thoroughly cleaned and rinsed out with a little strong sulphuric acid, and then thoroughly rinsed several times with the water to be examined before taking the specimen, no error will be introduced. The stopper of the bottle should be tied down with a thin layer of cotton-wool enclosed between two pieces of muslin, and the bottle should not be quite filled. In taking the specimen the following details should be attended to:

(1) If taken from a tap, the water should be allowed to flow for at least five minutes before the specimen is collected.

[1] See Savage, *Bacteriological Examination of Water Supplies* (Lewis, 1906); Thresh, *Examination of Water and Water Supplies* (Churchill, 1904); Houston, Gordon and others in *Reps. Med. Off. Loc. Gov. Board*, 1899–1904; Houston, *Reports to the Metropolitan Water Board*.

(2) The water from a cistern is not a representative sample of the water supply; to be so the specimen should be taken direct from the mains.

(3) If taken from a stream or pond, the bottle should be held about a foot below the surface and away from the edge before the stopper is removed.

(4) If taken from a well the conditions should be noted, *e.g.* whether the well has been recently disturbed or no, whether the pumps have been in operation, etc., for such may markedly influence the number of bacteria found.

The specimen should then be examined with as little delay as possible, for if allowed to stand for any time a large increase in the number of bacteria may take place. Frankland, for example, found that in distilled water, even at the ordinary temperature, organisms multiply enormously:

Hours	Number of organisms in 1 c.c.
0	1,073
6	6,028
24	7,262
48	48,100

In water of good quality the organisms are found to multiply much more rapidly during the first few days, after which time they become less and less numerous; but in impure water multiplication is slower, and the number more persistent, while in very impure water the number may diminish. It is essential, therefore, if reliable results are to be obtained, for the specimen to be examined at once (within three hours). If this cannot be done the specimen should be packed in ice; the cold will then inhibit multiplication to any extent. Special double-chambered metal boxes are made for this purpose: the bottle containing the sample (not less than 60 c.c.; the writer prefers to have not less than

200 c.c.) is placed in the inner chamber, the outer chamber (which surrounds the inner) being filled with a mixture of ice and sawdust, and the whole is packed in a wooden box with felt lining. According to Remlingler,[1] the addition of 10 per cent. of common salt to the sample preserves the original bacterial content of the water unaltered up to ninety-six hours after taking the sample, without icing. Besides the sample packed in ice, a "Winchester quart" of the water may also be collected for examination for the spores of the *B. Welchii* (*enteritidis sporogenes*).

The routine bacteriological examination of the specimen may be carried out according to the scheme (here somewhat modified) drawn up by a committee of the Royal Institute of Public Health.[2]

Procedures.—The following procedures should be carried out:

(*a*) Enumeration of the organisms which will develop aërobically in gelatin at 20° C.

(*b*) Enumeration of the organisms which will develop aërobically in agar at 37° C. (Enumeration is carried out by counting the number of colonies which develop in the plates [see p. 80].)

(*c*) Search for *Bacillus coli*, and identification and enumeration of this organism if present.

(*d*) Search for, and enumeration of, streptococci.

As a *routine* measure it is not necessary to search for the *Bacillus Welchii* (*enteritidis sporogenes*), but in special instances it may be desirable to do so.

The bottle must be well shaken to mix the sample. Before removing the stopper, it and the neck of the bottle should be swabbed with absolute alcohol, which is then carefully ignited and allowed to burn away.

[1] *Comp Rend. Soc. Biol.*, lxx, p. 64.
[2] *Journ. State Med.*, vol. xii, 1904, p. 471.

Media, Time of Incubation, etc.—For the gelatin count ordinary nutrient gelatin is employed, the period of incubation being seventy-two hours. In hot weather it may be necessary to use 15–20 per cent. gelatin (unless an incubator which can be *cooled* is available), but the development of the colonies is slower. For the agar count ordinary nutrient agar is used, the period of incubation being forty to forty-eight hours.

The media should preferably be recently prepared and be standardised to a reaction of + 10.

In addition to the actual numbers of organisms which develop in the gelatin and in the agar, a comparison of the ratio of the number of organisms developing in gelatin at 20° C. to those developing in agar at 37° C. also gives useful indications. With a pure water this ratio is generally considerably higher than 10 to 1; with a polluted water this ratio is approached, and frequently becomes 10 to 2, 10 to 3, or even less. The actual number of organisms growing at blood-heat is of considerable value apart from any question of ratio.

In certain instances it is true that this ratio may be unreliable. Thus with surface waters, especially in the tropics (as pointed out by Horrocks) varieties of the *B. fluorescens liquefaciens* and *non-liquefaciens* and *B. liquefaciens* may be abundant and grow well at blood-heat.

Distilled water gelatin and agar have also been recommended, but since the organisms of polluted water develop better in the ordinary *nutrient* media, the latter are preferable for routine use.

Amounts to be Plated, Size of Dishes, etc.—*Gelatin*—For an ordinary water amounts of 0·1, 0·2 and 0·3 c.c. may be plated in Petri dishes of about 10 cm. diameter, preferably done in duplicate.

Agar.—Two plates may be made with 0·1 and 0·2–0·3 c.c., and are preferably duplicated.

The desired volume of water should be run into the sterile Petri dish by means of a sterile 1 c.c. pipette graduated in hundredths. The tubes of gelatin should be melted in a water-bath at a low temperature (40° to 45° C.). A tube is taken out of the water-bath, wiped dry to prevent the adherent water running down into the Petri dish, its mouth well singed in the Bunsen flame to sterilise it, and the contents are then quickly poured into the dish and mixed with the water by tilting the dish several times.

The agar tubes must first be boiled, then cooled to about 45° C., and similarly treated, or surface plates may be made.

If waters are constantly being examined, it saves trouble to have the gelatin and agar in small flasks, 30–60 c.c. of the former and 20–40 c c. of the latter; a flask of each will then be used for an examination.

In dealing with an unknown water, and in all cases of doubt, additional plates should be prepared with a dilution of the water (made with sterilised tap-water) of ten or hundred fold, according to circumstances.

The amount of the medium in a plate should be 10 c.c.

The counting is done with the naked eye, preferably in daylight, any doubtful colony being determined with the aid of a lens or low-power objective. The number of liquefying colonies in the gelatin plates should also be noted. The plates should be inspected daily, in order that the count may be made earlier should liquefaction render this necessary.

In examining an ordinary drinking-water there is no need ever to dilute. As 1000 or 1500 colonies can be counted in a plate, and if the number on a plate should be, owing to crowding, uncountable, *ipso facto* this would be sufficient to

condemn without an actual count. Dilution is necessary when dealing with river or other water known to be polluted, and of which an estimate of the number of organisms present is desired. In order to count the colonies if very numerous, ink lines may be drawn across the bottom of the Petri dishes so as to divide them into sectors. Ruled paper discs (Pakes's discs) upon which the dishes are placed can also be obtained. The colonies in the sectors are then much more easily counted: or if the colonies be very numerous and evenly distributed, the number in two or three of the sectors may be counted, and the total number on the plate estimated by calculation.

Search for Bacillus coli, etc.—Various media may be employed for the detection, isolation, and enumeration of *B. coli*. The writer generally employs as a preliminary, glucose bile-salt peptone-water, but many other media may be employed, *e. g.* formate or neutral-red broth, or if the organism is abundant, neutral-red bile-salt agar.

As a routine, 50 c.c. should be the minimal quantity examined for the presence of the *Bacillus coli*, quantities from a minimum of 0·1 c.c. to a maximum of 50 c.c. being added to the tubes of culture media.

It is preferable to add the water directly to the tubes of culture medium, even with the larger amounts, and not to concentrate the bacteria by any method. The culture media may be diluted with at least an equal volume of the water without interfering with their cultural properties, and large tubes or small flasks are used for the larger amounts.

In the case of glucose or lactose bile-salt peptone-water, the medium may for the larger amounts be prepared of double strength. The glucose or lactose bile-salt peptone water should be incubated at 42° C. for not less than forty-eight hours.

For composition of glucose formate broth, glucose and lactose bile-salt media, and neutral-red broth, see p. 623, *et seq*. While a lactose medium has the advantage of excluding a number of forms which, though fermenting glucose, do not ferment lactose, and are therefore not typical *B. coli*, Houston has found that a glucose medium is more delicate than a lactose one. For general purposes, quantities of from 0·1 to 25·0 c.c. may be added to tubes of the medium selected. For the examination of an ordinary drinking-water, the writer usually employs five tubes with 1 c.c. of the water in each, two tubes (double strength) with 10 c.c. in each, and one tube (double strength) with 25 c.c. For the larger amounts large test-tubes and boiling tubes must be employed.

If the medium shows changes (acid + gas) suggestive of the presence of *B. coli*, it is only *presumptive* evidence of the presence of this organism. Occasionally other organisms produce a similar change, e. g. *B. lactis aërogenes*, *B. cloacæ*. Hence the necessity for the isolation and identification of the organism as recommended in the next section.

Isolation of Bacillus coli, if present.—If indications of the presence of the *Bacillus coli* be obtained in the preliminary cultivations (acid + gas), the organism must be isolated and identified. If several tubes show acid + gas, one or two of the tubes with the smallest quantities of the water should be used for this purpose.

This may be done by making *surface* cultures on plates (sloping tubes generally suffice) of either (*a*) litmus lactose agar, reaction + 10 ; (*b*) litmus lactose bile-salt agar ; (*c*) Conradi and Drigalski agar, which the writer generally employs ; or (*d*) ordinary nutrient gelatin. Agar media, incubated at 37° C., have the advantage of saving time. (For composition of media, see p. 624, *et seq*.)

Identification of, and Tests for, the Bacillus

COLI.—Having obtained coli-like colonies on the plates made from the preliminary cultivations of the water, various tests must be used for identification. The organism should conform in morphology, motility and staining reactions with the characters of the typical *B. coli* as given at pp. 401–405, and must be subjected to various cultural tests, *e. g.* the "flaginac" reactions of Houston (p. 405). The writer generally employs these, with the addition of the fermentation reactions given by dulcitol, mannitol, and adonit litmus peptone water, and gelatin for absence of liquefaction. If atypical *Bacilli coli* (see pp. 401 and 409) are met with, the fact should be noted, but their significance is not yet fully determined.

STREPTOCOCCI.—It is a distinct advantage to search for streptococci. They may be looked for by making hanging-drop preparations of the fluid media employed for the preliminary cultivation of the *B. coli* (glucose or lactose bile-salt peptone water, etc.) The presence or absence of streptococci in these tubes gives also a quantitative value to the examination, just as in the case of *B. coli*, and the result obtained should be stated. The streptococci can be readily isolated on Conradi-agar plates.

According to Houston (*loc. cit.*), fæces contain *at least* 100,000 streptococci per gram. The type of streptococcus generally present is one forming short chains, producing a uniform turbidity in broth, acid and clot in litmus milk within five days at 37° C., and non-pathogenic for mice. (See table, p. 248.)

BACILLUS WELCHII.—As already stated, it is not essential as a routine procedure to search for the *Bacillus Welchii* (*enteritidis sporogenes*), though in certain instances it may be of advantage to do so. A

negative result in such cases is probably of more value than a positive one.

For the isolation of *B. Welchii*, 500 c.c. of the water may be filtered through a Pasteur-Chamberland filter, the deposit is suspended in 5 to 6 c.c. of sterile water, and 1 c.c. of the suspension added to each of five to six tubes of sterile milk, which are then heated to 80° C. for ten minutes in a water-bath, and incubated anaërobically at 37° C. for forty-eight hours (filter-brushing method). A better method[1] is to employ large boiling tubes or small Erlenmeyer flasks, each containing 25 to 50 c.c. of sterile milk. To each tube a quantity of water equal to that of the milk is added, the tubes are then heated in a water-bath to 80° C. for fifteen to twenty minutes, some sterilised oil or melted vaseline is poured on the surface to exclude air, the tubes are cooled in water to 37° C. or thereabouts, and incubated for forty-eight hours at 37° C. Not less than 200 c.c. of the water should be used. The typical change in the milk (see p. 452) indicates the probable presence of the organism. To make sure that the change is due to the *B. Welchii* and not to the *C. butyricum*, 1 c.c. of the whey per 100 grm. of body weight should kill a guinea-pig in forty-hours when injected subcutaneously.

The virulence of a peptone-water culture has been suggested as an index of contamination, but in the writer's hands has not given reliable results. If sufficient peptone and salt be added to a measured volume of the water to form a 1 per cent. solution of the former and a ½ per cent. solution of the latter, the mixture incubated at 37° C. for twenty-four hours and injected intra-peritoneally into a guinea-pig, a bad water is stated to kill, whereas a good one does not. The amount to be injected is 2 c.c and death should ensue within forty-eight hours.

Interpretation of Results.—The interpretation of the results of the bacterioscopic examination of water is a difficult matter, for which experience is necessary. Just

[1] R. T. Hewlett, *Trans. Path. Soc. Lond.*, vol lv, 1904, p. 123.

as in chemical analysis, it is not possible to lay down an *absolute* standard, a knowledge of the source and surrounding conditions being of the greatest importance in forming an opinion. The ultimate aim is, of course, the detection of sewage or fæcal pollution; the bacterioscopic analysis does not give any information as to the suitability of the water for household, trade, or factory purposes.

Number of colonies on the gelatin plates.—The number of colonies represents approximately the number of organisms in the original sample capable of development aërobically at 20° C. in gelatin. This number in a good water rarely exceeds 100 or 150; in pure waters, particularly those coming from deep chalk-wells, there may be only a few—5 to 10 per c.c. (the results are always expressed in numbers per cubic centimetre of the original water). In waters of poorer quality the number may approach 500 per c.c. Anything over this casts suspicion on the water, and 1000 per c.c. or more should probably condemn the sample, always supposing, of course, that multiplication *in vitro* can be excluded by the proper storage of the sample bottle in ice. As a rule in water of good quality liquifying organisms are scanty, while in a polluted water they are numerous.

Number of colonies on the agar plates.—As mentioned before (see p. 612), it is the ratio of the number of organisms developing on the agar plates to those developing on the gelatin plates that is of importance.

Number of B. coli.—The detection and enumeration of *B. coli* are regarded by all as perhaps the most important part of water examination. The number of *B. coli* is estimated from the amounts of water that have been added to the tubes of media, which, however, assumes that the organism is regularly distributed

throughout the sample, and must so far as possible be ensured by thorough mixing. The results generally come out fairly concordantly, though irregularities exceptionally occur which can only be obviated by making duplicate sets of cultures. It is better to state the result as "*B. coli* present in . . . . . . c.c. of water" rather than to say that so many *B. coli* are present, though as a matter of fact the latter statement is approximately correct. Adopting the writer's methed for *B. coli* (p. 615), if *none* of the tubes contains *B. coli*, we say that "*B. coli* is absent from 50 c.c."; if the 25 c.c. tube contains *B. coli*, but not the remainder, "*B. coli* is present in 25 c.c. but not in less," and so on.

If nothing is known about the water, the following standards may be adopted:

(*a*) *Waters of good quality.*—*B. coli* absent in 50 c.c. of the water.

(*b*) *Waters of medium quality.*—*B. coli.* present in 50 c.c. but absent in 25 c.c.

(*c*) *Waters of poor quality.*—*B. coli* present in 50 c.c. and 25 c.c., but absent in 10 c.c.

(*d*) *Waters of suspicious quality.*—*B. coli* present in 50 c.c., 25 c.c., and 10 c.c., but absent in 1 c.c.

(*e*) *Waters unfit for drinking.*—*B. coli* present in 1 c.c. or less.

Waters which show no *B. coli* in 50 c.c. are of a high degree of purity, and therefore the proved absence of this organism in this amount, and still better in larger quantities, is of great value.

B. coli should be absent from at least 50 c.c. of spring or deep well water, possibly from greater amounts.

In upland surface waters the presence of *B. coli* in 40, 10, or even 2 or 1 c.c. means contamination, but not necessarily a contamination which it is essential to prevent. It may be

from contamination with the excreta of animals grazing on the gathering areas, and is by no means necessarily from sewage or other material containing specific organisms of infection. If *B. coli* are present in numbers greater than, say, 500 per litre (or even in that amount), such a water is suspicious, as it is rare to get so many *B. coli* in a water from the kind of animal contamination indicated, and further investigation is desirable. In filtered samples the number of *B. coli* is, as a rule, considerably reduced.

In surface wells *B. coli* in large numbers indicate surface or other contamination, generally very undesirable if not actually dangerous.

It must clearly be understood that the presence of the *B. coli* in water is used as an *index* of pollution, just as the organic ammonia is in a chemical analysis. This organism is not necessarily harmful in itself; it is what it indicates, viz. *pollution*, probably with human excremental matters, which may contain the organisms of specific disease, *e. g.* typhoid, dysentery, and cholera. As a *routine*, the typhoid bacillus is never looked for, and the statement sometimes seen in the report on the bacteriological examination of a sample of water that "no typhoid bacilli have been detected" is of little value. It is on the *general* results of the examination, as detailed in preceding pages, that a conclusion is arrived at respecting the purity or otherwise of a water.

Bacillus Welchii.—This organism being abundantly present in fæces and sewage, its presence in water has been suggested as an indication of pollution. Its spores, however, are very resistant, and it might, therefore, gain access to the water in ways other than by direct pollution —*e. g.* in dust—and for this reason the committee did not recommend the search for this organism as a routine procedure. On the other hand, Thresh[1] lays a good deal of stress on it, and the following are standards

[1] *Public Health*, 19[illegible]4.

suggested by him, based on an examination for, and detection of, *B. coli* and *B. Welchii*:

1. Water showing the absence of organisms capable of fermenting glucose, and of the *B. Welchii*. These we regard as being free from any evidence of pollution.

2. Waters showing the absence of organisms capable of fermenting glucose, but containing the *B. Welchii*, or its near ally. In the few cases of this kind which have come under our observation we have inferred the absence of sewage pollution, but the possible presence of water derived from fertile soil. This inference has been verified on more than one occasion.

3. Waters containing organisms capable of fermenting glucose, but not lactose, but free from the spores of the *B. Welchii*. These are regarded as unpolluted.

4. Waters differing from No. 3 only in containing spores of the *B. Welchii*. These we regard as free from sewage pollution, but as probably containing soil washings.

5. Waters containing lactose fermenters, none of which belongs to the *Bacillus coli* group, and free from the spores of the *B. Welchii*. These we do not regard as being sewage-polluted, but as containing surface water or subsoil washings.

6. Waters resembling No. 5, but containing the spores of the *B. Welchii*. These waters are usually from a source requiring careful watching, manurial matter probably being used on the collecting area.

7. Waters containing organisms of the colon group other than the *B. coli*, but no spores of the *B. Welchii*. These we do not regard as dangerously polluted, but as probably coming from a source such as that referred to under No. 6.

8. Waters containing organisms of the colon group other than the *B. coli*, and also spores of the *B. Welchii*. Pollution indicated, but possibly from a source not close at hand. The necessity for frequent examination is essential, especially after heavy rains, as such waters usually sooner or later show more serious signs of pollution.

9. Waters containing the true *B. coli*, but no spores of the

B. Welchii. Such waters are occasionally met with. No opinion can be expressed without an intimate knowledge of the source. We have had such water from a source absolutely free from the possibilities of contamination, but usually subsequent examination has revealed the presence of the spores of the *B. Welchii*. The proximity of manured soil is strongly indicated.

10. Waters containing the true *B. coli* and spores of the *B. Welchii*. These we regard as being decidedly contaminated with fæcal matter of recent origin.

Streptococci.—Streptococci are abundant in fæces and sewage, but are extremely rare, if ever present, in unpolluted natural waters; hence the value of their detection. Streptococci as a class are delicate organisms, and it was supposed that their presence indicates *recent* pollution.[1] Horrocks, on the other hand, believes that they maintain their vitality longer even than *B. coli*, and this is rather the opinion at present. We need further data before we can exactly estimate the value of streptococci as indicators of pollution. There can be no question, however, that the detection of many streptococci, together with *B. coli*, indicates serious pollution.

There can be no doubt of the value of the bacteriological examination of water, but it cannot entirely supplant chemical analysis, which on account of its rapidity and the valuable data it yields will probably always remain an integral part of the examination of potable waters. If the water be pure and uncontaminated, the bacteriological examination will occupy three days; but if contamination be present, though it may be *presumed* in the same time, ten days or a fortnight may be required to convert this presumption into a *certainty*, owing to the length of time necessary for determining the characters of the organism present.

[1] Houston, *Rep. Med. Off. Loc. Gov. Board* for 1898-99.

Media Employed for the Isolation of B. Coli.

(1) *Carbolised gelatin.*—Ordinary nutrient gelatin with the addition of 0·05 per cent. of phenol. (Hardly used now.)

(2) *Bile-salt peptone water* (MacConkey and Hill).—The composition of this medium is as follows: Sodium taurocholate 0·5 grm., glucose or lactose 1·0 grm., peptone 2·0 grm., water 100 c.c. The constituents are dissolved by heating; the mixture is filtered, and after filtration sufficient neutral litmus solution is added to give a distinct colour. The medium is then distributed into Durham's fermentation-tubes and sterilised by steaming for twenty minutes on three successive days. The medium may be put up in various sized tubes, a measured volume in each—*e. g.* 10 c.c., 20 c.c., 25 c.c., etc., according to the quantity of water which is to be added. For the larger quantities the medium may be made double the above strength. The inoculated tubes are incubated at 42° C. for forty-eight hours. The *B. coli* reddens and ferments both the glucose and lactose media, so that gas collects in the fermentation tube.

Lactose

(3) *Neutral-red broth* (Hunter, Makgill, Savage).—The dye known as neutral-red (Grübler's) is reduced by the action of the *B. coli*, the colour changing to a canary yellow, accompanied by a green fluorescence. The *B. enteritidis* (Gärtner) also reduces neutral-red, but the *B. typhosus* does not do so, nor do streptococci, *B. pyocyaneus*, and *Spirillum choleræ*. Some anaërobes also possess a reducing action. Glucose agar or broth (0·5 per cent. of glucose) is employed, and to every 10 c.c. of the medium 0·1 c.c. of a 0·5 per cent. aqueous solution of neutral-red is added. Savage recommends the following procedure: 10 c.c. of the water are added to a 10 c.c. tube of neutral-red broth; also to 40 c.c. of the water contained in a bottle or flask a 10 c.c. tube of the broth of *quadruple strength* is added. Both are incubated at 37° C., and examined daily up to eight days. If reduction occurs, *B. coli* is almost certainly present in the water; if reduction does not occur its presence is highly improbable.

(4) *Glucose formate broth* (Pakes).—To ordinary meat

infusion 1 per cent. peptone, 0·5 per cent. sodium chloride, 2 per cent. glucose, and 0·4 per cent. sodium formate are added. When these have been dissolved by heating, the medium is neutralised (indicator, litmus), and after neutralisation 2 c.c. of normal caustic soda solution per litre are added ; the broth is then steamed for twenty minutes, filtered, and distributed into test-tubes, 10 c.c. in each, which are steamed for twenty minutes on each of three successive days. These tubes are inoculated with the water, and incubated anaërobically at 42° C. for twenty-four to seventy-two hours. Tubes showing any growth at the end of twenty-four, forty-eight, or seventy-two hours are removed and examined microscopically and by plating.

(5) *Bile-salt lactose agar* (MacConkey).—This medium is prepared by adding to 1000 c.c. of tap-water in a flask 2 per cent. of peptone, 0·5 per cent. of sodium taurocholate, and 1·5 per cent. of agar. The mixture is autoclaved at 105° to 110° C. for 1½ hours, cleared with a small addition of white of egg, and filtered. To the filtrate 1 per cent. of lactose is added. The medium is then distributed into test-tubes, 10 c.c. in each, and sterilised by fifteen minutes' steaming on three successive days. Plates are made and incubated at 42° C. for forty-eight hours. The colonies of organisms which ferment lactose with the formation of acid are surrounded with a cloudiness or haze owing to the precipitation of the taurocholate. Neutral-red or krystal violet may be added (proportions, see Nos. 3 and 6).

(6) *Conradi-Drigalski agar.—Mixture A.*—To 1 litre of acid beef broth (p. 55) add :

Witte's peptone	10 grm.
Nutrose	10 ,,
Sodium chloride	5 ,,

Steam for one hour, and add 25 grm. of powdered agar. Steam for three hours, bring to a reaction of + 10, and filter through "papier Chardin."

Mixture B.—Boil for a few minutes 100 c.c. of Kubel-Tiemann's litmus solution, add 15 grm. of pure powdered lactose, and boil again for a few minutes.

Add *B* to *A*, and to this mixture add 2 c.c. of a hot 10 per cent. solution of anhydrous sodium carbonate and 10 c.c. of a 0·1 per cent. solution of krystal violet. The medium is then tubed, 10 c.c. being placed in each test-tube, and sterilised.

In using the medium it should be employed as *surface* plates. The required number of tubes are melted in a water-bath, and their contents poured out into sterile Petrie dishes and allowed to set. These sterile plates are then placed in the warm incubator for an hour or so with the lids slightly tilted at one edge, so that the surface of the medium may dry somewhat. The matter to be plated is sufficiently diluted, and from a few drops to 0·5 c.c. are run on to the surface and spread by means of a glass rod bent into a flattened hook, and sterilised by boiling. On this medium in forty-eight hours *B. coli* forms large red colonies, *B. typhosus* and *B. dysenteriæ* small blue colonies, and streptococci small delicate red colonies. Other organisms are to a large extent inhibited from developing.

(7) *S.D.S. rebipelagar* (Houston).—"Rebipelagar" has been much used by Houston[1] for the isolation of *B. coli*. It has the following composition: Agar 20 grm., taurocholate of soda 5 grm., lactose 10 grm. neutral-red 4 c.c. of a 1 per cent. solution, peptone 20 grm., water 1 litre. The S.D.S. rebipelagar has the following composition : Agar 20 grm., taurocholate of soda 5 grm., lactose 2·5 grm., neutral-red 4 c c. of a 1 per cent. solution, peptone 20 grm., saccharose 2·5 grm., dulcitol 2·5 grm., salicin 2·5 grm.

The Isolation of Specific Organisms from Water.

The principal disease-producing organisms conveyed by water are the *B. typhosus*, *B. paratyphosus*, *B. dysenteriæ*, and *Spirillum choleræ*.

The Isolation of B. typhosus, B. paratyphosus, and B. dysenteriæ from Water.—There is great difficulty in isolating the *B. typhosus* from water that has been very

[1] *First Rep. on Research Work*, Met. Water Board, 1908.

Coli
bile Red
Typh blue
Dep "
Strept
small red

copiously contaminated with specifically polluted sewage, there is, therefore, far greater difficulty when the specific pollution has been small in amount. The earlier records of the isolation of the *B. typhosus* must be accepted with much scepticism, as the methods of identification were formerly incomplete and unsatisfactory. It is necessary to bear in mind that usually, when drinking-water has suffered sewage-pollution, the amount of the pollution is relatively very minute when compared with the great bulk of the water supply. Moreover, allowing ten days as the average incubation period of typhoid fever, another week before the disease comes under notice, and another week before the fact that an epidemic is in progress is recognised, at least a month will have elapsed between the date of infection of the water supply (supposing this to have occurred on one occasion only, as may be the case) and the taking of the samples for examination, a period during which all the typhoid bacilli may have died out. The contamination of water may, however, be of an intermittent nature.

Numerous methods have been devised for the isolation of the typhoid bacillus from an infected water. With rare exceptions, it is impossible to detect the organism by direct plating; it is too scanty and too mixed with other organisms to admit of this, and therefore concentration of the bacterial content of the water must be attempted. The following are some of the methods which have been suggested for this purpose; they serve equally well for *B. paratyphosus* and *B. dysenteriæ*.

1. *Filtration through a porcelain filter.*—By passing one to two litres of the water through a sterile Pasteur-Chamberland filter, the whole of the organisms present may theoretically be collected in a few c.c.'s. Practically, however, a large proportion of the organisms are lost in the process: perhaps they get carried into and remain in the superficial layers of the filter-candle, and for this reason, though sometimes employed, this method has been largely given up.

[1] See H. S. Wilson, *Journal of Hygiene*, vol. v, 1905, p. 429; McWeeney, *Brit. Med. Journ.*, 1909, vol. ii, p. 866.

2. *Concentration.*—W. J. Wilson[1] has devised the following method: The water is placed in one or two Winchester quart bottles, and 10 c.c. of nutrient broth are added for every litre. The bottles are placed in a water-bath maintained at 37°–40° C., and are connected by rubber corks and tubing with a condenser (at a lower level) through which cold water continuously passes, and the tube of the condenser is connected to a large bottle (at a still lower level). This bottle is kept partially exhausted by means of a filter-pump. The water evaporates and is thus concentrated, the evaporated water being condensed and collected in the exhausted bottle. It requires twenty-one to twenty-two hours to evaporate a litre of water. The water remaining in the bottles, now concentrated to a few c.c.'s., is then plated on Conradi-Drigalski or malachite-green agar.

3. *Chemical precipitation.*—These methods depend on the formation in the water of a fine, inert precipitate, which entangles and carries down with it a large proportion of the bacteria present. Thus, in the Vallet-Schüder[2] method, to 2 litres of the water are added 20 c.c. of a 7·75 per cent. solution of sodium hyposulphite and 20 c.c. of a 10 per cent. solution of lead nitrate. The precipitate is allowed to settle or is centrifugalised off, is dissolved in a small volume of a saturated solution of the hyposulphite, from which plates are made in suitable media. Ficker[3] uses ferrous sulphate after making the water faintly alkaline with caustic soda; the ferrous hydrate formed carries down the micro-organisms (this must be a risky procedure, as the typhoid bacillus is very sensitive to caustic alkalies). Iron oxychloride may also be used as the precipitant. H. S. Willson (*loc. cit.*) employs alum. A stock solution of alum is prepared, containing 10 grm. per 100 c.c., and of this sufficient is added to the water to obtain 0·5 grm. to the litre. After the precipitate of aluminium hydrate has formed, the vessel is well shaken to mix its contents, and the mixture is centrifugalised for fifteen

[1] *Brit. Med. Journ.*, 1907, vol. i, p. 1176.
[2] *Zeitschr. f. Hyg.*, xlii, No. 2, p. 317.
[3] *Hyg. Rundschau*, xiv, No. 1, 1904, p. 7.

minutes at 2000 revolutions per minute. The clear, supernatant fluid is then syphoned or poured carefully off from the precipitate, and the mass of precipitate in the conical extremity of the tube stirred up with the little fluid (0·5 to 1 c.c.) remaining. The suspension is then plated out on Conradi-Drigalski, malachite-green or brilliant-green, agar. This seems to be a very promising method.

4. *Serum agglutination.*—An anti-typhoid serum—the serum of an animal which has been inoculated several times with the typhoid bacillus, having the power of agglutinating typhoid bacilli—if added to a water would presumably agglutinate any typhoid bacilli into masses which will sediment or may be centrifugalised off. The method has been used by Schepilewsky,[1] who adds 10 to 20 c.c. of the water to flasks containing 50 c.c. of nutrient broth, to which after three or four days' incubation at 37° C. an addition of the typhoid serum is made, and after standing for some hours and centrifugalising, the deposit is plated out.

5. *Method of enrichment.*—The principle of this method is to devise a medium which shall allow of the multiplication of the typhoid bacillus, and at the same time prevent, or at least retard, the growth of *B. coli* and allied forms. Almost all the methods which have been introduced for this purpose fail, inasmuch as, though they inhibit the growth of a great many organisms, they do not inhibit the growth of the *B. coli*, or, if they do, inhibit the *B. typhosus* to a still greater degree. Roth[2] found that caffeine in broth would retard *B. coli*, but allow *B. typhosus* to multiply. The method has been further elaborated by Hoffmann and Ficker,[3] who convert the water itself into a nutrient medium by the addition of 1 per cent. of nutrose, 0·5 per cent. caffeine, and 0·001 per cent. of krystal violet. The mixture is incubated at 37° C. for not more than twelve to thirteen hours, at the end of which time the typhoid bacilli should have multiplied to such an extent as to permit of direct isolation by plating, the

[1] *Centr. f. Bakt., Orig.*, xxiii, No. 5, 1903.
[2] *Hyg. Rundschau*, xiii, 1903, p. 489.
[3] *Ibid.*, xiv, 1904, p. 1.

B. coli being inhibited. Many observers have shown, however, that while caffeine may materially help, it cannot be entirely relied on to eliminate *B. coli* and allied form.

6. *Process of Cambier.*—Cambier[1] has devised a process based on the idea that an actively motile organism will find its way through the pores of a porcelain filter more quickly than feebly or non-motile forms. His procedure is to make use of a special alkaline peptone medium, which is placed in a glass jar. In this is immersed a Pasteur-Chamberland filter-candle half filled with the same solution, to which is added a little of the fluid to be examined, and the whole is incubated at 37° C. Sooner or later growth appears in the fluid outside the candle, and Cambier states that if typhoid bacilli be present they will make their appearance before *B. coli*. In hands other than those of Cambier, however, the method has not proved successful.

7. *Fuchsin Agar* (Endo).—One litre of 3 per cent. nutrient agar is made alkaline with 10 c.c. of 10 per cent. NaOH solution after neutralisation. Pure lactose 10 grm. and saturated alcoholic fuchsin solution 5 c.c. are added, and after mixing, 25 c.c. of fresh 10 per cent. solution of sodium sulphite are added. The medium when cold should be colourless. The medium is used as surface plates, and on it typhoid and paratyphoid colonies are colourless, coli colonies are red.

8. *Malachite-green Media.*—Löffler has found that malachite green (No. 120 Hoechst) in the proportion of about 1 in 5000 in media inhibits the growth of *B. coli* while still permitting the growth of *B. typhosus*. The dye may be added either to liquid or to solid media. The medium recommended by Löffler[2] is composed of 3 per cent. agar made with meat infusion, with 1 per cent. nutrose, and containing in every 100 c.c. 2–2·5 c.c. of a 1 per cent. solution of malachite green. On this medium the *B. typhosus* grows in twenty-four hours as delicate, slightly crinkled colonies, surrounded by a colourless zone (due to alkali formed by the bacilli). Thus

[1] *Rev. d'Hyg.*, 1902, p. 64.

[2] *Deutsch. med. Woch.*, 1906, No. 8.

it is possible to detect one colony of *B. typhosus* among 300 to 600 colonies of other bacteria. As a medium for "enriching"—*i. e.* for specially advancing the growth of the *B. typhosus*—Löffler recommends a 15 per cent. gelatin, prepared with beef-juice and peptone, and containing per 100 c.c. 3 c.c. of doubly normal phosphoric acid and 2 c.c. of 2 per cent. malachite-green solution. With the suspected matter, firstly, one series of malachite-gelatin plates is prepared and incubated at 25° C. for twenty to twenty-four hours; secondly, a tube of malachite gelatin is inoculated and incubated at 37° C. for twelve to twenty-four hours; from this a second tube is inoculated and incubated at 37° C., and then plated out on malachite gelatin and incubated at 25° C. The colonies of *B. typhosus* are well marked after twenty to twenty-four hours, as large as a pin's head, transparent, highly refractile, light grey and granular. Their shape is circular or oval, and they show characteristic offshoots resembling a bone-corpuscle or the body of an acarus. By using this 15 per cent. gelatin, which can be incubated at 25° C., there is the double advantage of speedy growth and formation of very characteristic colonies.

Houston recommends S.D.S. rebipelagar (p. 625) with the addition of malachite-green to the extent of 1 in 5000 (0·2 grm. to the litre). On this medium *B. typhosus* forms colourless colonies; most other bacteria do not grow, or appear as blue-black colonies.

9. *Werbitzki's China green agar.*—For this 3 per cent. nutrient agar (reaction +13) is used, and to every 100 c.c. of the agar 1·4–1·5 c.c. of a 0·2 per cent. aqueous solution of china green (Grübler's) are added.

10. *Brilliant green agar.*—Conradi devised an agar containing brilliant green and picric acid, and this has been modified by Fawcus[1] as follows: To 900 c.c. of tap-water are added sodium taurocholate, 5 grm.; powdered agar, 30 grm.; peptone, 20 grm.; and sodium chloride, 5 grm. Dissolve the constituents by steaming for three hours, filter through wool, and bring to a reaction of + 15 (by means of lactic acid or NaOH, as the case may be). In 100 c.c. of distilled water dissolve 10 grm.

[1] *Journ. Roy. Army. Med. Corps*, February, 1906, p. 147.

lactose and add this to the former, filter, distribute in flasks (100 c.c. in each), and sterilise. At time of using, melt and add to each 100 c.c., 2 c.c. of a 1–1000 aqueous solution of brilliant green and 2 c.c. of a 1–100 aqueous picric acid (extra-pure, Grübler's). Typhoid forms round, transparent refractile colonies of a light pale green colour by transmitted light, *B. coli* dark green colonies with an opaque spot at the centre.

Conclusion.—The writer would suggest for the isolation of *B. typhosus* from water: (1) Concentration of the organism by precipitation with alum (Wilson's method) or iron oxychloride, followed by plating of the precipitate on Conradi-Drigalski agar, or, better, on malachite-green agar (Löffler's or Houston's, No. 8 above), or brilliant-green agar (No. 10 above); (2) enrichment by Löffler's method and subsequent plating. In all cases the organism isolated must be examined as to its morphological, cultural, and biological characters, and should have its agglutination and Pfeiffer reactions tested with a high-grade typhoid serum. Two organisms which are likely to be mistaken for the *B. typhosus*, unless *all* tests are applied to them, are the *B.* (*fæcalis*) *alkaligenes* and *B.* (*aquatilis*) *sulcatus*. Both occur in the dejecta and in polluted water, and are very like the *B. typhosus* in morphology, motility, staining, and cultural reactions, but neither agglutinates with typhoid serum. The *B. alkaligenes* sometimes produces a brownish growth on potato, it renders litmus milk alkaline and produces alkali, but no gas, in glucose, lactose, dulcitol, mannitol, saccharose, and salicin. The *B. sulcatus* hardly grows at 37° C. and is almost a strict aërobe, little growth occurring in the depth of a stab. Some varieties of typical and of atypical *B. coli* agglutinate with typhoid serum, so

that a positive agglutination reaction does not necessarily prove that an organism is *B. typhosus*.

THE ISOLATION OF THE CHOLERA BACILLUS FROM WATER.—The detection of Koch's comma bacillus (*Spirillum choleræ*) in water, as in the case of the typhoid bacillus, is a matter of some difficulty, as this organism is rapidly overgrown by the ordinary water bacteria. In the examination of suspected water supplies, the best method to employ for the detection of this organism is to take advantage of the fact, first noted by Dunham, that the cholera spirillum multiplies with great rapidity in alkaline saline peptone solution. The suspected water is examined as follows: To 300–500 c.c. of the water are added 1 per cent. each of pure peptone and of common salt; the mixture is made faintly alkaline with sodium carbonate, distributed in a dozen small Erlenmeyer flasks having a layer not more than an inch deep in each, the flasks are loosely capped with caps of filter-paper, and incubated at 37° C. At intervals of ten, fifteen and twenty hours respectively, hanging-drop and cover-glass preparations are made from the top of the liquid, on which there is often a surface film, and care must be taken not to disturb this; these are then examined microscopically for vibrios and spirilla. At the same time agar, or, better, blood alkali agar (p. 471) plates are prepared and incubated at blood-heat. Any colonies that appear which resemble the cholera spirillum are examined microscopically; if the organisms are comma-shaped, they are at once subcultured into peptone water and other media. The original peptone water cultures are tested for the indole reaction with pure hydrochloric acid, withdrawing some of the contents of the flasks with a sterile pipette. Any likely spirillum isolated must have its cultural and biological reactions investigated and be tested for the agglutination and Pfeiffer reactions with a high-grade cholera serum.

On the survival of the typhoid and cholera organisms in water, see pp. 381 and 461 respectively.

Ice and *ice-creams* may be examined by methods similar to those used for water, the material being first melted at a low

temperature. Some of the fluid should also be centrifugalised and the deposit examined microscopically for gross contamination.

The infection in typhoid fever and cholera, and perhaps also in bacillary dysentery, is perhaps more frequently water-borne than conveyed in any other way. It might be supposed that the acid gastric juice would prevent this, and it may do so in many instances. Experiments by Macfadyen[1] showed that, whereas in fasting animals, to which suspensions in water of the cholera spirillum were administered, living spirilla pass into the intestine, when the vehicle is milk none could be detected in the intestines. The inference is that when there is no food there is no gastric juice secreted and the organisms are able to pass on into the intestine, but when food is present the gastric juice is secreted and the organisms are destroyed.

Sterilisation of Water.—This may be done on the small scale by heat, by the use of germicidal agents, or by filtration through a filter (see p. 635). Heat may be applied by simple boiling, or by the use of apparatus in which the water is heated to 65°–90° C., and the outgoing hot water is cooled by the ingoing cold water, which itself is thus warmed, thereby effecting economy in fuel (Griffiths' and other sterilisers). The chemical germicides that have been employed are (1) sodium bisulphate, 15 grains to the pint; (2) Potassium permanganate, sufficient to tinge the water deeply for at least half an hour; (3) chlorine gas or iodine tablets,[2] in both cases the taste of the agent being destroyed by the addition of sodium sulphite; (4) copper and copper sulphate. Sufficient metal is dissolved from bright copper in twenty-four hours to destroy typhoid and cholera. Copper sulphate 1 in 100,000 or less is similarly germicidal, and in still smaller quantities (1 in 1,000,000) destroys algæ, and has been used for the purifica-

[1] *Journ. of Anat. and Physiol.*, vol. xxi.

[2] Nesfield, *Journ. Prev. Med.*, vol. xiii, 1905, p. 623.

tion of reservoirs overgrown with algæ. On the large (also small) scale, chlorine derived from hypochlorites is one of the simplest and most efficient agents. Moor and Hewlett[1] showed that 0·25 part of chlorine (equivalent to about 0·75 part of good chloride of lime) per million parts of chalk water is sufficient to kill *B. coli* in half an hour. The taste disappears quickly in bright sunlight and on standing, or may be removed by an addition of sodium sulphite. If the water is organically polluted, more chlorine must be used.

Ozone produced by high-tension electric discharge is also employed on the large scale for the sterilisation of water supplies, *e. g.* at Chartres.

Examination of Shell-Fish.—Shell-fish may come from sewage-polluted layings (see p. 382). The following method may be employed for their examination (after Houston):

The outside of the shells are cleansed by thorough scrubbing and rinsing in tap-water, and a final rinse in sterile water. The fish after cleansing are laid on a sterile towel. The operator then cleanses his hands and opens the shells aseptically with a sterile oyster-knife, care being taken to avoid loss of their contained liquor. The liquor as each fish is opened is poured into a sterile litre cylinder, and the fish is cut up with sterile scissors and added to the liquor in the cylinder. Ten fish should be treated, the volume of fish + liquor noted, and sterile water is then added to make up to 1 litre; 100 c.c. liquid therefore corresponds to one fish. In addition, four dilutions of the liquid are prepared—1 in 10, 1 in 100, 1 in 1000, and 1 in 10,000. With the liquid and dilutions gelatin and agar plate cultivations are prepared for the enumerations of the organisms present. Cultures are also made in litmus lactose bile-salt peptone water and in milk for the enumeration and isolation of *B. coli* and *B. Welchii* respectively, taking 100 c.c. 10 c.c., and 1 c.c. of the liquid, and 1 c.c. of each of the four dilutions; in this way the contents of the fish, ranging from one fish to $\frac{1}{1000000}$ of a fish, are examined. The process and principles involved

[1] *Rep. Med. Off. Loc. Gov. Board* for 1909–10, p. 559.

correspond to those described for water. Houston has suggested for oysters as a lenient standard less than 1000, and as a stringent standard less than 100, *B. coli* per oyster. Even ten *B. coli* per fish should be viewed with suspicion, for Hewlett and others have shown that oysters from pure layings contain *no B. coli*.

Watercress, etc., may be examined in a similar manner, 100 grm. being weighed out and transferred bit by bit with sterilised forceps and scissors to a flask containing 900 c.c. of sterile water. The flask is shaken vigorously, and the washings examined in a manner similar to that employed for shell-fish.

Filters.—Reference has already been made to the removal of organisms in water by sand filtration. With regard to filters for domestic use, few of those in the market are capable of doing more than removing particles of suspended matter, while they allow from 5 to 50 per cent., or even more, of the bacteria present in the water to be filtered, to pass through. Such filters are, of course, useless for the prevention of disease—in fact, rather favour it, by engendering a false sense of security; and when in use for some time without cleaning, the water after filtration may be worse, bacteriologically and chemically, than before filtration.

Woodhead and Wood[1] found that the only filters which were capable of completely removing organisms were the Pasteur-Chamberland, Berkefield, and Porcelaine d'Amiante. The Berkefeld, while more rapid in action than the other two, after being in use for a few days may allow some organisms to appear in the filtrate. This, perhaps, is due rather to a growth of organisms through the pores of the filter-candle than to

[1] *Brit. Med. Journ.*, 1894, vol. ii, p. 1053 *et seq.*

a direct passage. Lunt[1] found that while the ordinary water bacteria, such as the *B. fluorescens liquefaciens*, appeared in the filtrate from a Berkefeld filter within a few days of the infection of the sample, the typhoid bacillus and the comma bacillus similarly introduced had not passed through the filter four or five weeks after infection.

Horrocks,[2] however, does not confirm this, and has found that when sterile water is inoculated with typhoid bacilli and run daily through a Berkefeld filter, the bacilli appear in the filtrate in one to two weeks, whereas this is not the case with the Pasteur-Chamberland. The writer has made some similar experiments, which partially, but not entirely, support Horrocks's conclusions. Much evidently depends upon the chemical composition of the water.

Messrs. Doulton have constructed a porcelain filter which seems to be perfectly efficient, like the Pasteur-Chamberland. All porcelain filters should be cleaned weekly by well scrubbing with a nail-brush and boiling in water containing some sodium carbonate.

The Bacteriological Examination of Water-Filters.

The large majority of water-filters at present in use are incapable of preventing organisms from being washed through into the filtrate. In order to ascertain whether this is the case with any particular filter, it should be sterilised in the steam steriliser, and water containing organisms of known species (*B. prodigiosus*, *B. violaceus*, and *M. agilis* are very suitable) should be passed through it for twenty-four hours. This water and the filter should during this period of the examination be maintained, if conveniently possible, at a temperature below 5° C. This will almost invariably prevent

[1] *Trans. Brit. Inst of Prev. Med.*, vol i, 1897.
[2] *Brit. Med. Journ.*, 1901, vol. i, p. 1471.

any growth or multiplication of the organisms. Samples should be taken immediately after the filtration has begun, and at intervals during the day, and again at the end of twenty-four hours. If they are all sterile, the filter is capable of preventing organisms from being directly washed through. In the case of filters of very great density or depth of filtering medium, it may be necessary to prolong the period of examination beyond the first day; but most ordinary filters which permit organisms to be washed through do so within the first few hours.

Protozoa and Algæ in Water.

The examination of water for the minute forms of life other than bacteria, and their enumeration, can be carried out by the Sedgwick-Rafter method.[1] A 6-inch glass funnel is plugged at the bottom of the stem with a perforated rubber cork, over the upper end of which a disc of fine silk bolting cloth, cut by a wad-cutter, is laid. Sharp, clean, dry quartz sand is then poured into the stem of the funnel to the depth of half an inch above the plug. The sand should be of such a size that the grains will pass through a sieve of 60 meshes to the inch, but not through one of 120 meshes. The sand is washed into place and well moistened with a little distilled water free from organisms.

The water to be examined is thoroughly shaken and 500 c.c. are poured into the funnel; it runs through the sand, which detains any organisms it may contain. After the water has all passed through, the rubber plug is carefully removed and the sand washed down into a test-tube with 5 c.c. of distilled water. The contents of the test-tube are agitated and the tube is allowed to rest until the sand has deposited. Immediately this is the case the supernatant fluid is decanted into a second test-tube, carrying with it the organisms. One cubic centimetre of this is withdrawn by a pipette from mid-way between the top and bottom and transferred to the

[1] Calkin, *Twenty-third Ann. Rep. State Board of Health, Massachusetts*, 1891.

counting plate. This consists of an ordinary glass slide on which a rectangular brass cell (20 × 50 mm.) is cemented, so enclosing exactly 1000 square mm. The brass cell is 1 mm. thick, so that the cell contains exactly 1 c.c. The preparation is covered with a cover-class and examined with a low power.[1]

The Bacteriology of Air.

Just as in water, the bacteria in the air vary considerably at different times and seasons, under different conditions, and in various localities. The species met with are mostly saprophytes, consisting largely of chromogenic forms. A number of moulds occur (as spores), and, in fact, ordinarily are in large excess, together with yeasts and torulæ.

It is not easy for micro-organisms to become diffused through the atmosphere; they are incapable of a voluntary rising, and cannot be torn from a fluid or moist solid medium by a strong current of air. The medium on which they are growing must dry up completely and crumble into fine dust before they can be distributed through the agency of air-currents (but see p. 385).

The number of organisms in the air varies with the season, with rain, with altitude, with movement, etc. At Montsouris, Miquel found in one cubic metre of air 49 organisms in winter, 85 in spring, 105 in summer, and 142 in autumn. After heavy rain the air is largely freed from organisms. Frankland found at Norwich Cathedral at an altitude of 300 feet 7 organisms in two gallons, while on the ground 18 were found; at the Golden Gallery at St. Paul's two gallons of air contained 11 organisms; in St. Paul's church-

[1] On the microscopy of water, see Whipple, *Microscopy of Drinking Water*.

yard the number was 70. On high mountains organisms are nearly absent from the air, and the same is the case at sea at a distance from land exceeding about 100 miles. Organisms are much fewer in the air of the country than in that of towns. At the entrance-hall, Natural History Museum, South Kensington, Frankland found in the morning 30 organisms; in the afternoon, when many visitors were present, the number had risen to 292, showing the influence of movement. By keeping a volume of air absolutely still, enclosed in a box the walls of which were smeared with glycerin, Tyndall was able to free it completely from particles and organisms. The writer found from 43 to 150 organisms per 10 litres of air in some of the principal streets of London during the daytime.

Gordon,[1] by exposing dishes of neutral-red broth to the air, or by aspirating air through neutral-red broth (p. 623), has been able to detect the presence of the *S. salivarius*, *M. epidermidis*, and scurf micrococcus (p. 241) in air subjected to human contamination. By these tests and by the use of *B. prodigiosus* as an indicator he concludes that particles of saliva are disseminated as far as 40 feet in the act of loud speaking, indicating the possibility of the wide distribution of such pathogenic organisms as the tubercle, plague, and influenza bacilli and the pneumococcus by speaking, and still more so by coughing.

The number of *dust* particles in the air may be enormous. In London Macfadyen and Lunt observed as extremes from 20,000 to nearly 600,000 per c.c. The ratio of micro-organisms to dust particles is therefore a very small one.

[1] *Reps. Med. Off. Loc. Gov. Board* for 1902–1904.

Bacteriological Examination of Air.

A number of methods have been devised for the estimation of the number of micro-organisms in the air, of which the following are the principal ones:

(1) *Plate method.*—Melted sterile nutrient gelatin is poured into a sterilised Petri dish, and allowed to set. The plate is then exposed to the air, by removing the lid, for a given time—one, five, ten, or fifteen minutes, etc.—the lid is replaced, and the plate incubated at 22° C. for some days. The number of colonies of moulds, bacteria, yeasts, etc., is counted, and, having estimated the area of the gelatin plate,[1] the result is expressed as the number of organisms falling per square foot per minute. The results obtained by this method are roughly comparative, but no estimate can be formed from it of the number of organisms contained in a given volume of the air.

(2) *Hesse's method.*—This is a quantitative method for estimating the number of organisms contained in a given volume of air. The apparatus consists of a glass tube 30 in. long by $1\frac{1}{2}$ to 2 in. in diameter. One end of this tube is plugged with a rubber cork through which a glass tube passes, the other end is covered with a piece of sheet rubber perforated with a hole $\frac{1}{4}$ to $\frac{1}{2}$ in. in diameter; over this is placed another sheet of rubber, unperforated. The small tube being plugged with cotton-wool, the whole is sterilised for an hour in the steam steriliser. Just before use 40 to 50 c.c. of melted sterile nutrient gelatin are poured into the tube, and its walls coated with the medium. The tube is then strapped horizontally on to a tripod stand, and the small tube connected by means of a piece of rubber tubing to an aspirator consisting of two flasks arranged so as to form a reversible syphon. A litre of water is poured into the flask connected with the tube, and the outer sheet of rubber having been removed from the end of the tube, the water is syphoned over to the second flask, placed at a lower level, and an equal volume of air is thus aspirated through the tube. The second

[1] The area of a circular dish is calculated by multiplying the square of the diameter by 0·785.

flask is then connected with the tube, and the position of the flasks being reversed the water is again syphoned over and a second litre of air passes through the tube, and this process is repeated until 5, 10, 15, or 20 litres of air have been drawn through the tube. The rate of flow is controlled by a screw-clamp on the rubber connecting-tube; it should not exceed half a litre per minute. With this rate of flow all the organisms are deposited on the gelatin-coated tube. The aspiration being completed the rubber tube is disconnected and the sheet of rubber replaced over the end of the tube, which is then incubated, and the colonies are counted when they have developed.

(3) *Petri's method.*—Petri aspirates the air through a glass tube containing sterilised sand, kept in place by fine wire-gauze wads. When the sample has been taken, the sand is

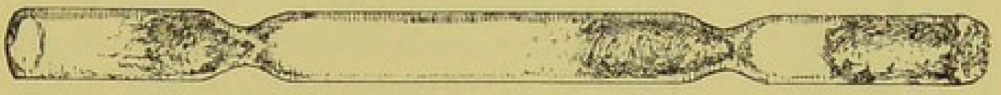


FIG. 67.—Frankland's tube for air analysis.

distributed in Petri dishes, and melted sterile gelatin is poured over it and allowed to solidify, plate cultures being thus prepared. The objection to this method is the presence of the opaque particles of sand in the culture medium.

(4) *Frankland's method.*—The air to be examined is aspirated through a tube 5 in. in length and ¼ in. in diameter (Fig. 67). One end of the tube is open, the other (C) is plugged with cotton-wool. At a distance of 1 in. from the open end the tube is slightly constricted to support a plug of glass wool (A). At a distance of 2½ in. from this plug the tube is again constricted to support a second plug (B), consisting of glass-wool and finely powdered cane-sugar, supported in front and behind by plugs of glass-wool. Several such tubes having been prepared, they are placed in a tin box and sterilised at 130° C. for three hours, and can then be easily transported without risk of contamination. When required for use, a tube is quickly removed from the box, being handled

by the plugged end, which is connected by stout rubber tubing to aspirating flasks such as are used in Hesse's apparatus. The tube is clamped horizontally to a retort stand, and by attaching the second flask to a small hand exhaust-pump, the water can be syphoned over from the first flask, a corresponding volume of air passing through the tube. When the desired volume of air has been aspirated through the tube, it is disconnected and placed in another sterile tin box. As many tubes as desired can be employed to control one another or to examine the air in different localities and under different conditions. All the samples having been taken, the tubes are manipulated on returning to the laboratory. The tubes, as before, being handled by the ends only, a file-mark is made across the centre of each tube, which is then broken in half and the plugs of glass-wool and sugar are shaken, or pushed by means of a sterile wire, into a sterile flask of about 250 c.c. capacity. Into this 10 or 15 c.c. of liquefied sterile nutrient gelatin are then introduced; the sugar dissolves, the glass-wool becomes disintegrated, and a roll-culture is made on the walls of the flask, which is incubated at 22° C., and the colonies are counted when they have developed.

Fig. 68.—Sedgwick and Tucker's tube for air analysis.

(5) *Sedgwick and Tucker's method.*—One of the best and most convenient methods for the bacteriological examination of air. A glass tube of special form is employed (Fig. 68); this consists of an expanded portion (A) about 15 cm. long and 4·5 cm. in diameter; one end of this is contracted so as to form a neck 2·5 cm. in diameter and in length; to the other end is fused a glass tube (B C) 15 cm. long and 0·5 cm. in diameter. The neck of the tube is plugged with cotton-wool, and two cotton-wool—or, better, glass-wool—plugs are

inserted in the narrow tube, one at its open end, the other (c) about 6 to 8 cm. from the wide part. The whole is then sterilised. When cool, the narrow part of the tube, from its origin at the wide part down to the first plug (c), is filled with powdered cane-sugar (No. 50, B.P. gauge) which has been carefully dried and sterilised at 120°–130° C. The tube is again sterilised at 120°–130° for two or three hours, the greatest care being taken not to melt the sugar. After sterilisation the tube is ready for use. The wool plug is removed from the mouth and a measured volume of air is aspirated through the layer of powdered sugar by means of a small hand air-pump, the volume of air being measured by the displacement of water in a flask. Having taken the sample (5 to 20 litres), the wool plug is replaced in the neck. The powdered sugar is then shaken down into the wide part of the tube (A), and 15 c.c. of melted sterile nutrient gelatin are poured in. The powdered sugar readily dissolves in the melted gelatin, and when solution is complete a roll-culture is made in the tube, just as in Esmarch's method (p. 83). The tube is then placed in an incubator at 20° C., and the colonies are allowed to develop.

In both Frankland's and Sedgwick and Tucker's methods the sugar, after powdering and sifting and before introducing into the tubes, should be thoroughly dried by keeping in the warm incubator for several days with occasional stirring. Unless this be done, the sugar is apt to cake and discolour during sterilisation.

Soil.

The upper layers of soil contain large numbers of organisms, chiefly bacilli. The species are very varied; among pathogenic ones may be named the bacillus of tetanus and of malignant œdema. The *B. mycoides* is very abundant, and the varieties of *Proteus*, the hay and potato bacilli, are common, while the nitrifying forms are of course present, but do not develop on ordinary media.

Below five or six feet aërobic organisms become scanty, but

the anaërobic and thermophilic ones are still met with. The number of organisms present in soil is variable, from 200,000 to 45,000,000 in ordinary earth, while in dirty and busy streets there may be as many as 1,000,000,000 per grm. According to Houston, uncultivated sandy soil averages 100,000, garden soil 1,500,000, and sewage polluted 115,000,000 per grm.

Houston[1] found that in virgin soils the *B. coli*, *B. Welchii*, and streptococci are practically absent, but that in soils polluted with animal excrement by manuring or otherwise the spores of *B. Welchii* are present in great abundance, also *B. coli* and streptococci if the pollution be of *recent* date.

The length of time pathogenic bacteria retain their vitality in buried corpses has been the subject of experiment by Lösener,[2] who injected cultures into the bodies of pigs, which were then wrapped in linen, placed in wooden coffins, and buried. The conclusions he arrived at were that, provided the soil has good filtering properties, there is practically no chance of the dissemination of a virus.

Klein,[3] experimenting with the bacilli of diphtheria, cholera, plague, typhoid fever, etc., also found that the vitality and infective power of these organisms passed away in a comparatively short time, in most cases within a month.

On the survival of the typhoid and cholera organisms in soil see also pp. 383 and 461 respectively.

Examination of Soil.

The bacteria in the soil may be examined by adding traces of the soil to sterile nutrient broth, thoroughly crushing and soaking it, and then making plate or roll cultures, aërobic and anaërobic.

To make anything like an accurate quantitative examination is almost impossible. Weighed amounts of the soil,

[1] *Rep. Med. Off. Loc. Gov. Board* for 1889–1900.
[2] *Centr. f. Bakt.* (1[te] Abt.), xx, 1896, p. 454.
[3] *Rep. Med Off. Loc. Gov. Board* for 1898-99, p. 344.

after thorough pulverisation is an agate mortar, may be introduced into sterile test-tubes and thoroughly exhausted by repeated washing with sterile water or broth, plate cultivations being made with the washings.

Various boring apparatus have been devised for withdrawing soil from different depths.

Sewage.[1]

Sewage is exceptionally rich in organisms, but the numbers present are variable. Jordan in Massachusetts found an average of 708,000 per cubic centimetre. Laws and Andrewes found from 905,000 to 11,216,000, the latter being the highest number obtained. The number of organisms naturally varies at different seasons and with the amount of dilution. The organisms present are very varied, but moulds, yeasts, and sarcinæ only occasionally occur. A few micrococci are met with and streptococci are present in considerable numbers, at least 1000 per c.c., but bacilli, especially liquefying forms, largely predominate. The commonest species are the *B. fluorescens liquefaciens* and varieties, several varieties of *Proteus*, the *B. filamentosus*, varieties of the *B. mesentericus*, *B. mycoides*, *B. subtilis*, *B. cloacæ*, and the colon bacillus. The latter numbers from 20,000 to 2,000,000 per c.c., and the other bacilli mentioned number 200,000 to 2,500,000 per c.c. Many anaërobic sporing bacilli are also found, especially the *B. Welchii*, the spores of which number from 30 to 2000 per c.c., averaging 500–600. Foreign bacteria introduced into sewage are probably soon suppressed by the predominant species of the sewage.

The air of well-ventilated sewers differs but little from that of the external air, and the organisms in it contrast with those of sewage by the abundance of moulds. Specific organisms may, however, gain access to it (p. 340).

The powerful liquefying and solvent actions of the bacteria

[1] See various *Reports to the London County Council* by Clowes, Houston, Laws and Andrews; Klein, Houston, *Reps. Med. Off. Loc. Gov. Board* for 1897–1904; *Rep. of the Sewage Commission*.

present in sewage have suggested a means of dealing with sewage so as to make use of these properties, and many bacterial systems of sewage disposal have been devised. The principle most widely adopted is to run the sewage into large covered reservoirs (septic tanks), where it remains at rest for twenty-four to forty-eight hours. Here it is under practically anaërobic conditions, and anaërobic bacteria exert their action on the solids, partly dissolving them, partly disintegrating them, with the formation of a sludge which has to be cleared out from time to time. From the septic tanks the sewage passes on to beds composed of broken brick, coke, or some similar material, through which it slowly percolates, and here it is subjected to the action of aërobic organisms, which complete the decomposition to such an extent that the effluent does not affect fish life nor putrefy, so that it may be run into a stream without causing a nuisance. Four sets of these aërobic bacterial beds are usually provided, each set being worked in turn for six hours and resting for eighteen hours during the twenty-four hours. The effluent from such bacterial beds may contain as many bacterias as, or more than, the sewage itself. Pathogenic organisms may be present in it, for Houston found that the *B. pyocyaneus* added to the beds soon appeared in the effluent.

On the survival of the typhoid and cholera organisms in sewage see pp. 383 and 461 respectively.

Examination of Sewage and Sewage Effluents.

To ensure a fair average sample, the sewage or effluent should be collected in small portions at intervals. The portions are mixed, strained through muslin, and dilutions of 1 in 10, 1 in 100, 1 in 1000, and 1 in 10,000 made with sterile tap-water. These are then examined according to the following scheme:

Tests.	Procedure.	Amount of sewage in c.c.
1. Total number of bacteria	Gelatin and agar plate cultivations	0·001, 0·0001, 0·00001
2. Number of spores of aërobes	Gelatin plate cultures with material previously heated to 80° C. for ten minutes	1·0, 0·1, 0·01
3. Number of spores of anaërobes	Agar plate cultures with material previously heated to 80° C. for ten minutes and incubated anaërobically	1·0, 0·1, 0·01
4. Number of organisms liquefying gelatin	*Surface* gelatin plates	0·001, 0·0001, 0·00001
5. Spores of *B. Welchii* (*enteritidis sporogenes*)	Milk cultures heated to 80° C. for ten minutes and incubated anaërobically	0·1, 0·01, 0·001
6. Number of *B. coli*	*Surface* plates of Conradi - Drigalski, or bile-salt media, etc., as described for water (p. 623)	0·001, 0·0001, 0·00001
7. Number of streptococci	*Surface*-plates of Conradi - Drigalski medium (p. 625)	0·01, 0·001, 0·0001

EFFLUENTS ONLY.

8. Incubate some of the effluent in beakers at 22° C. and 37° C. for some days. A good effluent should yield little or no unpleasant odour (an unpleasant odour indicates the presence of decomposable organic matter, and such an effluent might give rise to a nuisance).
9. Place a gold-fish or two in a bowl of the effluent. The fish will live in, and be unaffected by, a satisfactory effluent. (This may be done only by a licensee under the Vivisection Act.)

Milk.[1]

Milk is an admirable nutrient soil for the develop-

[1] See Houston, *Rep. to the London County Council*, No. 933, 1905; MacConkey, *Journ. of Hygiene*, vol. v, 1905, p. 333; Hewlett and Barton, *ibid.*, vol. vii, 1907, p. 22; Savage, *Rep. Med. Off. Loc. Gov. Board* for 1909–10, p. 474; Swithenbank and Newman, *Bacteriology of Milk.*

ment and multiplication of micro-organisms, and, though sterile in the udder,[1] as delivered to the consumer may contain an appalling number of bacteria. In milk as ordinarily supplied there are from one to five million bacteria per c.c., and it frequently contains ten to fifteen millions, with an average of about three to four millions. Hewlett and Barton found an average bacterial content of about 1,500,000 in London milk *as delivered at the railway termini* (the range was from a minimum of 20,000 to a maximum of 8,390,000), but this does not represent the condition of the milk *as delivered to the consumer*, for the bacteria present rapidly multiply in warm weather. Eyre[2] in the middle of summer found the following rate of multiplication :

	Microbes per c.c.
Initial content . . .	56,000
After 12 hours . . .	526,000
After 24 hours . . .	20,366,000
After 30 hours . . .	clotted

A similar specimen in the middle of winter gave the following results :

	Microbes per c.c.
Initial content . . .	20,000
After 12 hours . . .	24,000
After 24 hours . . .	43,000
After 30 hours . . .	280,000

In New York, Park estimated the average bacterial content of milk as supplied to the consumer at 1,000,000 per c.c. in winter and 5,000,000 per c.c. during the hot months. Eyre (*loc. cit.*) states that, as the result of his observations, the numbers are in London about 3,000,000

[1] The "fore" milk may contain organisms which have lodged in the milk-ducts, and it is extremely difficult to obtain completely sterile milk.

[2] *Journal of State Medicine*, vol. xii, 1904, p. 728.

to 5,000,000 in December, January, and February, and 20,000,000 to 30,000,000 in June to September, smaller numbers than these always being associated with the presence of boric acid or formaldehyde. Even in so-called sterilised milks bacteria are rarely completely absent.

Cream is even richer in bacteria than milk, and averages about 8,000,000, and may contain as many as 30,000,000 organisms per c.c.[1] Although all the ordinary species may be met with, milk has a bacterial flora largely its own, comprising many forms producing lactic and butyric acid fermentations. Organisms also occur having more or less specific effects, and giving rise to bitter milk, viscid milk, etc. The lactic ferments are mostly non-sporing, the butyric chiefly sporing, species. The commonest of the lactic ferments is the *B. acidi lactici*, which has some similarity to the colon bacillus (see table, p. 400). Another common lactic organism is the *Oïdium lactis*, a mycelial form, the colonies of which appear as little fluffy tufts. In addition to the organisms named, pathogenic species may be met with—viz. the tubercle, diphtheria, typhoid, paratyphoid, Gartner, dysentery, and comma bacilli, the *M. melitensis*, *M. pyogenes*, and the *Streptococcus pyogenes* (lactic-acid-forming streptococci are also common). The *B. coli* and *B. Welchii* are generally present in milk, and the *B. lactis aërogenes* is sometimes found (p. 410). Scarlatina (see "Scarlatina") and foot-and-mouth disease may likewise be conveyed by milk, and the diarrhœa of infants is largely due to the use of milk swarming with microbes, some of which in themselves may be harmful, and which also by the products they form tend to set up gastro-enteritis. The percentage of samples infected with tubercle bacilli

[1] Russell, *Centr. f. Bakt.* (2[te] Abt.), i, 1895, p. 741.

varies much: Barton and Hewlett found only one out of 26 samples taken at London railway termini. The supply of the large dairy firms is also comparatively free from tuberculous infection, as considerable precautions are taken to exclude tuberculous animals. For the quarter ending March 31st, 1911, of 760 samples examined for the London County Council, 106, or 13·9 per cent., were found to be tuberculous, and since 1907 of 5698 samples, 640, or 11·2 per cent., proved tuberculous (see also p. 337). A poisonous body, tyrotoxicon (p. 39) has been isolated from milk and milk products. Sources of contamination and infection are derived from the insanitary conditions of many farms and dairies and the dirty methods of those handling the milk. In order to render milk wholesome for infants and free from infective organisms under the *present* conditions of supply, two methods may be adopted—sterilisation and pasteurisation. To ensure sterilisation it is necessary to heat the milk to boiling-point for six hours, or to expose it for a shorter period to steam under pressure. Such treatment, however, markedly alters the flavour of the milk, and is said to diminish its nutritive value. If the milk be heated to a temperature not exceeding 70° C., the flavour and nutritive qualities are far less altered, while the pathogenic species are all destroyed. This method is termed "pasteurisation," and consists in heating the milk to about 68° C. for twenty to thirty minutes. Pasteurisation destroys 92–99 per cent. of the total organisms present. The objections to pasteurised milk are that the natural enzymes present in fresh milk are destroyed, the lactic-acid-forming organisms are killed, and if the treated milk be kept, the residuum of resistant putrefactive, etc., bacteria multiply enormously, without obvious change in the milk. Behring has advocated the

addition of formaldehyde to all milk used for the feeding of children. Another method for sterilising milk is the Budde process,[1] in which the milk, after the addition of hydrogen peroxide, is heated for three hours to 52–53° C. All non-sporing organisms are destroyed, and the added hydrogen peroxide is decomposed into H_2O and O.

All milk should be distributed in closed bottles, and pasteurised milk should be consumed within thirty-six hours of treatment.

The thermal death-point of pathogenic organisms in milk is as follows:[2]

Organism.	Temperature.	Period of Exposure.
B. tuberculosis	60° C.	20 min.
B. typhosus	60° C.	2 min.
B. diphtheriæ	60° C.	1 min.
Spir. choleræ	60° C.	1 min.
B. dysenteriæ	60° C.	10 min.
M. melitensis	60° C.	20 min.

The thermal death-point of tubercle bacillus, especially in milk, has been the subject of some controversy (see also p. 325). De Man found that an exposure of fifteen minutes at 65° C. was necessary to destroy the infective properties of tuberculous milk. Bang, of Copenhagen, considers that pasteurisation cannot always be relied upon, and recommends that milk should be heated to 85° C. The writer found that the vitality of the ordinary non-virulent laboratory cultures was destroyed by a temperature of 60° C. acting for ten minutes, and that the infective properties of tuberculous sputum, tested on guinea-pigs, were destroyed by a temperature of 65° C. acting for fifteen minutes in five out of six instances. Woodhead's experiments (First Royal Commission on Tuberculosis) gave irregular results which seem to be explained by

[1] Hewlett, *Lancet*, 1906, vol. i, January 27th.

[2] Rosenau, Hygienic Lab., Washington, *Bull.* 42, 1908.

Theobald Smith's careful work.[1] This showed that tuberculous milk was rendered non-infective by heating to 60° C. for ten to fifteen minutes, *provided there was no formation of a surface scum*; the latter seems to protect the bacilli. Russell and Hastings[2] confirmed Smith's experiments, and assert that it is sufficient to heat milk to 60° C. (140° F.) *in a closed receptacle* for a period of not less than twenty minutes in order to destroy the tubercle bacillus. The surface scum forms on milk only when it is heated in contact with air; all pasteurisers, therefore, should be closed vessels. The writer has devised a simple form of domestic pasteuriser, which is made by Messrs. Allen & Hanbury.

The occurrence of so-called leucocytes and pus-cells in milk must be considered. A certain number of cells resembling polymorphonuclear leucocytes are always present in milk, more numerous during the first week of lactation and then accompanied by colostrum corpuscles. An excess of these cells *may* indicate some local inflammatory affection of the udder, or, if streptococci and blood are present in addition, suppuration, but not necessarily, for Russell and Hoffman, and Revis have shown that a very large cell count (500,000–1,000,000, or even 10,000,000, per c.c.) may often be obtained from quite healthy cows. The nature of these cells has been the subject of an extended investigation by Hewlett, Villar and Revis.[3] Their conclusion is that the majority of these cells are not leucocytes, but are germinal cells of the secreting epithelium of the udder. Blood may also be present transitorily in health (Revis). The presence of squamous epithelial cells indicates desquamation from the teat or udder or from the hand of the milker—*i. e.* want of cleanliness.

There is no doubt that micro-organisms are far more

[1] *Journ. Exper. Med.*, vol. iv, 1899, p. 217.

[2] *17th Ann. Rep. Wisconsin Agricult Exp. Station.*

[3] *Journ. of Hygiene*, vols. ix, x, and xi,

abundant in milk as supplied to the consumer than should be. This arises from the ignorance and carelessness of those charged with the duty of providing and distributing this important article of diet. The udder and teats of the cow and the hands of the milker (who should wear a special dress) should be wiped before milking, and all vessels should be clean and steamed or scalded before use. The milk should be cooled at once, some more efficiently closed vessel than the present form of milk churn adopted, and the milk not stored, but forwarded without delay by the railway companies in special refrigerator vans. Distribution in bottles would be a great improvement.

The following might be suggested as a bacteriological standard for milk:[1] (*a*) Number of organisms not to exceed 1,000,000 per c.c.; (*b*) absence of excess of leucocytes or of pus-cells; (*c*) *B. coli*, *B. Welchii*, and streptococci should not be present in 1 c.c. or less; (*d*) the sediment after centrifugalising should be less than 100 parts per million; (*e*) the milk as delivered should not have a temperature above 10° C.; (*f*) absence of pathogenic organisms.

Sour milk.—Sour milk is used as an article of diet in many parts of the world, *e.g.* Bulgaria. In these sour milks a particular micro-organism or a variety of it, the *B. bulgaricus* or "bacillus of Massol," is generally present in association with lactic streptococci. It is a large, pleomorphic, Gram-positive bacillus, non-motile, non-sporing, growing best at about 40° C., but only in milk or in culture media made with milk or whey. It has been much employed for the preparation of a soured milk which is of considerable service in the treatment of certain disorders.[2]

[1] See "Rep. of a Committee on Milk Supply," *Philad. Med. Journ.*, October, 1900, p. 758; Park, *Journ. of Hygiene*, vol. i, 1901, p. 391; Houston, *loc. cit.*

[2] See Hewlett and others, *Brit. Med. Journ.*, 1910, vol. ii. (Bibliog.)

Examination of Milk.

Number of organisms per c.c.—This is carried out by diluting the milk to 1 in 1000—1 in 1,000,000 with sterile water, or preferably nutrient broth, as a better mixture is obtained. Plates are then made either in gelatin or in distilled water agar ($1\frac{1}{2}$ grm. powdered agar, distilled water 1 litre, Eastes), or preferably in both media.

B. coli, B. Welchii, and streptococci.—These are searched for quantitatively by the methods detailed for "Water" (pp. 609–617). Amounts of milk in decreasing decimal order from 100 c.c. to 0·000001 c.c. should be examined.

Pathogenic organisms.—The detection of these, with the exception of the tubercle bacillus, is difficult and uncertain. In all cases the milk should be centrifugalised and the deposit examined.

1. For the detection of the tubercle bacillus[1] staining methods are almost useless (except in cases of advanced tuberculosis of the udder) and inoculation must be performed. At least 250 c.c. of the milk should be centrifugalised at 2000 to 2500 revolutions per minute for an hour. As many organisms become entangled in the cream, it is advisable to stop the machine after half-an-hour, stir in the cream, and again centrifuge. The fluid is poured or pipetted off carefully, so as not to disturb the sediment, leaving about 3 c.c. in the tube. The sediment and the remaining fluid are then well mixed and about 1 c.c. is inoculated subcutaneously and intra-peritoneally into two guinea-pigs respectively (see also p. 304). For staining, a process of solution of the milk may be employed, 20 c.c. of the milk being mixed with 1 c.c. of a 50 per cent. potash solution, and heated in a water-bath until the solution turns brownish; 20 c.c. of acetic acid are then added. The mixture is shaken, heated in a water-bath for three minutes, and centrifugalised for ten minutes. The fluid is poured off, 30 c.c. of hot water are added to the sediment, and the mixture is again centrifugalised. Films are then

[1] See Delépine, *Rep. Med. Off. Loc. Gov. Board* for 1908-09, p. 134.

prepared from the sediment, and stained for the tubercle bacillus (see also p. 343).

Non-pathogenic acid-fast bacilli occur in milk (p 358).

2. The diphtheria bacillus is searched for by making serum cultures from, and inoculating guinea-pigs with, the sediment. If a diphtheroid organism is detected it must be isolated and examined by culture tests and animal inoculation.

In milk and cheese a bacillus is frequently met with closely resembling the diphtheria bacillus in its morphological and cultural characters; it is, however, quite non-pathogenic.[1]

3. The typhoid, paratyphoid, Gärtner, dysentery, and comma bacilli may be searched for by the methods given for "Water."

(4) The *M. pyogenes* and the *Streptococcus pyogenes* may be searched for by means of plate cultures on glycerin agar.

(5) *Examination of sediment.*—Houston and Savage (*loc. cit.*) have devised methods for the quantitative estimation of the sediment by centrifuging in special graduated tubes. For the microscopical examination of the sediment the milk is centrifugalised for twenty minutes at 1500 revolutions per minute, and the upper fluid is pipetted or syphoned off. Some of the sediment should be examined with the $\frac{2}{3}$ in. and $\frac{1}{6}$ in. objectives for the presence of "dirt," *e.g.* hairs, straw, etc. Three smear preparations are then made, each with four drops of the sediment, which are spread evenly over three fourths of the slide. The slides are air-dried, and may be treated with a mixture of absolute alcohol and ether for ten minutes. One slide is stained with Löffler's blue, another by Gram's method for streptococci, and a third by the tubercle method. The Löffler's blue specimen gives a general idea of the number of bacteria present, and of the presence of cells.

From what has been said above (p. 652), considerable caution must be exercised in stating the presence of pus-cells. Streptococci present are not necessarily pathogenic, as non-pathogenic lactic-acid-forming streptococci are common. For counting the number of cells present, Revis[2] employs a

[1] See *Scientific Bull.* No. 2, Health Dept., City of New York, 1895, p. 10.
[2] *Journ. of Hygiene*, vol. x, 1910, p. 58.

centrifuge tube of 10 c.c. capacity, the lower third of which is contracted to 0·8 cm. in diameter, and contains 1 c.c. The procedure is as follows:

In the tube are placed 5 c.c. of the well-mixed milk, diluted to the 10 c.c. mark with 0·8 per cent. salt solution. After inserting a rubber stopper the contents are well mixed. The tube is then centrifuged at about 2000 revolutions per minute for two minutes, the cream is broken up by violently shaking the upper part of the tube, and the rotation continued for four minutes longer. A glass rod, fitting roughly the narrow neck of the tube, is inserted, and the major part of the milk poured off, and the upper part of the tube well rinsed with water to remove cream, etc.; the contents of the narrow end down to within $\frac{1}{4}$ in. of the deposit are sucked out with a fine glass pipette, the upper part of the tube is wiped clean, and the tube is then filled to the 10 c.c. mark with salt solution. The tube, having been violently shaken till all the deposit is distributed through the liquid, is then rotated for four minutes, and the liquid down to within $\frac{1}{4}$ in. of the deposit again removed. In the case of small deposits, two to three drops of saturated aqueous solution of methylene-blue are added, and the deposit is stirred up by blowing through a fine glass capillary pipette (which is afterwards used for filling the counting chamber). After fifteen minutes, water is added to the 1 c.c. mark, and counting done in the usual way with a Thoma-Zeiss blood counter. Counting should not be restricted to the ruled spaces, but the field so arranged that a definite number of squares is included, and fields are counted all over the chamber. At least two different preparations should be made of the same deposit for counting.

Food Poisoning.—Apart from the presence of the ordinary poisons, food may be poisonous on eating—(*a*) naturally, *e. g.* certain fish, (*b*) from the results of the activity of micro-organisms with the formation of toxic products, the ordinary "ptomine poisoning," (*c*) from infection with certain organisms, particularly *B. enteritidis,* which generally induce gastro-enteritis. In the last named, symptoms do not usually

ensue until a lapse of twelve to forty-eight hours after the consumption of the food.

Meat is not likely to convey any infective disease with the exception of tuberculosis and anthrax. It may be examined by cultures and plate cultivations, and by inoculation and feeding experiments. *Tinned meats, etc.*, frequently contain sporing organisms of the *B. subtilis* and *mesentericus* groups. They may be examined by aërobic and anaërobic cultures, and by feeding mice. Poisonous ptomines are occasionally present. The *B. enteritidis* occurs in meat, and causes a form of poisoning (see p. 392).[1] In certain intoxications due to bad meat, known as "botulism," Van Ermengem isolated the *B. botulinus* (see p. 450).

Bread.—Troitzki states that new bread contains no micro-organisms, but Waldo and Walsh found that such organisms as the comma bacillus are not destroyed by passing through the ordeal of the baker's oven. Cut bread forms a good nidus for the development of pathogenic organisms.

The *Bacillus prodigiosus* may grow upon various food-stuffs, and give rise to suspicion of foul play. L. Parkes[2] describes cases of diarrhœa which he suggests were caused by this organism.

Butter contains from two to forty-seven millions of micro-organisms per gramme. Tubercle bacilli have been found in butter, and the comma bacillus artificially introduced survives for over a month. "Acid-fast" non-pathogenic forms also occur (p. 358).

For the isolation of the tubercle bacillus from butter and cheese the only certain method is by inoculation. Butter may be melted and allowed to stand in the incubator at 37° C. for some days, and the sediment inoculated. As this involves the multiplication of septic organisms, it is preferable to centrifugalise the melted butter, keeping it melted during the process, and to inoculate the sediment immediately.

Clothing, etc.—Attempts have been made to examine clothing, bedding, flock, etc., by bacteriological methods for filth contamination, but without much success.

[1] See Savage, *Rep. Med. Off. Loc. Gov. Board* for 1909–10, p. 446.
[2] *Brit. Med. Journ.*, 1905, vol. ii, 1330.

Common Organisms of Air, Water, and Soil.

Organism and its size.	Morphology.	Motility.	Spore-formation.	Gram-staining.	Growth on agar.	Growth on gelatin.	Liquefact'n of gelatin.	Growth on potato.	Litmus milk.	Glucose.	Habitat.	Other characters, etc.
1. *Micrococcus agilis*, 0·7–1·0 μ	Coccus, diplococcus, tetracoccus, chains and masses	+	–	+	Coral-pink creamy layer at 20° C.	Coral-pink	+	Coral-pink	–	–	Air, water	Does not grow at 37° C. General turbidity in broth, no film.
2. *Micrococcus candicans*, 0·8–1·0 μ	Cocci	–	–	+	White shining creamy layer	White porcelain-like	–	White porcelain-like	A	–	Air, water, milk	General turbidity in broth. Stated by MacConkey to produce acid and gas in bile-salt lactose media.
3. *B. filamentosus*, 3–5 μ	Anthrax-like	–	+	+	Wavy feathery greyish layer	Grey	+ +	Grey	A C	A	Sewage, water	Does not grow at 37° C. Strict aërobe. Broth clear with sediment.
4. *B. mycoides*, 3–5 μ	Somewhat anthrax-like	+	+	+	Grey creamy layer	Grey	+ +	Grey slimy	C *a*	A	Soil, water	Broth turbid. Colonies on agar plate woolly, tufted, and mould-like.
5. *B. megaterium*, 3–5 μ	Large rods and filaments	+	+	+	Grey creamy layer	Grey, thin, yellowish	+ +	Grey creamy	C *a*	A	Water	Broth turbid.
6. *B. mesentericus* (*vulgatus*), 2–4 μ	Slender rods and filaments	+	+	+	Dry grey wrinkled film	Grey with film	+ +	Grey or dry pinkish, crinkled, abundant	C *a*	A	Water, soil	The potato bacillus. Strict aërobe. Broth turbid with film. Varieties produce pigment (*fuscus*, brown; *ruber*, red; *niger*, black).
7. *B. subtilus*, 2–4 μ	Slender rods and filaments	+	+	+	Moist, grey, sometimes wrinkled	Grey	+ +	Grey, dull, thickish	C *a*	A	Hay, dust, soil	The hay bacillus. Strict aërobe. Spores germinate equatorially. Broth turbid with film.
8. *B. proteus* (*P. vulgaris*) 2–4 μ	Slender rods, filaments, and threads	+	–	–	Thin, moist, whitish	Grey	+ +	Slight grey	A C	A G	Soil, sewage, bowel, putrid matter	The *B. termo*. Occurs in putrefying matter. Colonies on gelatin wavy and motile. Varieties: *mirabilis*, slow liquefier: *Zenkeri*, non-liquefier.
9. *B. prodigiosus* 1–2 μ	Short rod, almost coccoid	+	–	–	Thick creamy, brilliant red	Red or pink	+	Red creamy	A C	A G	Air, water	Grows well at 37° C., but produces no pigment. Broth turbid.
10. *B. fluorescens liquefaciens* 2–4 μ	Slender rod	+	–	–	Thin creamy, fluorescent, greenish-yellow	Fluorescent, greenish-yellow	+ +	Brownish	C *a*	–	Water, sewage	Stated by Lehmann and Neumann to be identical with *B. pyocyaneus*, but non-pathogenic (p. 250). *B. fluorescens non-liquefaciens* similar, but non-liquefying.

+ = positive; – = negative, or no change; C = curdling; A = acid; *a* = alkaline; G = gas.

All the above forms are practically non-pathogenic except *B. proteus* (cystitis, abscesses, ? diarrhœa). *B. prodigiosus* is pathogenic to guinea-pigs by intraperitoneal inoculation. Chromogenic sarcinæ, *e. g. S. aurantiaca* and *flava*, torulæ, *e. g.* pink torula, and numerous other bacilli occur in air, water, and soil.

CHAPTER XXII.

Disinfection.[1]

HEAT—STEAM DISINFECTION—CHEMICAL DISINFECTANTS—THEORY OF DISINFECTION—METHODS OF DETERMINING DISINFECTANT POWER.

NATURAL agencies restrain the multiplication of disease organisms, but enough survive to determine the persistence of infective diseases, and to call for measures by which communities attempt to cope with them. These measures are broadly isolation, prophylactic inoculation, general improvement in sanitation and nutrition, and disinfection. In the present chapter the methods by which the fourth means of protection may be applied are considered. Disinfection implies the removal or the destruction of infective properties, but, for practical purposes, should be understood to mean the killing of the infective organisms to which those properties are due. For this purpose, the two agencies ordinarily used are heat and chemical action, though, in addition, other methods can occasionally be employed for destroying or excluding micro-organisms. Such are light, desiccation, and filtration.

HEAT.—*Fire* is the simplest and most efficient agent for destroying infective matter. Burning should always

[1] See Hewlett, "Milroy Lectures," *Lancet*, 1909, vol. i.

be employed where possible, as for rags, old clothing or bedding, native huts, etc.

For surfaces which would not be unduly injured, such as stables, pens, yards, etc., a torch-fire generated by means of the cyclone burner described by Forbush and Fernald has been favourably reported on by Stiles. The apparatus consists of a portable tank, from which paraffin gas oil is driven by a pump through a hose (such as is used for the delivery of oil) to which is attached a pole, consisting of an iron pipe 12 ft. long, which is protected by a covering of wood, and to the end of which is attached a cyclone nozzle. The fine spray from the nozzle is ignited, and the resulting fierce flame passed over the surfaces to be disinfected. The thorough wetting with water of all such surfaces would practically abolish danger from fire, and by proper adjustment of the power of the flame, and experience on the part of the operator, the method is an efficient one.

Dry heat may also be used, and forms the basis of some disinfectors (Ransome's), but is not nearly such an efficient means as moist heat. The objections to dry heat are, that to ensure the destruction of bacteria and spores the temperature must be high and the heating prolonged. Koch and Wolfhügel found that two hours at 150° C. did not always ensure sterilisation, and Gaffky and Löffler state that the spores of some organisms are killed only by exposure to hot air at 140° C. for three hours. Moreover, dry heat has little power of penetration, and it requires many hours for the centre of a mass of bedding, or the like, to attain the temperature requisite for sterilisation, while some articles and fabrics are distinctly injured by the prolonged heating. The highest temperature which can be safely adopted for a dry-heat disinfector is about

120° C., and then if large masses have to be treated the heating has to be continued for from eight to ten hours. A rise of 5° C. above this temperature is sufficient to damage many woollen goods, which enhances the objections to a dry-heat disinfector, as it is difficult to keep the temperature of a large chamber constant.

For the reasons given above, disinfection by dry heat is often impractible; on the other hand, *moist heat* is more effective, is found to work well in practice, and is now generally adopted. In the household, for articles which cannot be burnt, brisk boiling for an hour or so will suffice.

Steam disinfection.—For public disinfectors, steam under pressure—*i. e.* at a pressure greater than that of the atmosphere—is employed. Steam under pressure has not such a deleterious action on articles, with the exception of leather, as dry heat, while its penetrating powers are far greater. By "saturated steam" is meant steam at the temperature at which it can condense, and the temperature of the condensation point rises as the pressure increases. By "superheated steam" is meant steam at a temperature higher than that at which it can condense; therefore superheated steam has to be cooled down into the state of saturated steam before condensation ensues. If superheated steam is used for disinfection, it loses heat by conduction, and the rise in temperature of the articles treated approximately corresponds to the fall in temperature of the steam. With saturated steam, on the other hand, immediately it is cooled an enormous amount of latent heat is set free by the change in state from the gaseous to the liquid condition, therefore saturated steam is a far more efficient disinfectant than superheated steam. These considerations should always influence the choice

of a steam disinfecting apparatus for efficient working.

The Equifex disinfector is worked with saturated steam at 10 lb. pressure (239° F.). The chamber consists of a cylinder of mild steel, made without steam jacket, so as to avoid risk of superheating. The cylinder is lagged with non-conducting composition and wood, to reduce loss of heat by radiation, and, as usually supplied, is furnished with separate doors for infected and disinfected articles respectively. An arrangement can be supplied to prevent both doors being opened simultaneously. The Washington-Lyons apparatus, or its modifications, is an elongated cylindrical boiler with double walls, forming a jacket, and a door at each end. The chamber is of sufficient size to admit bedding, and is built into the partition wall between two rooms, so that each door opens into a different room. Into one of the rooms the infected articles are conveyed, and are placed in the disinfector as lightly packed as possible; when disinfected they are removed by the opposite door into the other room, thereby avoiding all chance of reinfection. Steam at a pressure of about 20 lb. is admitted into the jacket and then passes to the inner chamber, the object of the jacket being to warm the chamber, and so prevent condensation. For the same purpose hot air is sometimes injected beforehand to warm the chamber and articles, and after the steam disinfection, can again be injected for drying. The length of time required for disinfection does not exceed a half to one hour.

In Thresh's disinfector the steam is generated from a saline solution (calcium chloride), which has a boiling-point (105° C.) higher than that of water.

The thermal death-point of a number of organisms in pure

culture has been determined by many investigators. Eyre suggests the following as "standard conditions" for determining thermal death-points :

1. Length of "time exposure" to be ten minutes.
2. Emulsion to be prepared from "optimum cultivation."
3. The vehicle in which culture is suspended to be sterile salt solution or sterile distilled water.
4. Strength of emulsion to correspond to about 1 milligramme of culture per cubic centimetre.
5. Bulk of emulsion to be not less than 3 c.c.
6. Emulsion to be contained in test-tube of 1·5 cm. diameter with walls 1 mm. thick.
7. Emulsion to be exposed to moist heat in a water-bath regulated by a delicate and accurate thermo-regulator.
8. Broth cultivations and agar plates both to be used in determining the death of the bacteria, and the period of observation of these cultures to be extended, when necessary, to seven or fourteen days. The experiments to be repeated at least once.
9. Thermal death-point to be first roughly determined to within 5° C.
10. Thermal death-point to be finally determined to within 1° C., and to be defined as that temperature which causes the death of *all* micro-organisms exposed to it, within the ten minutes in these standard conditions.

Light is not used directly for disinfection, but indirectly in nature and in our homes may not be an unimportant factor. It has previously been referred to at p. 23. Sunlight, and artificial light rich in violet and ultra-violet radiations, such as that emitted by a *quartz* mercury vapour lamp, are efficient germicides. The latter has been tested by Barnard and the writer with excellent results, but, unfortunately, the germicidal rays have practically no power of penetration and are stopped even by thin glass.

Desiccation, although one of Nature's methods of

disinfection, is not made use of to any extent by man except as an inhibitory agent for the preservation of many articles of food.

FILTRATION is a method of disinfection by exclusion, and in the form of sand filtration and filtration through porous porcelain, as in the Berkefeld and Pasteur-Chamberland filters, is made use of for the sterilisation of water and other fluids.

CHEMICAL DISINFECTANTS.—A large number of chemical substances variously known as germicides, antiseptics, disinfectants, deodorants, etc., have the power of interfering with, or masking the results of, the vital activities of micro-organisms. Germicides are substances which kill bacteria or germs; antiseptics, by inhibiting bacterial development, prevent sepsis or putrefaction; and by "disinfectant" is meant a substance which prevents the action of, or destroys, infective matters, while deodorants destroy or absorb foul-smelling gases the result of putrefactive and similar processes. All germicides are disinfectant and antiseptic, but many antiseptics, though preventing or inhibiting the development of bacteria, are not necessarily germicidal.

Many *deodorants* act largely mechanically, and although often not germicidal, and hence not ideal disinfectants, are of some value in preventing the deleterious and depressing effects of the emanations from decomposing organic matter. Such are charcoal, ashes, dry mould, and peat (peat has also a germicidal action). Other deodorants, such as quicklime and chloride of lime, act chemically.

The germicides and antiseptics may be considered together, for although many antiseptics are not germicidal, all the germicides in small amounts act as antiseptics. The principal germicides and antiseptics are

the halogen elements, the mineral acids, a large number of metallic salts, phenol and many coal-tar derivatives, and various organic bodies and essential oils.

Theory of chemical disinfection.—The theory of chemical disinfection is not yet fully understood. It is probable, as suggested by Paul and Krönig, that the degree of ionisation of a solution may have an important bearing on its disinfecting efficiency.

Paul and Krönig[1] made a number of experiments on the *M. pyogenes*, and spores of anthrax, with a view of determining the effects of various acids, bases, oxidising agents, and metallic salts on bacteria. The salts of mercury, gold and silver exert a marked germicidal action, strongest in the case of mercury, while the platinum salts are almost inactive. The efficiency of mercuric chloride is markedly lessened by the presence of sodium chloride or other chlorides. Of the oxidising agents, nitric, chromic, chloric, and permanganic acids act in the order stated; chlorine has the most powerful action of the halogens. Phenol acts better in a 5 per cent. solution than in higher concentrations, and the efficiency is increased by the addition of sodium chloride, but diminished by the presence of alcohol, and under the most favourable conditions it is not such a powerful germicide as mercuric chloride. Mercuric chloride dissolved in absolute alcohol has little or no efficiency, and the addition of sodium chloride reduces its activity. Organisms in masses are less readily acted upon by antiseptics than when they are isolated.

The efficiency of a germicidal salt in solution seems to vary with its dissociation. It is believed that the molecules of a salt in solution are more or less dissociated into constituent electrified atoms or "ions," and the greater the dissociation the more active will the

[1] *Zeitschr. f. physikal. Chem.*, 1896, xxi, p. 414.

substance be as a germicide. Taking mercuric chloride, bromide and cyanide, it is found that the ionisation of the chloride is greater than that of the bromide, and this is more ionised than the cyanide, and the following results show that the germicidal power of the three is in this order :[1]

Solution.	Number of colonies which developed. After 20 minutes' treatment.	After 85 minutes' treatment.
1 mole $HgCl_2$ in 64 litres .	7	0
1 ,, $HgBr_2$,, ,, .	34	0
1 ,, $Hg(CN)_2$ in 16 litres	8	33

Since the amount of this dissociation may be greatly influenced by the presence of other substances, much caution should be exercised in adding salts, etc., to increase solubility or prevent precipitation, as the addition may seriously impair germicidal or antiseptic power (see p. 672).

The disinfection process is a gradual one. In the early stages of disinfection large numbers of organisms are killed, but the rate of killing becomes slower and slower as time elapses. Madsen and Nyman and Miss Chick[2] have found that if the results be plotted, ordinates representing the numbers of surviving bacteria, and abscissæ the corresponding times, the points lie on a logarithmic curve. The curve so obtained, in fact, appears to be similar in form to that of a " unimolecular reaction," and may be expressed by the formula $\frac{1}{t_2-t_1} \log \frac{n_1}{n_2} = K$, where n_1 and n_2 are the numbers of bacteria surviving after times t_1 and t_2 respectively, and K is a constant. In the case of disinfection of anthrax spores with phenol, Miss Chick found the mean

[1] Findlay, *Physical Chemistry*, 1905.

[2] *Journ. of Hygiene*, vol. viii, 1908, p. 92. (Summary and Bibliog.)

value of K to be 0·44. In the case of *B. paratyphosus*, however, the course of the disinfection is different unless the culture is very young, and Miss Chick concluded that the older individuals are less resistant than the younger. The progress of heat disinfection apparently follows the same course. Miss Chick asserts that the act of disinfection is a unimolecular reaction, but it is difficult to accept this view. Disinfectants in emulsion tend to be more efficient than when in solution.

Factors modifying disinfectant action.[1]—The efficiency of a disinfectant liquid partly depends on its concentration. The rate of penetration into bacterial cells decreases as the concentration increases above a certain limit. Most disinfectants yield, therefore, a greater amount of disinfectant energy per gramme-hour in dilute than in strong solutions. In oil, glycerin, or alcohol, disinfectants lose some or most of their activity. Of fats, lanolin alone seems compatible with disinfectant efficiency. Some disinfectants form an emulsion on the addition of water, and their efficiency for a given amount of active material may vary within wide limits according to the manner in which they are emulsified. The temperature at which the organism is exposed to the disinfectant has a considerable influence on the extent or rate of disinfection. Up to the optimum temperature at which the organism to be disinfected grows on the medium in which it is exposed the activity of a disinfectant may fall off as the temperature rises, owing to the increased vigour which the organism derives from the improvement in its conditions in respect of temperature. A relatively small difference of temperature—two or three degrees—may make an appreciable difference in the activity of the disinfectant,

[1] This section is largely taken from *Applied Bacteriology*, Moor and Hewlett, 1907.

and in the examination of disinfectants the failure to remember this fact has led to serious error. Above the optimum a rise of temperature increases the activity of the disinfectant, sometimes to an enormous extent. The same is sometimes the case even at temperatures below the optimum, when the organism is in unfavourable conditions for growth. A mixture of disinfectants in many cases has a more powerful effect than can be produced by either separately (Chamberland). The resistance of bacteria to disinfection by chemical agencies is extremely variable and is also selective. Bacteria of one class may be many times more sensitive to one disinfectant than to another when both substances exert an equal effect on bacteria of another class. The presence of organic matter may profoundly modify the action of chemical disinfectants, particularly those acting by oxidation, considerably reducing their efficiency.

Requirements for an efficient disinfectant.—The conditions which should be satisfied by an efficient disinfectant for general use are simple, but not easy to obtain. Because a disinfectant effect depends on the strength of the solution, the substance should have an approximately definite efficiency for particular organisms in given conditions, and for the same reason it should be permanently homogeneous. In practice disinfectants must be used with water or in an aqueous solvent; it should, therefore, yield a stable solution or uniform emulsion in all proportions. Because bacteria as presented for practical disinfection usually have some organic coating, it should be stable in the presence of organic matter; and as this coating is often of a greasy character, it should, especially if intended for use on dirty or greasy surfaces, have high solvent power for grease. For use when heat can also be applied, whereby

its activity is enhanced, unless it breaks up, it should be stable at all reasonable temperatures. These conditions may be considered to be indispensable It is further desirable that it should have a sufficiently high specific efficiency to allow of its being used in a readily diffusible dilution; that it should yield a cheap solution or emulsion, not act on metals, and be neither caustic nor toxic. Some disinfectant substances may now be considered more in detail.

Acids.—All acids have disinfectant action, and their relative values are interesting in the respect that for them a general law has been fairly well established by Von Lingelsheim, and confirmed by Boer—namely, that the efficiency varies with the degree of acidity. Solutions of acids not of equal percentage concentration, but of *equal acidity*, have approximately the same disinfectant efficiency, whatever may be the acid, and whether it be inorganic or organic.

The acids have no great practical application in disinfection. That which has been most commonly used is sulphurous acid, applied either direct from burning of sulphur (in which case it will also contain SO_3 if there is sufficient moisture to hold the sulphur dioxide in solution) or by the use of the liquefied gas. It produces a slow superficial disinfection of a weak and uncertain character even under laboratory conditions. Such experiments avoid, however, to a far greater extent than is possible in practice the difficulty of diffusion, and the unequal diffusion of sulphurous acid in air and its small power of penetration make it less efficient in practice. To obtain even the poor efficiency which is its maximum possible it is necessary for the air to be damp and the room most carefully sealed, and in these conditions it is often more injurious to the objects under treatment than to the bacteria against which it is directed.

One of the most efficient methods of applying sulphurous acid disinfection is by means of the Clayton apparatus. The gas is generated by burning sulphur in a current of air at a high temperature, and contains, in addition to SO_2, traces of higher oxides of sulphur. It is also a very efficient vermin-killer, destroying rats, cockroaches, bugs, fleas, flies, etc.

Alkalies and soaps.—The degree of alkalinity of a solution affects, but does not by itself altogether determine, its germicidal power, which is also dependent on the nature of its metal. The hydrates of thallium, lithium, barium, calcium, potassium, sodium, and ammonia have widely different efficiencies, roughly in the order named. For practical purposes only those of potassium, sodium, and calcium need be considered.[1] They exhibit notably the characteristic of all disinfectants that they work much more vigorously in hot than in cold solution. It is to the hydrates or alkaline carbonates of potassium and sodium that the soaps owe such power as they possess against naked organisms. The relative efficiency of soaps in practical disinfection may be understated by the results of comparative experiment on laboratory cultures because the resistance of the microbe itself to disinfection by chemical substances, and, indeed, by other agencies, may be small compared with the resistance offered by the envelope of grease or greasy dirt, derived from perspiration, pus, fat, and the oily grime which pervades cities and is everywhere caused by handling. A disinfectant of greater efficiency than soap on a laboratory culture may, therefore, be of much less efficiency on an infection in actual practice. Soaps are incompatible with most disinfectant substances, but not with all. Biniodide of

[1] See Forrest and Hewlett, *Journ. Roy. Army Med. Corps,* February, 1904.

mercury can be prepared with soap, and for surgical purposes is a disinfectant of high value. The "carbolic soaps" of commerce are, for the most part, worthless.

Caustic lime, used generally as a 20 per cent. milk, has considerable disinfectant power, and has been applied to the disinfection of fæces. For this purpose care has to be taken to break up any lumps of excreta, and whenever practicable a heat process, of which the efficiency and rapidity may be greatly increased by an alkaline disinfectant, is much to be preferred. Lime is inefficient against the more resistant organisms, and lime-washing cannot be considered a sufficient precaution against them or against infections, such as those of scarlet fever and smallpox, of which the exciting organism is unknown.

Halogens.—The disinfectant values of dry chlorine, iodine, and bromine are low. Both in a dry and a damp state chlorine is inconvenient, and the others are costly; and the use of halogens is therefore practically confined to solutions, notably "chloride of lime" (a mixture of calcium hypochlorite, hydrate, and chloride) and hypochlorite of soda (chloros). These have a powerful effect on laboratory cultures, but in practice need to be used in excess proportionate to the amount of organic matter which may be present. Thus, for instance, a 1 per cent. solution of hypochlorite of soda mixed with an equal volume of urine loses the whole of its available chlorine almost immediately, and becomes inert as a germicide. Where the amount of organic matter is small, and the objects are not likely to be injured, the hypochlorites are among the best of known disinfectants, provided they are used fresh. The slow addition of hydrochloric acid, yielding nascent chlorine, increases the activity of a hypochlorite considerably. A solution of iodine is now used for skin disinfection in surgical

practice. Iodine trichloride is a powerful disinfectant, of which the use has been suggested, among other purposes, for the sterilisation of water. Nessfield has suggested the use of chlorine for sterilising water on the large scale, and iodine for the same purpose on the small scale (p. 633). Chloride of lime or other hypochlorite may be used for sterilising water on the large scale (p. 634).

Other inorganic substances.—Solutions of salts of mercury exercise a powerful disinfectant action in proportion to the amount of dissolved metal which they contain. The most commonly used is the perchloride (corrosive sublimate). Apart from its extremely poisonous character, it has the disadvantage of forming with albuminoid substances both insoluble and soluble compounds of little or no germicidial value, sulphuretted hydrogen converts it into the insoluble and inert sulphide, and it acts on some metals. The addition of acids or salts (*e. g.* hydrochloric or tartaric acid or sodium or ammonium chloride) prevents or largely reduces the formation of insoluble compounds; but it does not prevent the reactions resulting in soluble substances, it may reduce the germicidal power, and the action of perchloride in the presence of albuminoids is therefore very variable. The reduction in germicidal power by addition of sodium chloride is well seen from the following results (Finlay, *loc. cit.*):

16 litres of solution contained	Number of colonies developing after treatment for 6 minutes.
1 mole $HgCl_2$	8
1 ,, $HgCl_2$ + 1 mole NaCl	32
1 ,, $HgCl_2$ + 2 moles NaCl	124
1 ,, $HgCl_2$ + 4 ,, NaCl	382
1 ,, $HgCl_2$ + 10 ,, NaCl	1087

Extremely high values were at one time given for the

germicidal efficiency of corrosive sublimate. This is now known to have been due to its powerful *inhibitory* action, traces of the substance carried over into the subcultures preventing growth (see p. 680).

The Local Government Board recommended the following solution of corrosive sublimate for disinfecting purposes :

Corrosive sublimate . . .	$\frac{1}{2}$ oz.
Hydrochloric acid . . .	1 oz. fl.
Anilin blue	5 gr.
Water	3 gals.

This forms a solution of 1–900 nearly ; it would be preferable to use 1 oz. of corrosive sublimate.

The biniodide is also a powerful disinfectant when dissolved in potassium iodide. It is not affected by albuminoids nearly as much as is perchloride, and may be incorporated with soap.

Soluble silver salts are powerful disinfectants, weaker than mercuric chloride, but far less sensitive to albuminoids ; in blood-serum, for instance, silver nitrate is several times as powerful as corrosive sublimate. They are incompatible with chlorides, except in certain organic combinations, from which silver chloride is only partially precipitated. Silver salts are poisonous, though less so than those of mercury.

Iron and zinc salts have been credited with useful disinfectant action ; but, in fact, their value is very small, and no practical account need be taken of them. A very strong antiseptic power has been attributed to copper salts, which, according to some experiments, exercise a sufficient disinfectant action on sporeless organisms, such as the *B. typhosus*, to enable drinking water to be sterilised from such infections by the small quantity of copper which it dissolves (p. 633).

There is some ground for connecting the disinfectant

action of metallic salts with a reducing action on some forms of protoplasm, as pointed out by Loew.

The permanganates have considerable germicidal power when in strongly acid or alkaline solution, but the readiness with which they are are affected by organic substances makes them unsuitable for practical use. Peroxides and ozone are open to the same objection, and have less disinfectant power. Hydrogen peroxide is used in the Budde process for sterilising milk (p. 651), and ozone has been practically applied in the sterilisation of water supplies (p. 634).

Organic substances.—The methane and the aromatic series furnish the disinfectants which are most important in practice.

Alcohol itself possesses some disinfectant power for sporeless organisms, but only when absolute or in very strong solution.

Formaldehyde is by far the most important of the methane group. It can be applied either as a solution (formalin) or as gas. The gas can be produced by the incomplete combustion or oxidation of methyl alcohol, by the evaporation, with or without pressure, or spraying of formalin, either alone or mixed with calcium chloride or glycerine, by the depolymerisation by heat of the solid polymer paraformaldehyde, or by mixing this substance with potassium permanganate. Many apparatus have been designed for the production of formaldehyde gas for disinfection. In any form the gas seems to give little more than superficial disinfection, and to require precautions to ensure diffusion throughout the atmosphere of a room. The conditions desirable for disinfection by formaldehyde gas are saturation of the air with moisture, maintenance of a good room temperature, sealing of the room, the use of at least 60 grm. of formaldehyde per 1000 cubic feet (preferably more, up

to 120 grm.), and in the case of large rooms mixture of the gas with the air of the room, either mechanically or by the provision of a multiplicity of inlets for the gas into the atmosphere. By the use of a vacuum formaldehyde can be evaporated in a closed chamber at temperatures indifferent to many substances which will not stand steam at 100°, and considerable penetration can be obtained (Defries process). As a spray formalin can be be used in any ordinary apparatus. Formalin seems to have a very slow germicidal action, for tested by the Rideal-Walker method, its carbolic co-efficient is only about 0·7 for the *B. typhosus.* Yet 2 per cent. formalin kills anthrax spores in two or three days and gaseous formaldehyde is similarly active.

Of the aromatic series, the number of substances and preparations is extraordinarily large. The standardisation of methods of examination will, it is to be hoped, eliminate the less efficient.

The best known is phenol (carbolic acid). Its saturated solution contains about 9 per cent. It is only slightly affected by albuminoids, and generally is stable in the presence of organic matter at ordinary temperatures. Its compounds, when it forms any, have themselves some disinfectant action. With acids this action is usually greater than that of pure phenol, with alkalies less. Light tends to decompose it, but the efficiency is not affected. It is poisonous and caustic. For practical uses its chief value is as a standard, as its disinfectant value is comparatively low, and for spore-bearing organisms it is practically useless. Like the cresols, its efficiency is greatly increased by the addition up to saturation of common salt or hydrochloric acid. The following results well demonstrate the increased germicidal power of phenol by additions of sodium chloride (Findlay, *loc. cit.*):

Solution	Anthrax spores treated. Number of colonies developing after treatment (days)			
	0	1	3	7
3 per cent. phenol	6300	1390	1260	950
3 ,, ,, + 1 per cent. NaCl	5720	1450	1320	360
3 ,, ,, + 8 per cent. NaCl	1940	150	50	0

Probably the addition of salt alters the distribution of the phenol between the water and the cells, the salt increasing the concentration of the phenol in the bacterial cells.

"Crude carbolic acid" consists mainly of cresols and higher phenols in proportions largely dependent on the source of the tar from which they are prepared; phenol is nearly absent from it. By themselves the cresols are extremely insoluble in water; in oil or alcohol they have little or no disinfectant value. Cresols are much reduced in efficiency by albuminoids. In saturated salt solution the disinfectant value of crude carbolic acid is greatly increased.

Ordinarily neutral tar oils with no appreciable disinfectant value are left in, or mixed with, tar distillate, and the saponified product produces an emulsion with water. Innumerable products of this type are made. Their efficiency varies not only with their active ingredients, but also with the character of the emulsions which they form, from about the same as that of phenol to about three times as much. Commercially they are known as soluble carbolic acid, soluble creosote, etc. Creolin is a type of numerous preparations of the same character. They are all poisonous and sensitive to albuminoids. If naphthalene is present in excess it is deposited in cold weather on standing. Lysol is mainly a solution of the cresols in fat or linseed oil, saponified, with addition of alcohol. It gives a clear solution with

water, having slightly less efficiency on naked bacteria than cresol, much superior solvency for grease, and equal sensitiveness to albuminoids. A number of proprietary disinfectants of high germicidal power are now to be obtained. Such are cyllin, McDougall's M.O.H. fluid, izal, kerol, etc. The active agents appear to be oxidised hydrocarbons without phenol and cresol, in emulsion in glue, soaps, oils, etc., and they are comparatively non-toxic. The active principle of cyllin is an oxidised hydrocarbon, having a di-phenyl nucleus in place of the single phenyl present in carbolic acid; it is insoluble in water, hence for the purpose of even distribution in water it is emulsified with a neutral hydrocarbon oil. The finished product contains 50 per cent. of the active principle, and is free from carbolic acid and its homologues. The active principle of kerol consists of oxidised hydrocarbons with a di-phenyl nucleus and contains no phenol or creosol. The germicidal efficiency, expressed as the carbolic-acid coefficient (p. 682), of a number of substances is given in the table on the next page.

Some of the *anilin dyes*, especially purified methyl violet or pyoctanin, have been claimed to be powerfully antiseptic in solutions of 1–500 to 1–1000.

Chloroform is a powerful antiseptic, but at least 1 per cent. must be present to act as a germicide; it is costly, and not much used as a practical disinfectant, but in bacteriological and physiological chemistry is a useful antiseptic for preserving solutions which putrefy easily.

Iodoform is valuable for dusting wounds, though its penetrating odour is objectionable, and has led to the introduction of many substitutes. Its value as an antiseptic has been greatly discussed; micro-organisms will develop in nutrient media containing a considerable

[1] Fowler, *Journ. Roy. Army Med. Corps*, July, 1907.

Carbolic Acid Co-efficients obtained by the Rideal-Walker Method[1] (*p.* 680).

Disinfectant.	Observer.	Date of experiment.	Organism.	Carbolic acid co-efficient (carbolic acid = 1).
Absolute alcohol . .	Fowler	8·05	*B. typhosus*	0·03
Boric acid . . .	Walker	10·04	,,	0 (?)
Chinosol . . .	Fowler	11·03	,,	0·15
Chloros . . .	,,	1·04	,,	21·0
,, (with 50 per cent. urine) . .	Walker	7·06	,,	8·0
Copper sulphate . .	,,	6·04	,,	0·04
Cyllin*. . . .	Fowler	11·06	,,	14·0
,, (with 50 per cent. urine) . .	,,	5·06	,,	11·0
Cyllin	Klein	5·05	*M. pyogenes*	9·3
,, ...	Simpson and Hewlett	6·06	*B. pestis*	34·0
Formalin . . .	Fowler	3·05	*B. typhosus*	0·7
Hydrochloric acid .	Walker	2·05	,,	11·0
Izal*	Fowler	3·06	,,	11·0
Kerol*	,,	9·06	,,	12·0
,, (with 50 per cent. urine) . .	,,	8·06	,,	8·5
Little's phenyle . .	,,	5·04	,,	2·0
Lysol	,,	2·06	,,	2·5
Mercuric chloride .	,,	8·05	,,	1000·0
,, ,, .	Walker	8·05	,,	400·0
Potass. permanganate	Fowler	8·05	,,	42·0
,, ,, (with 3 per cent. organic matter) . . .	Walker	1·07	,,	1·0
Zinc chloride . .	,,	1·06	,,	0·15

* The germicidal efficiency of these substances has been increased since the date of the experiments recorded, and they now have a carbolic-acid co-efficient of from 16 to 20–22.

proportion, but probably when in contact with living cells a decomposition is effected, free iodine being liberated, hence its value.

The essential oils, *peppermint, mustard, cloves, thymol,* and *menthol,* are powerfully antiseptic.

Disinfectant powders at best exert but a superficial

[1] Fowler, *Journ. Roy. Army Med. Corps,* July, 1907.

action. They act chiefly as deodorants, but may be useful in preventing the breeding of flies in garbage, etc.

It is useless to add a small quantity of disinfectant to a large volume of fluid or solid; the disinfectant must be added in sufficient amount so that the mixture contains the minimum percentage which has been found by experiment to be efficient. For this reason the attempt to disinfect sewers, sewage, streets, etc., by relatively small quantities of disinfectants is useless, and the money so wasted would be far better employed in providing more water for flushing purposes.

In medical practice, while antiseptics can be applied locally with success and, to some extent, for disinfecting the alimentary tract, no substance has yet been discovered which can be administered with safety to such a degree as to saturate the body, and so exert a general germicidal action in infective diseases.

In surgical practice no unbiased observer can doubt the efficacy of antiseptic treatment, but many so-called "antiseptic operations" are marred by faults of omission and commission which render them far from being perfectly antiseptic. There has been some controversy between the advocates of "antiseptic" and of "aseptic" surgery. Undoubtedly antiseptics do diminish the vitality, and therefore the reparative power of the tissues and aseptic methods should so far as possible replace antiseptic ones. The skin of the patient and the hands of the operator having been disinfected as far as possible, no antiseptic should be permitted to come into contact with the wound, which may be irrigated with *warm* sterile physiological salt solution. A *dry* wound is an important element to success, and a dry, sterile, unirritating dressing should be employed. Instruments, sponges, etc., may be kept in sterile salt solution after the preliminary disinfection—by heat (not sponges) or chemicals. But the aseptic system requires more care to ensure success than the antiseptic one, and unless the assistants can be trusted and the details rigorously carried out, the latter seems preferable.

The Determination of the Germicidal Power.

For determining germicidal power on sporing organisms anthrax spores are generally used, on non-sporing organisms cultures of the *B. typhosus* are usually employed.

(1) *Thread method.*—Sterilised silk threads are impregnated with sporing and non-sporing organisms, lightly dried, and then exposed to the action of the antiseptic solution of a known strength for a given time. After treatment the threads are thoroughly washed with distilled water to remove the antiseptic, and sown on the surface of agar or other suitable culture medium. If no growth occurs the organisms are assumed to have been destroyed. As a matter of fact, however, it is extremely difficult to get rid of the last traces of the antiseptic, which may inhibit growth although the organisms may yet be alive, a fallacy which caused an exaggerated value to be assigned to many substances—for example, corrosive sublimate. The thread method may still be employed, but after treatment the threads should be sown in broth, or, better still, if pathogenic organisms be the subject of experiment, inoculated into a susceptible animal. The writer finds that in disinfection experiments with anthrax spores, surface agar is a much better medium than broth.

In experiments with corrosive sublimate, by whatever method, the last traces of the substance must be converted into the inert sulphide by treatment with hydrogen or ammonium sulphide.

(2) *Garnet method.*—Small garnets the size of a pea are sterilised, soaked in a suspension or a broth culture of the organism, removed and dried. The garnets with the organisms attached are then soaked in solutions of the disinfectant of known strengths for various periods of time; they are then removed from the solution, well washed with sterile water, and finally placed in tubes of broth.

(3) *Rideal-Walker or drop-method.*—Moor first suggested that the germicidal efficiency of a disinfectant might be compared with that of a standard solution of carbolic acid, which has a definite composition, is stable, and can be accurately standardised, and Rideal and Walker devised an

ingenious and simple method for carrying this out. A special test-tube rack is very convenient (Fig. 69), in which the lower tier has five holes which hold three or four tubes containing the solutions of decreasing strengths of the disinfectant to be tested, and two tubes or one tube containing standard carbolic acid solution of known strength for comparison. The upper tier has thirty holes in two rows, and spaced into six sets of five holes each. These hold tubes of sterile nutrient broth which are numbered from 1 to 30. The test is usually made with a broth culture of *B. typhosus*,

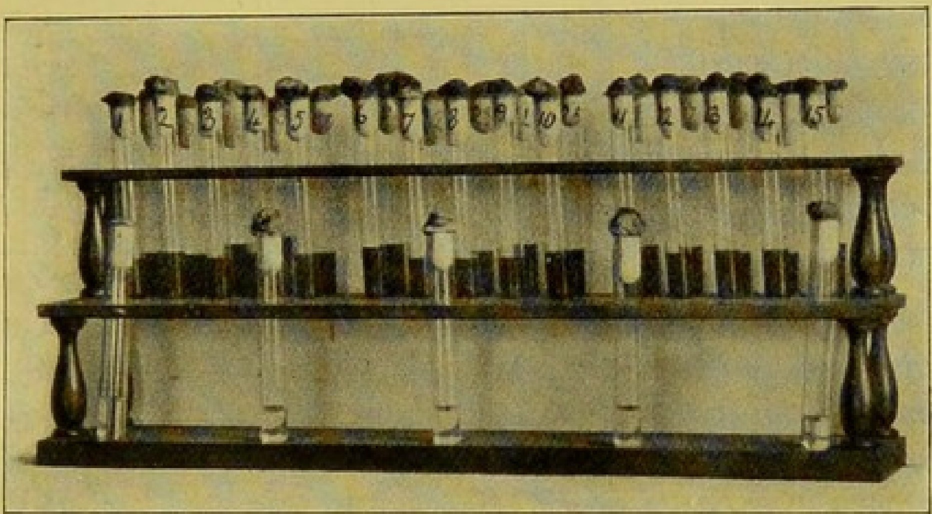


Fig. 69.—Test-tube rack with test-tubes arranged for the Rideal-Walker method of testing disinfectants.

but other organisms may be employed. The process is as follows: The five tubes in the lower tier each contain 3 c.c. of the disinfectant and carbolic solutions. Into each in succession, at intervals of half a minute, three drops of the typhoid broth culture are added with a pipette. Half a minute after the *last* tube has been inseminated, a loopful is taken from the *first* tube and inseminated into the first broth tube, and this process is repeated at half-minute intervals until all the broth tubes have been inoculated. The inoculated broth tubes are then incubated at 37° C. for three days, and the occurrence or not of growth is taken as indicating the killing or non-killing of the organism respectively. Obviously the first set of five broth tubes inoculated are subcultures in

which the organism has been acted upon by the disinfectant and carbolic solutions for two and a half minutes, the second set for five minutes, and so on. The results (taken from an actual test) may be charted as follows:

B. typhosus, 24-hour broth culture at 37° C.

Room-temperature 60° F.

Disinfectant.	Dilution.	Time culture exposed to action of disinfectant (in minutes).						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of incubation.	Temperature.
X	1–1400	+	*	*	*	*	*	3 days	37° C.
X	1–1500	+	+	*	*	*	*		
X	1–1600	+	+	+	*	*	*		
X	1–1700	+	+	+	+	*	*		
Carbolic	1-100	+	+	+	*	*	*		

+ = growth in the sub-cultures. * = no growth in the sub-cultures.

From this it will be seen that the disinfectant X in a solution of 1 in 1600 kills in the same time (7½ minutes) as carbolic 1 in 100. This result is expressed as a coefficient obtained by dividing the strength of disinfectant by the strength of carbolic which kills each in the same time; in the present instance the co-efficient is $\frac{1600}{100} = 16{\cdot}0$, and this figure is known as the "carbolic acid coefficient."

If nothing is known about the strength of the disinfectant, some preliminary experiments should be performed with dilutions at wide intervals as regards strength (*e. g.* 1–100, 1–500, 1–1000, 1–1500, 1–2000, etc.), and when the limit has thus been approximately ascertained, the test is performed as above.

Precautions to be taken in carrying out the test.—(1) The *culture* should be a broth one about twenty to twenty-four hours old, and should be free from clumps; this may be attained by filtration through paper.

(2) The *carbolic acid* should be kept in the form of a 5 per cent. aqueous solution standardised by the bromine method. Failing this, the solutions may be made with the *acidum carbolicum liquefactum* of the Pharmacopœia, which contains

100 parts of phenol in 110, but is not absolutely constant in composition.

(3) All *measures*, *pipettes*, and *test-tubes* used for making dilutions should be sterile.

(4) The *dilutions* of the disinfectant and carbolic should be made with sterile distilled water.

(5) The *broth* used for culturing and subculturing should have the following composition:

Lemco	20 grm.
Peptone	20 grm.
Salt	10 grm.
Water	1000 c.c.

The medium should be standardised to a reaction of + 10 (Eyre's scale).

(6) The *loop* used for subculturing should have an internal diameter of 3 mm., and be made with platinum wire of 27–28 B.W.G.

(7) *Growths* in the subcultures should be obtained in those taken at not less than two and preferably at three of the time intervals ($2\frac{1}{2}$, 5, and $7\frac{1}{2}$ minutes) from both the disinfectant and the carbolic solutions which correspond.

(8) The *temperature* at which the determination is made should be noted, and the strength of carbolic varied accordingly (1–100 for 56°–62° F., 1–110 for 62°–67° F., and 1–120 for 67°–73° F. for *B. typhosus*), or the determination may be made at a standard temperature (*e. g.* 20° C.) by warming (or cooling) the disinfectant and carbolic tubes in a water-bath.

(9) When the organism does not form a uniform culture in broth, a suspension of an agar or other culture must be made in water and filtered. Sub-culturing in some cases (*e. g.* with *B. pestis* and *B. anthracis*) must be made on agar or other suitable culture medium.

The method is an admirable one for determining the relative efficiencies of disinfectants on *naked* organisms in the *absence* of organic matter. But in practice disinfection is almost always carried out in the presence of organic matter, and various suggestions have been made with a view of

introducing this factor into the test, for the presence of organic matter may reduce the carbolic-acid coefficient of many disinfectants (see pp. 668, 674, and table, p. 678). Among the substances suggested are urine, fæces, 2 per cent. suspension of dried and sterilised fæces (Martin and Chick), and milk. Kenwood and Hewlett found that the presence of urine or fæces reduced the carbolic acid coefficient of some proprietary disinfectants to a greater relative extent than that of carbolic.

The method is also sometimes somewhat erratic in practice, and a number of determinations may be needed before the strengths of disinfectant and carbolic which coincide are found. Occasionally also two strains of *B. typhosus* may differ widely as regards the germicidal action of the disinfectant on them, while they are practically identical as regards the germicidal action of the carbolic.

Woodhead and Ponder have proposed a modification of the method. In this, *B. coli* is used as the test-organism and bile-salt peptone water as the culture medium, a platinum spoon being used for culturing, and more cultures at shorter intervals up to half-an-hour are made.

4. Volatile disinfectants may be tested by moistening the wool plug of an agar tube, inoculating the agar, and capping with a rubber cap, and observing whether any growth occurs.

5. Volatile disinfectants may also be tested by exposing silk threads, pieces of paper or fabrics, splinters of wood, etc., impregnated with organisms, some free, others done up in packets of cotton-wool, in a room or chamber of known cubic capacity, to the action of the gas, a known amount of which is present in the chamber. After exposure for a given time, the threads are sown in broth tubes, and the tubes incubated.

On the Rideal-Walker method, etc., see Rideal and Walker, *Journ. Sanitary Inst.*, vol. xxiv, 1903, p. 424; Kenwood and Hewlett, *ibid.*, vol. xxvii, 1906, p. 1; Firth and Macfayden, *ibid.*, p. 17; Kenwood, *Public Health*, 1908; Fowler, *Journ. Roy. Army. Med. Corps*, July, 1907; Partridge, *Bacteriological Examination of Disinfectants*; Woodhead and Ponder, *Lancet*, 1909, vol. ii.

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25 millimetres	=	1 inch.
1 centimetre	=	0·39 inch.
2·5 centimetres	=	1 inch.
5 centimetres	=	2 inches.
1 gramme	=	15½ (15·432) grains.
4 grammes	=	1 drachm (apothecaries'), nearly.
28 grammes	=	1 ounce (avoirdupois), nearly.
1 kilogramme	=	2·2 pounds (avoirdupois).
0·5 kilogramme	=	1 pound (avoirdupois), nearly.
1 cubic centimetre	=	16 minims, nearly (16·23 minims).
3½ cubic centimetres	=	1 fluid drachm, nearly.
28 cubic centimetres	=	1 fluid ounce, nearly.
568 cubic centimetres	=	1 pint ($\frac{4}{7}$ litre).
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